RESUMOS

Competição de Estudantes

Student Competition
ABSTRACT 1

PRODUCTION AND PARTITION OF IVF DERIVED TRANSGENIC EMBRYOS

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Techniques routinely used to produce transgenic embryos show the disadvantage of being expensive and quite inefficient. The objective of this work was to determine the feasibility of producing genetically modified bovine embryos by IVF, through injection of oolema-cytoplasm fragments shortly incubated with exogenous DNA. Additionally, the potential of these embryos to develop after blastomere separation was evaluated with the objective of duplicating transgenic embryo production. Oolema cytoplasm fragments were incubated with two different plasmid concentrations (50 and 500 ng/ul) and were injected into oocytes prior and after IVF. Small oolema cytoplasm fragments were obtained from donor oocytes by microsurgery and transferred containing 50 or 500 ng/ul pCX-EGFP plasmid. Afterwards, oolema cytoplasm fragments were microinjected into MII oocytes (Injection Prior IVF) or into presumptive zygotes (Injection Post IVF). IVF was performed following Brackett and Oliphant protocol (1975) with 15x10⁶ spermatozoa/ml. All presumptive zygotes were cultured in SOF medium and rates of cleavage and blastocysts were evaluated on days 2 and 7. Some cleaved embryos produced by Injection Post IVF were removed of their pelliculae zone and blastomeres were cultured individually in the WOW system. In all cases, EGFP expression was determined with a fluorescence microscope under blue light (488nm) and data was analyzed by Fisher test (P<0.05). When 50 ng/ml pCX-EGFP were injected, Injection Post IVF resulted in higher rates of blastocysts than Injection Prior IVF (36/67 (54%) vs 20/65 (31%) respectively (p<0.05)). However, when 500 ng/ml were injected no blastocysts were obtained both in Prior (0/63) and Post (0/43) IVF treatments. The highest EGFP early expression rates were obtained with 500 ng/ml Injection Prior IVF (49%; 31/63); nevertheless the highest EGFP blastocysts rates were obtained after 50 ng/ml plasmid Injection Post IVF (29%; 26/36). Blastomere separation of 5 cleaved embryos resulted in 3 blastocysts (60%), 2 of them transgenic (25%; 40%). Our results show that Injection Post IVF of oolema cytoplasm fragments incubated with 50 ng/ul pCX-EGFP plasmid is the best condition both for development to blastocysts and for transgenic blastocysts production. In addition, high rates of transgenic blastocysts were obtained by our partition experiments. IVF mediated transgenesis by oolema-cytoplasm fragments injection could be considered a simplified and low costing alternative for transgenesis in livestock. As well, the blastomere separation technique could help to increase transgenic embryo production.

ABSTRACT 2

DEVELOPMENT OF BOVINE XENOTRANSPLANTED PREANTRAL FOLLICLES IN IMMUNODEFICIENT MICE SUBMITTED OR NOT TO HORMONAL STIMULATION

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Xenotransplantation of ovarian tissue has been considered a promising technique for the recovery of oocytes from domestic and wild animals. For this purpose, immuno-deficient mice are needed. Further, considering that reduction of number of animals used is desirable according to ethical principles of animal experimentation, this study aimed first to develop a technique of ovarian tissue xenotransplantation followed by single or successive biopsies (experiment I) and to verify the grafts responsivity to two different treatments of hormonal stimulation (experiment II). Twenty one ovaries were obtained from different bovine fetuses at a local slaughterhouse and were then transported to the laboratory. Small fragments of ovarian cortex of approximately 1 mm³ were divided in control or transplanted (three to five pieces) without vascular reanastomosis under renal capsule of a total of 50 immunodeficient female mice. In Experiment I, transplanted tissues were assessed by biopsy versus time after transplantation in three groups: (a) 30 (n = 11); (b) 60 (n = 07); and (c) 30 and 60 days after transplantation (n= 05), when the viability of follicular growth were evaluated by histology. In Experiment II, 32 recipient females (five of them previously submitted to two successive biopsies received hormonal stimulation of 10 IU of eCG (n=18) or 10IU of r-hFSH (N=14). Antral follicles between 2 and 6 mm were counted and oocytes were collected to IVF. In both experiments the percentages were compared by chi-square test (P<0.05). The results showed successive and spontaneous growth of preantral follicles during time of transplantation, with higher percentage of antral follicles at 60 days when compared to control ovaries (P<0.05). Results of exogenous stimulation showed antral follicles in 46% (r-hFSH) and 38% (eCG) of recipients (P>0.05). Five oocytes from each treatment were submitted to IVM and 40% of oocytes per treatment presented adequate signs of cumulus expansion and oocytes clear and homogeneouse for IVF. No cleavage was observed. In conclusion, preantral follicles xenotransplanted from bovine fetus can grow and rise, spontaneously or after stimulation, to appropriate size of antral stage and can provide oocytes for in vitro maturation, including in cases of repeated biopsies. The implementation and consolidation of this research may be an important tool for studies regarding folliculogenesis in association with other reproductive and molecular biotechniques. Keywords: xenotransplantation, ovarian biopsy, preantral follicles, immunodeficient mice, IVF.
ABSTRACT 3

SEXING BY FLOW CYTOMETRY CHANGES MORPHOMETRIC CHARACTERISTICS AND SURFACE MEMBRANE OF BOVINE SPERMATOZOA

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The atomic force microscopy (AFM) provides three-dimensional data images in nanometric scale from surface of natural structures, kept closely to its physiological state. Therefore, it is a tool to accurately evaluate the morphometry of sperm head. To date, the effects of sexing on the dimensions of the sperm head in such level of details have never been reported in the literature. The objective this study was evaluate whether sexing by flow cytometry changes the dimensions and surface of the sperm head, and whether these measurements differ between cells that bearing the X or Y chromosome. Frozen semen from four Nellore bulls was used. For all the bulls the same ejaculate was used to produce non-sexed semen (NS), sexed X (SX) and sexed Y (SY). For AFM were analysis the groups NS, SX, SY and a fourth group formed by pooling SX and SY samples (SXY). Quantitative analysis of the 400 images for groups in MFA was performed using software that considered one, two and three-dimensional morphological and structural measurements. The analysis took into account a total of 17 variables, such as mean radius, perimeter, surface area, maximum diameter and volume of spermatozoa heads. Moreover, a fourth set with 12 variables was formed using mathematical formulas and uni- and bi-dimensional measurements, which were identified as descriptors of shape. For comparisons among groups, analysis of variance was performed (P<0.05). Then, simultaneous evaluation of features by discriminator analysis was conducted, to determine to which group belong each individual cell, which had been submitted to the identical evaluations and measurements. No differences were observed between groups for all variables. However, using different parameters simultaneously in discriminator analysis, in an attempt to differentiate groups, the greatest discriminator model had 100% accuracy in separating NS, SX, SY and SXY. In that model, the features with greatest relevance to NS, SX, SY and SXY were descriptors of shape, roughness of membrane (1.39 ± 0.01 1.37 ± 0.02, 1.37 ± 0.02 and 1.37 ± 0.02), circularity ratio (0.72 ± 0.01, 0.73 ± 0.01, 0.73 ± 0.01 and 0.73 ± 0.01) degree of roundness (2.06 ± 0.01, 2.02 ± 0.03, 2.01 ± 0.02 and 2.03 ± 0.08), elongation (17.50 ± 0.02, 17.23 ± 0.07, 17.24 ± 0.21 and 17.03 ± 0.3). The distinct separation of the groups was possible due to high precision of the equipment associated with the discriminator analysis. This study identified that the process of sexing affects the shape of sperm. Moreover, it has demonstrated that sperm containing X or Y chromosome showed different changes in morphometry and surface membrane.

ABSTRACT 4

UTERINE DOPPLER INDEXES AND VASCULAR PERFUSION DURING THE EARLY GESTATION IN MARES

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Doppler ultrasonography has been recently used to study the uterine hemodynamics of pregnant and nonpregnant mares. Earlier study in mares using color-mode Doppler imaging described an increased vascularity just after day 11 of gestation (Silva et al.; 2005; BOR: 72; 755-761). However, there are no reports of color-mode Doppler imaging to produce spectral waveforms before day 10 of pregnancy. According to Ginther (2007; ed.1; 39-57), power-mode Doppler has greater sensitivity to weak blood flow and reduced blooming artifacts when compared to color-mode Doppler. The purpose of the present study was to determine the variations on Doppler indexes and to describe the vascular perfusion of the uterus of mares during the early gestation phase. Uterine vascular perfusion and mesometrial doppler indexes of pregnant mares (n=18) were evaluated every day from D0 (day of ovulation) to D20 using Power and Spectral modes Doppler, respectively. Vascular perfusion from both uterine horns (left and right) was estimated subjectively as described previously for Ferreira et al. (2008; Reproduction; 135; 541-550). To determine resistance (RI) and pulsatility (PI) indexes in spectral mode, the sample-gate cursor was placed on an artery of the mesometrial attachment as previously described (Silva et al.; 2005). Two Way Repeated Measure was used to analyze main effects of group and time. Differences between two means were evaluated by Tukey’s test. Lower RI and PI were detected (P<0.05) from D3 to D6, and a progressively decrease (P<0.05) for both indexes was observed after D8. Uterine vascular perfusion was higher (P<0.05) between D3 and D4, with a progressive increase observed (P<0.05) after D11. This is the first description of mesometrial spectral-Doppler measurements of mares during the first 10 days of gestation. These findings have potential use on the diagnoses of uterine vascular dysfunction in pregnant mares. Greater uterine and lower mesometrial vascularity doppler indexes beginning on D3 might be associated with an uterine adjustment for the entrance of embryonic vesicle into the uterus between D5 and D6. This earlier detection of increased uterine vascularity may be a result of the use of a technique with higher sensitivity to weak blood flow associated to the evaluation of both, endometrium and myometrium, as suggested previously by Ferreira et al. (2008). In conclusion, pregnant mares had a gradual increase and decrease in uterine vascular perfusion and mesometrial doppler indexes, respectively, through the first 20 days of gestation. Mesometrial arteries were successfully used to evaluate uterine hemodynamics of pregnant mares. This study was supported by FAPESP.
ABSTRACT 5

EMBRYO PRODUCTION AFTER IN VITRO GROWTH, MATURATION AND FERTILIZATION OF OOCYTES FROM GOAT PREANTRAL FOLLICLES CULTURED IN PRESENCE OF GROWTH HORMONE (GH)


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The aim of this study was to evaluate the effects of growth hormone (GH) on the survival, growth, maturation, and fertilization of goat preantral follicle oocytes that were cultured in vitro. Preantral ovarian follicles were isolated from the ovarian cortex of goats and individually cultured for 18 days in basic medium in the absence (α-MEM supplemented with bovine serum albumin (BSA), insulin, transferrin and selenium, glutamine, hypoxanthine, ascorbic acid and FSH at growing concentrations) or the presence of GH at concentrations of 10 (GH 10) or 50 ng/mL (GH 50). Follicle development was evaluated on the basis of follicular survival, antral cavity formation, follicular diameter increase, and the presence of healthy cumulus-oocyte complexes (fluorescent staining with calcein-AM and ethidium homodimer-1) and mature oocytes (labeled with Hoechst 33342). At the end of the culture, the oocytes were used for in vitro maturation (IVM) and then in vitro fertilization (IVF). Data regarding follicular survival, retrieval of grown oocytes for IVM, antrum formation and meiotic resumption after in vitro culture were compared using Chi-square test. The treatments were compared using Student-Newman-Keuls (SNK) test and for comparisons between values recorded on different days of culture, Kruskal-Wallis test was used (SAS, 1999). Results were expressed as mean ± standard deviation (SD) and differences were considered to be significant when p<0.05. The survival rates decreased significantly from days 6 to 12 but did not change from day 12 to day 18 (p<0.05). The results demonstrated that the rate of antrum formation after day 6 was significantly higher in the groups treated with GH than the control (p<0.05). The daily growth rate and the percentage of oocytes grown from follicles that were acceptable for IVM were also higher in the GH-treated groups than in the control (p<0.05). A higher percentage of oocytes in the GH 50 group underwent meiotic resumption (p<0.05), produced mature oocytes and enabled the production of a viable embryo after IVF than the control group. In conclusion, GH plays an important role in the in vitro growth and maturation of goat preantral follicle oocytes and the subsequent successful production of an embryo.

ABSTRACT 6

EVALUATION OF DNA DAMAGES FOR c’H2A.X DETECTION IN EARLY BOVINE EMBRYOS PRODUCED BY IN VITRO FERTILIZATION (IVF), PARTHENOGENESIS AND SOMATIC CELL NUCLEAR TRANSFER (SCNT)

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Embryos produced from in vitro manipulation presented DNA damages from exposure to artificial conditions. Phosphorylated H2A.X (c’H2A.X) focus formation is used as a marker for this damage. The aim of the study was to detect quantitatively c’H2A.X in early bovine embryos produced from in vitro manipulation. Cumulus-oocyte complexes from slaughterhouse ovaries were matured in modified TCM199 for 22–24 h at 39 °C and 5% CO2. Embryos were obtained by IVF, parthenogenesis or SCNT. In the case of IVF, matured oocytes were incubated with 15x106 spermatozoa/mL. Parthenotes were produced by activation of matured oocytes using 5 µM ionomycin (4 min) and 2 mM 6-DMAP (3 h). Zona free SCTN embryos were obtained by the method described by Oback et al. (2003, Cloning Stem Cells 5, 3–12). Reconstructed embryos were activated as described for parthenotes. All types of embryos were incubated in SOF at 39 °C and 5% CO2. Zona free embryos were cultured in WOW system. The detection of c’H2A.X was carried for indirect immunofluorescence on pronuclear (PN) and 2 cell (2C) embryos. Samples were evaluated by the use of confocal microscopy and were images analyzed using EZ-C12.20 and ImageQuantTL softwares. At least 10 structures were obtained for each type and stage of embryo development. Both the number of c’H2A.X focus and cromatin area with damage were analyzed using ANOVA following Tukey test (P < 0.05). For PN embryos, the mean number of c’H2A.X focus/nucleus (309.5 ± 70.2; 164.6 ± 38.2; 147.4 ± 35.1) and cromatin area with damage/nucleus (7007.1 ± 3547.7; 2837.2 ± 728.0; 3074.3 ± 659.6 pixels) did not differ among IVF; parthenotes and SCNT embryos, respectively. However, 2C embryos derived from SCNT presented a cromatin area with damage twice as large as parthenotes (P < 0.05). Moreover, SCNT embryos showed an increase in the number of c’H2A.X focus/nucleus during the development of PN to 2C embryos (147.4 ± 35.1 vs. 553.3 ± 216.0, P < 0.05). This same pattern was observed for damage cromatin area (3074.3 ± 659.6 vs. 12780.8 ± 742.6 pixels). This suggests that although DNA damage occurs, the repair mechanisms in this transition are not able to restore these embryos. Further research is necessary to elucidate the deficiencies of these mechanisms. This knowhow will help understand the processes involved in the initial stages of IVF, parthenogenesis and SCNT developments, besides being also important for other technologies such as transgenesis.
Fisiologia da Reprodução no Macho e Tecnologia do Sêmen

Male Reproductive Physiology and Semen Technology
ABSTRACT 7

EFFECT OF THE ADDITION OF ANTIOXIDANTS IN THE COLLING EXTENDERS ON THE MORPHOFUNCIONAL CHARACTERISTICS OF REFRIGERATED DOG SEMEN

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The technique of artificial insemination (AI) can be used in dogs, when natural breeding is not possible and the use of refrigerated semen is an option for this clinical application. However, the spermatozoa submitted to refrigeration and storage present a lost in there viability and suffers lipid peroxidation, losing some important characteristic for the preservation of the fertilizing capacity. The addition of antioxidant molecules in the cooling extender could reduce the oxidative stress during the storage process, that way prolonging the viability of the spermatozoa. The goal of this research is analyze the effects of the addition of an enzymatic antioxidant (catalase), comparing with a non enzymatic antioxidant (vitamin C) on the quality of the refrigerated dog semen. Semen from five dogs in perfect health and nutritional conditions of different owners was collected by digital manipulation of the penis. After collection, the semen was divided in three samples: the first was diluted in Tris extender without antioxidants (control), the second was diluted in Tris extender with the addition of catalase (300U/ml) and the third was diluted in Tris extender with addition of vitamin C (1mM) and then submitted to 96 hours of refrigeration at 5ºC. Sperm movement parameters was evaluated using computed analyses (CASA) and the plasma membrane integrity was evaluated using fluorescent probes, in moments T0 (fresh semen) and T96 (after 96 hours of refrigeration). The statistical analyses were done by the comparison of the treatment means by Tukey test, using a significance level of 5%. Plasma membrane integrity of the fresh semen (T0) had significantly better results than the refrigerated samples (T96). Referring to spermatic movement parameters, the track speed (VCL) of the refrigerated semen with the addition of catalase had a tendency to present lower results compared to the fresh semen (p= 0.0812). The beat cross frequency (BCF) of the fresh semen was superior compared to the refrigerated control and had a tendency of superiority in relation of the refrigerated sample with the addition of catalase (p= 0.0739). The other characteristics analyzed did not present significant differences between the groups. Thus, it can be conclude that the addition of antioxidant substances in the cooling extender did not had and positive effect in the quality of the refrigerated semen.

ABSTRACT 8

DNA INTEGRITY EVALUATION BY THE COMET TEST OF OVINE SPERMATOZOA CRYOPRESERVED WITH GLYCEROL AND DIMETHYL ACETAMIDE AND ADDED TREHALOSE

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The sperm DNA damage may not affect the process of fertilization and cleavage, however, changes in this structure are responsible for development defects and embryonic death. Given the importance of DNA to sperm fertility, substances that contribute to the maintenance of its integrity after thawing must be studied. Then, eleven semen samples of Santa Ines rams (n=11) were cryopreserved with the objective of verifying the effect of the dimethyl acetamide (DMA, 3%) or glycerol (GLY, 6%) in a hyperosmotic extender with trehalose (TRE, 100mOsmol) on the post-thaw DNA sperm integrity. The base extender was the Tris-egg yolk (TRIS). Then, they were formed four experimental groups: TRIS+GLY; TRIS+GLY+TRE; TRIS+DMA; TRIS+DMA+TRE. The base extender was the Tris-egg yolk (TRIS). Then, they were formed four experimental groups: TRIS+GLY; TRIS+GLY+TRE; TRIS+DMA; TRIS+DMA+TRE. The spectrum of DNA sperm damage was assessed by single cell gel electrophoresis (Comet assay). For this assessment, aliquots containing 1x10⁴ post-thawed cellwells were mixed with 120 µL 0.5% low melting point agarose at 37ºC. This mix were dispensed on a slide with 1.5% normal melting point agarose, covering it with a 2x490mm coverslip. The slides had the coverslips removed after five minutes at +4ºC and placed in lysis buffer(+4ºC, 2.5 M NaCl, 100 mM NaEDTA, 10 mM Tris, pH 10 with 1% Triton X-100 and 10mM de DL-Dithiothreitol). After 1.5h in lysis buffer, the slides were placed in a horizontal electrophoresis unit filled with alkaline electrophoresis solution at 5ºC (1 mM EDTA, 300 mM NaOH, pH 13) for 20 minutes. The electrophoresis was performedfor 15 min at 17V and 300 mA. The slides were then washed with a neutralizing solution (0.4 M Tris, pH 7) for 15 minutes, fixed with absolute ethanol before being stained with the fluorescent DNA binding dye SYBR Green I (Molecular Probes, Oregon, USA). The Comet measurements performed were tail moment (TMOM) and percentage tail DNA (TINT) using the Comet Assay II software (Perceptive Intruments, Haverhill, UK). One hundred cells were analyzed per semen sample. All the statistical analyses were performed using the SAS software version 5.0 (1996) (Proceeding MEANS and GLM – SNK test, with P<0.05). The values of TINT and TMOM were of 14.9 and 3.9 (TRIS+GLY); 14.7 and 2.9 (TRIS+DMA) and 16.6 and 4.4 (TRIS+DMA+TRE) (P> 0.05). No influence on the sperm chromatin integrity was observed by the presence of trehalose and both cryoprotectants protected similarly the ovine sperm DNA.
ABSTRACT 9

SPERM PARAMETERS OF SEMEN DOSES SUBMITTED TO THE THERMORESISTANCE TEST

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Pentoxifylline (PTX) has been successfully used in semen extenders, as a stimulant of sperm motility by promoting the increase of cAMP. The objective was to compare sperm parameters of thawed bovine semen doses, which were fractionated and diluted in four different extenders, with or without glycerol (GLY) and PTX and submitted to thermoresistance test (TRT). For each of the five replicates performed, we used ten commercial semen doses (0.5mL straws), which were thawed at 37°C for 30 seconds, forming a pool of doses. Four aliquots of 0.5mL this pool were rediluted with 1.5 mL of extenders: Botu-bov® (Biotech, Botucatu, Brazil), Botu-bov®+PTX, Botu-bov®+GLY and Botu-bov®+GLY+PTX and aliquots 2mL served as control group. The samples of each treatment were subjected to a slow TRT remaining incubated at 37°C for 5 hours. The evaluation of sperm parameters was proceeded on two times, immediately after the fractionation and after TRT, for this, was assessed a total motility (TM), progressive motility (PM), spermatic vigor (VIG). For the statistical analysis it was used SAS statistical package, version 5.0 (Procedures MEANS and LM with P <0.05). The means for TM, PM and VIG soon after the fractionation were respectively of 62.5%; 57.5% and 3 for the control group; 60.5%; 56.5% and 3 for the Botu-bov® group; 64.5%; 59.0% and 3 for the Botu-bov®+PTX group; 52.0%; 47.5% and 2.8 for the Botu-bov®+GLY group and 65.0%; 60.0% and 3 for the Botu-bov®+GLY+PTX group. At the end of TRT were noticed the following results for TM, PM and VIG, respectively, 28.0%; 23.0% and 1.2 for the control group. At the end of TRT were noticed the following results for TM, PM and VIG, respectively, 28.0%; 23.0% and 1.2 for the Botu-bov®+GLY group. The objective was to compare sperm parameters of thawed bovine semen doses, which were fractionated with the use of extenders tested, except for the Botu-bov®+GLY, without the involvement of sperm parameters evaluated. However, as there are contradictions regarding the correlation of TRT with fertility rates in vivo, studies should be conducted with the use of extenders for the fractionation of the thawed semen and in artificial insemination programs.

ABSTRACT 10

BIOCHEMICAL AND MOLECULAR CHANGES INDUCED BY LOW INTENSITY LASER THERAPY IN BOVINE SPERMATOZOA

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The low intensity laser therapy (LILT) is effective in increasing sperm metabolism with subsequent improvement in embryo rates and quality. However, it still requires better understanding of the biochemical changes resulting from LILT in these cells. The aim of this study was to characterize these changes induced in bovine sperm by LILT with Fourier transform infrared spectroscopy (FTIR). Semen straws were thawed in a water bath at 37°C for 30s and diluted at final concentration greater than 70x106 sperm/mL. For irradiation the diode laser with wavelength in the red region (λ=650nm) was chosen as the light source with power of 5, 7.5 and 10mW and irradiation times of 1, 5 and 10 minutes. Evaluations were performed immediately after irradiation and after 30 minutes of incubation. Spermatozoa were irradiated in 4 wells plates and controls were subjected to the same conditions but without light. For FTIR evaluation, samples were transferred to plates of platinum and air dried. The infrared absorption spectra was measured by the Varian 620-IR FT-IR Spectrochemical Imaging Microscope system which provided quantitative and qualitative data of proteins, lipids, carbohydrates and nucleic acids. The spectroscopic measurements were evaluated using multivariate analysis strategies applied to bioanalysis through the software Multinab. From the collected data was possible to identify the regions 1540cm⁻¹, 1580, 1740 and between 2800 and 3000cm⁻¹ as the most variable bands among groups. These regions are related to phenyl groups, amide II and specific lipid structures, respectively, suggesting a possible influence of LILT in the generation/degradation of reactive oxygen species with subsequent effects on the structure of sperm membrane. The 7.5mW power laser applied for 1 minute and the 10mW power laser applied for 10 minutes were the ones that caused major changes at the analyzed regions, and the 7.5mW showed greater heterogeneity among spectra. Based on these data we conclude that LILT with 7.5mW power laser applied for 1 minute and 10mW power laser for 10 minutes were able to induce changes in sperm metabolism, but further studies are needed to verify the role of such changes in fertility.
ABSTRACT 11

BREEDING SOUNDNESS EVALUATION OF WATER BUFFALOES FROM BAIXADA MARANHENSE: PARTIAL RESULTS OF TESTICULAR EVALUATION

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Water buffalo farms found space in Baixada Maranhense due to the species’ great adaptability to flooded fields, common in the region. Add to that, the low costs/high productivity that water buffaloes offer in an extensive and rather precarious system make its farming a favorable business to the region. However, buffalo farming needs technical support to improve its productivity rates. Reproduction is one of the key elements and has breeding soundness evaluation as its first step. This research intended to evaluate the breeding soundness of bulls servicing buffalo bulls in Baixada Maranhense. 31 buffaloes, 24 months old or older, were evaluated, presenting body score (BS) of 2.7±0.4. Partial results showed that mean scrotal circumference (SC) of these buffaloes were of 28.09cm±2.28cm (varying from 22cm until 39cm). Average right testicle height and width were of 11.01cm±1.79 and 5.07cm±0.44 respectively. And average left testicle height and width were of 11.03cm±1.61 and 5.21cm±0.57. Average consistency (in a scale of 1-5, being 1 - less consistent and 5 - most consistent) of right and left testicles were respectively of 2.38±0.56 and 2.48±0.47, being 3 the consistency most frequent (n=14), out of a variation of 1 to 3. 12 of the 31 bulls showed minor testicular alterations, such as superficial lesions (n=3), ticks (n=3). Mobility, thickness, temperature, symmetry and pain sensibility were considered normal for all bulls. The other genital organs were also evaluated and no abnormalities were found. These results showed that buffalo bulls in Baixada Maranhense have similar testicular parameters to those described by Valle (2008), however to prove that these animals are fully sound in their breeding capabilities, further analysis such as semen evaluation must be conducted. Financial support: CNPq.

ABSTRACT 12

ROUTES AND MECHANISMS OF ACTION OF NITRIC OXIDE ON THE INTEGRITY OF BOVINE CRYOPRESERVED SPERM MEMBRANE AFTER IN VITRO CAPACITATION

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The aim of this study was to evaluate the effects of nitric oxide (NO) in in vitro capacitation induced by heparin (H) of in cryopreserved spermatozoa from 3 bulls, by adding L-arginine (L-arg, precursor synthesis NO), 8-bromo-cGMP (cGMP analog) and 1H-(1,2,4) oxadiazole-(4,3-a) quinoxaline-1-one (ODQ, inhibitor of cGMP soluble). The control consisted of capacitated sperm in TALP medium-sp plus H (10 mg/mL). It was added different concentrations of L-arg (1, 2.5, 5, 10 and 50mM), 10mM cGMP and 0.1mM ODQ incapacitation medium(200mL- final volume).The plasma membrane integrity (PM), acrosomal (AM) and mitochondrial potencial (MP) were assessed using fluorescent probes: propidium iodide (PI-0.5mg/mL) and Hoechst 33342 (10mg/mL); lectin pisum sativum agglutinin (FITC-PSA-100mg/mL) and JC1 (153mM) respectively, after 4h of capacitation, in six replicates. Lipid peroxidation was evaluated using kit peroxidetect in the capacitation medium and in the sperm after 4h of capacitation, in three replicates. All reagents and kits used were from Sigma (St Louis, MO, USA). Data were analyzed by analysis of variance and means compared by the Tukey test at a 5% level of probability. The addition of 1 and 2.5mM L-arg in the capacitation medium increased PM, and AM integrity, and MP (P <0.05) compared to control. High concentrations of L-arg (10 and 50mM L-arg), increased PM and AM injuries and decreased the MP (P<0.05), compared to control and other L-arg concentrations. Addition of 0.1mM ODQ increased membrane damage and decreased the MP (P=0.05) compared to control and others treatments. The addition of 1mM L-arg to the capacitation medium previously treated with 0.1mM ODQ partially reverted of the inhibitory effect of ODQ. Ten mM cGMP were added to evaluate whether L-arg had a similar role. Addition of cGMP increased PM and AM integrity, and MP compared to control and others L-arg concentrations (P <0.05). After the addition of 10mM cGMP + 0.1mM ODQ, there was total reversal of the inhibitory effect of ODQ (P<0.05). The addition of 1mM L-arg and 0.1mM ODQ decreased lipid peroxidation in control and other treatments (P<0.05). These results indicate that: 1) NO is involved in the control of plasma membrane integrity and acrosomal and mitochondrial potential, 2) there was a dose-response effect of L-arg in reducing lipid peroxidation, suggesting that lower concentrations of cGMP may also decrease lipid peroxidation, 3) ODQ decreased plasma membrane integrity and acrosomal and mitochondrial potential by means of lipid peroxidation, but not by NO/L-arg/cGMP.
EFFECT OF HOECHST 33342 IN THE KINETICS OF BOVINE SPERM CELLS ANALYSED BY COMPUTER SYSTEM (CASA)

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ABSTRACT 13

Computerassisted semen analysis (CASA) provides an objective analysis of sperm cell movement, being currently the most used method in experimental evaluations. To use that system, the sperm cells can be stained with H33342. This dye has a high affinity for the DNA of cells, and therefore avoids the interference of debris present in the samples during evaluation. However, some reports have speculated that H33342 may be toxic to sperm. Therefore, this study evaluated the influence of H33342 in the kinetics of cryopreserved bovine sperm. Semen was collected from four Nelore bulls, diluted with Botu-bov® (Biotech Botucatu) and packed for freezing. For each bull three replicates were performed. In each replicate, the samples were fractionated into four groups: 1) Immediate evaluation without dye (WDO0), 2) Evaluation after incubation for 15 min at 39°C without dye (WOD15), 3) Immediate evaluation with dye (WD0), 4) Evaluation after incubation for 15 min at 39°C with dye (WD15). After evaluation of each group, the remaining semen was subjected to Percoll gradient (45:50%) and centrifuged at 5400 G for 5 minutes for sperm selection. Then, the same evaluations were performed after Percoll. All treatments underwent CASA (Model Ivos Ultimate-12, Hamilton Thorne Biosciences, France); whereas total motility (TM: %), progressive motility (PM: %), curvilinear velocity (VCL: µm/s), progressive velocity (VSL: µm/s) and beat cross frequency (BCF: Hz) were evaluated. Data were analyzed as repeated measures using PROC MIXED. Results were presented as least square means ± pooled standard error. In general, Group WD0 presented the lowest and WD15, intermediate values compared to WDO0 and WOD15 before moving to Percoll for TM (34.5*, 64.1*, 58.2*, 57.7* ± 8.7), PM (12.3*, 19.8*, 26.0*, 24.7* ± 5.8), VAP (39.2*, 45.3*, 52.3*, 52.4* ± 4.0), VSL (34.0*, 40.1*, 43.7*, 45.7* ± 3.2), VCL (58.3*, 68.0*, 82.6*, 80.6* ± 6.8) and BCF (28.8*, 39.7*, 29.7*, 35.9* ± 2.0). The difference between the WDO and the others was maintained after passage through Percoll gradient, being the WD15 group not different from WDO0 and WOD15 groups: TM (30.2ª, 54.4b, 63.7b and 57.3b ± 8.6), PM (15.4*, 32.6*, 42.4* and 37.5* ± 7.2), VAP (41.1*, 54.1*, 57.2* and 60.2* ± 7.2), VSL (36.1*, 49.8*, 52.4* and 55.0* ± 6.2), VCL (59.8*, 75.0*, 81.3* and 83.7* ± 10.4) and BCF (22.4*, 35.9*, 32.0* and 34.7* ± 3.0). It can be concluded that the use of H33342, especially without respecting the incubation period, affects some characteristics of the movement of cryopreserved bovine sperm cell evaluated by CASA either before or after Percoll selection.

EFFECT OF ORAL SUPPLEMENTATION WITH VITAMIN C AND E ON SEMINAL PLASMA FROM FERTILE AND SUBFERTILE DOGS

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ABSTRACT 14

Seminal plasma contains reactive oxygen species (ROS) produced by testicular tissue and spermatozoa. High ROS concentration on seminal plasma has been correlated with subfertility, since ROS can cause alteration on sperm morphology and increase sperm apoptosis during spermatogenesis. Oxidative stress is due to a high production of ROS and a deficit from antioxidant, leading to a loss on fertility capability from spermatozoa. The seminal plasma is the main source of antioxidant, leading to a loss on fertility capability from spermatozoa. The seminal plasma is the main source of antioxidant against lipid peroxidation, and its role is to scavenge ROS and regenerate vitamin E. The aim of this work was to evaluate the oxidative stress on seminal plasma from fertile and subfertile dogs after oral supplementation with vitamin C and E on a period of 60 days, by measuring TBARS. Thirteen (n=13) healthy, from different breeds and age, were used in this study. The animals were separated into two groups: G1- fertile dogs (n=7), they had normal sperm count and less than 20% of sperm pathology. G2- subfertile dogs (n=6) had low sperm count (<20x10⁶ spz/mL) and/or more than 30% of total sperm pathology. The dogs were fed with a commercial dog food Maxi adulto – Royal Canin® supplemented with vitamin C and E (500 µg each) for 60 days. Semen from those dogs were diluted in TRIS/egg-yolk extender and then centrifuged, in order to obtain seminal plasma. The concentration of TBARS was measured using a spectrophotometer on a wave length of 532nm, on three moments: M1: before the supplementation, M2, after 30 days of supplementation and M3, 60 days of supplementation. Results were analyzed using ANOVA and tukey’s test. Between groups, from fertile and subfertile animals, a difference was verified on M1 (735.2±153.8ng/mL x 507.9±161.1ng/mL, p= 0.041). On moments 2 and 3 no difference was verified between groups, 554.7±154.5ng/mL x 499.1±142.4ng/mL (p= 0.516) on M2 and 531.6±158.9ng/mL x 461.97±136.51ng/mL (p= 0.419) to M3. The fertile group had a superior TBARS concentration than subfertile group, but after the adding of vitamins C and E on diet, this difference has not been maintain. It can be concluded that supplementation with vitamin C and E for 60 days, did not influence the oxidative stress on seminal plasma from fertile and subfertile dogs. Acknowledges: FAPESP, Royal Canin, Polícia Militar de Bauru
The use of intratesticular injection of chemical sclerosing agents to prevent fertility in male and female dogs is being researched. However, very often those drugs presented an uneven distribution and the combination with dimethylsulfoxide (DMSO) can increase intratesticular penetration. The objective of this study was to evaluate the efficacy of a double intratesticular injection of zinc gluconate and DMSO for chemical neutering in male dogs. Twenty two male dogs were selected in a double-blind experimental design with seven animals considered control group and fifteen as treated group. The animals received a twice 1 mL intratesticular injection (with a monthly interval) of zinc gluconate and DMSO or saline solution for treated or control group, respectively. The first application was performed in a dorsum-cranial orientation of the testis and the second application in a ventral-cranial orientation. The animals were evaluated every 15 days during 2 months before the first injection and after 5 months. Males were submitted to general physical examination, scrotal inspection and palpation, testicular perimeter measurement and a breeding soundness exam (libido test and seminal analysis). Monthly, the dogs were submitted to an ultrasonographic examination of testes and epididymides. At the end of the trial, all dogs were orchietomized and the testes histologically examined. The results were compared within groups by ANOVA and Tukey test with p < 0.05. No behavioral and clinical change were observed. Also, no increased sensitivity was detected by scrotum palpation. In regards to libido, a significant reduction was observed in 60% of the treated group, especially after the second application; while only a 14% reduction was verified in control group. Progressive motility and sperm vigor of the treated group suffered a significant reduction. Sperm concentration and percentage of live sperms was also reduced and an increase in sperm defects was observed. Changes in testicular consistency were detected in 87% of the treated dogs and in 15% of the control after the first application. There was a statistical decrease in the right testicular major length and the left testicular minor length in the treated group. In the ultrasonographic examination, 87% of the treated group showed changes in testicular echogenicity after the first application. All treated dogs presented macro or micro alterations of distinct degrees in histological analysis, specially restricted to the injections areas. In conclusion, intratesticular administration of zinc gluconate and DMSO showed to be a potent chemical agent for neutering, as it decreases reproductive performance through testicular morphological and functional changes. Sperm alterations may lead to progressive further sterilization.

**ABSTRACT 16**

**EFFECT OF DISTINCT ANTIBIOTICS ON POST-THAWING VIABILITY OF RAM SPERM**


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Sperm cryopreservation would be a suitable alternative for the preservation of ovine breeds at risk of extinction, such as the Crioula Lanada breed in Brazil. However, that strategy may be limited by the usually reduced post-thawing viability of ram sperm. Among the many additives present in extenders for frozen ram sperm, antibiotics are commonly added to prevent disease transmission through semen without jeopardizing its viability. The objective of this study was to evaluate the effects of distinct antibiotics commonly included in extenders for frozen semen on the viability of ram sperm after thawing. Ejaculates were collected twice a week during six weeks from five rams from the Crioula Lanada breed (n = 12), using an artificial vagina. At the time of collection, ejaculates were diluted 1:1 in a TRIS-egg yolk-based extender. Ejaculates were pooled with a fixed spermatozoa concentration of 50x10⁶ sperm/straws. Thirty minutes lates, glycerol was added to the extender. The pool was split in 5 treatments: T1, including no antibiotics (control); T2 (gentamicin, tylosin, spectiomicine and lincomycin); T3 (penicillin and streptomycin); T4 (Sodium cefiticiot); and T5 (enrofloxacin). Sperm was packed in 0.25 mL straws, cooled up to 5°C, frozen in vapor of liquid nitrogen and stored. After thawing, sperm motility was determined with optic microscope and integrity of sperm membrane, acrosome and DNA were determined with an epifluorescent microscope using, respectively, the following fluorescent probes: prodium iodide and carboxyfluorescein, prodium iodide and PNA-FICT and acridine orange in Carnoy. Sperm motility was lower (P < 0.05) for T5 (17.1%) than for both T1 (40.9%) and T2 (25.4), but no further differences in sperm motility were observed among treatments. Sperm membrane integrity did not differ (P > 0.05) for T1 (20.1%), T2 (18.5%), T3 (19.1%), T4 (20.1%) and T5 (13.3%). There were also no differences among treatments with regard to acrosome integrity (T1 = 83.2%, T2 = 82.0%, T3 = 85.3%, T4 = 82.0% and T5 = 80.0%). Sperm DNA integrity was lower (P < 0.05) for T2 (84.1%) than for T3 (92.0%), with no further differences observed among treatments. Therefore, the use of enrofloxacin would not be recommended due to its negative effect on post-thawing sperm motility. Also, the association of penicillin and streptomycin was associated with reduction in the sperm DNA integrity.
ABSTRACT 17

IMMUNOHISTOCHEMICAL LOCALIZATION OF ESTROGEN α AND β RECEPTORS AND AROMATASE CYTOCHROME P450 IN ADULT STALLION TESTICLES

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Steroid hormones like testosterone and estradiol are primary produced in the testes. Innumerable studies suggest the role of estrogens in the male reproduction via their specific receptors named estrogen receptor α (ERα) and estrogen receptor β (ERβ). In adult stallions, it have been found the presence of high levels of plasma estrogens, supporting the importance of the estrogen receptors and also of cytochrome P450 aromatase, the enzyme responsible for aromatization of androgens into estradiol. The present study aimed to confirm the presence of both ERs and cytochrome P450 aromatase enzyme via immunohistochemistry in adult stallion testes. Tissue specimens were fixed in 10% buffered formalin for 24 h and stored in 70% ethanol until they were embedded in paraffin. 4µm-tissue-section slides were deparaffinized and rehydrated. For antigen retrieval, sections were either microwaved for 15 minutes (ERα) or in a water bath at 96°C for 30 minutes (cytochrome P450 aromatase) in sodium citrate solution 10mM (pH=6.0). Endogenous peroxidase activity was quenched with 9% peroxidase solution for 20 minutes followed by incubation with a 3% milk solution for 1 hour. Slides were incubated with the primary mouse anti-human ERα monoclonal antibody (clone PPG5/10, Dako, USA), diluted 1:100 or rabbit anti-human cytochrome P450 aromatase (Hauptman Woodward Medical Research Institute Inc., USA), diluted 1:1000. Both in a humidified chamber for 18 h at 4°C. Then, slides were incubated with the secondary antibody (Advance, Dako, USA). DAB was added as chromogen staining substrate and counterstained with Mayers hematoxylin. The slides were dehydrated and preserved using Permount mounting medium. For negative controls, another section was incubated with either mouse or rabbit immunoglobulin (Dako, USA) instead of primary antibody. All slides were evaluated for positively stained nuclei (ERα) or cytoplasmatic immunostaining (aromatase). It could be observed a strong immunostaining for cytochrome P450 aromatase in almost all interstitial area, demonstrating that the Leydig cells' cytoplasm were positive for this enzyme. It was also observed for the nuclei of Leydig, Sertoli and germ cells an ERα immunostaining. However, for Leydig cells it could be visualized a mixed pattern, with some positive and other negative cell regions. The majority of Sertoli cells were positive with strong immunostaining. The majority of round germ cells were also positive. However, elongated germ cells were negative. At the present moment we failed to find immunostaining for ERβ and new protocols are yet to be performed. Data presented support the concept the estrogens play an important role in function of stallion reproductive system and the expression of aromatase and ERα in testicular cells could indicate that estrogen are important for sperm maturation.

ABSTRACT 18

FERTILITY OF EPIDIDYMAL SPERM FROM SUBFERTILE STALLIONS

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Epididymal sperm harvest aims the recovery of genetic material from valuable stallions that gone dead, euthanized or with obstructive processes. However, subfertile stallions may also benefit from this technique, since epididymal sperm has no contact with potentially deleterious seminal plasma (SP). The goal of the present study was to compare sperm viability between ejaculated (Group 1, G1) and epididymal cauda sperm (Group 2, G2) and to test fertility of epididymal sperm post-thaw from subfertile stallions. Two stallions from Mangalarga Marchador breed were used. Three ejaculates from each stallion were collected and frozen in G1. One week later the stallions were castrated and sperm were harvested from epididymis using retrograde flushing (G2) with Botu-Sêmen® and then frozen with Botu-Crio®. For fertility trial, ejaculates from each stallion were collected and frozen in G1. One week later the stallions were castrated and sperm were thawed from subfertile stallions. Two stallions from Mangalarga Marchador breed with history of subfertility were used. Three no contact with potentially deleterious seminal plasma (SP). The goal of the present study was to compare sperm viability or with obstructive processes. However, subfertile stallions may also benefit from this technique, since epididymal sperm has no contact with potentially deleterious seminal plasma (SP). The goal of the present study was to compare sperm viability.
ABSTRACT 19

EFFECT OF SEMINAL PLASMA ON CRYOPRESERVATION OF EQUINE SEMEN AFTER COOLING FOR 12 HOURS

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One of the difficulties in using the semen freezing technique is the lack of infrastructure for this procedure in most stables. Thus, cooling the semen for 12 hours allows the transport of the stud to a laboratory for cryopreservation. This study aimed to compare the effect of seminal plasma on cryopreservation of semen after refrigeration for 12 hours. We used five stallions, three ejaculates from each, total 15 ejaculates. After collection with an artificial vagina, the semen was separated into two groups: group I, frozen according to Papa et al. (2002), group II, diluted (2:1) with Botu-Sêmen® (Biotech, Botucatu, Brazil) and cooled to 5 °C/12 hours and group III, diluted (1:1) with Botu-Sêmen®, centrifuged for removal of seminal plasma, again diluted (2:1) and cooled to 5 °C/12 hours. After refrigeration, the semen was centrifuged (600 xg / 10 minutes) the supernatant discarded and the pellet resuspended with Botu-Crio® (Biotech, Botucatu, Brazil) for freezing. After loading in 0.5ml straws, they were subjected to chilling at 5 °C for 20 minutes followed by freezing in nitrogen vapor for 15 minutes and finally immersed in liquid nitrogen and stored until evaluation. Thawing was performed at 46 °C for 20 seconds and sperm parameters were analyzed using CASA. The plasmatic membrane integrity was evaluated by fluorescent probes Carboxyfluorescein and Propidium Iodide. Statistical analysis was performed using ANOVA and Tukey test. The averages for post-thawing total motility were: 69.6%, 48.9%, 55.9%; for progressive motility were: 32.7%, 20.1%, 24.5% and for membrane integrity were: 39.7%, 25.3%, 30.5% for groups I, II and III, respectively. The results of variance analyses showed that total motility in group II was significantly less (p <0,05) than the group I, with no differences (p> 0.05) between groups I and III; and II and III. We concluded that the cryopreserved semen cooled for 12 hours was necessary to remove seminal plasma. *Allowance of CNPq. Acknowledgements: Biotech Botucatu.

ABSTRACT 20

ASSESSMENT OF BOVINE SPERMATOZOA VIABILITY USING DIFFERENT COOLING PROTOCOLS PRIOR TO CRYOPRESERVATION


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The objective of this study was to evaluate the influence of different cooling rates upon the post-thawing quality of bovine spermatozoa. Ejaculated semen from a 24 mo old Jersey bull was collected twice weekly for two weeks using an artificial vagina, immediately transported to the laboratory and maintained in a water bath at 30°C for evaluation. Semen was diluted in a commercial extender with a final concentration of 25x10⁶ spermatozoa/0.25mL straw. Sperm concentration and motility were evaluated subjectively before cooling and freezing and after thawing. Morphological characteristics were evaluated by phase contrast microscopy before and after freezing. Straws were allocated to four different cooling curves: rapid (RD; conventional cooling curve), semi-rapid (SRD), semi-slow (SSLW) and slow (SLW). The temperature was decreased from 25°C to 4°C in 10, 50, 110 and 135 min, which represents a cooling rate of 2.06, 0.40, 0.18 and 0.15°C/min, for the four treatments respectively. After cooling, straws were frozen in nitrogen vapor for 12 min and stored in liquid nitrogen (-196°C). Frozen straws were thawed in water bath at 37°C for 1 min and one aliquot of each straw was used for sperm evaluation. Plasma and acrosomal membrane integrity and mitochondrial function were evaluated using a combination of fluorescent probes containing 100 mg/mL FITC-PSA, 0.5 mg/mL PI, and 153 mM JC-1. Results were analyzed using repeated measure and ANOVA tests using SAS software, for P<0.05. At the end of cooling period, sperm motility did not differ among RD (63.3%), SRD (66.7%), SSLW (66.7%) and SLW (80.0%) treatments. However, normal sperm morphology was lower in SRD (84.8%) compared to RD (91.7%), SSLW (91.7%) and SLW (90.3%) treatments (P<0.05). In thawed semen, sperm motility and normal morphology did not differ among RD (40.0%; 88.8%), SRD (43.3%; 82.5%), SSLW (4.0%; 87.2%) and SLW (36.7%; 88.0%) treatments. The percentage of damaged sperm, including plasma and acrosome membranes damage and low mitochondrial potential, was higher in the RD compared to other curves (P<0.05). However, to confirm these changes, we need to investigate the spermatozoa integrity using large sample sizes. In conclusion, the cooling rate during the semen freezing process affects the sperm viability. A rapid cooling curve is detrimental to the sperm cells and affects the post-thaw sperm integrity of bovine frozen semen.
ABSTRACT 21

COMPARISON BETWEEN TWO COOLING EXTENDER MEDIUM FOR EQUINE SEMEN BY 12 HOURS PRIOR TO CRYOPRESERVATION

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The methods of equine semen cryopreservation prior to cooling to 20 to 60 minutes depending on the extender medium used. But, usually it is necessary to transport ejaculated from stud to laboratory, which often takes more than this period, and then, the ejaculated can be refrigerated for several hours before cryopreservation. The aim of this study was to compare two semen coll exenders prior to cryopreservation. A total of 15 ejaculates obtained from five stallions (three ejaculates from each), and two semen extenders for cooling were used: Botu-Sêmen® (Biotech, Botucatu, Brazil) and Botu-Turbo® (Biotech, Botucatu, Brazil). The semen from each collection was divided into equal volumes and subjected to three treatment groups: group I, frozen according to Papa et al. (2002); group II, diluted (2:1) with Botu-Sêmen® and refrigerated at 5°C/12 hours and group III, diluted (2:1) with Botu-Turbo® and refrigerated at 5°C/12 hours. After refrigeration, the semen was subjected to centrifugation at 600 xg for 10 minutes, the sediments were resuspended with Botu-Crio® (Biotech, Botucatu, Brazil) and packaged in 0.5mL straws. These were subjected to chilling at 5°C for 20 minutes, placed at 6 cm above nitrogen for 15 minutes and after that dipped in it and stored in a Cryobiology Canister. For analysis of samples, these straws were thawed in a water bath at 46°C for 20 seconds. They were then examined for computerized sperm motion (CASA) and plasmatic membrane integrity by fluorescent probes Carboxyfluorescein and Propidium Iodide. Statistical analyses were performed using ANOVA and the Tukey test. The averages for post-thaw total motility were: 69.6%, 48.9%, 52%; for progressive motility were: 32.7%, 20.1%, 22.5% and for plasma membrane integrity were: 39.7%, 25.3%, 26.5% for groups I, II and III, respectively. The results of variance analyses showed significant differences (p<0.05) only in total motility between groups I and II. We concluded that the Botu-Turbo® was better for cooling the semen, because of no significant difference (p>0.05) in group I. *Allowance of CNPq. Acknowledgements: Biotech Botucatu.

ABSTRACT 22

EFFECT OF CONCENTRATION OF SEMINAL PLASMA ON CRYOPRESERVATION OF EQUINE SPERMATOZOA

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Seminal plasma is generally removed during the processing of semen cryopreservation, but according to Aurich et al., the presence of seminal plasma may be beneficial to cryopreserved sperm (1996; Theriogenology, 46:791-797). The aim of this study was to evaluate the effect of concentration of seminal plasma on cryopreservation of equine semen. Two matches were used from 5 stallions. After collection, each ejaculate was diluted 1:1 with Botu-Sêmen® (Botucatu, Brazil) and centrifuged at 600g for 10min. Seminal plasma was removed and the pellet resuspended with Botu-Crio® (Botucatu, Brazil) with different concentrations of seminal plasma: 0% (CT), 10% (a) 20% (b) and 50% (c), and the seminal plasma proceeding from semen of a stallion with high freezability (Group 1) and other with a low freezability (Group 2). The samples were packaged in straws of 0.5mL and submitted to the process of cooling to 5°C for 20min. Then, the straws were placed horizontally on a holder 6cm from the level of liquid nitrogen in a styrofoam box for 20min and soon after, were immersed in liquid nitrogen. After thawing, specimens from groups CT, G1a, G2a, G1b, G2b, G1c and G2c were analyzed, respectively, for the following parameters (mean ± standard deviation): total motility (TM) = 62.0±17.49, 47.3±20.67, 14.19±57.3, 47.2±20.86, 10.00±46.2, 40.3±16.73, 35.9±20.70; progressive motility (PM) = 30.8±11.16, 12.4±19.4, 23.1±11.72, 17.4±12.95, 16.6±10.38, 20.6±12.23, 13.2±12.75, average path velocity (VAP) = 89.3±9.12, 78.3±8.79, 79.7±9.33, 72.0±8.51, 71.9±8.89, 72.4±9.00, 70.3±10.36; progressive linear velocity (VSL) = 72.2±7.66, 63.4±6.98, 63.9±7.22, 59.3±8.69, 59.4±8.35, 61.0±7.94, 58.4±8.50; curvilinear velocity (VCL) = 163.9±11.20, 147.0±12.86, 149.7±14.02, 136.0±11.49, 137.6±14.71, 137.8±13.35, 131.4±14.99; fast sperm (RAP) = 47.1±18.07, 18.7±29.80, 36.4±17.39, 25.3±16.60, 22.0±12.66, 18.4±16.92 and membrane integrity (MI) = 34.4±13.02, 33.8±12.92, 37.0±11.29, 32.9±17.32, 27.9±10.28; 22.9±7.42, 17.1±6.29. The statistical analysis was performed by ANOVA followed by Tukey. The results showed that the addition of 50% seminal plasma of stallions with low semen freezability had a deleterious effect on MT and IM sperm. The addition of seminal plasma did not affect MP in any of the groups. The addition of 20 and 50% seminal plasma decreased the VAP, VSL and VCL, while RAP was lower only when added 50% of seminal plasma. In conclusion, seminal plasma added to the diluent at 6 cm above nitrogen for 15 minutes and after that dipped in it and stored in a Cryobiology Canister. For analysis of samples, these straws were thawed in a water bath at 46°C for 20 seconds. They were then examined for computerized sperm motion (CASA) and plasmatic membrane integrity by fluorescent probes Carboxyfluorescein and Propidium Iodide. Statistical analyses were performed using ANOVA and the Tukey test. The averages for post-thaw total motility were: 69.6%, 48.9%, 52%; for progressive motility were: 32.7%, 20.1%, 22.5% and for plasma membrane integrity were: 39.7%, 25.3%, 26.5% for groups I, II and III, respectively. The results of variance analyses showed significant differences (p<0.05) only in total motility between groups I and II. We concluded that the Botu-Turbo® was better for cooling the semen, because of no significant difference (p>0.05) in group I. *Allowance of CNPq. Acknowledgements: Biotech Botucatu.
SWIM UP AS A STRATEGY FOR THE IN VITRO EVALUATION OF FROZEN RAM SEMEN


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The in vitro evaluation of semen is used to predict male fertility. However, for the ram, in especial for frozen semen, no positive correlations exist between parameters of in vitro sperm viability and pregnancy rates. Methods to assess only the motile sperm population may generate more reliable in vitro information that may possibly better correlate with fertility. This study evaluated the use of swim up as a sperm segregating method for the in vitro evaluation of frozen ram semen. Semen samples were allocated into two groups: Group 1 (G1), incubated for 5 h at 37°C, after sperm segregation by swim up, and Group 2 (G2), in which semen was directly incubated for 6 h. For the swim up, 100 IL of semen was deposited in 500 IL of TRIS-glucose-citric acid solution and incubated for 1 h at 37°C. Then, 400 IL of supernatant containing migrated sperm cells was removed and re-incubated in a water bath at 37°C for 5 h. Parameters evaluated included: (a) progressive motility (%), (b) vigor (1-5), and (c) membrane functional integrity (%). Evaluations were performed each hour over a 6-h incubation period. The motility degradation rate (TDM) was calculated using the equation: TDM = (initial motility – final motility) x 100/initial motility. Four replicates were performed and data were subjected to ANOVA with repeated measures (PROC MIXED of SAS), and the percentage of TDM data was submitted to ANOVA, for p<0.05. Results observed in the six evaluations between 1 and 6 h of incubation differed significantly between G1 and G2 (p<0.01) for the three parameters and in all stages of evaluation: (a) motility: G1 (82.5%, 78.8%, 77.5%, 73.8%, 66.3%, 62.5%) vs. G2 (42.5%, 40.0%, 33.8%, 30.0%, 23.8%, 18.8%), (b) vigor: G1 (3.5, 3.5, 3.5, 3.1, 3.0, 3.0) vs. G2 (3.0, 2.6, 2.4, 2.0, 2.0, 1.5), (c) membrane integrity: G1 (86.0%, 82.8%, 82.5%, 77.0%, 68.3%, 61.8%) vs. G2 (44.3%, 36.8%, 34.8%, 28.0%, 24.8%, 17.5%) for 1, 2, 3, 4, 5 and 6 h of incubation, respectively. Additionally, data distribution over time have slopes for motility, vigor and membrane integrity that are different between G1 and G2 (p<0.05). Moreover, TDM was lower (p<0.01) in G1 (25.8%) than in G2 (55.9%). These results demonstrated that the parameters had a distinct behavior over time, with higher maintenance of sperm viability in G1. As the swim up procedures mimics some in vivo conditions, where motile sperm are naturally selected, we infer that this method enables a more physiological and perhaps reliable in vitro semen evaluation, with a stronger correlation with fertility. Further in vivo studies should be carried out to confirm this hypothesis.

ABSTRACT 24

SPERM DNA FRAGMENTATION INFLUENCE ON BOVINE IN VITRO EMBRYO PRODUCTION

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Sperm chromatin integrity is essential for the transmission of paternal genetic information. Changes in sperm DNA can lead to failures in the reproductive process. However, information on the influence of sperm DNA integrity in the process of fertilization and embryonic development in cattle are very scarce. Due to the lack of information this study aimed to evaluate the sperm DNA fragmentation rate in a population of bulls and the influence of possible sperm DNA damage on in vitro production (IVP). Towards this aim, frozen semen from 221 bulls was evaluated by sperm chromatins structure assay (SCSA). Afterwards animals were divided in 6 groups, according to DNA fragmentation rate observed (group 1: low DNA fragmentation; group 2: medium DNA fragmentation; group 6: high DNA fragmentation). Seven animals from each group were randomly assigned for sperm evaluation: motility, sperm DNA fragmentation by the SCSA and Alkaline Comet and oxidative stress by thiobarbituric acid reactive substances (TBARS). After sperm evaluations embryo IVP was performed. Cleavage and blastocyst rates were assessed and the obtained blastocysts were submitted to TUNEL for apoptosis evaluation. Regarding sperm motility, group 1 had lower motility when compared to group 4 (36.67 ± 7.15 and 58.33 ± 4.77, respectively, p < 0.05). There was no significant difference in motility between the other experimental groups. The SCSA revealed that group 1 showed lower susceptibility to sperm DNA fragmentation when compared to group 5 (4.78 ± 1.43 and 7.77 ± 1.26, respectively, p<0.05). Group 2 was less susceptible to sperm DNA fragmentation, when compared to groups 3, 4, 5 and 6 (6.07 ± 0.69, 5.73 ± 0.59, 7.77 ± 1.26 and 5.57 ± 0.59, respectively, p<0.05). For the alkaline comet, the assessment of oxidative stress and embryo IVP no significant difference was observed between experimental groups. However, the TBARS was negative correlated to cleavage (r=-0.417; p=0.009) and blastocyst (r=-0.367; p=0.023)rates. In addition, TBARS correlated positively to TUNEL (r=0.417; p=0.009) and SCSA (r=0.416; p=0.009). Considering the results we concluded that embryonic development was not influenced by sperm DNA fragmentation. However, embryonic development was negatively influenced by sperm susceptible to oxidative stress.
ABSTRACT 25

BIOCHEMICAL AND MOLECULAR EVALUATION OF ANTIOXIDANTS TREATMENT ADDED TO THE EXTENDER ON CRYOPRESERVATION OF EPIDIDYMIS BULL SEMEN

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The study of the several factors that may affect the sperm recovery from the epididymis quality is still necessary. The cryopreservation may induce chemical and physical stress in these cells, which reduces their viability. This is associated with molecules called reactive oxygen species (ROS). ROS are involved in energy production, signaling and synthesis of intercellular substances. Its excess may cause membrane lipids peroxidation and degradation of proteins and enzymes. To avoid the damaging effects of ROS several antioxidants can be used like vitamin C, E and reduced glutathione (GSH). Vitamin C acts as a pro or antioxidant molecule, mainly at the DNA. However, the mechanisms of this action still remain unknown. Vitamin E and GSH act protecting the sperm from lipid peroxidation. This study aimed to evaluate the efficiency of antioxidants treatment added to the extender on cryopreservation of epididymis bull semen by means of infrared spectroscopy (FTIR). In FTIR, IR radiation is passed through a sample and the resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. FTIR can result in a positive identification (qualitative analysis) and the size of the peaks in the spectrum is a direct indication of the amount of material present. For this study, epididymal semen samples cryopreserved (n = 6 bulls) treated or not (control group) with non-enzymatic antioxidants - vitamin E (0,5mM, 1,5 mM and 2,5 mM), vitamin C (1,5mM, 3mM and 4,5mM) and GSH (0,5mL, 1mL and 5mL) - were thawed and an aliquot was reserved and kept under refrigeration. The samples were air dried on a platinum surface and analyzed by FTIR. The data were processed by the software “Fityk” and “Origin 8”. The most important differences were observed between 1080cm⁻¹-1122cm⁻¹ (referring to nucleic acids) and between 2800cm⁻¹-3000cm⁻¹ (referring to lipids structural vibrations). All samples treated with vitamin C presented variations mainly on the nucleic acids region, which can be explained by the protective action of this compound on DNA/RNA structure. All treatments presented variation on lipids region in comparison to the control group. The samples treated with 2,5mM and 1,5mM Vitamin E and vitamin C presented a decrease in the intensity of the region of 2916cm⁻¹ (the region of stretching vibrations of CH₂/CH₃ cholesterol and phospholipid) when compared to the other samples. Thus, we conclude that antioxidants induced significant changes in sperm structure which could be verified by changes in nucleic acids and lipids.

ABSTRACT 26

FAVORING THE BIRTH OF FEMALE PUPPIES FROM FRENCH BULLDOG BITCHES AFTER ARTIFICIAL INSEMINATION USING SEMEN DILUTED WITH POWDERED COCONUT WATER (ACP 106C)

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The possibility to choose the gender of the puppies to be born seems to be an important tool for those who work with canine genetic improvement. For most of the breeds the demand for female puppies is superior to that for males. The main process of gender selection, developed for most species, is the use of sperm previously sorted by flow cytometry. However, this technique results in low fertility, high costs and operational difficulties (Fayer-Hosken, 1997, Proceedings of Canine Male Reproduction Symposium, 53-57). To our knowledgement, coconut water based extender is the only extender able to cause a divergence in sexual proportion. There are reports that a greater proportion of female goats were born following AI with fresh coconut water based extender or ACP 106C, with no statistical differences between the tested extenders. Researches reporting similar results from the use of other extenders had not been found. The aim of this work was to evaluate the influence of powdered coconut water extender (ACP 106C) on the proportion of female puppies born. Sixteen French Bulldog bitches were subjected to NM and, during the subsequent estrus, they were inseminated using the same males in order to determine the influence of ACP 106C extender, on pregnancy and parturition rates and the proportion of female puppies. Fresh semen was evaluated according to the parameters of motility, vigor and membrane functionality. Afterwards, it was cooled down to 5°C, for six hours, in a refrigerator. After completing six hours of refrigeration, the semen was heated up to 37 °C, for 30 seconds, the same parameters were reassessed and AI was performed. Pregnancy and parturition rates following NM were both 100% and following AI were both 87.5%. Litter size for NM was 5.31 ± 1.14 and for AI was 5.57 ± 0.9, exhibiting no statistical differences. The number of born male and female puppies, after NM, was 2.81 ± 1.0 (male) and 2.5 ± 0.63 (female), and after AI, was 2.07 ± 1.07 (male) and 3.50 ± 1.10 (female). The number of females born through NM (51.9%) was similar to the number of males (48.1%). The number of females born through AI (62.8%) was significantly higher than the number of males (37.2%). In conclusion, extending and chilling the semen with ACP 106C provided good pregnancy and parturition rates and favored the birth of a higher proportion of female offspring from French Bulldog bitches.
Foliculogênese, Oogênese e Superovulação

Folliculogenesis, Oogenesis and Superovulation
ABSTRACT 27

GENE EXPRESSION OF FIBROBLAST GROWTH FACTOR 2 IN GOAT OVARIAN

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The fibroblast growth factor 2 (FGF-2) has an important role in the regulation of various ovarian functions such as mitosis, steroidogenesis, differentiation and apoptosis of granulosa cells. Although the FGF-2 importance in follicular development, to our knowledge there are no data on this factor presence in goats (Capra hircus) ovarian cells. Thus, this study aimed to quantify FGF-2 mRNA in different categories of follicles isolated from goats by real time polymerase chain reaction (PCR). For this purpose, ovaries were collected from 30 adult crossbred goats. Of these, ten were used to isolate preantral follicles (primordial, primary and secondary) and 20 were used to collect COC and mural granulosa and theca cells of small (<3 mm) and large (>3 mm) antral follicles. Isolation of total RNA was performed by Trizol purification kit (Invitrogen, São Paulo, Brazil) with subsequent reverse transcription to obtain cDNA, which was subjected to amplification and quantification by real time PCR. We used the primers for amplification of mRNA for FGF-2, GAPDH and ß-actin, the latter two being used as endogenous controls for normalization of expression. The delta-delta-CT method (Livak & Schmittgen, 2001; Methods, 25:402-408) was used to transform the values of CT in relative normalized expression levels. Was detected FGF-2 mRNA expression in all goat follicles categories. However there was no statistical difference in mRNA expression between the different classes of preantral follicles, COCs or among granulosa / theca cells of small and large antral follicles (P>0.05). However, when compared COCs with granulosa / theca cells, large antral follicles, COCs showed expression levels significantly below their respective granulosa / theca cells (P<0.05). Thus, we concluded that mRNA for FGF-2 is expressed in follicle cells throughout follicular development.

ABSTRACT 28

MORPHOLOGICAL QUALITY, RECOVERY AND MATURATION RATES OF CAPRINE OOCYTES FROM SLAUGHTERHOUSE ANIMALS

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The oocytes quality is evaluated for their ability to fertilize and develop to the blastocyst stage, allowing, besides the establishment of pregnancy, getting an alive product (LONERGAN et al., 2001, Reprod Nutr Dev, 41:427-437). The use of animals for slaughter is an important source of material for the development and refinement of assisted reproductive biotechnologies. However, are still low rates of maturation, fertilization and in vitro embryo production in goats. Reports on the recovery rate of oocytes from slaughterhouse animals for the procedures of in vitro maturation are still scarce, creating difficulties in the in vitro production of embryos. Thus, this study aimed to evaluate quantitatively and qualitatively the oocytes recovered by follicular aspiration from goat ovaries and their ability to undergo nuclear maturation in vitro. For this, 60 adult mixed-breed goats have had their ovaries aseptically collected from local abattoirs, and in the laboratory, the ovaries were punctured to verify the quantity and quality of cumulus oocyte complexes (COCs). The COCs selected were classified as grade I (more than three layers of compact cumulus cells, homogeneous cytoplasm and uniform staining), II (less than three layers of cumulus cells) and III (or denuded oocytes with irregular cytoplasm). The COCs grades II and III were cultured together. After aspiration, COCs were washed in TCM199 buffered with HEPES, and then in maturation medium (TCM199 supplemented with 1% BSA, 5 mg/mL LH and 0.5 mg/mL rFSH®, 10 ng/mL EGF, 50 ng/mL IGF-I, 0.911 mMol/L pyruvate e 1 µg/mL estradiol). The culture was grown in an incubator at 39°C and 5% CO2 in humid atmosphere for 27h. After the maturation period, oocytes were labeled with 10 mM of Hoechst 33342 at 37°C for 15 minutes for subsequent evaluation of the metaphase plate in a fluorescence microscope. The percentage of oocytes in metaphase II was evaluated by chi-square test and the differences were considered significant when P<0.05. A total of 227 COCs (1.89 COCs per ovary) was cultured in maturation medium: 55 (24.23%) grade I and 172 (75.77%) grade II and III. These results demonstrate clearly the low quality of oocytes obtained from slaughterhouse ovaries goat, since almost two-thirds of these oocytes were grade II and III. As expected, grade I oocytes had a higher (58.18%) percentage of metaphase II than oocytes grade II e III (34.88%) (P<0.05). Thus, it can be concluded that goat oocytes retrieved from ovaries of animals abattoir have low quality, but grade I oocytes showed rates of nuclear maturation in vitro relatively satisfactory.
ABSTRACT 29

EPIRREGULIN GENE EXPRESSION IN EQUINE OVARIAN FOLLICLES AFTER EPE AND hCG TREATMENT

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Anatomic particularities of the equine specie difficult oocyte recovery using ovum pick up (OPU). The best oocyte recovery rates is obtained in preovulatory follicle, since in these, there are cumulus cells expansion, induced by the preovulatory follicular wave of LH. The LH causes the final oocyte maturation and changes in the cumulus cells organization and secretory properties. Among the ovarian growth factors that act as possible paracrine mediators of the LH action in preovulatory follicles, the Epidermal Growth Factor (EGF) family has shown great importance. Between these, the epirregulin (EREG) is involved in the cumulus cells expansion and nuclear maturation in humans and mice. The aim of this study is to characterize the gene expression profile of EREG in equine preovulatory and immature follicles subjected to superovulatory treatment with equine pituitary extract (EPE) and induction of ovulation with hCG. Ten mares was randomly distributed in 4 treatment groups: 1) Preovulatory control: OPU was performed 24 hour after the detection of a follicle with 35 mm; 2) immature control: the OPU was performed 24 hour after the detection of a follicle with 30 mm; 3) EPE/hCG preovulatory: the EPE treatment (6mg/twice daily/m.) started in the seventh day after ovulation, and continued until the larger follicle reached 35 mm, when hCG (2500 UI, i.v.) was administered. OPU was performed after 24 hours; and 4) EPE/hCG immature: the treatment was similar to group 3, but the hCG was administered when the largest follicle reached 30 mm, and the OPU was done after 24 hours. The granulosa cells obtained with the aspirations was centrifuged in 4000 rpm during 15 minutes, the pellet was removed and re-suspended in 1 ml of Trizol®, to be storage at -80°C until the moment of total RNA extraction and reverse transcription. Primers to epirregulin and α-actin (ACTB) were used to quantification for real time PCR. The data was expressed in relation to a standard sample to each gene, and the relative gene expression was determined by the Pfaffl equation, analyzed by ANOVA. The gene expression of EREG was similar among the experimental groups (p>0.05), suggesting that, at the evaluated moment, gene expression of this factor is not influenced by the hormonal treatments utilized. The characterization of the expression of the same gene in another moments, and of another genes that possibly are involved in the final follicular maturation in mares, in different moments after the induction of ovulation still need to be done. Financial Support: FAPESP (08/56804-2). Acknowledgments: CAPES.

ABSTRACT 30

EFFECTS OF IGF-1 AND FSH ON IN VITRO DEVELOPMENT OF CAPRINE PREANTRAL FOLLICLES AND LEVELS OF IGF-1 AND FSHR mRNA


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This study aimed to assess the effects of IGF-1, with or without FSH on the survival, growth and antrum formation of goat preantral follicles in vitro cultured and on mRNA levels for IGF-1 and FSH receptor (FSHR) after in vitro culture. For this purpose, secondary follicles were isolated and individually cultured for 6 days in α-MEM medium (control) or in α-MEM containing (IGF-1 (50 ng/mL), FSH (100 ng/mL) or IGF-1 + FSH. Each treatment was repeated three times using approximately 40 follicles. After culture period, three groups of 7 follicles were used in real-time PCR, while the remainder was intended to viability analysis using the technique of fluorescence microscopy. The data of survival and antrum formation were evaluated by chi-square, while the results of diameter and mRNA levels for IGF-1 and FSHR were subjected to ANOVA followed by Kruskal Wallis test, all with a significance level lesser than 5% (P<0.05). The percentage of surviving follicles after culture was 91.49% in control and 91.30%, 95.00% and 97.67% in the treatments containing only FSH, IGF-1 (12.9±7.6) to the addition of FSH (3.8±3.3) or IGF-1 (11.2±7.6) to the culture medium in vitro (P>0.05). In conclusion, this study demonstrated that IGF-1 and FSH promotes follicular growth in vitro. Furthermore, the interaction between FSH and IGF-1 promotes an increase in both IGF-1 and FSHR mRNA levels in caprine preantral follicles cultured.
ABSTRACT 31

EFFECT OF ANGIOTENSIN II IN SURVIVAL OF CAPRINE PREANTRAL FOLLICLES CULTURED IN VITRO


The aim of this study was to evaluate the effect of angiotensin II (ANG II) on the survival, activation and growth of caprine preantral follicles cultured in vitro. The ovarian cortex from adult mixed-breed goats (n=4) was divided into small pieces and one fragment was immediately fixed (fresh control) and the remaining fragments were cultured in vitro for 1 or 7 days at 39°C and 5% CO₂, in supplemented á-Minimum Essential Medium (áMEM⁺) alone or with different concentrations of ANG II (1, 5, 10, 50 or 100 ng/mL). Non-cultured (fresh control) and cultured ovarian fragments were processed for histological analysis. Follicles were classified as primordial or developing (transition, primary and secondary), as well as normal or degenerated. The data were subjected to analysis of variance followed by Dunnett and Student t tests, both at a significance level of 5%. Our findings indicate that when compared with áMEM⁺ alone, the addition of 10 or 50 ng/mL of ANG II resulted in significantly higher percentages of normal preantral follicles after 7 days of culture. With the progression of the culture period of 1 to 7 days, all the treatments kept the percentage of normal follicles, except the áMEM⁺ alone or with 100 ng/mL of ANG II. The culture of ovarian tissue for 1 or 7 days increased the percentage of follicular activation in all treatments when compared with fresh control. In relation to follicular and oocyte diameters after 7 days of culture, all the treatments with ANG II had equivalent values to áMEM⁺ alone. In conclusion, this study demonstrated that after 7 days of in vitro culture, 10 or 50 ng/mL of ANG II maintains the morphology of preantral follicles.

ABSTRACT 32

EXPRESSION OF mRNA FIBROBLAST GROWTH FACTORS (FGFs) AND COGNATE RECEPTORS (FGFRs) DURING BOVINE COC IN VITRO MATURATION

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The oocyte actively participates in the regulatory mechanisms of cumulus-oocyte complex (COC) maturation via secretion of paracrine factors. Recently, we detected the expression of fibroblast growth factors (FGFs) in the oocyte and their receptors in cumulus cells (CC; FGF-10 and its receptors FGFR-1b and-2b; FGF-8, -16 and -17 and their receptors FGFR-2c and -3c), suggesting the involvement of the FGF system in the regulation of CC differentiation. The aim of this study was to determine the mRNA expression pattern for these FGFs and receptors in oocytes and CC, respectively, during bovine COC in vitro maturation. Immature COCs (grades 1 and 2) were obtained from 2-8 mm follicles from abattoir ovaries (predominantly Bos indicus). For the immature group, oocytes and CC from 20 COCs were separated and then stored at -80°C. Groups of 20 COCs were cultured for 4, 8, 12, 16 and 20 hours with (10 ng/mL) or without FSH. After culture oocytes and CC were separated and stored at -80°C. Total RNA was extracted from pools of 20 oocytes and their corresponding CC using the RNeasy® kit (Qiagen). Only oocytes from cultures with FSH were used to assess FGF mRNA expression. Expression of target genes was assessed by real time RT-PCR and normalized by Cyclophilin (CYC-A). Relative quantification of mRNA were determined by the Pfaffl equation. Effects of time in culture and of FSH treatment were tested by ANOVA and groups were compared by Tukey-Kramer HSD test. Level of significance was P<0.05. Our findings indicate that when compared with áMEM⁺ alone, the addition of 10 or 50 ng/mL of ANG II resulted in significantly higher percentages of normal preantral follicles after 7 days of culture. With the progression of the culture period of 1 to 7 days, all the treatments kept the percentage of normal follicles, except the áMEM⁺ alone or with 100 ng/mL of ANG II. The culture of ovarian tissue for 1 or 7 days increased the percentage of follicular activation in all treatments when compared with fresh control. In relation to follicular and oocyte diameters after 7 days of culture, all the treatments with ANG II had equivalent values to áMEM⁺ alone. In conclusion, this study demonstrated that after 7 days of in vitro culture, 10 or 50 ng/mL of ANG II maintains the morphology of preantral follicles.
ABSTRACT 33

INCIDENCE OF ATRESIA AND APOPTOSIS BY CASPASE-3 DURING FOLLICLE MOBILIZATION AND GROWTH IN MICE OVARY

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Follicular atresia is a key event in the selection of ovulatory follicles, and was shown to occur in all developmental stages. Differences in follicular dynamics among species, however, can account for a higher incidence of atresia in specific follicular stages. The aim of this study was to evaluate the incidence of atresia (by caspase-3 activation) and morphological atresia in different follicle developmental stages in mice and its relationship with follicular population and follicular mobilization patterns. Ovaries were obtained from Swiss (n = 6, G1), F1 Swiss x C57BL/6 (n = 6, G2), inbreed strain C57BL/6 (n = 6, G3), and F1 C57BL/6 x Swiss (n = 6, G4), all with 60 days old, fixed and processed for histology (follicle quantification). The ovaries of F1 Swiss x C57BL/6 (n=8) was used for active caspase-3 immunolocalization, by immunohistochemistry. The parameters were tested by Tukey's or Kruskal-Wallis's test. Primordial follicles population of the animals from G3 was significantly lower than other groups (7565±1845 vs. 17180±3159, 14785±3319 and 13325±2685 for G3, G1, G2 and G4, respectively; P < 0.05). However, G3 showed follicular mobilization rate significantly higher (29.2% vs. 18.2%, 17.3% and 13% for G3, G1, G2 and G4, respectively; P < 0.05), which led homogenization in the number of antral follicles between groups (P > 0.05). Coherently, the lower follicular pool in G3 animals was also associated a lower rate of atresia than the other groups (11.4% vs. 17.2%, 16.7% and 13% for G3, G1, G2 and G4, respectively; P < 0.05). In immunolocalization of active caspase-3 during follicular development was observed higher (P < 0.05) number of follicles immunopositives in the final stages of pre-antral folliculogenesis (24 %), suggesting that in mice atresia via caspase-3 occurs early when compared to large animals. In conclusion, the relationship between follicular pool and number of antral follicles is regulated by the balance between mobilization and follicular atresia, which was also observed that the predominant form of atresia occurs at the end of pre-antral development. Financial Support: CNPq/Fapemig/Embrapa
ABSTRACT 35

EXPRESSION OF FIBROBLAST GROWTH FACTOR 22 (FGF22) AND ITS RECEPTOR, FGFR1B, DURING LUTEAL DEVELOPMENT IN CATTLE

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There are evidences indicating the participation of intra-ovarian factors in the control of corpus luteum (CL) formation and regression. Fibroblast growth factors (FGFs) are involved in the control of ovarian angiogenesis, steroidogenesis and apoptosis. FGF7 and FGF10 belong to the same FGF subfamily together with FGF22, and all three activate the same receptors (FGFR1B and 2B). FGF7 and FGF10 expression was demonstrated throughout the CL lifespan without signs of regulation, whereas FGFR2B mRNA expression was highest in the regressing CL and transiently decreased by PGF2α treatment. The aims of the present study were to determine the expression pattern of FGF22 and FGFR1B mRNA and to assess the presence of the respective proteins in the bovine CL. Bovine CLs were obtained from abattoir ovaries and classed into four stages of development (stage 1= corpus hemorrhagicum, stage 2= developing CL, stage 3= mature/early functional luteolysis CL, and stage 4= structural luteolysis). Tissue samples were submitted to total RNA extraction. Expression of FGF22 and FGFR1B mRNA was measured by real-time RT-PCR with oligodT in the RT and bovine-specific primers in the PCR. Expression of cyclophilin (CYC-A) was used as the internal control. Presence of FGF22 and FGFR1 proteins was assessed by immunohistochemistry using commercial antibodies (human anti-FGF22 and anti-FGFR1; Abcam). Negative controls were performed using antibodies pre-absorbed in excess of protein. Effect of developmental stage on mRNA abundance was tested with ANOVA. Group means were compared using Tukey test. FGF22 mRNA was expressed in all stages of CL development, and at lower levels in stage 4 in comparison with stage 3. FGFR1B mRNA was detected in all stages of CL development, and its abundance did not vary with time. Immunohistochemical analysis revealed the presence of FGF22 and FGFR1 in the bovine CL. In conclusion, FGF22 and FGFR1B are expressed along the CL lifespan phases suggesting their participation in the formation, differentiation and regression of the CL. Furthermore, the expression pattern of FGF22 mRNA suggests that its suppression may be important for luteal regression. Supported by FAPESP

ABSTRACT 36

ROLE OF BONE MORPHOGENETIC PROTEIN 15 IN IN VITRO SURVIVAL AND DEVELOPMENT OF CAPRINE PREANTRAL FOLLICLES

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The objectives of this study were to investigate the effects of Bone Morphogenetic Protein 15 (BMP-15) in vitro survival, activation and growth of caprine preantral follicles. From each ovarian pair (n=4) fragments were obtained, of which one was immediately fixed, constituting the fresh control (non cultured), and the other fragments were cultured for 1 and 7 days in minimum essential medium (MEM) supplemented with different concentrations of BMP-15 (0, 1, 10, 50, 100 e 200 ng/mL). Thereafter, the ovarian fragments were processed for histological analysis and the follicles were classified in terms of integrity of caprine preantral follicles after 7 days of culture, but also promotes the follicular growth and transition to secondary follicle stage.

ABSTRACT 35

EXPRESSION OF FIBROBLAST GROWTH FACTOR 22 (FGF22) AND ITS RECEPTOR, FGFR1B, DURING LUTEAL DEVELOPMENT IN CATTLE

Castilho, A.C.S.; Machado, M.F.; Lima, P.F.; Price, C.A.; Buratini Jr., J.
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There are evidences indicating the participation of intra-ovarian factors in the control of corpus luteum (CL) formation and regression. Fibroblast growth factors (FGFs) are involved in the control of ovarian angiogenesis, steroidogenesis and apoptosis. FGF7 and FGF10 belong to the same FGF subfamily together with FGF22, and all three activate the same receptors (FGFR1B and 2B). FGF7 and FGF10 expression was demonstrated throughout the CL lifespan without signs of regulation, whereas FGFR2B mRNA expression was highest in the regressing CL and transiently decreased by PGF2α treatment. The aims of the present study were to determine the expression pattern of FGF22 and FGFR1B mRNA and to assess the presence of the respective proteins in the bovine CL. Bovine CLs were obtained from abattoir ovaries and classed into four stages of development (stage 1= corpus hemorrhagicum, stage 2= developing CL, stage 3= mature/early functional luteolysis CL, and stage 4= structural luteolysis). Tissue samples were submitted to total RNA extraction. Expression of FGF22 and FGFR1B mRNA was measured by real-time RT-PCR with oligodT in the RT and bovine-specific primers in the PCR. Expression of cyclophilin (CYC-A) was used as the internal control. Presence of FGF22 and FGFR1 proteins was assessed by immunohistochemistry using commercial antibodies (human anti-FGF22 and anti-FGFR1; Abcam). Negative controls were performed using antibodies pre-absorbed in excess of protein. Effect of developmental stage on mRNA abundance was tested with ANOVA. Group means were compared using Tukey test. FGF22 mRNA was expressed in all stages of CL development, and at lower levels in stage 4 in comparison with stage 3. FGFR1B mRNA was detected in all stages of CL development, and its abundance did not vary with time. Immunohistochemical analysis revealed the presence of FGF22 and FGFR1 in the bovine CL. In conclusion, FGF22 and FGFR1B are expressed along the CL lifespan phases suggesting their participation in the formation, differentiation and regression of the CL. Furthermore, the expression pattern of FGF22 mRNA suggests that its suppression may be important for luteal regression. Supported by FAPESP

ABSTRACT 36

ROLE OF BONE MORPHOGENETIC PROTEIN 15 IN IN VITRO SURVIVAL AND DEVELOPMENT OF CAPRINE PREANTRAL FOLLICLES

1Faculdade de Medicina Veterinária, LAMOFOPA, PPGCV, Universidade Estadual do Ceará, Fortaleza, CE; 2Instituto de Pesquisa e Tecnologia, Universidade Tiradentes, Aracaju, SE; 3Núcleo de Biotecnologia Aplicada ao Desenvolvimento Folicular Ovariano, Universidade do Vale de São Francisco, Petrolina, PE; 4Centro de Biotecnologia de Sobral (NUBIS), Universidade Federal do Ceará, Sobral, CE - Brasil

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The objectives of this study were to investigate the effects of Bone Morphogenetic Protein 15 (BMP-15) in vitro survival, activation and growth of caprine preantral follicles. From each ovarian pair (n=4) fragments were obtained, of which one was immediately fixed, constituting the fresh control (non cultured), and the other fragments were cultured for 1 and 7 days in minimum essential medium (MEM) supplemented with different concentrations of BMP-15 (0, 1, 10, 50, 100 e 200 ng/mL). Thereafter, the ovarian fragments were processed for histological analysis and the follicles were classified in terms of survival in normal or degenerated, and for the activation in primordial or growing follicles (transition, primary and secondary). The data were subjected to analysis of variance followed by Dunnett and Student Newman Keuls (SNK) tests, both at a significance level of 5%. The results showed that, after 7 days of culture, the concentrations 10, 50 or 100 ng/mL of BMP-15 maintained the percentage of normal follicles similar to fresh (non cultured) control (83%). Moreover, culture in medium supplemented with 200 ng/mL of BMP-15 significantly reduced the percentage of normal follicles in comparison to fresh control and other concentrations of BMP-15, except 1 ng/mL. From the first day of culture, the presence of BMP-15 in all concentrations significantly reduced the percentage of primordial follicles and increased concomitantly the percentage of growing follicles. The percentage of primary follicles after 7 days of culture significantly increased when BMP-15 was tested at concentrations 10, 50 or 100 ng/mL compared to fresh control and MEM alone. Moreover, a significant increase in percentage of secondary follicles was observed when BMP-15 was tested at 100 ng/mL compared to fresh control and MEM alone. In all the concentrations of this factor, except 200 ng/mL, it was observed an increase in oocyte and follicle diameters after 7 days of culture. In conclusion, this study demonstrated that BMP-15 at 100 ng/mL not only maintain the morphologic integrity of caprine preantral follicles after 7 days of culture, but also promotes the follicular growth and transition to secondary follicle stage.
ABSTRACT 37

FIBROBLASTIC GROWTH FACTOR -10 PLAYS A ROLE IN THE GROWTH AND SURVIVAL IN VITRO OF GOAT PREANTRAL FOLLICLES

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Recent studies have evidenced the involvement of different fibroblast growth factors (FGF) on ovarian follicular development control. FGF-10 modulates cellular activities in several tissues, but it has not been investigated in goat ovary yet. Therefore, the aim of the present study was to investigate the effects of FGF-10 on the survival, the activation and the growth of goat preantral follicles cultured in vitro. Ovaries (n = 12) from adult, non-pregnant, cyclic, mixed-bred goats with age between 1 to 3 years were collected from a local slaughterhouse. In the laboratory, the cortex of each ovarian pair was sliced and the fragments were immediately fixed (fresh control) or cultured for 1 and 7 days in the absence or presence of FGF-10 (0.1, 1, 10, 50, 100 and 200 ng/mL). Each treatment was repeated six times. Non-cultured and cultured tissues were processed and analyzed by classic histology, transmission electron microscopy and viability testing using fluorescent marker (calcein-AM–ethidium homodimer-1). The data were subjected to analysis of variance and compared using the Dunnett’s, SNK and ÷2 tests, for P<0.05.
The results showed that, after 7 days of culture, a higher percentage (80%) of morphologically normal follicles was observed in treatment with 50 ng/mL of FGF-10 and that this treatment was significantly higher than the other concentrations of FGF-10 (0 ng/mL – 67%; 1 ng/mL – 68%; 10 ng/mL – 63%; 100 ng/mL – 64%; 200 ng/mL – 53%). Ultrastructural analyses and viability testing confirmed the follicular integrity of FGF-10 (50 ng/mL) treated fragments after 7 days of culturing. In fresh control, we found 74% of primordial follicles and 26% of development follicles. After 7 days, all FGF-10 concentrations reduced the percentage of primordial follicles (0 ng/mL – 30.1%; 1 ng/mL – 25%; 10 ng/mL – 24%; 50 ng/mL – 21%; 100 ng/mL – 24%; 200 ng/mL – 21%) and increased the percentage of developing follicles (0 ng/mL – 70%; 1 ng/mL – 75%; 10 ng/mL – 78%; 50 ng/mL – 78%; 100 ng/mL – 76%; 200 ng/mL – 79%). In the presence of 50 ng/mL of FGF-10, follicles increased in diameter (80% ± 2.2) after 7 days of culturing when compared with other concentrations tested (0 ng/mL – 72% ± 2.1; 1 ng/mL – 73% ± 1.3; 10 ng/mL – 70% ± 2.1; 100 ng/mL – 70% ± 2.0; 200 ng/mL – 68% ± 1.6) (P<0.05). In conclusion, this study demonstrated that FGF-10 maintains the morphological integrity of goat preantral follicles and stimulates the growth of activated goat preantral follicles in culture.

ABSTRACT 38

LEVELS OF mRNA FOR BONE MORPHOGENETIC PROTEIN 7 (BMP-7) IN GOATS OVARIAN FOLLICLES AND IN VITRO EFFECTS OF BMP-7 ON SECONDARY FOLLICLES DEVELOPMENT

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Bone morphogenetic protein 7 (BMP-7) promotes activation and growth of primordial follicles in mice (LEE et al., 2004, Mol Reprod Dev, 69:159-163). However, the effect of BMP-7 and FSH on growth and expression of mRNA for FSH-R and BMP-7 in the goat secondary follicles is not known. The objective of this study was to quantify the mRNA levels for BMP-7 in goat ovarian follicles at different stages of development, and to evaluate the effects of FSH and BMP-7 on growth and expression of FSH-R and BMP-7 in secondary follicles. Then, groups of 10 primordial, primary and secondary follicles were isolated from goat ovaries (n=10) using a tissue chopper and stored at -80°C. Groups of 10 cumulus-oocyte complexes (COCs) of small (<3 mm) and large antral follicles (3-6 mm) were aspirated from ovaries (n=16) and stored at -80°C. For samples of mural granulosa cells/theca from large antral follicles, small and large antral follicles were microdissected from 10 ovarian follicular wall was collected and stored. For culture, secondary follicles (~200μm) were mechanically isolated and cultured in incubator with 5% CO2 at 39°C for 6 days in MEM supplemented with FSH (50ng/mL), BMP-7 (50ng/mL) or the combination of both. After, we evaluated the formation of follicular diameter and antral follicles and groups of 8 follicles were frozen at -80°C to evaluate the expression of mRNA for BMP-7 and FSH-R. Then, extraction of total RNA and cDNA synthesis were carried out. The levels of mRNA of BMP-7 and FSH-R were quantified by real time PCR. Kruskal-Wallis was used to compare the follicular diameter and the levels of mRNA for BMP-7 and FSH-R, chi-square test was used to compare the percentage of follicles that formed antrum (P<0.05). The expression of mRNA for BMP-7 was absent in primordial follicles, primary, secondary, as well as in COCs from antral follicles, but present in mural granulosa cells/theca of antral follicles. Compared to small antral follicles, the level of mRNA for BMP-7 was higher in follicular cells of large antral follicles. The addition of BMP-7 or FSH to the culture medium stimulated the growth of secondary follicles. The addition of FSH and BMP-7 was important to significantly increase the percentage of follicles that formed antrum, and the levels of mRNA for BMP-7 and FSH-R in follicles cultured in vitro. In conclusion, the levels of mRNA for BMP-7 are higher in mural cells of the granulosa/theca from large antral follicles. FSH and BMP-7 affect the growth of secondary follicles after in vitro culture, but the formation of the antrum and expression of mRNA for BMP-7 and FSH-R is stimulated only in the presence of FSH.
At device insertion and 5 days later, animals received 2 mL (0.15 mg) of PGF (Veteglan®, Calier, Spain). A dose of 500 µg of activin-A were similar to the MEM. Only follicles cultured with activin-A showed increased levels of mRNA for FSH-R, compared to MEM alone. However, when activin-A or both activin-A and FSH were added to medium the levels of mRNA for FSH-R, while the chi-square test was used to compare the percentage of follicles that formed antrum (p<0.05). The results normalize the data. Kruskal-Wallis test was used to compare the follicular diameter and the levels of mRNA for activin-A and FSH-R, while the chi-square test was used to compare the percentage of follicles that formed antrum (p<0.05). The results showed that after culture of secondary follicles for 6 days in MEM alone or supplemented with FSH, activin-A increased the follicular diameter, as compared to day 0. Moreover, the presence of activin-A, alone or in combination with FSH did not increase follicular diameter, as compared to MEM. When follicular diameter was compared between treatments, the presence of FSH significantly increased follicular growth (p<0.05). The percentage of follicles that reached antrum formation did not differ between treatments. The culture of secondary follicles with FSH increased the levels of mRNA for activin-A, when compared to MEM alone. However, when activin-A or both activin-A and FSH were added to medium the levels of mRNA for activin-A were similar to the MEM. Only follicles cultured with activin-A showed increased levels of mRNA for FSH-R, compared to MEM. In conclusion, FSH promotes increased follicular diameter and this effect was inhibited by the presence of activin-A. In addition, FSH increased the levels of mRNA for activin-A, and activin-A increased the levels of mRNA for FSH-R in goat secondary follicles.
ABSTRACT 41

INDUCTION OF PUBERTY IN HEIFERS WITH PGF₂α

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Methods to improve early reproductive function in heifers have been developed. In one study, one to two week-old calves already showed follicular developmental patterns similar to pubertal heifers (SILVA et al., 1973, Pesq Agrop Bras. Sér Vét. 8:81-83; EVANS et al., 1994, J Rep Fert, 102: 463-470). Puberty can be induced through the use of hormones such as progesterone (P₄), which reduces the number of estradiol receptors in the hypothalamus, leading to increased frequency of LH pulses after the end of treatment (IMWALLE et al., 1998, Biol Rep, 58: 1432-1436). Prostaglandin (PGF₂α) can also hasten the onset of puberty by increasing the pituitary response to GnRH and consequently, increasing LH release in postpartum cows (RANDEL et al, 1996, Theriogenology, 45:643-654). This study evaluates the effect of exogenous PGF₂α, with or without previous P₄ treatment, on the ovulation rate of pre-pubertal heifers. The hypothesis was that PGF₂α applied at the end of the growth phase of the dominant follicle induces ovulation in pre-pubertal heifers. Forty Red Angus pre-pubertal heifers, averaging 250 kg, were randomly assigned into three groups. The Progesterone + PGF₂α group (PPG, n = 12) received an intravaginal progesterone releasing insert (CIDR) plus an intramuscular (IM) injection of 500ìg cloprostenol upon removal of the CIDR; the PGF₂α group (PG, n = 15) was treated with 500ìg cloprostenol IM five days after the follicular wave began; the control group (CG, n = 13) received no treatment. Immediately after CIDR insert, the PPG heifers received 50 mg of progesterone IM and 2 mg of estradiol benzoate. On the fifth day after the emergence of a new follicular wave, CIDRs were removed. The results were analyzed using One-way ANOVA followed by Fisher's exact test. The groups had different ovulation rates (P<0.001). Only one ovulation was observed in the control group (7.7%), while 11 ovulations were detected in the PPG group (91.66%) and 10 ovulations in the PG group (66.66%, P < 0.0001). There was no difference in the ovulation rate between the groups that received progastaglandin (P>0.05); similarly there was no difference in the diameter of the ovulatory follicle between heifers from the PPG (12.45 ± 0.31 mm) and the PG group (12.45 ± 0.33 mm, P > 0.05). The daily follicular growth rate was greater in the PPG group (1.1 ± 0.05 mm / d) than in the PG group (0.96 ± 0.06 mm / d) and control group (1.00 ± 0.05 mm / d; P = 0.02). This study indicates that PGF₂αinduces ovulation in pre-pubertal heifers, with or without exogenous progesterone, at the end of follicular growth stage.

ABSTRACT 42

CULTURE OF FELINE PREANTRAL FOLLICLES

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Follicles from domestic cat (Felis silvestres catus) have been used as an experimental model for endangered wild cats. The definition of an adequate system for growing cat follicles will enable the expansion of assissted reproduction techniques. However, the domestic cat has a low rate of follicular development in vitro (JEWGENOW, 1998; Theriogenology 49:1567-1577). Therefore, this study aimed to evaluate the performance of advanced preantral follicles isolated from cats in culture (6 days). Five adult cats underwent ovariohysterectomy Ovaries were washed in 70% ethanol and in PBS supplemented with penicillin (100 g/ml) and streptomycin (100 mg/mL) and transported to the laboratory at 1 ha 4 ° C. Fine fragments were removed from ovarian cortex (1 mm³) for the isolation of preantral follicles e⁰ 150 lm microdissected using 26G needles attached to 1 mL syringes with magnifying glass (Nikon SMZ 645, Tokyo, Japan). Only follicles with a spherical oocyte, surrounded by two or more compact layers of granulosa cells without apparent damage to the basal membrane, were used. The culture medium was a-MEM supplemented with BSA (3 mg/mL), glutamine (2 mM), hypoxanthine (2 mM), insulin (10 ig/ ml), transferrin (5.5 µg/mL) selenium (5 ng/mL) ascorbic acid (50 mg/mL). Follicles (n = 57) were cultured individually in drops of 100 mL of medium under mineral oil in an incubator at 39 ° C and 5% CO₂ and 60 μl of the medium changed every two days. At the end of the growing follicular, viability was assessed by simultaneous marking with 4 mM calcein-AM and 2 mM ethidium homodimer-1 (Molecular Probes, Invitrogen, Karlsruhe, Germany) to live (green) and dead (red), respectively, using fluorescence microscope (Nikon, Eclipse 80i, Tokyo, Japan). At the end of cultivation were observed high rates of follicular viability (100%) found by the marking of follicles in the green calcein-AM. Based on these results, we can conclude that feline preantral follicles can be cultured and remain viable in vitro. However, although more research is needed about the optimum period for the in vitro development of preantral follicles from cats and addition of substances that stimulate their growth.
ABSTRACT 43
ASSOCIATION OF KIT LIGAND (KL) AND FOLLICLE STIMULATING HORMONE (FSH) IN DYNAMIC MEDIUM INFLUENCES SURVIVAL AND ACTIVATION OF CAPRINE PREANTRAL FOLLICLES AFTER LONG-TERM IN VITRO CULTURE


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The aim of the present study was to verify the role of KL, alone or associated with FSH, in in situ culture of caprine preantral follicles. For this purpose, ovaries from adult mixed-breed goats were collected at a local slaughterhouse and transported to the laboratory in Minimum Essential Medium (MEM) at 4°C. Thereafter, an ovarian fragment was randomly removed and fixed in Carnoy (fresh or non-cultured control) to histological analysis. The other fragments were cultured for 1, 8 or 16 days in a-MEM medium supplemented or not with KL (50 ng/mL), FSH (50 ng/mL) or FSH+KL (50 ng/mL) each. The substances tested (KL or FSH), or their association, were maintained from day 0 until day 16 of culture or were combined (e.g. KL added from day 0 to day 8 followed by culture with FSH from day 8 to day 16 – KL/FSH), totaling 10 different treatments. After each culture period, the ovarian fragments were fixed and analyzed by classical histology. In this analysis, the follicles were classified regarding survival in normal or degenerated, and in relation to activation in primordial or growing follicles (transition, primary and secondary). The percentage of morphologically normal follicles and the different follicular stages were subjected to ANOVA followed by SNK test to compare means. The data were expressed in mean ±SEM and the differences were considered statistically significant when P<0.05. The results demonstrated that, after 1 day of culture, only the treatments with KL or FSH maintained the percentage of morphologically normal follicles similar to fresh control (P>0.05). At day 16, all treatments cultured with KL until day 8 (KL/KL, KL/FSH and KL/FSH+KL), as well as the treatment cultured only with FSH until the end of culture (FSH/FSH) showed a higher percentage of normal follicles compared to cultured control (a-MEM) (P<0.05). Regarding the results of follicular activation, at days 1 and 8 all treatments initially cultured with KL (KL/KL, KL/FSH e KL/FSH+KL) demonstrated a higher percentage of growing follicles compared to a-MEM* and other treatments (P<0.05). After 16 days, a high percentage of follicular activation was verified in KL/FSH (98.89 ±1.10) (P<0.05). In conclusion, a dynamic medium containing KL and FSH maintains the follicular survival and stimulates the activation of caprine preantral follicles after long-term in vitro culture.

ABSTRACT 44
ROLE OF LEUKEMIA INHIBITORY FACTOR ASSOCIATED WITH FOLLICLE STIMULATING HORMONE FOR IN VITRO DEVELOPMENT OF CAPRINE PREANTAL FOLLICLES ISOLATED

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The aim of the study was to investigate the effects of leukemia inhibitory factor (LIF), with or without follicle stimulating hormone (FSH) on the formation of the antrum and in vitro growth of preantral follicles isolated from goats. Goat ovaries of goats were carefully dissected, and the secondary follicles (> 150 mm) were visualized under a stereomicroscope and mechanically isolated with the aid of needles. Growing follicles surrounded by granulosa cells, oocytes with visible and intact basement membrane were selected and cultured individually in drops of 100 mL of medium. The base medium used consisted of a-MEM supplemented or not with LIF (10 or 50 ng/ml) in the presence or absence of sequential FSH (day 0-6: 100 ng/mL, days 6-12: 500 ng/mL and days 12-18: 1000 ng/mL). Culture was performed at 39°C and 5%CO2 for 18 days, and 60 µL per drop of medium was changed every 48 h. It is noteworthy that, every six days, total return was performed for the change in concentration of FSH. It was used for each treatment a minimum of 35 follicles. The follicular development was observed by the formation or absence of antral cavity and increasing the diameter measured in the follicles were growing every six days with the aid of an ocular micrometer. The rate of formation of the antrum was compared by chi-square, while the follicular diameter was subjected to ANOVA followed by Kruskal-Wallis and Student Newman Keuls test (P<0.05). The results showed that on day 6 of culture, the addition of FSH promoted high percentage (80%) of antrum formation. It was observed that, at the end of growth period, the follicles treated with the interaction of LIF and 50 FSH show antrum formation rate significantly higher than the group treated only with LIF 50. In relation to follicular diameter, on the 6th of culture, the addition of FSH to the basic medium and medium containing LIF 50 caused a significant increase in this parameter. However at the end of culture all treatments were similar. When comparing the different days of evaluation, we could see a significant increase of follicular diameter until day 12 in all treatments. In conclusion, this study demonstrated that the LIF 50 ng/mL associated with FSH, not only enhances the antrum formation rate of goat preantral follicles isolated, as it promotes an increase in follicular diameter until day 6 of culture.
ABSTRACT 45

OVARIAN FUNCTION IN OVERCONDITIONED NELORE COWS (*Bos taurus indicus*) AFTER INDUCTION OF OVULATION

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Nutrition has been shown to affect postpartum reproduction. Currently, an increasing number of evidences are indicating that overconditioning cows may be hazardous to reproduction. The present study aimed to evaluate ovarian function in postpartum Nelore (*Bos taurus indicus*) beef cows with different body condition score (BCS). Twenty non-suckled cows (BW = 420.8 ± 39.9 kg and DPP = 129 ± 41.7 days) received an auricular implant (3mg norgestomet - Crestarâ, Intervet) and an IM injection of 2mg of estradiol benzoate. Nine days later, implant was withdrawn and 2mg of estradiol benzoate (IM) were given after 24 hours. Thirteen cows had their ovulation confirmed through ultrasound and remained in the trial. Females were split into two groups according to their BCS (1-9 scale), as follows: normally conditioned cows group (GBCS=5.7; n=7; BCS = 5.7 ± 0.4) and overconditioned cows group (GBCS=6.7; n=6; BCS = 6.7 ± 0.6). Ultra-sound examination of ovaries and blood sampling were carried out 48 hours after implant removal to the subsequent ovulation on a daily basis and observation of estrus behavior was made twice a day. Collected blood was processed to plasma and circulating progesterone concentration ([P4]) was determined through a validated RIE. Results were analyzed through ANOVA. The dominant follicle of the third wave in overconditioned cows persisted longer (P<0.05) than normally conditioned cows. Estrous cycle length and interovulatory interval were not affected by BCS. On the other hand, several attributes of luteal function differed (P<0.05) between groups. Overconditioned cows showed shorter lutel phase (13.3 ± 4.3 vs. 17.2 ± 1.7 days, respectively for GBCS=5.7 and GBCS=6.7), lowest value for maximum [P4] within the estrous cycle (5.24 ± 1.1 vs. 7.55 ± 1.7 ng/mL respectively for GBCS=5.7 and GBCS=6.7), lowest value for mean [P4] throughout the estrous cycle (3.69 ± 0.7 vs. 4.74 ± 1.2 ng/mL/day, respectively for GBCS=5.7 and GBCS=6.7). Nonetheless, corpus luteum (CL) maximum volume of overconditioned cows were larger than normally conditioned cows (5,837 ± 639 vs. 2,020 ± 692 mm³, respectively for GBCS=5.7 and GBCS=6.7). The steroidogenic capability of the luteal tissue of overconditioned cows might have been negatively affected by their higher nutricional status, measured as BCS. Indeed, it is a paradox that CL was larger in overconditioned cows but their progesterone secretion was smaller. It was concluded that overconditioned Nelore cows present suboptimal luteal function as compared to normally conditioned postpartum females.

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INDUCTION OF MULTIPLE OVULATIONS IN MARES USING LOW DOSES OF DESLORELIN ACETATE


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The induction of multiple ovaries in mares have been studied by many researchers with the main purpose of improve the index of embryo collection and increase the offer of oocytes. The rate of embryo recovery in non-superovulated mares ranges around 65%, in other words, less than one embryo per donor per collection. Many drugs and protocols are used in superovulation programs and embryo transfer (ET), such as Equine Pituitary Extract, equine FSH, porcine FSH, recombinant equine FSH, GnRH and immune-neutralization against inhibin. Nevertheless, the drugs utilized show inconstant results and/or a high cost, making difficult their utilization in commercial systems of ET. This work aimed to induce multiple ovulations in mares using low doses of deslorelin acetate (Bachem - USA - handled by the laboratory of animal reproduction - FMVZ). Twenty six mares were submitted to an embryo collection eight days after ovulation. Then, received 5mg of Dinoprostone (Lutalyse® Pfizer Brazil), and were randomly divided into two groups, one was treated and the other one was the control group. The animals were monitored daily by the use of ultrasound and at the moment of the detection of a follicle with diameter ranging between 23-25mm and at least a second follicle ≥ 20mm of diameter, was initiated the superovulatory protocol with 100µg of deslorelin acetate in the treated group and saline solution in control group. The administrations were done each 12 hours intra-muscular (IM) until the detection of a follicle which diameter ranged from 33-35mm. At this moment, 1mg of deslorelin acetate IM associated with 1000UI of hCG IV (Vetercor®, Laboratórios Hertape Calier, Brazil) was administrated to induce the ovulations. All mares were inseminated between 24 to 48 hours after the ovulation induction, and the embryos were collected at day 8 (D8). On the follow cycle mares were swited on control and treatment group. The data were evaluated by Graphic Pad using Fisher Exact Test and the differences were considered significantly when p<0.05. Based on the obtained data, it was verified that the percentage of mares that responded to the treatment with at least two ovulations per cycle was 85% and the embryo recovery per ovulation did not differ statistically between the groups (p>0.05), however the number of ovulations per cycle (26 x 48) and the percentage of embryo recovery per cycle (55% x 90%), for control and treated group respectively, were significantly different (p<0.05). Based on the results of the present experiment we can concluded that administration of low doses of deslorelin acetate was efficient to increase the number of ovations and the rate of embryo recovery per cycle and is a good alternative to increase the efficiency and decrease the costs of Equine ET programs.
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PLASMA AND URINARY CONCENTRATION OF FSH AND LH IN NELLORE HEIFERS

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This study evaluated FSH and LH concentration from heifer's blood and urine samples, exploring new ways of samples for hormone analysis in these animals. Blood samples (10 mL) were collected daily for 7 consecutive days always at 6:00 am, from jugular vein using vacuum tubes and urine samples (approximately 10 mL) from spontaneous urination using nine Nellore heifers (Bos taurus indicus). Blood samples were mixed with 200µl de anticoagulant EDTA, and centrifuged at 1500 rpm for 15 min to obtain plasma. Serum and urinary concentration of the LH and FSH were determined using radioimmunoassay. Data analysis used paired t test (GraphPad, Instat). LH plasma (0.77±0.07 ng/mL) and urine (0.57±0.05 ng/mL) concentration were different (p=0.0059). Despite of difference there was (p=0.0153) a weak positive correlation (r=0.2842) between plasma and urinary LH concentration. On the other hand urinary FSH concentration negatively correlated (p= 0.0022, r=-0.3982) with plasma concentration. As for LH, urinary FSH concentration (0.81±0.38 ng/mL) was higher (p < 0.0001) than plasma concentration (0.36± 0.07 ng/mL). Some considerations can be performed: first it is shown the possibility of protein hormone quantification from bovine urine; second LH urinary concentration is 36% and FSH 125% higher than plasma concentration, showing that although renal protein excretion is limited, after urine volume reduction, the both LH and FSH urinary concentration increases; third weak correlation either negative or positive shows that urinary concentration does not reflect blood concentration at that moment, more researches are needed to evaluate the needed time for a blood hormone increase to appear at urine. Acknowledgments: FAPESP #2008/56680-1

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EFFICIENCY OF PROTOCOL P-36, ASSOCIATED WITH eCG OR LH ADMINISTRATION, IN THE LAST DAY OF SUPERESTIMULATORY TREATMENT, IN NELLORE COWS

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The objective of this study was to evaluate the use of pLH in replacement of eCG on the last day of P-36 superstimulatory treatment in Nellore donors. Recent studies have demonstrated improvement on embryo production when the last two doses of FSH were replaced by eCG. However, consecutive use of eCG in bovine superstimulatory protocols may induce antibody against eCG, decreasing embryo production. Twenty-five Nellorecows were randomly allocated in four groups: P-36 (Control), P-36/eCG, P-36/LH2, and P-36/LH4. All animals underwent four treatments in a cross over design. Donors received an intravaginal device (IVD, Primerâ, Tecnopec, São Paulo, Brazil) at a random stage of the estrous cycle containing 1.0g of progesterone and estradiol benzoate (2.0 mg, i.m.; Estroginâ, Farmavet, São Paulo, Brazil; D0). Cows from Control group were superestimulated with decreasing doses of pFSH (133 mg, i.m., Folltropin-Vâ, Bioniche, Ontario, Canada; D5-8). In P-36/eCG group the last two doses of pFSH were replaced by 2 doses of eCG (200 IU each dose, i.m, Folligonâ, Intervet, Netherlands). In P-36/LH2 and P-36/LH4 groups the last two doses of pFSH were replaced by two doses of 1 and 2 mg of pLH respectively (i.m., Lutropinâ, Bioniche, Ontario, Canada). All animals were treated with PGF2α(150 µg d-cloprostenol, i.m., Proliseâ, Tecnopec, São Paulo, Brazil) on D7, and the IVD was removed 36 h after. Ovulation was induced with 12.5 mg of pLH (i.m.), on D9, and all animals received fixed-time artificial insemination (FTAI) 12 and 24 h after pLH. Embryo flushing was performed on D16. Data were analyzed by ANOVA (Proc Mixed, SAS). There was asignificant difference in the number of CL in eCG group (19.2±2.4) when compared to LH2 (12.7±2.0) and LH4 groups (12.3±1.5; p<0.05). Additionally, there was a tendency of lower ovulation rate in LH2 as compared to eCG group (50.6 and 67.8%, respectively; p=0.06). However there was no difference in viable embryo yield among groups P-36 (3.3±0.7), P-36/eCG (4.5±0.5), P-36/LH2 (3.7±0.8) and P-36/ LH4 (4.2±1.0); P>0.05. In conclusion eCG can be replaced by pLH (4.0 mg), in the last day of P-36 protocol, without affecting the production of viable embryos in Nellore cows. Acknowledgments: Hildergard G. V. Pritzewitz Experimental Station for the facilities used in the present experiment, and FAPESP (São Paulo – Brazil) for funding and fellowships (A.C.S. Oliveira, M.C.C. Mattos and M.R. Bastos).
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FOLLICLE DYNAMIC IN BRAZILIAN BREEDING JENNIES IN TWO DIFFERENT PERIODS OF YEAR

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Aiming to study the ovarian dynamic during estrous cycle of jennies and to verify possible seasonal effects, 10 estrous cycles of Brazilian breeding jennies (n=10) were monitored during the period of increasing natural luminosity (phase 1), 1st of August to 15th of September. Other estrous cycle (n=10) of the same animals were monitored during period of high luminosity (phase 2) 1st of December to 20th of January) at a latitude 20°43'05"S and longitude 48°32'38"W. In both phases, jennies were evaluated by daily transrectal ultrasonography during an interovulatory interval (IOI). Ovaries were mapped during each evaluation and position and diameter of follicles ø14 mm were registered as at the end of experiment, it was possible to retrospectively analyze the dominant follicle (DF) growing profile and the follicle dynamic. The number of major primary and secondary waves (Ginther et al, 1993, Eq. Vet. Sci., 13: 18-25) per IOI, the moment of wave emergence (fol.14mm), follicle deviation, maximum diameter and growth rate of DF were identified. Data from phases 1 and 2 were analyzed by paired-samples t Test at a 5% of significance. A major follicular wave was detected in 80% (n=8) and 60% (n=6) and two waves in 20% (n=2) and 40% (n=4) in the jennies in phases 1 and 2, respectively. In animals of one wave estrous cycle, emergence occurred at day 13.2±3.0, follicular deviation at day 18.6±1.7 post ovulation, maximum diameter of DF was 37.5±4.1 mm with daily growth rate of 2.7±0.7 mm during phase 1, these findings were similar (p>0.05) to results found for phase 2, which emergence occurred at day 13.2±3.2, follicular deviation at day 20.0±0.0 post ovulation, maximum diameter of DF 36.9±2.3 mm with daily growth of 2.2±0.7 mm. IOI of 24.6±1.8 days in phase 1 and 26.2±2.6 days in phase 2 were also similar. The absence of a seasonal influence on the studied variables allowed grouping the cycles in two phases for comparison of the cycles of one (n=14) and two (n=6) major follicular waves by ANOVA, concerning the wave emergence and follicular deviation that occurred on an average of days 13.2 and 14.3 and 19.0 and 18.7, respectively (p>0.05). Maximum diameter of DF was 37.3 mm with a daily growth rate of 2.5 mm for cycles of one wave and 33.3 mm with a daily growth rate of 2.9 mm for cycles of two waves (p>0.05). IOI was also similar (p>0.05), 25.3 and 25.2 days for cycles of one and two waves, respectively. In conclusion, jennies maintained in the mentioned latitude/longitude were not influenced by seasonal variations in the studied periods. However, if the lower natural light period makes influence on the estrous cycles need to be confirmed. The majority of estrous cycles demonstrated one follicular wave and a lower percentage with two waves. Knowlodgement: FAPESP and EMBRAPA-OEPAS.

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QUANTIFICATION OF mRNA FOR BMP-15 IN GOATS OVARIAN FOLLICLES AND IN VITRO EFFECTS OF BMP-15 ON SECONDARY FOLLICLES DEVELOPMENT

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Bone morphogenetic protein 15 (BMP-15) is found in oocytes of all categories and in granulosa cells of goat primary, secondary and antral follicles (Silva et al., 2006, J. Endocrin., 189: 113-125). However, the levels of mRNA for BMP-15 in different follicular categories, as well as the effects of BMP-15 and FSH on the growth of goat secondary follicles are not known. The objective of this study was to quantify the levels of mRNA for BMP-15 in different follicular categories and to evaluate the effects of BMP-15 and FSH on the growth of secondary follicles. To achieve these aims, groups of 10 primordial follicles, primary and secondary were isolated from goat ovaries (n=10) and stored at -80° C until extraction of total RNA. Another group of ovaries (n=16), cumulus-oocyte complexes (COCs) from small (<3mm) and large antral follicles (3-6 mm) were aspirated and stored at -80°C until extraction of total RNA. The mural granulosa cells/theca, small and large antral follicles were microdissected from 10 ovarian follicular walls, collected and stored at -80°C until extraction of total RNA. The levels of mRNA for BMP-7 were performed by real time PCR. For in vitro culture, secondary follicles were mechanically isolated from ovaries (n=20) cultured in incubator with 5% CO2 at 39°C for 6 days in MEM supplemented with FSH (50ng/ml) combined or not with BMP-15 (100ng/ml). After culture, follicular diameter and antrum formation were analyzed. Kruskal-Wallis test was used to compare the follicular diameter and the levels of mRNA for BMP-15, and the chi-square test was used to compare the percentage of follicles that formed antrum (p<0.05). Secondary follicles have higher levels of mRNA for BMP-15 than primordial and primary follicles (p<0.05). There were no significant differences in levels of mRNA for BMP-15 when COCs and granulosa cells/theca from small and large antral follicles were compared (p>0.05). The expression of mRNA for BMP-15 in COCs of small and large antral follicles was higher when compared to their respective granulosa cells/theca. After culture, a significant increase in follicular diameter was observed in all treatments when compared to day 0, and follicular diameter did not differ between treatments. The percentage of follicles that formed antrum did not differ between treatments. In conclusion, during transition from primary to secondary follicles occurs an increase in the levels of RNAm for BMP-15 and COCs are the main sources of RNAm for BMP-15 in antral follicles. In addition, BMP-15 did not affect growth of secondary follicles in vitro.
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A NEW APPROACH OF STUDYING THE DEVELOPMENTAL PATTERN OF SMALL ANTRAL FOLLICLES IN THE BOVINE OVARY


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Although the current knowledge about the pattern of antral follicular development is well documented for follicles larger than 4 mm, very little is known about the follicular growth of smaller antral follicles. Recently, an ultrasound instrument (Ultrasound biomicroscope; UBM) with resolution comparable to a stereomicroscope has become available for reproductive biology research. Applications of this imaging technique could result in a better understanding of biological events such as follicle wave emergence and developmental patterns of small antral follicles. The objectives of this study were to: 1) evaluate the number of small antral follicles (<3 mm); 2) compare the number of follicles of different categories; and, 3) compare the follicular dynamics between conventional ultrasound and UBM ultrasound exams, in the first follicular wave after ovulation. Hereford crossbred heifers (14-16 months old, n=12) were used in the study. Heifers were given 500 µg cloprostenol, im, in order to induce the ovulation. Conventional ultrasonographic (Aloka SSD-900, Aloka, Japan, equipped with 7.5 MHz a transducer) along with ultrasound biomicroscopy examinations (Vevo 660; Visual Sonics Inc., Toronto, Canada, equipped with a 20 MHz end-fire transducer for tranvaginal examinations) were performed from Day -4 to Day 10 (Day 0 = ovulation day). In the conventional US examination was possible to detect that the number of small (1 – 3 mm) and large (> 4 mm) follicles changed over days (P = 0.036 and P < 0.001, respectively). Same effect in number of follicles over days was detected in very small follicles (< 1 mm) and large follicles (P = 0.036 and P = 0.03, respectively) in the UBM examinations. The peak in number of small follicles was detected on Day -4, -1 and 0 of follicular wave on the conventional examinations, whereas in the UBM evaluation the peak in number of small follicles was detected on Day -4 and -2. The very small antral follicles (<1mm) were detected only with UBM US. In addition, this follicle category maintain quite constantly in number of follicles during the follicular wave, having a slightly increased in number of follicles on Day -2. When the number of small and large follicles were compared between techniques (UBM vs Conventional US), there were an effect of day (P < 0.001) and technique (P = 0.05) on the number of small follicles during the first follicular wave. The higher number of small follicles detected with the UBM technique was of 23.71 ± 4.06 on Day -4, whereas in the Conventional US examinations the number of small follicles detected from Day -4 to Day 1 was constant, on average 13.1 ± 2 (range 12 - 14.1). In conclusion the conventional US evaluation showed that the number of follicles detected is underestimated, since the UBM demonstrated higher number of very small (< 1 mm) and small follicles (1-3 mm) throughout a follicular wave. This study was supported by NSERC and CIHR, Canada. Luiz Pfeifer was supported by CAPES foundation, Brazil.

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QUANTIFICATION OF MESSENGER RNA FOR c-KIT IN GOATS OVARIAN FOLLICLES


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Kit ligand (KL) and its receptor c-kit are important for migration, proliferation and survival of primordial germ cells (ZAMA et al., 2005, Biol Reprod, 73:639-47). YOSHIDA et al. (1997, Dev Biol, 184:122-137) reported that the c-kit receptor is required for the activation of primordial follicles. In addition, the levels of mRNA for KL have been quantified in the different categories of goat ovarian follicles (CELESTINO et al., 2010, Mol Reprod and Dev, 77:231-40), but the levels of mRNA for c-kit in the different follicular categories are still unknown. The objective of this study was to quantify mRNA levels for c-kit in different categories of goats ovarian follicles. For this, 10 goat ovaries were used, and primordial follicles, primary were mechanically isolated mechanically using a tissue chopper, while secondary follicles (~ 200um) were isolated by microdissection. From another group of ovaries (n = 16), cumulus-oocyte complexes (COCs) from small (<3mm) and large antral follicles (3-6 mm) were punctured and selected according to the morphology of oocyte and granulosa cells. Then, groups of 10 follicles in each category and groups of 10 COCs were collected and stored at -80°C until extraction of total RNA. For the collect of mural granulosa cells and theca, small and large antral follicles were microdissected from 10 ovaries and the follicular wall was collected and stored at -80°C. We carried out reverse transcription and quantification of levels of mRNA for c-kit by PCR in real time. Data of mRNA expression for c-kits in primordial, primary and secondary follicles were analysed by Kruskal-Wallis, while paired t-test was used to compare data from small and larde antral follicles(P<0.05). The mRNA for c-kit was expressed in primordial, primary, secondary follicles, and COCs and granulosa cells and theca in antral follicles. The results showed that levels of mRNA for c-kit were significantly lower in secondary follicles when compared with the primordial and primary follicles. Levels of mRNA for c-kit in COCs from large and small antral follicles were similar. No significant differences in levels of mRNA for c-kit in granulosa cells and theca of small and large antral follicles were observed. However, the levels of mRNA for c-kit were significantly higher in COCs from large and small antral follicles when compared with their granulosa and theca cells. In conclusion, the mRNA for c-kit was detected in all follicular categories, being less expressed in secondary follicles. In addition, COCs from small and large antral follicles show higher levels of mRNA for c-Kit than that of granulosa and theca cells.
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QUANTIFICATION OF MESSENGER RNA FOR VASOACTIVE INTESTINAL PEPTIDE (VIP) IN GOATS OVARIAN FOLLCLES

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The vasoactive intestinal peptide (VIP) is a neuropeptide that has been identified in bovine ovarian follicles (HULSHOF et al., 1994, Biol Reprod, 50:553-560). In addition, VIP promotes survival of granulosa cells inhibited apoptosis (LEE et al., 1999, Endocrinology, 140:818-826). However, few studies quantifying the levels of mRNA for VIP in the ovarian follicles exists. The objective of this study was to quantify the levels of mRNA for VIP in different categories of goat ovarian follicles. To achieve these aims, primordial, primary and secondary follicles were mechanically isolated from goats ovaries (n=10) using a tissue chopper. Then, groups of 10 follicles in each category were collected and stored at -80°C until extraction of total RNA. From another group of ovaries (n=16), cumulus-oocyte complexes (COCs) from small (<3mm) and large antral follicles (3-6 mm) were microdissected from 10 ovaries. The follicular wall was collected and stored at -80°C until extraction of total RNA with the aid of TRizol® (Invitrogen, São Paulo, Brazil). After reverse transcription, quantification of levels of mRNA for VIP was performed by PCR in real time. The data were normalized using the reference genes ß-actin and ß-tubulin. Data from the mRNA for VIP were compared by Kruskal-Wallis and t-test (p<0,05).

Quantification of mRNA for VIP showed that the secondary follicles present levels of mRNA significantly larger than primordial follicles (p<0,05). There was no significant difference between when the levels of mRNA for VIP in primordial and primary follicles were compared (p=0,05). In addition, there was no significant difference between mRNA for VIP in COCs from small antral follicles (<3 mm) and large (3-6 mm), but granulosa and theca of large antral follicles showed a significant increase in the levels of mRNA for VIP than the COCs from small antral follicles (p<0,05). However, there was no significant difference between the levels of mRNA for VIP in COCs from small antral follicles (<3 mm) and large (3-6 mm), but granulosa and theca of large antral follicles showed a significant increase in the level of mRNA for VIP as compared to COCs from small antral follicles. Real-time PCR showed that in COCs from small and large antral follicles, the levels of mRNA for VIP are significantly higher than their respective granulosa cells/ theca. In conclusion, during transition from primary to secondary follicles, as well as from small to large antral follicles occurs a increase in the levels os of RNAm for VIP. In addition, COCs from small and large antral follicles had higher levels of mRNA for VIP than their respective granulosa cells and thecal.

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EFFECT OF VOLUME OF MEDIUM ON IN VITRO DEVELOPMENT OF ISOLATED GOATS PREANTRAL FOLLICLES

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Several factors may influence the in vitro development of preantral follicles, among which stands out the volume of medium, since the number of factors and nutrients present in the culture medium directly influence follicular growth. Thus, the aim of this study was to evaluate the effect of different volumes of culture medium on in vitro development of caprine preantral follicles isolated. Ovaries (n=36) from adult non-pregnant mixed-breed goats were collected at a local slaughterhouse and transported in HEPES-buffered Minimum Essencial Medium (MEM) supplemented with antibiotics (100 mg/l streptomycin and 1% ITS, 50 mg/ml ascorbic acid, 1% glutamine, 1% hypoxanthine and 1000 ng/ml FSH). Isolated follicles were randomly divided into two groups: Group I (drops of 25 µl) and Group II (drops of 100 µl). In all groups, the follicles were cultured individuallly for 18 days in drops under mineral oil at 37°C and 5% CO2 in air. Every other day the culture medium was changed and measurement of follicular diameter was performed, and the following parameters were analysed: diameter and follicular morphology, membrane integrity and antrum formation. Intact and clear follicles surrounded by granulosa cells were considered viable and those with irregular or absent basement membrane, and dark areas with retraction of the oocyte were considered degenerated. Folicles in Group II presented a significantly higher rate of viability when compared to Group I. With respect to rates of growth and antrum formation, no significant differences between groups. In conclusion, 100 µl drops resulted in higher follicular viability after 18 days of culture.
EFFICIENCY OF PROTOCOL P-36, ASSOCIATED WITH eCG OR LH ADMINISTRATION, IN ANGUS DONORS

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The objective of the present study was to verify the efficiency of protocol P-36/eCG in Angus breed, and to test the replacement of eCG by LH in the last day of superstimulatory treatment. In experiment (exp.) 1, 22 Angus cows were allotted to 4 groups: LH60, LH60/eCG, LH60/LH and LH60/FSH+LH. Each donor was superovulated 3 times, in such a way that each animal received 3 of 4 treatments (incomplete block). At a random stage of the estrous cycle the donors received an intravaginal device (IVD) containing 1.0g of progesterone and estradiol benzoate (3.0mg, i.m, Day 0). In the LH60 group the animals were superstimulated with decreasing doses of FSH, whereas in groups LH60/eCG and LH60/LH the last 2 doses of FSH were replaced by eCG (i.m, each dose=200IU) or LH (i.m., each dose=1.0mg), respectively. Finally, the cows from group LH60/FSH+LH received 2 doses of 1.0mg of LH simultaneously with the last 2 doses of FSH. All animals were treated with PGF2αanalog on D6, and the IVD was removed 36h afterwards. Ovulation was induced with 12.5mg of LH (i.m.), on D8, and the animals were FTAI 12 and 24 h after LH. In exp.2, 17 cows were allocated in 3 groups: LH48, LH60 and LH48/FSH+LH. The difference between the first and the second exp. is that in groups LH48 and LH48/FSH+LH the hormone used to induce ovulation was administered 48h after PGF2α, instead of 60h. Each donor was superstimulated 3 times (cross-over). In a thirddexp. the dose to induce ovulation (12.5vs25.0mg) was tested in LH60/FSH+LH protocol (n=12 cows, cross-over).Embryo flushing was performed on D15, in all exp., and data were analyzed by ANOVA (Proc Mixed, SAS). In exp.1, replacement of eCG by LH (LH60/LH4), resulted in a decline (p<0.05) on number of total embryos and transferable embryos, when compared to the others. However, addition of LH to the last 2 doses of FSH (LH60/FSH+LH) improved embryo quality and numerically increased the total embryo yield (87) when compared to LH60 (40) and LH60/LH (13). In exp.2, there was no difference among treatments for embryo yield. Nevertheless, group LH48/FSH+LH had a higher number of excellent and good embryos than the others (p<0.05). In exp.3, it was observed ovulation rate of 78% in donors treated with 25mg of LH and 54% in the receiving 12.5mg. It is concluded that eCG can be replaced by FSH+LH, in the last day of superstimulation treatment of P-36 protocol, without affecting viable embryos yield. Additionally, the dose of 25mg is better than 12.5mgLH to induce ovulation in Angus donors. Acknowledgments:CAPES for scholarship to Rosa FS and ABN Agropecuária for providing the animals for the exp.

EFFICIENCY OF LOW DOSE OF EQUINE PITUITARY EXTRACT ON THE RATES OF EMBRYO RECOVERY

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Equine Pituitary Extract (EPE) has been consistent to induce multiple ovulation in a dose of 25 mg. However, when evaluating the embryo recovery per follicle ovulated, that number is still low. Thus, the purpose of this study was to test the efficiency of low dose of EPE (8.0 mg) to induce two or three ovulations and get the recovery of at least one embryo per cycle. For this, six Margalarga Marchador embryo donors, between three and 18 years, weighting between 350 and 450 kg, cycling regularly were used. The ovarian cyclicity was monitored by ultrasonic examinations, performed daily during estrus to determine the day of ovulation (D0). We used two consecutive estrous cycles, the first of untreated-control, (n = 6) and the second treated cycle (n = 6). Treatment consisted of administration of 8.0 mg of EPE twice daily, starting at D8, until at least two follicles reached 35 mm. At this time, were given 1000 IU hCG intravenously to induce ovulation. The mares received 5.0 mg of Tromethamine Dinoprost the first day of treatment. All mares were inseminated with fresh semen collected from a stallion of proven fertility using 500 million of viable spermatozoa 24 hours after the time of ovulation induction, and then every 48 hours until ovulation occurred with the semen of stallions of proven fertility. The embryo recovery was conducted eight days (D8) after the last ovulation. The results were evaluated by the non-parametric Mann Whitney test, at 5% significance level. Embryo recovery rates for the control and treated cycles were 83% (5/6) and 150% (9/6), respectively, showing a significant difference (P<0.05) between groups. The embryo recovery per ovulation for untreated-control cycle and treated cycle were, respectively, 71% (5/7) and 75% (9/12). There was no significant difference between groups (P>0.05). In the treated cycle, it was observed the development of 12 follicles and 12 ovulations (one mare showed triple ovulation and three embryos were recovered; four mares showed double ovulation and one animal did not respond to treatment). Only one mare did not ovulate 48 hours after the induction. This animal was re-inseminated later. In the untreated-control cycle, one mare presented double ovulation and one embryo was recovered. With these results, it is clear that the EPE was effective in increasing the rate of embryo recovery per animal. However, concerning to recovery rate per ovulation, it was not possible establish difference between groups due to low number of animals and the high embryo recovery for control group. Financial support from FAPERJ.
The aim of this study was to develop a dynamic culture medium with FSH, LH and EGF to promote in vitro development of oocytes obtained from goat preantral follicles until complete maturation and to improve the capacity of their oocytes for in vitro fertilization (IVF) and embryo production. For the culture, preantral follicles (~150 µm) were isolated from ovarian fragments and cultured for 18 days. To experiment 1, it was utilized α-MEM-supplemented with increased concentrations of FSH throughout culture periods, corresponding to control medium or treatment 1-T1 (FSH 100 ng/mL-until day 6, FSH 500 ng/mL-until day 12 and FSH 1000 ng/mL-until day 18). The other treatments consisted of adding LH alone or associated with EGF to the control medium from day 12 of culture: treatments T2 (LH 50 ng/mL), T3 (LH 50 ng/mL + EGF 50 ng/mL), T4 (LH 50 ng/mL + EGF 100 ng/mL), T5 (LH 100 ng/mL), T6 (LH 100 ng/mL + EGF 50 ng/mL) and T7 (LH 100 ng/mL + EGF 100 ng/mL). For experiment 2, preantral follicles were cultured only in the culture medium used on treatment 7 and after 18 days their oocytes underwent in vitro maturation followed by IVF. Follicle development was evaluated on the basis of antral cavity formation, follicular and oocyte growth, and cumulus oocyte complex health. At the end of culture period, the association of both EGF concentrations with LH 50 ng/mL and EGF 100 ng/mL as well as the association of LH 100 ng/mL and EGF 100 ng/mL (T7) had a positive influence on the daily follicular growth rate (P < 0.05). After 32 hours of IVF, oocytes grown in the presence of EGF 100 ng/mL associated with LH in both concentration (T4 and T7) had a meiosis resumption percentage significantly superior to the other treatments (P < 0.05). Two embryos were obtained in the experiment 2, in which preantral follicles were cultured for 18 days in medium supplemented with 100 ng/mL LH and 100 ng/mL EGF (T7). In conclusion, the sequential culture system was able to promote in vitro growth of preantral follicles, promoting their oocyte maturation and caprine embryo production from preantral follicles. Keywords: Preantral, sequential medium, FSH, LH, EGF, goat.

ABSTRACT 58

EFFECT OF GDF-9 AND FSH ON GROWTH AND EXPRESSION OF mRNA FOR FSH-R AND GDF-9 IN GOAT SECONDARY FOLLICLES CULTURED IN VITRO

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Growth and Differentiation Factor 9 (GDF9) is a member of the transforming growth factor-α family and plays an important role in ovarian folliculogenesis. The deletion of GDF-9 gene in mice blocked folliculogenesis at the primary stage, demonstrating the importance of this factor in early follicular development (Dong et al., 1996, Nature, 383: 531-535). However, no one knows the effect of GDF-9 and FSH on the growth and expression of mRNA for FSH-R and GDF-9 in secondary follicles goat. The objective of this study was to evaluate the effect of GDF-9 alone or in combination with FSH on the growth and expression of mRNA for FSH-R and GDF-9 in goat secondary follicles after 6 days of in vitro culture. For this, secondary follicles (~200µm) were mechanically isolated from ovaries (n=20) and cultured in incubator with 5% CO₂ at 37°C for 6 days in MEM supplemented with 50 ng/mL FSH, 200 ng/mL GDF-9 or the association of FSH and GDF-9. After culture, follicular diameter and antrum formation were evaluated. Then, groups of 8 follicles were collected and stored at -80°C. Then, we carried out the extraction of total RNA followed by reverse transcription of samples from all treatments. Levels of mRNA for GDF-9 and FSH-R were quantified by real time PCR. The Kruskal-Wallis test was used to compare the follicular diameter and the levels of mRNA for GDF-9 and FSH-R, while the chi-square test was used to compare the percentage of follicles that reached the antrum formation (P < 0.05). The results showed that when compared to day 0, the culture of secondary follicles for 6 days in MEM alone or supplemented with FSH, GDF-9 or both significantly increased follicular diameter in all groups. When the follicular diameter was compared between treatments, it was observed that the presence of FSH significantly increased follicular growth when compared to MEM alone (P < 0.05). Moreover, the presence of GDF-9 alone or in combination with FSH did not increase follicular diameter. In addition, the percentage of follicles that reached the antrum formation did not differ between treatments. Regarding mRNA expression, the culture of goat secondary follicles in medium supplemented with FSH, GDF-9 or both did not influence the levels of mRNA for FSH-R alone or in combination with FSH. In conclusion, FSH promotes the growth of goat secondary follicles after culture in vitro, but the GDF-9 alone or in combination with FSH did not promote follicular development. Furthermore, GDF-9 alone or in combination with FSH did not influence the levels of mRNA for GDF-9 and FSH-R in goat secondary follicles after culture in vitro.
The aim of the present study was to determine the effect of activin-A on the capacity of oocyte development enclosed in caprine isolated preantral follicles. For this purpose, ovaries (n=16) from adult goats were collected at a local slaughterhouse, for follicular isolation. Only preantral follicles with diameter > 150 µm and visible oocytes, surrounded by granulosa cells, basement membrane and without antrum were selected for in vitro culture. The culture medium was α-MEM® added with FSH in a sequential manner (100 ng/mL until day 6, 500 ng/mL until day 12 and 1000 ng/mL until day 18) and/or activin-A (50 or 100 ng/mL). Preantral follicles were cultured for 18 days in an incubator with 5% air CO2 at 39°C. Every other day, 60 µL of the culture medium were replaced. Antrum formation and follicular survival rates were evaluated every 6 days during culture (days 0, 6, 12 and 18). Antral cavity formation was defined as a visible cavity between the granulosa cell layers. Follicles were considered morphologically normal when it had translucent membrane intact basal oocytes and granulosa cells surrounding homogeneous and no signs of degeneration. The rate of resumption of meiosis was calculated by dividing the number of oocytes undergoing germinal vesicle breakdown of the number of oocytes used for in vitro maturation x 100.

Antrum formation, survival and meiosis resumption rates were compared using the Chi-Square test. Data were considered significant when P<0.05. During 18 days of culture, in different treatments, no significant differences were found on the percentage of preantral follicles surviving. However, for each treatment, it was verified that activin-A 50 ng/mL maintained the percentage of health follicles, from day 12 onward. At day 6, it was verified that activin-A in both concentrations (50 and 100 ng/mL) increased significantly the percentage of antrum formation compared to control (P<0.05). The percentage of oocytes resuming meiosis was significantly higher in activin-A 50 ng/mL compared to control (P<0.05). In conclusion, the addition of activin-A to the medium not only maintains follicular viability and induces antrum formation, but also is essential for the oocyte meiotic resumption after in vitro culture of isolated caprine preantral follicles.

ABSTRACT 60

OVARIAN FOLICLE DYNAMICS AND PROGESTERONE CONCENTRATIONS DURING ESTROUS CYCLE IN ANGLONUBIAN GOATS

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Female goats present estrous cycles with 21 day regular intervals (GALINA et al., 1995, Small Rum. Res., 18:249-253) and a defined estrous period, with 30 hour average duration (CHEMINEAU et al., 1992, Anim. Reprod. Sci., 30:157-184). In sheep, the number of follicular waves is three or four (BARTLEWSKI, 2000, Anim. Reprod. Sci., 58: 273-29) while in goats, four or five were registered (GINTHER & KOT, 1994, Theriogenology, 42:987-1001). De CASTRO et al (1999, Theriogenology, 52:399-411) observed that Saanen goats presenting four follicular waves have higher progesterone concentrations during luteal phase, than goats presenting three or two waves. The present work aimed to evaluate follicular growth dynamics and progesterone concentrations during estrous cycle in Anglonubian goats. Ten female goats with 18 months old, 40 kg average weight, kept in semi-intensive breeding management were used. For estrous synchronization, they received intravaginal sponges containing 50 mg of MGA (Progespon®, Intervet Schering-Plough, Brazil) for 11 days and, 48 hours before sponges removal by intramuscular way, 50 Ig of PGF2α (Ciosin®, Intervet Schering-Plough, Brazil) and 400 IU of eCG (Novormon® Intervet Schering-Plough, Brazil). After sponges removal, estrous was daily monitored and oocytes were evaluated by rectal ultrasonography, for follicular growth dynamics study during 46 days, stabilized time to register the occurrence of three ovulatory events, and consequently, 2 estrous cycles. The ovaries evaluation consisted in measuring e 3mm diameter follicles to determine the number of follicular waves per estrous cycle. Blood samples were daily collected and centrifuged (3018,2 G/10 minutes) for serum obtaining; then, stored at -20°C, until progesterone concentrations were determined. The data average numbers and deviations were: interovulatory period =22,00±0,00 days (12 cycles); follicular waves per cycle =3,66±0,57 (12 cycles); pre-ovulatory follicle maximum diameter =11,58±1,94 mm (21 ovulations); follicular phase duration (P<1ng/ml) =6,00±1,00 days (3 cycles); luteinlic phase duration (P>1ng/ml) =15,92±0,67 days (12 cycles); follicular phase P concentrations =0,02±0,04 ng/ml (3 cycles); luteal phase P concentrations =7,12±17,54 ng/ml (12 cycles). The averages obtained in this experiment, for Anglonubian females, were similar to those obtained in other caprine race females (GINTHER & KOT, 1994; GALINA et al., 1995; DE CASTRO et al, 1999).
ABSTRACT 61

USE OF SEXED SEMEN IN A SUPEROVULATION PROTOCOL FOR NELORE (Bos indicus - Preliminary Results)


Use of sexed semen versus conventional semen in a superovulation protocol for Nelore (Bos indicus) heifers: preliminary results.

The objective of the present study was to evaluate the effect of a six hour delay on the fixed-time artificial insemination (FTAI) with sexed and conventional semen on the production of viable embryos in Nelore donors (Bos indicus, n=16) submitted to multiple ovulation. Donors were treated with intravaginal progesterone device (1gr: Sincrogest®, Ouro Fino, Brazil) for 7 days. At the moment of device insertion (Day 0) Estradiol Benzoate (2mg i.m.; Sincrodiol®, Ouro Fino, Brazil) was administered. From the 4th to the 7th day, 8 decreasing doses of FSHp (133mg i.m.; Folltropin-V®, Bioniche, Canada), were administered in a 12 hours interval in order to superstimulate follicular growth. On the 6th day of treatment, two equal doses of Prostaglandin F2αwere administered (0.53mg of Cloprostenol sodic i.m.; Sincrocio®, Ouro Fino, Brazil) and on the 8th day (12 hours after the last FSH), ovulations were induced by LHp (25mg armour i.m.; Lutropin-V®, Bioniche, Canada). The animals were divided into four groups, according to the moment of FTAI (12 and 24 vs. 18 and 30 hours after ovulations induction) and type of semen used [conventional (2x10⁶ sperm/dose) vs. sexed (4.2x10⁶ sperm/dose)].

Semen was produced from the same bull and batch were used on all inseminations. Embryos were harvested on day 15 by non-surgical method of uterine flushing, and the recovered embryos were quantified and classified according to morphology and quality (IETS, 1998). The design was completely randomized factorial 2x2, in four replicates with an interval of 42 days and 16 repetitions per treatment, aiming to minimize the individual effects on the final result (all animals were submitted to all treatments). The variables were analyzed by Proc GLM procedure of SAS®. Preliminary results showed no interaction between the type of semen and sexed and the moment of insemination, which allowed the analysis of main effects. There were no differences regarding the type of semen (conventional vs. sexed) and to the FTAI moment (12 and 24 vs. 18 and 30 hours) in the total of embryonic structures (3.46 ± 0.81 vs. 3.79 ± 1.16 and 3.79 ± 1.17 vs. 3.46 ± 0.79), transferable embryos (2.88 ± 0.73 vs. 2.04 ± 0.82 and 2.67 ± 0.95 vs. 2.25 ± 0.57), degenerated (0.50 ± 0.20 vs. 0.83 ± 0.40 and 0.38 ± 0.12 vs. 0.96 ± 0.42) and unfertilized (0.08 ± 0.06 vs. 0.88 ± 0.59 and 0.75 ± 0.59 vs. 0.21 ± 0.10; P>0.05).

Preliminary results from this study indicate the possibility of using sexed semen associated with multiple ovulation protocol without the necessity of delay in six hours the FTAI.

ABSTRACT 62

FOLLICULAR DEVELOPMENT EVALUATED BY ULTRASONOGRAPHY DURING DIFFERENT STIMULATION TREATMENTS IN CANINDÉ GOATS


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The aim of the present study was to evaluate the effect of different hormonal stimulation treatments on follicular antral population in naturalized Canindé goats monitored by transrectal real-time ultrasonography (RTU). Seventeen cyclic goats were treated with intravaginal sponges containing 60 mg MAP (medroxiprogesterone acetate, Progespon, Syntex, Buenos Aires, Argentina) for 7 days. At the moment of device insertion (Day 0) Estradiol Benzoate (2mg i.m.; Sincrodiol®, Ouro Fino, Brazil) for 7 days. At the moment of device insertion (Day 0) Estradiol Benzoate (2mg i.m.; Sincrodiol®, Ouro Fino, Brazil) for 11 days and a single im injection of 50 µg d-cloprostenol (Ciosin, Schering Plough, São Paulo, Brasil) on the morning of the eighth day. Additionally, the females received following treatment regimens for ovarian stimulation: in multi-doses (MD) and triple (TD) groups. Donors were treated with intravaginal progestrone device (1gr; Sincrogest®, Ouro Fino, Brazil) for 7 days. At the moment of device insertion (Day 0) Estradiol Benzoate (2mg i.m.; Sincrodiol®, Ouro Fino, Brazil) was administered. From the 4th to the 7th day, 8 decreasing doses of FSHp (133mg i.m.; Folltropin-V®, Bioniche, Canada), were administered in a 12 hours interval in order to superstimulate follicular growth. On the 6th day of treatment, two equal doses of Prostaglandin F2αwere administered (0.53mg of Cloprostenol sodic i.m.; Sincrocio®, Ouro Fino, Brazil) and on the 8th day (12 hours after the last FSH), ovulations were induced by LHp (25mg armour i.m.; Lutropin-V®, Bioniche, Canada). The animals were divided into four groups, according to the moment of FTAI (12 and 24 vs. 18 and 30 hours after ovulations induction) and type of semen used [conventional (2x10⁶ sperm/dose) vs. sexed (4.2x10⁶ sperm/dose)].

Semen was produced from the same bull and batch were used on all inseminations. Embryos were harvested on day 15 by non-surgical method of uterine flushing, and the recovered embryos were quantified and classified according to morphology and quality (IETS, 1998). The design was completely randomized factorial 2x2, in four replicates with an interval of 42 days and 16 repetitions per treatment, aiming to minimize the individual effects on the final result (all animals were submitted to all treatments). The variables were analyzed by Proc GLM procedure of SAS®. Preliminary results showed no interaction between the type of semen and sexed and the moment of insemination, which allowed the analysis of main effects. There were no differences regarding the type of semen (conventional vs. sexed) and to the FTAI moment (12 and 24 vs. 18 and 30 hours) in the total of embryonic structures (3.46 ± 0.81 vs. 3.79 ± 1.16 and 3.79 ± 1.17 vs. 3.46 ± 0.79), transferable embryos (2.88 ± 0.73 vs. 2.04 ± 0.82 and 2.67 ± 0.95 vs. 2.25 ± 0.57), degenerated (0.50 ± 0.20 vs. 0.83 ± 0.40 and 0.38 ± 0.12 vs. 0.96 ± 0.42) and unfertilized (0.08 ± 0.06 vs. 0.88 ± 0.59 and 0.75 ± 0.59 vs. 0.21 ± 0.10; P>0.05).

Preliminary results from this study indicate the possibility of using sexed semen associated with multiple ovulation protocol without the necessity of delay in six hours the FTAI.
ABSTRACT 63

ABSENCE OF GREEN FLUORESCENCE IN CUMULUS OOPHORUS CELLS, IN CUMULUS-OOCYTE-COMPLEXES WITH FLUORESCENT OOCYTES FROM C57/BL6/EGFP MOUSE

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Generation of animal models, as GFP transgenic mice, is an important tool to understand the morphology and physiology of cells, isolated or in association to other cells. The functional unity of the cumulus-oocyte-complex (COC) is of essential importance to oocyte growth, maturation and fertilization understanding. The fluorescence of cumulus oophorus cells could be explored (coupled with nonfluorescent oocytes) to studies of the influence of those cells on denuded oocytes. The aim of this report was describe the lack of complete green fluorescence of the COC from EGFP mice. Four C57BL/6-Tg (CAG-EGFP) C14-Y01-FM132 Osb females, weighting around 35g, were sacrificed to other purposes (i.e., embryo recovery). Ovaries were collected and manipulated in PBS with 0.6% BSA. They were manually fragmented (23G needle) in order to release COCs. After 3 to 4 washes, groups of COCs and eventually primordial follicles, were evaluated with bright and UV light under an inverted microscope (Eclipse Ti-S, Nikon, Japan). Control COCs from four ovaries (Swiss Webster strain) were used to standard absence of fluorescence. Images were captured (NIS-Elements Advanced Research, Nikon, Japan) with both light sources and merged.

As expected, control COCs did not fluoresce with specific UV light to EGFP (488nm). However, oocytes from C57/BL6 EGFP fluoresced with high intensity, independently if they came from primordial or antral follicles. Interestingly, cumulus oophorus cells did not fluoresce even with their counterpart (i.e., oocyte) strongly glowing. When put together control COC and C57/BL6 EGFP COC, no difference was detected between cumulus oophorus cells from both COCs under UV light. Herein it was reported that the cumulus-oocyte-complex from C57/BL6 EGFP did not follow the constitutive gene expression of EGFP, as related previously when the Tg mouse was generated (“Green mice” as a source of ubiquitous green cells; Okabe et al., FEBS Letters, 1997:407;313-9). Cumulus cells should be put together with hair and red blood cells as the only cells(structures) that do not fluoresce on described EGFP mouse. The authors wish to acknowledge Dr. Masaru Okabe (Genome Information Research Center, Research Institute for Microbial Diseases, Osaka University, Japan) for C57BL/6-Tg (CAG-EGFP) C14-Y01-FM132 Osb mice and FAPESP (São Paulo, Brazil) for funding (06/06491-2) and fellowships (09/15919-4, 09/15919-4, 09/17605-7 e 07/07705-9).

ABSTRACT 64

OVULATORY DYNAMICS OF TOGGENBURG GOATS SUBMITTED TO ESTROUS INDUCTION WITH AUTOCLAVED REUTILIZED INTRAVAGINAL DEVICES

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Reutilization of progesterone intravaginal devices may cause health risks. The aim of the study was to evaluate the influence of the autoclaving process in intravaginal devices on the ovulation parameters in Toggenburg goats. It was conducted in October of 2008 (non-breeding season), Piau-MG, Brazil (latitude 21° 35’ and longitude 43° 15’). Twenty-one goats were selected and distributed according to weight and body condition scores, respectively, into three treatments: animals received in October of 2008 (non-breeding season), Piau-MG, Brazil (latitude 21° 35’ and longitude 43° 15’). Twenty-one goats were selected and distributed according to weight and body condition scores, respectively, into three treatments: animals received

The results are described as mean ± SD. All goats showed subluteal (≤1ng/mL) progesterone concentrations seven days before receiving the devices, reflecting the reproductive seasonality of this breed at that altitude. No difference was detected (P>0.05) among treatments on the following parameters: females ovulating rate (%) for NC (100.0; 6/6), C6 (87.5, 7/8) and C12 (100.0; 7/7); Number of ovulations for NC (1.5 ± 0.5, 9/6), C6 (1.9 ± 0.7, 13/7) and C12 (1.7 ± 0.8, 12/7); Interval from device removal to ovulation (h) for NC (72.0 ± 13.1), C6 (61.7 ± 4.5), C12 (72.0 ± 17.0); Interval from estrus to ovulation (h) for NC (40.0 ± 9.8), C6 (29.1 ± 13.6), C12 (41.1 ± 11.7); Diameter of dominant follicle (mm) for NC (7.6 ± 1.0), C6 (7.3 ± 0.4), C12 (7.4 ± 0.3) and Diameter of co-dominant follicle (mm) for NC (7.1 ± 1.1), C6 (6.9 ± 0.5), C12 (6.5 ± 0.2). It is concluded that reused autoclaved devices present similar efficiency regarding goats reproductive parameters associated to ovulation. Financial Support: CNPq, Embrapa Goats and Sheep Keywords: Caprine, CIDR, Follicular Dynamics, Reproductive Efficiency
EFFECTS OF FGF-2 AND FSH ON THE DEVELOPMENT AND EXPRESSION OF FSH-R AND FGF-2 SECONDARY FOLLICLES GOATS CULTIVATED IN VITRO

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Fibroblast growth factor-2 (FGF-2) is an important regulator of various ovarian functions such as mitosis, steroidogenesis, differentiation and apoptosis of granulosa cells (SKINNER, 2005, Human Reprod Update 11:461-471). In goats, FGF-2 (50 ng/mL) increased follicle development and maintenance of follicular viability during 5 days of culture (MATOS et al., 2007, Reprod. Fertil. Dev, 19:677-684). However, the effect of FGF-2 and FSH on the growth and expression of mRNA for FSH-R and FGF-2 in caprine preantral follicles is not yet known. The objectives of this study were to evaluate the effect of FGF-2 alone or in combination with FSH on the growth and expression of mRNA for FSH-R and FGF-2 in preantral follicles after 6 days of in vitro culture. For this, secondary follicles (~ 200lm) were mechanically isolated from goat ovaries (n = 20) and cultured in an incubator with 5% CO2 in air at 39°C for 6 days in minimum essential medium (MEM) supplemented with 50 ng/mL FSH, 50 ng/mL FGF-2 or the combination of FSH and FGF-2. After the culture period, follicular diameter and antrum formation were evaluated. Then, groups of eight follicles were frozen at - 80°C to evaluate the expression of mRNA for FGF-2 and FSH-R. Then, extraction of total RNA and reverse transcription were carried out for follicles from all treatments. Levels of mRNA for FGF-2 were quantified by real time PCR. Data of mRNA expression for c-kit were compared by Kruskal-Wallis and t-test (P<0.05). The results showed that after culture of secondary follicles for six days in MEM alone or supplemented with FSH, FGF-2 or both FSH and FGF-2, a significant increase in follicular diameter was observed in relation to day 0. When the follicular diameter was compared between treatments, the results showed that the presence of FGF-2 significantly increased the follicular growth, when compared to MEM alone or MEM supplemented with FSH and FGF-2 (P<0.05). Furthermore, FSH alone or combined with FGF-2 did not increase follicular diameter. Moreover, the percentage of follicles which have reached formation of antrum did not differ between treatments. The culture of goat secondary follicles in medium supplemented with FSH, FGF-2 or both did not influence the levels of mRNA for FGF-2. However, the secondary follicles cultured in medium supplemented with FSH showed levels of FSH-R mRNA significantly higher than those follicles cultured in medium supplemented with FGF-2. In conclusion, FSH and FGF-2 stimulate the growth of secondary follicles after 6 days of in vitro culture. In addition, FSH increased levels of mRNA for FSH-R after culture of goat secondary follicles compared to follicles cultured with FGF-2.

THE EFFECT OF ECG IN MULTIPLE OVULATION PROTOCOLS FOR HOLSTEIN HEIFERS EMBRYO DONORS

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The aim of this study was to evaluate the multiple ovulation response (number of corpora lutea – CL, number of recovered embryos and embryo quality) in Holstein heifers treated with eCG in the end of the protocol for multiple ovulation (MO) with FSH. The study was conducted in two periods (30 to 40 days interval). A total of 36 donors were used in a cross over design, ending up with 72 MO. The heifers were allocated in two experimental groups, according to the administration or not of eCG. Heifers from group FSH (n=35) received a norgestomet ear implant (Crestar®, Intervet, Brazil) in the morning (AM) of a random day of the estrous cycle (D0) + 2 mg estradiol benzoate (Gonadiol®, Intervet, Brazil) IM. From recovered embryos and embryo quality) in Holstein heifers treated with eCG in the end of the protocol for multiple ovulation (MO) protocol response. Acknowledgments: Fazenda Santa Rita (Agrindus) and Intervet Schering-Plough.
IATF/TETF/IA

Fixed-time AI, Fixed-time ET, AI
ABSTRACT 67

REUSE OF VAGINAL DEVICES ADDED WITH PROGESTERONE FOR THE ESTRUS CONTROL

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One of the limiting factors for the use of fixed timed artificial insemination is the cost of the hormones used in this technique. Therefore some authors work with the reuse of vaginal devices or reducing the hormone levels. The aim of this study was tested the reuse of vaginal devices added with progesterone. The experiment was conducted in Iguatemi, Mato Grosso do Sul, Brazil. There were used 62 heifers, divided into three groups. All animals were submited to estrus control protocol, based on the insertion of a vaginal device. On day 0, it was administered 1.5 mg of estradiol benzoate. After eight days, the vaginal device was removed and it has been applied 150 mg of d-cloprostenol and 200UI of equine chorionic gonadotrophin (ECG). On the ninth day 9 (D9), 0.008 mg of Buserelin was administrated. All animals were inseminated on the tenth day. Vaginal devices differ between groups. In group 1 (n = 20), it was used a new commercial device (CIDR®). The commercial vaginal devices, which had been used previously 3 times, were reused in group 2 (n = 21) and in group 3 (n = 21). For these reuse, 500 mg (group 2) or 750mg (group 3) of alcoholic solution of based progesterone were directly injected with a syringe into devices. Thirty-five days after the artificial insemination, the pregnancies were diagnostics with ultrasound. The results were analyzed by Chi-square. The animals in groups 1, 2 and 3 showed 35.0%, 61.9% and 47.6% of pregnancies. There was no statistical difference between treatments (p> 0.05), however the number of animals in each treatment is insufficient to eliminate the type one error. In the conditions of these experiment, the reuse of vaginal devices for estrus control after a further addition of progesterone showed to be efficient.

ABSTRACT 68

EFFECT OF ARTIFICIAL INSEMINATION MOMENT ON CONCEPTION RATE IN A PROTOCOL FOR FTAI

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The fixed-time artificial insemination is a very widespread technique among the beef cattle farms that use the AI, attracted by the potentialities that your use can provide in relation reproductive management and genetic improvement of livestock. If used properly the results will not differ from those found at natural mating or the AI itself. The challenge is make the technology a less onerous procedure. Thus, was put to rebuttal the effect of flexibility of time of AI on conception rate of Nelore cows subjected to FTAI protocol of three managements. To perform this study 322 females were submitted the following protocol: In a random stage of the estrous cycle (D0) at 8:00 a.m., each animal received an intravaginal progesterone device (Sincrogest®, Ouro Fino, Sao Paulo, Brazil) and an application 2,0mg of estradiol benzoate (Sincrodiol®, Ouro Fino, Sao Paulo, Brazil) im and an application 500µg of cloprostenol (Sincrocio®, Ouro Fino, Sao Paulo, Brazil) im. On day eight at 8:00 a.m., the device was removed and applied 500µg of cloprostenol (Sincrocio®, Ouro Fino, Sao Paulo, Brazil) im. On day ten, animal were allocated according to animal category (lactating cow, heifers, single cows) and body score into three groups: AI-M (AI in the morning, n=128), AI-A (AI in the afternoon, n=162) and AI-N (AI in the night, n=32), inseminated according to the group and measured the diameter of the preovulatory follicle by transrectal ultrasonography. The pregnancy diagnosis occurred 50 days after the start of the protocol and the results between groups were analyzed statistically by chi-square. For diameters of the follicle was applied analysis of variance (PROC GLM) and the means were compared by SNK (SAS). The means for conception rate and the diameter of the follicle were 52.80% and 12.53 ± 3.03mm, respectively. The average diameter of follicles varied little in relation to groups (P>0.05) AI-M (12.47 ± 3.07mm), AI-A (12.64 ± 2.97mm) and AI-N (12.27 ± 3.31mm); similarly, the conception rate did not differ statistically between the groups AI-M (53.13% - 68/128), AI-A (51.23% - 83/162) and AI-N (59.38% - 19/32). The results show that using a simplified protocol, of three managements, it is possible flexibilize the time of artificial insemination can be performed by the morning, afternoon and/or night; besides, increasing the number of animals on group treatment provided by this protocol FTAI, can be considered an increment on the practical applicability of the technique.
ABSTRACT 69

INCIDENCE OF PREGNANCY LOSS AND EFFECT OF VACCINATION AGAINST REPRODUCTIVE DISEASES ON PREGNANCY RATE IN BEEF COWS SUBMITTED TO FIXED TIMED ARTIFICIAL INSEMINATION.

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The aim of this study was to determine the incidence of pregnancy loss in beef cows, and to evaluate the effect of vaccination against reproductive diseases in pregnancy rate, and maintenance of pregnancy. In experiment 1 and 2 were used cows never vaccinated against reproductive diseases and in experiment 3 cows received immunization for Leptospirosis semiannually. In all experiments the cows received the same TAI protocol: [D0- 2mg of estradiol benzoate (Estrogên®); 1.9g de progesterone (CIDR®); D7-dinoprost tromethamine (PGF2á, 12.5mg, Lutalyse®); D9- device removal CIDR® and 1mg of estradiol cypionate (ECP®) and calf removal; D11-TAI]. The evaluation of pregnancy was performed at 30 and 120 days post TAI.In experiment 1, no vaccination was carried out and were evaluated 4289 cows on 20 farms to determine the rate of pregnancy loss between 30 and 120 days. In experiment 2, were used 2384 cows, randomly divided into the same group to receive or not the vaccine against IBR / BVD / Leptospirosis (5.0 mL, ir, Cattle Master ® 4 + L5, Pfizer Animal Health, Lincoln, USA) following this vaccination schedule: first dose at the beginning of the TAI protocol and the second dose (booster) at the time of first diagnosis of gestation (30 days post TAI). In experiment 3, were used 299 cows randomly distributed within the same group to receive or not the vaccine against IBR / BVD / Leptospirosis, the first dose thirty days before the start of the TAI protocol and the second dose at the beginning of the TAI protocol. The data were analyzed in SAS Proc Logistic.In Exp.1, were detected farm effect (results ranging from 1.45% to 12.16%, P < 0.05) and order effect (Primiparous: 9.03%; Multiparous: 5.06%, P<0.05) on the rate of pregnancy loss between 30 and 120 days.In Experiment 2, treatment affected (P < 0.01) pregnancy rate at 30 (Control: 53.2% and Vaccinated: 57.4%) and 120 days (Control: 48.3% and Vaccinated: 53.5%). In Experiment 3 was detected a tendency of effect (P < 0.10) of treatment on pregnancy rate at 30 (Control: 52.9%; Vaccinated: 59.7%) and 120 days (Control: 50.0%; Vaccine: 57.7%). These results show that there are farms with different rates of pregnancy loss and the vaccination with Cattle Master ® 4 + L5 (Pfizer Animal Health, Lincoln, USA) increased the pregnancy rate in beef cows.

ABSTRACT 70

COMPARISONS THE CONCEPTION RATES AND PREGNANCY IN CROSSBREDEDEWES SUBMITTED TO ARTIFICIAL INSEMINATION IN FIXED TIME AFTER NORGESTOMET TREATMENT BY FIVE, NINE AND FOURTEEN DAYS

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The aim of this work was to compare conception rates at AIFT and pregnancy late in crossbred ewes treated with Norgestomet for fourteen, nine and five days, in two different experimental periods. The first experimental period (E-I) was conducted in april and may 2008 and the second period (E-II) in march of 2009. Were used in E-I 70 adult sheep, crossbred, non pregnant, with ECC 2.5 to 3.5. For E-II, 62 ewes were used under previous EI, under the same conditions. In both experimental periods, the animals received 1.5 mg of Norgestomet (Crestar®, Intervet, Holanda) on D0 and this was maintained for 14 days in the G14 (E-I, n=24, E-II, n=21), 9 days in the G9 (E-I, n=23, E-II, n=21) and 5 days in the G5 (E-I, n=23, E-II, n=20). On the withdrawal of Crestar®, were administered in all groups 22.5 ugdoprostrenol (Prelobar®,Intervet, Holanda) and 400 IU of eCG (Folligon®, Intervet, Holanda). All animals were subjected to cervical IA with semen pool of cooled, 54 hours after the withdrawal of the Crestar®. Detention of estrus was performed in 72 hours, to detect by the use of ruffians. After 15 days of the ending the protocols, was introduced three males for the realization accomplishment, during 45 days. The pregnancy diagnosis was performed at 30 and 60 days after AIFT. The results were tested for Fisher (p<0.05). The rate of presentations of estrus was similar between treatment groups in periods E-I e E-II [E-I: G5=87% (20/23), G9=95.6% (22/23), G14=75% (18/24), E-II:G5=95% (19/20), G9=100% (21/21), G14=90.5% (19/21)] (p>0.05). In EI period, rates of conception in G9 (60.9%, 14/23) and G5 (47.8%, 11/23) were similar (p> 0.05) and the last lower the G14 (83.3%, 20/24) (p<0.05). In E-II period, the rate of pregnancy after the pass was 76.2% (16/21), 95.2% (20/21) and 95.0% (19/20) for G14, G9 and G5, respectively (p<0.05). It was concluded protocol based Norgestomet administered for fourteen, nine and five days in crossbred ewes, provided adequate conception rates after cervical AIFT with cooled semen, in both experimental periods.
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EFFECT OF MILK PRODUCTION IN CONCEPTION RATE OF DAIRY COWS INSEMINATED AFTER HEAT DETECTION OR AFTER PROTOCOL FOR FIXED TIME ARTIFICIAL INSEMINATION

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The objective of this experiment was to evaluate the effect of milk production on conception rate in dairy cows inseminated after heat detection or after protocol for TAI. The experiment was conducted in five farms, from July to December, 2009. In AI group after heat detection, 672 cows with average production of 31.7 ± 1.61 kg of milk per day and 191 ± 5.4 days in milk were inseminated in the schedule AM/PM 12 hours after heat detection. In group that received TAI, 1073 cows with average production of 31.1 ± 1.09 kg of milk per day and 173 ± 4.2 days in milk were inseminated with the following protocol for TAI: D0 - 2mg of estradiol benzoate (Estrogin®, Farmavet, Brazil) and insertion of intravaginal device containing 1.9g of progesterone (CIDR®, Pfizer Animal Health, Brazil); D7 - injection of prostaglandin (25mg of Lutalyse®, Pfizer Animal Health, Brazil); D8 - blood sampling to determine concentrations of progesterone before removal of the intravaginal device and administration of 1mg estradiol cypionate (ECP®, Pfizer Animal Health, Brazil); D10 - TAI. The binary data was analyzed by logistic regression procedure and continuous data for the GLM of SAS. Cows inseminated at fixed time had better (P=0.02) conception rate (26.6%) when compared with inseminated after heat detection (21.3%), and cows inseminated in winter had higher (P=0.02) conception (26.2%) compared to cows inseminated in the spring (21.5%). Milk production influenced negatively the conception, independent of the reproductive strategy used (P<0.05). In animals subjected to TAI, the concentration of progesterone on the day of the intravaginal device removal did not affected conception (P=0.42). The number of CIDR uses - without prior use (1st use), pre-used by eight (2nd use) or sixteen days (3rd use), influenced (P<0.01) the concentration of progesterone on the day of removal the intravaginal device, however, did not affected (P=0.07) conception (CIDR1: 1.55 ± 0.04 ng/ml and 26.2%; CIDR 2: 1.10 ± 0.04 ng/ml and 25.8%; CIDR 3: 1.02 ± 0.06 ng/ml and 27.2%), respectively. Thus, the protocol used for TAI was efficient, because improves the conception rate compared to AI after heat detection.

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CONCEPTION RATES WITH ARTIFICIAL INSEMINATION IN A DAIRY HERD GIROLANDO IN THE MUNICIPAL DISTRICT OF RIO CLARO, RIO DE JANEIRO STATE

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This field study is based on the analyze of the conception rates of the Girolando breed females in a dairy farm at municipal district of Rio Claro, Rio de Janeiro State, during years 2005 to 2009. The goal of was to evaluate IA reproductive efficiency, through the conception rates, considering periods, inseminators and bull breeds. The cows were milked two times a day, managed in rotational grazing system occupying an area of 60 ha, being 11ha with Brachiaria decumbens, Tifton, Mombaça (Panicum maximum cv) and supplied sugar cane (Saccharum officinarum), elephant grass (Pennisetum purpureum) and corn silage in the feeding trough, still receiving proportionally to the milk production, a balanced concentrate ration and not receiving mineral in the last 2 years. The herd was composed by 196 Girolando cows and heifers with average productions from 4500 to 6000 kg/lactation. The estrus detection was accomplished with teaser utilization, in 3 daily observations, and IA was accomplished second Trimberger's outline. The heifers were inseminated when they reached 340 kg of alive weight and the cows from 60 days post parturition. During the evaluation periods were registered 1449 IA, 34 bulls of 4 breeds (Gir, Girolando, Holstein and Jersey) and 8 inseminators (A, B, C, D, E, F, G and H). For statistical analysis was used the test of two proportions (P=0.05) in the Triola Statdisk software. Were obtained 53,4a% (n = 215), 45,7a% (n = 221), 46,9a% (n = 349), 40,9b% (n = 354) and 32,5b% (n = 310) for the conception rates during the periods of 2005, 2006, 2007, 2008 and 2009, respectively. The conception rates for inseminators were 50,5a% (n = 91), 50,0a% (n = 2), 50,0a% (n = 2), 43,6ab% (n = 1125), 44,8ab (n = 49), 43,7ab% (n = 16), 30,4b% (n = 82) and 12,1c% (n = 82). The conception rates for the breeds Gir, Girolando, Holstein and Jersey were 100a% (n = 2), 46,1b% (n = 39), 44,9b% (n = 1033) and 37,0b% (n = 375), respectively. The differences in the conception rates found for the evaluated parameters, they can be partially explained by individual variations, breeds, ability inseminators, and the reductions observed in the two last periods, be relative the minerals deficiency possible effects, what in dairy animals of larger productive potential has negative reflex in the reproduction.
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EFFECT OF TIME OF INSEMINATION IN THE CONCEPTION RATE OF NELORE COWS SUBMITTED TO TAI PROTOCOLS IN COMMERCIAL PROGRAMS IN THE STATE OF BAHIA

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Reproductive performance, is five times more important than weight gain and up to 10 times larger than carcass characteristics (Segui et al., Arch. Vet. Sc., 2002., 2:173-178). Several technologies are available to producers to achieve better results (Johnson et al., Theriogenology, 2005. 64:639-656). The use of fixed timed artificial insemination (TAI) protocols allows the cows to be inseminated 48 hours after removal of progesterone implants and ovulation induction, which allows groups to inseminate at morning and afternoon allowing a larger number of cows inseminated per day, with a better ovulation synchrony. The aim of this study was to investigate the effect of time of artificial insemination (AI) on pregnancy rate in beef cows subjected to TAI synchronization protocols in commercial programs at the state of Bahia. In this study 5422 cows originated from nine farms subdivided into 28 programs during the years 2008, 2009 and 2010 were evaluated. All animals were examined before, being discarded those that presented problems. All cows were synchronized with progesterone device associated with estradiol benzoate, estradiol cypionate cloprostenol and eCG. Groups were divided randomly and were AI in the morning (GM) and afternoon (GT) with an average difference of 6 hours ± 20 minutes between groups with each group always starting 48 hours after progesterone device removal and induction of ovulation. Pregnancy diagnosis was performed 45 days after AI by rectal palpation and ultrasonography (FALCON VET, Esaoste Pie Medical). Statistical analysis was performed by chi-square (c2). The total results obtained was 45.88% (2488 pregnant cows from 5422 inseminated cows). The pregnancy rate was significantly higher in GM compared to GT (48.58% vs. 40.88%, respectively p<0.05). Some factors such as higher temperatures during afternoon, fatigue of inseminators (particularly if greater than 70 cows per group) or herd stress expecting the time of insemination in the same group that could influenced negatively the results obtained in GT. In 5422 cows, 7.7% difference represents a considerable number of 417 pregnant cows. Based on these results in order to increase reproductive efficiency, we can suggest to reduce number of inseminated cows per turn or to increase the number of insemination technicians in order to avoid AI failures or fatigue from them.

ABSTRACT 74

EFFECT OF DIFFERENT DOSAGE OF eCG ON SUPEROVULATORY RESPONSE OF NELORE DONOR (Bos indicus) SUBMITTED TO FIXED TIME ARTIFICIAL INSEMINATION

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The objective of this study was to evaluate the effect of different dosage of eCG on superovulatory response of Nelore donor (Bos indicus) submitted to fixed-time artificial insemination. In this experiment accomplished in random design in cross-over arrangement (12 donors x 3 treatments = 36 superovulation), 12 Nelore cows were treated with a progesterone intravaginal device (DIB®, Intervet-Schering Plough, Brasil) and 2 mg of estradiol Benzoate (Gonadiol®, Intervet-Schering Plough, Brasil) in random day of estral cycle (DO). On day 4, the donors were randomly allocated in one of three groups. The superstimulation was accomplished with the administration i.m. of 1500IU(one injection) of eCG (Folligon®, Intervet-Schering Plough, Brasil) in Group eCG1500IU, 2000IU(one injection) of eCG in Group eCG-2000IU and 133mg of FSH (Folltropin-V®), Bioniche, Canadá) in Group FSH in 8 decreasing doses each 12 hours. In the morning of Day 6, 150µg of D-Cloprostenol (PROLISE®,Syntex, Argentina) were administered. The progesterone intravaginal devices were withdrawal 36 hours after D-Cloprostenol (with the last FSH) administration. On Day 8 (12 hours after the last FSH) 25mg of LH (Lutropin-V®, Bioniche Canadá) were administered. The progesterone intravaginal devices were withdrawal 36 hours after D-Cloprostenol (with the last FSH) administration. On Day 8 (12 hours after the last FSH) 25mg of LH (Lutropin-V®, Bioniche Canadá) were administered. The progesterone intravaginal devices were withdrawal 36 hours after D-Cloprostenol (with the last FSH) administration. On Day 8 (12 hours after the last FSH) 25mg of LH (Lutropin-V®, Bioniche Canadá) were administered. The progesterone intravaginal devices were withdrawal 36 hours after D-Cloprostenol (with the last FSH) administration. On Day 8 (12 hours after the last FSH) 25mg of LH (Lutropin-V®, Bioniche Canadá) were administered. On Day 15, the flushing was performed. An index (embryo quality index or EOI) for embryo quality was used such that, EOI = (Excellent x 1 + Good x 2 + Regular x 3 + Poor x 4 + Degenerate x 5) / total oocytes/embryo. Absolute values closest to 1.00 indicate better quality. The variables were analyzed by GLIMMIX procedure of Statistical Analysis System (SAS). There was no statistical difference between the treatments for the variables recovery rate (P=0.84), number of recovery oocytes/embryo (FSH=9.42±1.17, eCG-1500IU=8.58±1.56 and eCG-2000IU=7.58±0.93; P=0.97), EOI (FSH=2.19±0.25, eCG-1500IU=2.17±0.18 e eCG-2000IU=2.27±0.24; P=0.85), number of transferable (FSH=5.33±0.81, eCG-1500IU=6.58±0.97 e eCG-2000IU=6.42±1.01; P=0.59) and frozen embryos (FSH=5.50±1.04, eCG-1500IU=4.75±0.68 and eCG-2000IU=4.58±0.96; P=0.81). The results indicate that the eCG (1500IU or 200IU) show similar efficient the treatment with 133mg of FSH at embryo production in Nelore cows submitted to superstimulation with fixed-time artificial insemination.
COMPARISON OF CONCEPTION RATES IN LACTATING NELORE COWS SUBMITTED TO FTAI AND TREATED WITH PITUITARY EXTRACT SWINE, EQUINE CHORIONIC GONADOTROPIN OR TEMPORARY CALF REMOVAL

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The aim of this study was to evaluate the efficiency of different stimulators to lactating Nelore cows inseminated using different strategies of estrous synchronization. 190 Nelore cows were used, with 35 days postpartum and body condition score ranging from 3.0 to 3.5 (range = 1-5 ECC) in Alto Paraíso, Paraná State. The animals, in each experiment, were separated in 3 groups related to condition. In the first experiment, the first group (G-RB, n = 61) received an intravaginal device containing 1.9 g progesterone (CIDR®, Pfizer, Brazil) and an application of 2 mg of estradiol benzoate (EB), (Estrodin®, Farmavet, Brazil), intramuscularly (IM). On the eighth day the devices were removed while the application of 150µg of cloprostenol (Veteglan®, Hertape-Cailler, Brazil) intramuscularly and 1 mg of estradiol cypionate (ECP, Pfizer®; Brazil). The calves were removed for 48 hours from the removal of the progesterone device. In the second group (G-EHS, n = 66), cows were treated similarly to the G-RB, were excluded the removal of calf and was given 50 IU of porcine pituitary extract (Pluset®, Hertape-Caill, Spain) for IM, simultaneos CIDR removal. In the third group (G-eCG, n = 63) cows were treated similarly to the G-RB, were excluded the removal of calf and was given 300 IU of equine chorionic gonadotropin (Novomorn®, Intervet-Scherling, Brazil) by IM, simultaneos CIDR removal. All animals were inseminated 45 hours after the CIDR removal. In the second experiment, was used the same protocol described above to the groups G-RB, n = 218, G-EHS, n = 104 e G-eCG, n = 114, respectively to the first, second and third group. Pregnancy diagnosis was made 30 days after artificial insemination by transrectal ultrasonography (Aloka SSD 500, 5 MHz). The results were compared by chi-square test with significance level of 5%. The conception rates for groups G-RB, G-G-EHS and eCG were respectively (48%, 29/61), (42%, 28/66), (41%, 26/63) (P > 0.05). From the observed results, we conclude that the porcine pituitary extract showed conception rate comparable to those obtained with the use of eCG or temporary calf removal, becoming an option in the composition of TAI protocols in cows in Nelore postpartum period.

BUFFALO HEIFERS OVULATION SYNCRONIZATION WITH GnRH OR EB TO FTAI DURING THE OFF BREEDING SEASON (PARTIAL RESULTS)

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The aim of this study was to evaluate the efficiency of different inducers of ovulation - EB and GnRH - to be used in the protocol of fixed-time artificial insemination (FTAI) in buffalo heifers during the off-breeding season (spring and summer). It had been used twenty-nine buffalo heifers divided into two Groups (GEB, n = 16; GGNRH, n = 13) according to age, ovarian activity, body condition score and weight. At random stage of the estrous cycle (Day 0 = D0; afternoon), all heifers received an intravaginal progesterone device of a second use (DIB®, Intervet/Schering-Plough, Brazil) plus 2.0mg of Estradiol Benzoate (i.m.; EB; Sincrodiol®, Ouro Fino, Brazil). In D9 (afternoon), females received 0.53mg of an analogue of PGF2α (i.m.; Cloprostenol sodic, Sincrocio®, Ouro Fino, Brazil) plus 400IU of eCG (i.m.; Novomorn®, Intervet/Scherling-Plough, Brazil), followed by the device removal. In D11 (morning) ovulation of heifers of GEB was induced with 1.0mg of Estradiol Benzoate (i.m.; Sincrodiol®, Ouro Fino, Brazil), while in GGNRH ovulation was induced eight hours later (D11 - afternoon) through the application of 20mg of GnRH (i.m.; Buserilin acetate, Sincrocort®, Ouro Fino, Brazil). Heifers were submitted to the FTAI 24 (GEB) or 16 (GGNRH) hours after administration of inducers of ovulation (D12, morning). For the FTAI was necessary to use applicator and sheath specific for sheep and goats (IMV, France) because the buffalo heifers have thinner cervix than adult animals, which complicates the introduction of the conventional applicator of semen. The ultrasonographic evaluation (MindrayDP2200Vet, China) was performed on D0 to check ovarian activity, in D9 to check the follicular diameter, from D11 to D14 (12/12 hours per 60 hours) to establish the moment of ovulation and in D42-30 days after the FTAI - for pregnancy diagnosis. The variables were analyzed by Proc GLM procedure of SAS®. The diameter of the dominant follicle (1.2 ±0.0 vs. 1.1 ±0.1 cm), the diameter of ovulatory follicle (1.3 ±0.0 vs. 1.3 ±0.1 cm), the interval between administration of EB or GnRH and ovulation (32.0 ±2.1 vs. 33.0 ±5.4 hours), the ovulation (86.0% (12/14) vs. 67.0% (8/12)) and conception ([43.7% (7/16)] vs. 53.8% (7/13)) rates did not differ (P > 0.05) between groups GEB and GGNRH. Partial results of this study are indicative that the treatment with progesterone associated to Estradiol Benzoate (D0), followed by administration of eCG more PGF2α (D9) and the induction of ovulation with EB or GnRH (D11) resulted in satisfactory follicular response and conception rate in buffalo heifers submitted to FTAI during the off-breeding season.
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PREGNANCY RATES AND BODY MORPHOMETRY IN NELORE COWS SUBMITTED TO PROGESTERONE AND TEMPORARY WEANING OF CALVES

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Pregnancy rate for suckling cows is around 25%, according to Cachapuz (1997, EMATER, 52p.). The use of progestagen treatment associated with calf removal makes the use of Artificial Insemination (AI) easier to carry out under extensive conditions in suckling cows (Jaume; Moraes, 2001, EMBRAPA, Documentos, 37). Animals of body condition score (BCS) 3 (1 to 5 scale) (Radostitis; Blood, 1986, Veterinary Clinics, 2nd ed., 127-132) were used throughout the breeding season and submitted to progestagen treatment and temporary weaning (TW). The expected pregnancy rate should be around 50% (Moraes et al., 2007, Pesquisa Agropecuária Brasileira, 42:104-111). The present study aims to evaluate the body morphometry and temporary weaning (TW) associated with two protocols of fixed-time artificial insemination (FTAI) using intravaginal progesterone releasing device (IPRD) upon pregnancy rates. Nelore cows, 45-59 days post-partum and body condition score (BCS 3) were kept in extensive Brachiaria decumbens fields. Cows were randomly divided in group 1 (n=147) and group 2 (n=197). Group 1 received: 1g progesterone (IPRD) and 2mg estradiol benzoate on day 0 (EB-D0); 150mg D-cloprostenol on day 7 (PGF-2a-D7); 0.5mg estradiol cipionate and (TW-48 hours) on day 9 (EC+TW-D9); FTAI on day 11 (D11) and group 2: IPRD+EB (D0); PGF-2a+EC+TW (72 hours) on D8; FTAI (D11). Results were analysed using the X2 test, as significance was set as P<0.05. The pregnancy rate was higher in cows submitted to TW (72 hours) than in cows with TW (48 hours) 49.74% vs 30.60%, respectively (P<0.05). There was statistical difference (P<0.05) in groups 1 and 2 between pregnant or no pregnant cows to body weight (412kg vs. 400kg and 419kg vs. 390kg) and body condition score “BCS” (3.33 vs. 3,08 and 3,53 vs. 3.32) respectively. The weaning of 72 hours associated to the applied protocol may have improved the pregnancy rate and the evaluation of the body condition score (BCS) during post-partum can be used to adjust the start of the breeding season.

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REPRODUCTIVE PERFORMANCE OF PREPUBERTAL BOS INDICUS HEIFERS AFTER PROGESTERONE-BASED TREATMENTS

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The objective was to evaluate the effects of exogenous progesterone (P4) on reproductive performance of prepubertal Bos indicus heifers. Prepubertal Nelore heifers (n = 589; 24.0 ± 1.1 mo; 298.0 ± 1.9 kg; BCS 3.2 ± 0.3 in a 1 to 5 scale; mean ± SEM) were randomly assigned to receive, between experimental Days -12 and 0: no treatments (CIDR0; n = 113); a new intravaginal insert (CIDR) containing 1.9 g of P4 (CIDR1; n = 237); or a similar insert previously used three times, with each use occurring for 9 d (CIDR4; n = 239). An additional treatment group was pubertal heifers given 12.5 mg dinoprost tromethamine im on Day 0 (PGF; n = 346), and used as controls for evaluation of conception rates. On Day 0, transrectal palpation was done for uterine score evaluation (UtS; 1 to 3 scale), blood samples were taken for serum P4 concentrations, and follicle diameter (FD) was measured. The breeding season (BS) started on Day 1 and consisted of AI after detection of estrus between Days 1 and 45, and exposure to bulls between Days 46 and 90. There were effects of treatment (P < 0.05) on serum concentrations of P4 on Day 0 (0.37 ± 0.16, 2.31 ± 0.11, and 1.20 ± 0.11 ng/mL for CIDR0, CIDR1, and CIDR4, respectively; mean ± SEM), FD on Day 0 (9.45 ± 0.24, 9.72 ± 0.17, and 11.42 ± 0.16 mm), UtS on Day 0 (1.49 ± 0.06, 1.88 ± 0.04, and 2.24 ± 0.04), estrus detection rates at 7 d (19.5, 42.6, and 38.3%) and 45 d (52.2, 72.1, and 75.3%) of the BS, and on pregnancy rates at 7 d (5.3, 14.3, and 18.4%), 45 d (27.4, 39.2, and 47.7%) and 90 d (72.6, 83.5, and 83.7%) of the BS. Conception rate 7 d after the start of the BS was greater (P < 0.05) in heifers from the CIDR4 (46.8%) and PGF (43.8%) groups than in the CIDR0 (27.3%) and CIDR1 (33.7%) groups. In conclusion, exogenous P4 hastened puberty and improved pregnancy rates at the beginning of the BS in prepubertal Bos indicus heifers. Furthermore, previously used CIDR inserts were better than new inserts.
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Evaluation of equine chorionic gonadotrophin (eCG of) efficiency in Cattle


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It was evaluated the efficiency of eCG OF on ovarian response in synchronization of ovulation protocols. It was used 147 crossbred heifers (Bos taurus x Bos indicus) with 24 to 36 months and 2.78±0.03 of body condition score (1 to 5 scale). In random day of estrous cycle (D0), the animals were treated with an intravaginal progesterone device (Sincrogest®, Ourofino Agronegócio, Brazil) and 2mg of estradiol benzoate (Sincrodio®, Ourofino Agronegócio, Brazil). In same day, an ultrasonographic examination (US) was performed to evaluate presence of CL or the diameter of bigger follicle when a CL was not present. Five days later (D5), the animals were allocated in one of four experimental groups considering BCS and ovarian status on D0 and received: no treatment(Control Group; CG; n=30); 300IU of eCG (300eCG-OF; eCG OF, Ourofino Agronegócio, Brazil; n=39); 500IU of eCG (500eCG-OF; eCG OF, Ourofino Agronegócio, Brazil; n=41); 500IU of eCG (Market-eCG Folligon®, Intervet, Holand; n=37). On Day 8, the device was removed and animals were treated with 0.530mg of Cloprostenol (Sincrocio®, Ourofino Agronegócio, Brazil) and 1mg estradiol cypionate (ECP®, Pfizer, Brazil). At same day, an US was performed to identify the number of follicles (NFol) at ovarian and measure the diameter of dominant follicle (DF). On Day 17, other US was performed to evaluate the ovulation rate, to identify the number of CL (NCL) and measure the diameter of CL. The statistical analysis was performed using the software SAS. The explanatory variables (diameter of DF on D8, NFol, diameter of CL on D17 and NCL on D17) were analyzed using PROC GLM. The ovulation rate was analyzed using PROC GLIMMIX. It was observed difference on ovulation rate [p=0.0001; CG=33,3%b(10/30); 300eCG-OF=69.2%a(27/39); 500eCG-OF=92.7%a(38/41) AND Market-eCG=89.2%a(33/37)], in NFol (p=0.001; CG=0.75b, 300eCG-OF=1.21b, 500eCG-OF=3.68a, Market-eCG=3.81a) in CL diameter (p=0.006; CG=9.13mmb, 300eCG-OF=11.0mma, 500eCG-OF=11.28mma, Market-eCG=10.58mma), in NCL (p=0.03; CG=0.31 c, 300eCG-OF=1.02b, 500eCG-OF=3.68a, Market-eCG=3.81a) in CL diameter (p=0.03; CG=0.66mma, 300eCG-OF=20.0mmma, 500eCG-OF=19.8mma, Market-eCG=21.1mma). We can conclude that eCG-OF used in synchronization of ovulation protocols on dosage of 300IU is efficient in increase the ovulation rate of dominant follicle and on dosage of 500IU it increases the number of dominant follicle on D8 and CL on D17.

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PREGNANCY RATE OF NELORE HEIFERS SUBMITTED TO FTAI PROTOCOLS WITH DIFFERENT FSHp (FOLLTROPIN-V®) DOSES AFTER CYCLICITY INDUCTION PROTOCOL

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Follicle stimulating hormone (FSH) is used in fixed time artificial insemination (FTAI) protocols to induce follicular growth, promoting ovarian activity of adult bovine anestrous females in a dosage of 10mg (Santos et al., Acta Scientiae Veterinariae, 35 suppl 3, 1151, 2007). It is well known that heifers are more responsive to gonadotropins than adult females. The aim of this study was to evaluate the effects of different doses of FSHp (Folltropin-V®, Bioniche, Canadá) on pregnancy rates of Nelore heifers submitted to FTAI protocols. The experiment was conducted at Cocalinho, Mato Grosso, using 187 Nelore heifers from 18 to 25 months of age and 280 Kg average weight. All animals received a cyclicity induction protocol to standardize reproduction development of these heifers and it consisted of an injection of 2 mg of oestradiol benzoate (RIC-BE®, Tecnopec, Brasil) plus a insertion of 1gr progesterone intravaginal device (Primer®, Tecnopec, Brasil). Eight days later, the device was removed and 1 mg of oestradiol benzoate (RIC-BE®, Tecnopec, Brasil) plus a insertion of 1gr progesterone intravaginal device (Primer®, Tecnopec, Brasil) and 112,5µg de d-Cloprostenol (Prolise®, ARSA, Argentina) plus 1 mg of oestradiol benzoate (RIC-BE®, Tecnopec, Brasil). On day 8, heifers were treated with 0, 5 or 10 mg of Folltropin-V®. At day 10, all heifers were treated with 3,12mg (2,5 ml) of LH (Lutropin-V®, Bioniche, Canadá) and inseminated with 2 distinct bull semen in a random mating. Data were analyzed by Chi-square procedure. Sire did not (p=0,7) affect pregnancy rates (50,0% vs. 53,6%). Pregnancy rate for FSH 5mg, FSH 10mg and control group were, respectively, 58,7%, 54,8% and 41,9% (p=0,06). The results showed that dose of 5mg of Folltropin-V® improved pregnancy rates of FTAI-LH protocols when compared with protocols without follicular inductor hormone, proving the efficiency of ovarian stimulation with this dose. Acknowledgements: Fazenda Santa Marta.
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DIAMETER OF PREOVULATORY FOLLICLE IN THE MOMENT OF FIXED TIME ARTIFICIAL INSEMINATION IN NELORE COWS

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Several hormonal protocols able to regulate the follicular growth and ovulation were developed in recent decades, making possible the fixed-time artificial insemination (FTAI) and its applicability in Brazilian beef cattle. In this context, several factors reflect on the efficiency of the protocols employed among which stands out the diameter of the preovulatory follicle. The objective was to evaluate the relationship between the diameter of the preovulatory follicle and the conception rate in Nelore cows submitted to a protocol of FTAI with three managements. So, 308 female Nelore cows were evaluated and were considered able to reproduce. On a random day of the estrous cycle (D0) the animals received an intravaginal device of progesterone (Sincrogest®, Ouro Fino, São Paulo, Brazil) associated with 2mg of Estradiol benzoate (Sincrodiol®, Ouro Fino, São Paulo, Brazil) by the intramuscular route (im). On the eighth day (D8) it was administrated 500 ìg of Cloprostenol (Sincrocio®, Ouro Fino, São Paulo, Brazil) im, 0,6 mg of Estradiol cypionate (ECP®, Pfizer, São Paulo, Brazil) im and 300 IU of eCG (Folligon®, Shering Plough, São Paulo, Brazil) im, after removing the source of progesterone. On the tenth day (D10) immediately before each insemination, all animals were examined by transrectal ultrasound to measure the diameter of the preovulatory follicle. The pregnancy diagnosis was performed by transrectal ultrasound 30 days after FTAI. The data analysis was performed by PROC GLM and the means diameters of follicles were compared by Student-Newman-Keuls (SNK) in SAS statistical package. Of the total 308 animals, 163 became pregnant giving a conception rate of 52.92%. The overall mean diameter of the preovulatory follicle at the time of FTAI was 12.52 ± 3.0 mm. Comparing the sizes of follicles, the pregnant cows had diameters (13.52 ± 2.44mm) significantly higher (p=0.0001) than nonpregnant cows (11.41 ± 3.24mm). The results of this study demonstrated that the diameter of the preovulatory follicle influence the conception rate in Nelore cows submitted to a synchronization protocol with three managements. These results reassure the importance of adopting strategies aimed at increasing the diameter of the follicle at the time of artificial insemination, in addition, suggest as another possibility the direction of mating, so, the use of semen of high economic value in cows which have larger follicular diameter.

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COMPARISONS BETWEEN EC GCC OR FSH TREATMENTS ON ESTROUS SYNCHRONIZATION AND PREGNANCY RATE IN WOOLESS EWES

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The aim of present work was to compare protocols of estrus synchronization using eCG or FSH in ewes. Eighty-seven ewes received intravaginal dissipatory containing 60 mg of MAP (medroxyprogesterone acetate) (day 0). On day 5 of the protocol each animal was treated with 0,5 mL (37,5 ìg) of the PGF2α and then the sponge was removed (day 10), when eCG and FSH was administrated. Animals were randomly divided into 3 groups: at Group I (control group, n=21) it was administrated (IM) 2,0 mL of the saline solution; at Group II (n=33), 400 IU of eCG (IM); and in the Group III it was administrated (IM) 25 IU of FSH. Three vasectomized rams were used to detect females in estrus. Ewes were inseminated by laparoscopy, 55 hours after sponge withdrawal, using fresh semen diluted in extender Tris-Gema containing 150 x 10⁶ viable sperms per dosage. Thirty five days after inseminations, females were submitted to ultrasound examination to detect pregnancy. Chi-square test was performed for statistical analysis, with 5% of level of significance. Concerning females in estrus, it was not significant difference (P>0.05) among FSH (97%) and control (86%) groups but it was significant difference (P<0.05) between eCG (100%) and control (86%) groups. For pregnancy rate, was obtained 42.9%, 60.6% and 42.4% for the groups I, II and III, respectively, there is no difference among groups (P>0.05). It was concluded that eCG can be replaced by FSH in protocols of estrus synchronization in ewes to prevent formation anti-eCG antibodies and decrease costs with hormones.
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PREGNANCY RATE AND EXPRESSION OF ESTROUS IN HIGH PRODUCING DAIRY CATTLE TREATED WITH OVSYNCH OR PROGESTERONE/ESTRADIOL PROTOCOL

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The aim of the present study was to evaluate the effect of different protocols for timed-artificial insemination (Ovsynch vs P4+BE) on expression of estrous and pregnancy rate of high producing dairy cattle. Four hundred nineteen lactating Holstein cows with average milk production of 33.5±0.5 L/day, 168.4±5.5 days in milk, 2.92±0.41 lactation number and 2.09±0.11 service number were used. At the beginning of the experiment cows were submitted to ultrasonographic examination to determine the reproductive status and to divide the experimental groups considering the presence or absence of corpus luteum and cysts. Holstein cows were randomly allocated to two groups (Ovsynch and P4+BE Groups): 1) Group Ovsynch – on D0, cows received 10ìg buserelin acetate (GnRH - Sincroforte®, Ourofino, Cravinhos, Brazil). On D7, 500ìg of cloprostenol was administered (PGF - Sincrocio®, Ourofino, Cravinhos, Brazil) and 56 hours after the PGF cows received 10ìg de GnRH. The FTAI was performed 16 hours after the second GnRH; 2) Group P4+BE – on D0, cows received 2mg of estradiol benzoate (Sincrodol®, Ourofino, Cravinhos, Brazil) and an intravaginal progesterone device (Sincrogest®, Ourofino, Cravinhos, Brazil). On D7, cows received 500µg of PGF. On D8, the intravaginal devices were removed and 1mg of estradiol cypionate (SincroCP®, Ourofino, Brazil) was administered. The FTAI was performed 48 hours after the implant withdrawal. Pregnancy diagnoses were performed by ultrasound 28 days after the FTAI. The statistical analysis was accomplished by GLIMMIX procedure of the Statistical Analyses System (SAS). It was found no interactions between the treatments and the explanatory variable. There were, however, differences between treatments on rate of estrous expression [Ovsynch - 42.8% (89/208) and P4+E2 - 66.4% (140/211); P=0.0001] and pregnancy [Ovsynch - 27.5% (57/208) and P4+E2 - 40.8% (86/211); P=0.03]. Also, cows that showed estrous behavior had greater pregnancy rate [No estrous - 25.8% (49/190) and Estrous - 41.4% (94/227); P=0.004]. These results indicate that high-producing dairy cows submitted to treatment with intravaginal progesterone device in association with estradiol had greater estrous behavior and pregnancy rate compared to animals synchronized with Ovsynch protocol.

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RUMEN-PROTECTED FAT SUPPLEMENTATION, PROGESTERONE PRODUCTION, LUTEOLYSIS MOMENT AND PREGNANCY IN NELORE COWS

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Four experiments were designed to evaluate the possible mechanisms related to the increased pregnancy rates in cows supplemented with PF, as previously found by Lopes et al. (2009). In Experiment 1, 51 ovulated non-lactating Nelore multiparous cows were used to evaluate if PF supplementation affects circulating progesterone (P4) concentrations and timing of luteolysis. In Experiment 2, 43 ovulated non-lactating Nelore multiparous cows were used to evaluate if PF supplementation alters the sensitivity of a 6-d corpus luteum to exogenous prostaglandin treatment. In Experiment 3, 27 ovulated postpartum Nelore cows were used to evaluate if PF supplementation affects the incidence of premature luteolysis. In Experiment 4, we evaluated if the length of PF supplementation in different times after timed-AI (14, 21 or 28 days) alters the pregnancy rate. Beginning at the d of estrus, the daily treatments in these experiments were: Control (0.1 Kg Mineral + 0.1 Kg corn + 0.1 Kg kaolin); SF (0.1 Kg Mineral + 0.1 Kg Megalac-S® [7-9% linoleic acid] + 0.1 Kg corn), this group was used just in experimental 1 and 2; PF (0.1 Kg Mineral + 0.1 Kg Megalac-E® [40-42% linoleic acid; 2-3% linolenic acid] + 0.1 Kg corn). No effect was detected on P4 concentration, luteolysis or short cycle (P>0.1), but when the cows of exp. 1 and 2 were grouped had higher (P=0.01) concentration of P4) on cows that were supplemented with PF compared with SF or control (4.45; 3.25; 3.48 ng/ml, respectively; SEM=0.278. Cows supplemented with PF during 21 (PF21) or 28 d post-AI (PF28) had higher pregnancy rates (50.38%; P < 0.05) than cows from other treatments (42.38%). There was no difference between PF21 (50.99%) and PF28 (49.81%) treatments. These experiments indicated that the possible mechanism for greater conception with PF supplementation post-AI may be due to effects on embryo development, animals supplemented for more than 21d had greater pregnancy rates. Key words: Nelore, P4, luteolysis, PF, pregnancy rate. Lopes, C.N., et al. 2009. JAS. 87: 3935-3943.
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EQUINE SEMEN EXTENDER WITHOUT ANIMAL DERIVED INGREDIENTS: PRELIMINARY RESULTS

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The use of cooled semen in artificial insemination (AI) programs has been widely accepted as a tool for genetic improvement in the equine industry. Most of the semen extender in the market are derived from animal ingredients. The cooling processes produce physical and chemical stress on the sperm membrane that reduces sperm viability and their fertilizing ability (Gadea et al., 2005, J. Andro. 26:396-404). Glutathione Peroxidase (GPx) (Irvine DS. 1996 Rev Reprod. 1:6-12) and Cisteyne (C) (Pasqualotto et al., 2009, Hum Fertil. 12(3):166-71), non enzymatic antioxidants plays an important role as an intracellular defense mechanism against oxidative stress. The aim of this study was to evaluate the efficacy of a lyophilized coconut water extender (ACP-105®, UECE - Fortaleza, Ceará - Brazil) in equine cooled semen viability at 5°C and to look the effect on the addition of GPx and C to this extender. A total of four Quarter Horse Stallions ranging from 4 to 8 years of age was used. The ejaculate was splitted into three aliquots for Time 0 (T0), T12 and T24. The T0 was evaluated immediately after collection for motility and vigor. The 3 aliquots was subdivided into two groups: ACP-105 here called ACP and a positive control with skim milk extender (Botusemen™, Biotech - Botucatu, São Paulo - Brazil), here called BS. Each aliquot was also splitted into 3 different aliquots being ACP control without antioxidants (ACP), ACP with 5 IU/mg GPx (GP) and ACP with 5 mM cisteyine (C) and in the other group BS without antioxidants and BS associated to GP and C. There were 4 replicates. No difference (P > 0.05) in motility and vigor were seen between treatments, however there was a decrease at T0 in the ACP control group compared with BS control T0 - 80% vs. 60% (stallion 1) 60% vs. 50% (stallion 2), 70% vs. 60% (stallion 3) and 60% vs. 40% (stallion 4). No difference (P>0.05) were seen in the addition of GPx and C when comparing extenders, however the addition of C to ACP-105 kept the motility stable in 3 out of 4 stallions up to 24 hours showing a longevity effect. In conclusion, it is possible to use as an alternative, an extender that does not containanimal derived ingredients. The association of a lyophilized coconut extender with cisteyne suggests a longevity effect up to 24 hours. Further in vitro and in vivo studies are being performed in order to confirm these preliminary data.

ABSTRACT 86

FOLLICULAR DYNAMICS OF CYCLIC ADULT MARES TREATED WITH DIFFERENT OVULATION INDUCTORS

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The present study aimed to evaluate the follicular dynamics of cyclic adult mares with follicles bigger than 35mm of diameter treated with different ovulation inducers (hCG and deslorelin). It was used 144 adult mares, in estrous, were evaluated by ultrasonographic examinations (ALOKA SSD 500) twice a day. When it was observed a follicle e*35mm of diameter, the mares were randomly allocated in one of four treatments and received: no additional treatment (CG; n=36); 750µg of Deslorelin (D750; Deslorelina OF , Ourofino Agronegócio, Brazil; n=36); 1000µg of Deslorelin (D1000; Deslorelina OF, Ourofino Agronegocío, Brazil; n=37) and 2500UI of hCG (H2500; Chorulon, Intervet, Holand; n=35). Ultrasonographic examinations were performed at each 12 hours until ovulation or until 72 hours after treatment. It was evaluated the follow parameters: follicular diameter at treatment, ovulatory follicle diameter, interval form treatment to ovulation, ovulation rate and interval between estrus. Data were analyzed using GLM and GLIMMIX procedures of SAS. The follicular diameter at treatment (CG=36.6±0.2mm; D750=36.3±0.1mm; D1000=36.7±0.2mm and H2500=36.3±0.1mm) and the interval between estrus (CG=21.1±0.3 days; D750= 20.6±0.3 days; D1000=20.9±0.3 days and H2500=20.99±0.3 days) presented no difference between treatments (P=0.10 and P=0.74, respectively). However, it was observed difference between Control Group and others treatment groups to ovulatory follicle diameter (CG=42.9±0.3:mm; D750=41.2±0.3:mm; D1000=41.5±0.3:mm and H2500=41.2±0.2:mm; P=0.001), interval between treatment and ovulation (CG=60.0±2.2:h; D750= 46.1±1.4:h; D1000=46.2±1.4:h and H2500=45.7±1.2:h; P=0.001) and ovulation rate [CG=61.1% (22/36), D750=88.9% (32/36), D1000=89.2% (33/37) and H2500=88.6% (31/35); P=0.007]. We can conclude that hCG and Deslorelin has similar efficiency as ovulation inducer in cyclic adult mares and with follicle equal or bigger than 35mm.
ABSTRACT 87

Incidence of twin pregnancy in mares treated with hormones

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Twin pregnancies in mares rarely reach the end successfully, and when it occurs, there are risks of death to the mare or newborns. Therefore, in most cases, the option is the elimination of the fetal vesicles and the manual crushing method is the most commonly used (Mattos et al., 1994, Arquivos da faculdade de Veterinaria - UFRGS, v. 22, p. 11-16). Factors such as age, reproductive status, breed and size can influence the number of embryos of the pregnancy, and the multiple ovulation are responsible for 93% of twin equines cases (GINther, 1986 Ultrasonic imaging and reproductive events in the mare. 378p.). Currently, the use of ovulation-inducing hormones for assisted reproduction can improve the ovulation rate with a consequent increase in the number of twin pregnancies (GINther, 1987, J. Equine Vet Sci, vol 7, p.82-88). The aim of this study was to determine the incidence of twin pregnancy in mares treated with hormones to induce the ovulation previously the bred. There were used 159 Thoroughbred mares in positive photoperiod, divided in four treatments groups. The animals of treatment 1 (T1, n = 39) received the intramuscular injection of 1.0 mg of deslorelin. In treatment 2 (T2, n = 26), they received 1.5 mg of deslorelin intramuscularly. Mares in Treatment 3 (T3, n = 63) were received HCG 2500 intravenously. The treatment 4 (T4) was the control group (n = 31). Hormone treatments were performed when the largest follicle reached 30 mm. Dynamics of follicular growth was daily monitored in all mares after estrus detection. Pregnancy diagnosis was performed by ultrasound examination and by assessing the number of twin pregnancies in association with hormonal treatment applied to each animal. The results were analyzed by Chi-square, p <.05. There were no statistical differences between T2, T3 and T4, and the percentage of twin pregnancies, for these groups, were respectively 38.46%, 44.44% and 45.84%. There were statistical differences between T1 and the other treatments, and outcomes for this treatment were 54.84%. The conclusion of this work was that increased rate of twin pregnancies may vary according the drug and dose hormone applied.

ABSTRACT 88

EFFECT OF FSH AND eCG ON FOLLICULAR DYNAMIC AND PREGNANCY RATE IN FIXED-TIME AI PROTOCOL IN ANESTROUS BEEF COWS

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The objective of this study was to evaluate the effect of treatment with FSH and eCG on follicular dynamics and pregnancy rate of lactating beef cows submitted to fixed time artificial insemination (FTAI). The study was conducted with 588 lactating Bos indicus cows (multiparous within 45 to 70 days post-partum) and body condition score (BCS) of 2.6±0.3 (1 to 5 point scale). At unknown stage of the estrous cycle (D0), all animals received 2mg of oestradiol benzoate (RIC-BE®, Tecnopec, Brazil) and an intravaginal progesterone device (Primer®, Tecnopec, Brazil). At the same day, an ultrasound exam (US; CHISON 600vet) was performed to verify presence or absence of CL. At P4 device removal day (D8), cows were submitted to an US exam to measure follicular diameter and they were homogenously allocated according to follicular size in one of three treatment groups (FSH Group, eCG Group and Control Group). Cows with a CL on D0 or D8 (n=55) were removed of the experiment. At D8, cows received 112.5 ìg of d-cloprostenol (Prolise®, ARSA, Argentina) and 1mg of oestradiol benzoate Animals from eCG Group received 300IU of eCG (Folligon®, Intervet, Netherland), animals from FSH Group received 10mg of FSH (Folltropin-V®, Bioniche, Canada) and animals in Control Group did not receive any additional treatment. At D10 (insemination moment) and D20, US exam were accomplished to evaluate follicular diameter and ovulation rate, respectively. Cows were fixed-time inseminated 45 hours after progesterone device removal. Data were analyzed by the GLIMMIX procedure of SAS. There was a significative effect (P=0.003) of treatment on ovulation rate. Control group data were inferior than other groups: 71.8% (127/177) vs. 85,5% (153/179) in FSH Group and 82,5% (146/177) in eCG Group. Also there was an effect of treatment on pregnancy rate (p=0.008). Control group showed inferior results than other groups: 38,9% (69/177)vs. 51,4% (92/179) in FSH Group and 55,9% (99/177) in eCG Group. There was no treatment effect in diameter of dominant follicle (mm) at D8 (P=0.96 – 9.9±0.3mm) and at D10, FTAI moment, (P=0.46 - 12.5±0.3mm). The results of this experiment show that FSH or eCG use in FTAI protocols were similar and they significantly improved ovulation and pregnancy rates of lactating anestrous zebu cows, comparatively to control group.
ABSTRACT 89

EFFECT OF eCG ADDITION ON FIXED TIMED ARTIFICIAL INSEMINATION PROTOCOL ON PREGNANCY RATE OF LACTATING DAIRY COWS

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This experiment was designed to evaluate the effect of adding 300 IU of equine chorionic gonadotropin (eCG), on the day of removal of intravaginal progesterone (P4) device, in pregnancy rate of dairy cows of average production. Animals with more than 30 days postpartum were synchronized with the following protocol: D0-Ultrasonographic examination (Mindray, model DP3300 VET) to evaluate the presence or absence of corpus luteum, evaluation of body condition score (BCS), injection of estradiol benzoate (2.0 mg, Estrogin®) + CIDR®; D7 – dinoprost trometamina (PGF2α, 12.5mg, Lutalyse®); D9 – removal of the device + estradiol cypionate (0.5 mg, ECP®); D11 – TAI + rectal temperature. Lactating Holstein or crossbreed (Girolandas) cows (n = 292) were synchronized in 23 farms, producing 23.62 ± 8.32 Kg of milk per day, in D0 69.61% had presence of corpus luteum with BCS 2.96 ± 0.52 (1 to 5). In day 9 of the protocol the animals were randomly divided into the same lot to receive (treated group; n = 144) or not (control group; n = 148) 300IU of eCG (Folligon®). The pregnancy rate to TAI was evaluated by ultrasound at 30 and 60 days after insemination. The data were analyzed in PROC LOGISTIC of SAS and in the model were included the variables: treatment, days in milk, milk production, body condition score, rectal temperature and interactions. Milk production affected negatively (p<0.05) pregnancy at 30 and 60 days and rectal temperature trend negatively influence the rate of pregnancy at 30 days (p<0.10).The pregnancy rate of control and treated group were respectively at 30 days: 45.3% (n=148) and 48.6% (n=144), and at 60 days: 37.5% (n=136) and 39.8% (n=138). The addiction of eCG did not alter the pregnancy rate of cows of average production.

ABSTRACT 90

EFFECT OF CICLICITY INDUCTION PROTOCOL ON NELORE HEIFERS

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This experiment aimed to evaluate the rate of corpus luteum on Nelore heifers after treatment with a ciclicity induction protocol. The study used 636 Nelore heifers, within 24 to 28 month of age and 290 Kg of average weight, from Engano farm, Camapuá county, Mato Grosso do Sul. Animals were classified by ultrasound exam as cyclic (corpus luteum presence; n=199) and without corpus luteum (n=437). Cyclic heifers were put away of the study. Heifers without CL were randomly allocated in 4 groups: control (without hormone treatment) and treated group (puberty induction protocol). These groups varied the previous condition of device use: New Primer group–new devices; 2nd use Primer group – device previously used for 8 days; 4th use Primer group – device previously used for 8 days; 4th use Primer group – device previously used for 24 days. Protocol consisted in insertion of an intravaginal of 1gr progesterone device (PRIMER®, Tecnopec, Brasil) and injection of 2 mg of oestradiol benzoate (RIC-BE®, Tecnopec, Brasil) at D0. Eight days later (D8), devices were removed and were administrated 1mg of oestradiol benzoate. At D20, heifers were evaluated by ultrasound exam to detect corpus luteum in the ovaries. Data were analyzed by GLM procedure of SAS. Results analyzes showed that treated groups had higher percentage of corpus luteum 20 days after treatment initiation. Ciclicity rates for control group, New Primer group, 2nd use Primer group and 4th use Primer group were, respectively, 35,45% (39/110), 50,93% (55/108), 56,36% (62/110) and 65,14% (71/109). The results demonstrated that ciclicity induction treatment enhanced the number of females with corpus luteum 12 days after protocol ending, being a efficient tool of management to include zebu heifers in reproductive programs.

In conclusion, P/AI is increased when the TAI using sex-sorted is performed closer to synchronized ovulation in suckled cows. A total of 339 suckled multiparous Nelore cows from an experimental farm to 96 hours afterwards, to evaluate ovarian follicular dynamics and interval from device removal to ovulation. All females were examined for pregnancy 30 days after AI. The data were analyzed using the SAS program. Incidence of ovulation after the estrous synchronization protocol was 92.0 % (312/339). Diameter of ovulatory follicle was 14.7 ± 2.3 mm and the interval between the P4 device removal and synchronized ovulation occurrence was 71.8 ± 7.7 hours. The distribution of the synchronized ovulation relative to the device removal was: 48 hours (6.73%; 21/312), 60 hours (0.64%; 2/312), 72 hours (80.77%; 252/312), 84 hours (11.22%; 35/312), and 96 hours (0.64%; 2/312). The pregnancy per artificial insemination (P/AI) was increased (P <0.001) when the TAI was delayed 36 hours (5.8%; 5/86), 48 hours (20.8%; 27/130) and 60 hours (30.9%; 38/123). Higher P/AI was achieved on TAI performed closer to ovulation (0 to 12 hours before ovulation = 37.9 %; 35/95) than TAI performed on 12.1 to 24 hours (19.4%; 21/108; P = 0.05) or > 24 hours (5.8%; 5/87; P = 0.0001) before the synchronized ovulation. In conclusion, P/AI is increased when the TAI using sex-sorted is performed closer to synchronized ovulation in suckled Nelore cows.
ABSTRACT 93

EFFECT OF hCG ON FOLLICULAR DYNAMICS IN SANTA INÊS EWES SUBMITTED TO FTAI

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The aim of the present study was to investigate the effect of human chorionic gonadotrophin (hCG) injection on a synchronization protocol in Santa Inês ewes. The experiment was conducted in two consecutive steps, 9 ewes at each stage. The animals were randomly divided in one of two treatments: Control Group (GC) and hCG Group (GhCG). The ewes in the GC received an intravaginal progesterone device (Primer-PR®, Tecnopec, Brasil) on D0. The progesterone device was removed on D9, injected 100µg of d-cloprostenol (Prolise®, Syntex, Argentina) and 250 UI of eCG (Folligon®, Intervet, Holanda). The ewes in the GhCG, received the same protocol of GC, but 24 h after device withdrawal 500 UI of hCG was injected (Vetecor® - Hertape Calier-Espanha). Ultrasound examinations (Aloka SSD-500) were performed every 12 h from D9 until ovulation. Moreover, blood samples were taken 11 days after device removal for progesterone concentration analysis. Statistical analysis was performed by GLM of the Statistical Analyses System (SAS). Data were tested for normality of residuals and homogeneity of variances and transformed when necessary. There was no difference between groups in the diameter of the largest follicle at time of device withdrawal (GC: 4.56±0.99 mm vs. GhCG: 4.39±0.65 mm; P=0.68) and the maximum diameter of the preovulatory follicle (GC: 5.78±0.30 mm vs. GhCG: 5.36±0.69 mm; P=0.09). But there was difference between experimental groups in the interval between device removal and ovulation (GC: 79.9±15.4 h vs. GhCG: 54.7±4.9 h; P=0.001). Besides, ovulation occurred more synchronized in animals that received hCG. Moreover, the progesterone concentration 11 days after device removal was higher in GhCG (10.9±3.4 ng/ml) than GC (8.22±1.3 ng/ml). It can be concluded that hCG administration in Santa Inês ewes induced earlier and synchronized ovulation, and improved progesterone production on Day 11 after device removal. Acknowledgments: Hertape-Calier - FAPESP: Proc. No. 08/05175-5

ABSTRACT 94

SERUM PROGESTERONE PROFILE AND EFFICIENCY OF LONG PROTOCOLS WITH AND WITHOUT CIDR REPLACEMENT IN SANTA INÊS EWES DURING ANOESTRUS SEASON

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The aim this study was to evaluate serum progesterone (P4) concentration during long protocols with and without CIDR replacement and the efficiency of treatments for ovulation rate and quality of the corpus luteum in Santa Inês ewes. The experiment was conducted in southeastern Brazil (21º15'18"S e 48º19'19"W) during anoestrus season. Twenty-three ewes were randomly divided into two groups (GI, n=12 e GII, n=11). Estrus was synchronized with a P4 device (CIDR™) for 14 days. However, in GII, the CIDR was replaced by a new one on D7 (D0 = P4 administration). Doses of 2.5mg of dinoprost (PGF2α) i.m. were administered on D0 and 14. All ewes received 300 IU of eCG on D14. Blood samples for serum P4 determinations were taken daily during the protocol, one day after the end of treatment and on days 5, 10 and 15 post-ovulation. Data were analyzed by ANOVA by using SAS. Although the study has been performed during anoestrus season, 62.5% (GI) and 37.5% (GII) of females had corpus luteum of the previous cycle, at onset of treatment. On D0, P4 concentration was 2.56±0.49 and 2.60±0.66ng/ml for the respective groups (P>0.05). There was an increase (P<0.01) of P4 concentration for D1, reaching 5.49±0.56 and 5.07±0.65ng/ml, for the respective groups. In GI, P4 concentrations decreased continuously (P>0.05), reaching 1.06±0.11ng/ml on D14 and, 0.16±0.03ng/ml on D15. However, in GII, P4 concentration declined progressively (P>0.05) until D7, when was observed the value of 2.08±0.35ng/ml. Due to CIDR replacement in this group, was observed increase (P<0.01) P4 concentrations to 4.67±0.49ng/ml on D8, with subsequent decrease (P>0.05) until 1.59±0.26 and 0.30±0.09ng/ml on days 14 and 15, respectively. There was no statistical difference between groups for this variable, even for days after CIDR replacement in GII (P>0.05). Additionally, all ewes ovulated after the end of the protocols. The ovulation per animal was 1.33±0.49 and 1.36±0.67 for GI and GII, respectively (P>0.05). The diameters of CL at Day 5, 10 and 15 post-ovulation were 11.52±1.81, 13.28±1.51 and 12.79±1.34 in GI (abP<0.01) and 11.48±1.74, 12.94±1.87 and 12.94±1.66 in GII (P>0.05) respectively. The P4 levels at these times were 2.47±1.21, 4.62±2.82 and 4.30±1.82 ng/ml in GI (P>0.04) and 2.65±1.54, 3.90±2.06 and 3.82±1.96ng/ml in GII (P>0.05). We concluded that P4 profile showed an expected pattern for each group. However, there was no significant variation among groups. Finally, the protocols were effective to promote ovulation and formation of corpus luteum functional. Acknowledgments: FAPESP.
Due to its reproducibility and robustness, progesterone (P4) is a steroid hormone of fundamental importance in bovine reproduction, due to its involvement in estrus synchronization, superovulation and pregnancy diagnosis protocols. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is considered an auto-confirmatory technique, because an analyte with a specific retention time can be identified by its mass-to-charge ratio (m/z) and additionally by its fragmentation profile, eliminating cross-reaction occurrence. Due to these reasons, the mass spectrometry is nowadays considered to be the analytical technology of choice for the identification and quantitation of drugs and hormones in human clinical practice and for agropecuary products regulatory offices. The objective of this work is to develop a method for bovine serum P4 quantitation using atmospheric pressure photoionization (APPI), the most efficient ionization source for low polarity compounds such as steroid hormones. For sample extraction protocol and analytical-instrumental optimization, we used male bovine serum filtered in activated charcoal, in order to obtain a biological matrix free of the analyte of interest. Before extraction, medroxiprogesterone acetate (2.5 ng mL⁻¹) has been added as an internal control, and also known concentrations of progesterone (0.25 to 10 ng mL⁻¹) for the analytical curve. For liquid phase P4 extraction, 0.4 mL of serum was transferred to a polypropylene tube, followed by the addition of 1 mL of hexane. Samples were vortexed for 1 min, and then centrifuged for 2 min at 10 000rpm (Minispin, Eppendorf). The volume of 0.7 mL of the organic phase was transferred to a 2 mL clean vial and evaporated until dry under a nitrogen stream. Samples were reconstituted in 0.25 mL of methanol prior to analysis in LC-MS/MS system. Liquid chromatography was performed using a 1100 series HPLC (Agilent Technologies), with a Agilent Eclipse XDB-C18 5 um 4.6 x 150 mm column and methanol as the mobile phase. Ionization was performed in positive ion mode in a triple quadrupole-linear ion trap mass spectrometer 4000Q-TRAP (Applied Biosystems/MD Sciex) and was used as dopant toluene. For P4, transitions of m/z 315.2 > 109.2 were monitored for quantitation, and 315.2 > 123.2 for confirmation. For medroxiprogesterone acetate, transition of m/z 387.2 > 123.1 has been monitored. Determined limit of detection (LOD) for P4 was 0.05 ng mL⁻¹, and limit of quantitation (LOQ) was 0.25 ng mL⁻¹. Sample extraction efficiency was 85%, and recovery efficiency was 95%. The proposed analytical methodology presents high specificity to the target analyte, involved a simple and fast bovine serum P4 extraction protocol. This method may contribute to increase analytical parameters in veterinary reproductive medicine, since it will allow comparing the quantitative data from studies performed in different laboratories due to its reproducibility and robustness.

ABSTRACT 96

VACCINATION AGAINST REPRODUCTIVE DISEASES ASSOCIATED WITH TIMED ARTIFICIAL INSEMINATION ON REGNANCY RATE IN LACTATING DAIRY COWS


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The aim of this study was to evaluate the effect of vaccination against reproductive diseases on the reproductive performance of lactating dairy cows (Holsteins or Girolandas). In the experiment 01 were used 1140 cows with milk production of 21.29 ± 8.19 Kg/day in 37 properties that had never use vaccine against IBR/BVD/Leptospirosis and in the experiment 02 were used 820 cows with milk production of 24.52 ± 8.69 Kg/day in 16 properties that utilize vaccination against IBR/BVD/Leptospirosis in their annual sanitary program. In both experiments all cows were timed artificially inseminated (TAI) with the protocol: D0-estradiol cipionate injection (2mg, ECPA) and insertion of intravaginal progesterone device containing 1.9g of P4 (CIDR®); D7-prostaglandin injection (12.5mg, Lutalyse®); D9-device withdrawal, application of estradiol cionipate (1mg, ECPA) and; D11- TAI. At the beginning of the TAI protocol cows were randomly divided into two groups to receive or not one dose of vaccine (5.0 mL i.m., CattleMaster®4 + L5, Pfizer Animal Health, Lincoln, USA). At 30 days after TAI, was performed the first pregnancy diagnosis and revaccination of cows of experiment 01. The second diagnosis of pregnancy was performed after 41 days to determine the pregnancy rate of day 71 and pregnancy loss between 30 and 71 days of gestation. Data were analyzed by Logistic Regression in SAS. In experiment 01, animals that received CattleMaster®4 + L5 had a higher (P=0.02) pregnancy rate [44.4% (257/579) vs. 37.6% (211/561)] on day 30; higher (P=0.001) pregnancy rate [41.1% (238/579) vs. 31.7% (178/561)] on day 71 and lower (P=0.004) pregnancy loss [7.4% (19/257) vs. 15.6% (33/211)] between 30 and 71 days of gestation than the control group. In experiment 02, the vaccination in the TAI protocol did not improve (P=0.813) the pregnancy rate on day 30 [34% (131/385) vs. 34.7% (151/435)]; on day 71 (P=0.886) [30.6% (118/385) vs. 30.1% (131/435)] and in the pregnancy loss (P=0.189) [13.2% (20/385) vs. 9.9% (13/385)] compared with the control group. These data show that the use of CattleMaster®4 + L5 had a positive impact on pregnancy rate, and in cows that already received CattleMaster®4 + L5 in their annual sanitary program, another vaccination in the beginning of the TAI protocol is not necessary.
**ABSTRACT 97**

**EFFECT OF ANTIPARASITICIDE TREATMENT AFTER WEANING ON CYCLICITY RATE OF NELORE (Bos indicus) HEIFERS**

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The objective of this study was to evaluate the effect of the injection after weaning of Ivermectin 2.25% associated to Abamectin 1.25% (Solution 3.5% LA) on fertility rate of Nelore heifers in the beginning of breeding season. At the Experiment, 470 calves with 8 months old were divided in two experimental groups according to weight gain during the 60 days before the treatment. The Ivermectin Group (G-IV; n=235) was treated s.c. with 630 µg/Kg of Ivermectin 3.15% (Ivomec Gold, Merial, Brazil) and the Ivermectin + Abamectin Group (G-Sol; n=235) with the association of 450 µg/Kg Ivermectin 2.25% + 250 µg/Kg Abamectin 1.25% (Solution 3.5% LA, Intervet Schering-Plough, Brazil). The animals were allocated in same lots during entire experimental period. The antiparasitics were injected in May, July and September of 2007 and 2008. Ultrasonographic evaluations (Aloka SSD 500, Tokyo, Japan) were performed in May, July and October of de 2008. The weight gain and the cyclic rate (CL presence) were evaluated before the breeding season. The statistical analysis was performed using software SAS. It was observed that the females of G-Sol showed bigger weight in the beginning of breeding season (P=0.05). Furthermore, it was observed that the heifers treated with Ivermectin and Abamectin association presented bigger cyclic rate before the breeding season (G-IV=42.9%; G-Sol=53.6%; P=0.02). We can conclude the use of Ivermectin and Abamectin association promoted the bigger weight gain from weaning during the experimental period and consequently increased number of cycling animals in the beginning of breeding season. Thus, the treatment with the associated antiparasitics increased the number of heifers used in FTAI program because the presence of CL is one of the criteria to obtain satisfactory results in this animal category.

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**EFFECT OF FSHp (FOLLTROPIN-V®) ADMINISTRATION NUMBER ON SUPEROVULATORY RESPONSE AND EMBRYO PRODUCTION IN BRAHMAN DONORS**

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The reduction of FSHp administration number (3 injections) in Nelore donors superovulation protocols showed similar results than standard protocol with 8 injections (Martins et al., Acta Scientiae Vet., 36 suppl 2, 636, 2008). The aim of this study was to evaluate superovulatory response of Brahman donors submitted to protocol with 8 (standard) and 3 injections of FSHp. The trial was conducted with 12 donors from Fazenda Iguaçu, Paraná. It was used cross-over experimental designing and all donors received two treatments. At first day (D0), females received 1q intravaginal progesterone device (Primer®, Tecnopec, Brasil) and 2mg of oestradiol benzoate (RIC-BE®, Tecnopec, Brasil); at D4, superovulation started. On 8 FSH group, cows received 133mg of FSHp (Folltropin-V®, Bioniche, Canadá) in 8 decreasing dosages each 12 hours. On 3 FSH group, the same FSHp amount was shared in 3 injections: 39%-D4am, 39%-D5pm and 22%-D7am. At D7am, 150µg d-cloprostenol (PROLISE®, ARSA, Argentina) was administered and device was removed. At D8am, cows received 25mg of LH (Lutropin-V®, Bioniche, Canadá) and insemination was performed after 12 and 24. It was used the same crossing (bull/cow) in all replicates. The embryo collectors were performed at day 15. The variables were analyzed by GLM procedure of SAS. Mathematics model tested individual effects of cows, replication and treatment for variables: Total structures (TS), Non-Fertilized (NF), Degenerate (DEG), Transferable Embryos (TE), Frozen-thawed Embryos (FTE), Follicle number at D4 (NFOld4), Follicle number at LH injection (NFordLH), Corpus luteum number at D15 (NCLD15), ovulation rate (OR – calculated by division of NCLD15 by NFordLH) and recovery rate (RR – calculated by division of TE by NCLD15). The results showed that the number of TE was significantly higher in the 8 FSH group, with an average of 77.9±11.6 vs. 59.6±10.3 for OR and 75.2% vs. 69.8% for RR. These results showed there weren’t differences between treatments with 8 and 3 FSH injections for any analyzed variables in Brahman donors. Number reduction of FSHp dose within 36 hours of interval in superovulation protocols is a viable alternative for embryo transfer programs, with the advantage of reducing labor and minimizing zebu females management.
IMPORTANCE OF PRIOR GYNECOLOGICAL EVALUATION IN FEMALE SUBMITTED TO A PROTOCOL OF FIXED TIME ARTIFICIAL INSEMINATION WITH eCG ADMINISTRATION

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The objective was analyse the importance of prior gynecological evaluation to determine the need to use or not of eCG in FTAI protocol. Were used 307 female Nelore previously assessed gynecological rectal palpation and ultrasonography, on the uterine tonus (TON1- relaxed uterus, TON2 - moderately relaxed uterus and TON3 - contracted uterus), presence or absence of corpus luteum (CL) and body score condition (BSC 1-5). On a random day of the estrous cycle (D0), animals received an intravaginal progesterone device (Sincrogest®, Ouro Fino, Sao Paulo, Brazil) and an application of 2.0mg estradiol benzoate (Sincrodiol®, Ouro Fino, São Paulo, Brazil), intramuscular (im). On day eight, the intravaginal device was removed and were administered 500ìg of cloprostenol (Sincroci®, Ouro Fino, Sao Paulo, Brazil) im and 0.6mg of estradiol cypionate (ECP®, Pfizer, São Paulo, Brazil) im. At the time of implant removal, the induction of puberty and heat detection, with an increase of cycling heifers at the beginning of the breeding season.

To the results can be stated that the use of eCG or eCG + ECP in Nellore heifers induced with CIDR 4th use, improve the rates of G3b. There was no treatment effect in CR (P=0.5587) G1: 46.67% (7/15), G2: 34.7% (17/49) and G3: 33.33% (27/81). According to the results obtained dividing the number of pregnant heifers by the total of inseminated heifers. In EXP1 was effect of treatment (P < 0.0001) 53,33% (240/450) G1 and 53.33% (240/450)G2 and US (P = 0.0003) 57,59% (315/547)US1; 69,35% (215/310)US2 and 80%(28/114) US3 in IR. There was no treatment effect in heat detection rate (HDR) (P = 0.3637), G1 45,1% (69/153) and G2 43,55% (54/120). In EXP2 was effect of treatment in IR (P < 0.0001), 45,57% (36/80) G1b, 43,55% (54/124) G2b and 48,89% (63,64/131) G3b. There was no treatment effect in CR (P=0.5587) G1: 46.67% (7/15), G2: 34% (17/49) and G3: 33.33% (27/81). According to the results can be stated that the use of eCG or eCG + ECP in Nellore heifers induced with CIDR 4th use, improve the rates of induction of puberty and heat detection, with an increase of cycling heifers at the beginning of the breeding season.

USE OF eCG AND ESTRADIOL CYPIONATE IMPROVE THE RESPONSE TO INDUCTION PROTOCOLS WITH CIDR PREVIOUSLY USED FOR 27 DAYS IN NELLORE HEIFERS

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The aim of this study was to evaluated the effect of the addition of eCG and estradiol cypionate (ECP) in response to the induction protocol with CIDR previously used for 27 days (CIDR 4th use) and conception rate in heifers. For this purpose, we performed two experiments (EXP1 and EXP2). In EXP1 was used 896 heifers aged 24 months and body condition score (BCS) average of 2.99 ±0.23. The heifers were evaluated by ultrasonography (Mindray - 2200VET DP) to determine the presence of corpus luteum (CL) in D-19 and D-12 protocol. The heifers with CL in one of the tests were taken from the experiment. In D-12 was assessed body condition score (BCS), uterus score (US) in 1 (smaller diameter and without uterine tone), 2 (smaller diameter and uterine tone) and 3 (with larger diameter and uterine tone) animals. D0, the CIDR was removed and applied in a random 200 IU of eCG i.m. at half of the animals (G1), the rest has done no additional treatment (G2). In D1 began on estrous detection and heifers detected in estrus were inseminated according to the Trimberger system for 7 days. In D8 ultrasonographic evaluation was performed to detect the presence of CL in heifers that were not detected in estrus. In EXP2 were used 401 heifers with an average of 24 months of age and BCS average of 2.88 ±0.16. The design was as described in EXP1, except that in D0, 20% of the animals received no treatment (G1b), 40% received 200 IU of eCG im (G2b) and 40% 200 IU of eCG im + 0,5 mg ECP (G3b). Data were analyzed by logistic regression using PROC LOGISTIC of SAS. The rate of induction (RI) was calculated dividing the number of pregnant heifers by the total of inseminated heifers. In EXP1 was effect of treatment (P < 0.0001) 71,97% (321/446) G1 and 53.33% (240/450)G2 and US (P = 0.0003) 57,59%(315/547)US1; 69,35% (215/310)US2 and 80%(28/114) US3 in IR. There was no treatment effect in heat detection rate (HDR) (P = 0.3637), G1 45,1% (69/153) and G2 43,55% (54/124). In EXP2 was effect of treatment in IR (P < 0.0001), 45,57% (36/80) G1b, 43,55% (54/124) G2b and 48,89% (63,64/131) G3b. There was no treatment effect in CR (P=0.5587) G1: 46.67% (7/15), G2: 34% (17/49) and G3: 33.33% (27/81). According to the results can be stated that the use of eCG or eCG + ECP in Nellore heifers induced with CIDR 4th use, improve the rates of induction of puberty and heat detection, with an increase of cycling heifers at the beginning of the breeding season.
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REPRODUCTIVE EFFICIENCY OF HIGH-PRODUCING HOLSTEIN RECIPIENTS SYNCHRONIZED FOR FTET USING PROTOCOLS WITH OR WITHOUT ESTRADIOL AND/OR ECG DURING SUMMER AND WINTER

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The aim of this study was to evaluate the percentage of recipients selected for embryo transfer (RS), pregnancy rate (PR) and embryo loss (EL) of high-producing Holstein cows synchronized for fixed time embryo transfer (FTET) using protocols with or without estradiol (E2) and/or eCG during the summer and winter. Cows (n=784) with 318.3±5.0 DIM, 26.6±0.3 Kg milk/day, 4.7±0.1 services, 2.10±0.04 lactations and BCS of 3.19±0.02 (average±SE) were allocated in four experimental groups, during summer and winter, according to the administration or not of E2 and eCG. Cows from group E2 received a norgestomet ear implant (Crestra®, Intervet, Brazil) on a random day of the estrous cycle (D0) + 2mg estradiol benzoate (Gonadrol®, Intervet, Brazil) IM; implant removal + 0.150mg D-sodic cloprostenol (Preloban®, Intervet, Brazil) + 1.0mg estradiol cypionate (E.C.P®; Pfizer, Brazil) on D8, and FTET on D17. Cows from group E2+eCG received similar treatment with the addition of 400IU eCG (Folligon®, Intervet, Brazil) on D8. In groups GnRH and GnRH+eCG, the E2 given on D0 was replaced with 100µg Gonadorelin (Fertagyl®, Intervet, Brazil) IM, the implant was removed on D7 and E.C.P® was replaced with 100µg Gonadorelin on D9. FTET was done on D16. Pregnancy diagnosis was done at 25 and 42 d of pregnancy. Statistical analysis was done by logistic regression using PROC GLIMMIX of SAS. No interaction was found among eCG and treatment with E2/GnRH for any variable nor among E2/GnRH and season for RS, EL and P4 concentration on D7 (P4; P=0.05). Using or not eCG had no effect on RS ([59.0% (232/393) vs 65.5% (256/391)], 25d conception rate [CR25; 33.6% (78/232) vs 36.3% (93/256)], 25d PR ([PR25; 19.9% (78/393) vs 23.8 % (93/391)] and 42d PR [PR42; 14.5% (57/393) vs 16.6% (65/391)], PG [26.9% (21/78) vs 30.1% (28/93)] and P4 (3.2±0.22 vs 3.03±0.09ng/ml). An interaction among E2/GnRH and season was found for CR25 [E2 summer=30.5% (32/105), GnRH summer=36.2% (50/138), E2 winter=42.7% (49/115), GnRH winter=30.8% (40/130)], PR25 [E2 summer=16.2% (32/198), GnRH summer=25.6% (50/198), E2 winter=25.5% (49/192),GnRH winter=20.4% (40/196)], and PR42 [E2 summer=12.6% (25/198), GnRH summer=16.7% (33/200), E2 winter=19.8% (38/192), GnRH winter=13.3% (26/196)]. The RS was greater when cows received GnRH (68.0%; 268/394) than E2 (56.4%; 220/390; P<0.01), but EL was also (GnRH=34.4% (31/90) e E2=22.2% (18/81); P=0.06). In conclusion, the addition of 400IU eCG to a FTET protocol of high-producing Holstein cows had no effect on the evaluated reproductive parameters. Treatments with E2 and GnRH had similar PR25 during the winter, however, during the summer PR25 was lesser when E2 was used. Acknowledgments: Faz. Santa Rita and Intervet.

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OPTIMIZING THE USE OF SEXED SEMEN AND REDUCING ANIMAL HANDLING DURING TIMED ARTIFICIAL INSEMINATION PROGRAMS IN SUCKLED NELORE COWS

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The aim of this project was to evaluate two strategies to optimize the use of sexed semen (Experiment 1) and to reduce the animal handling (Experiment 2) during timed artificial insemination (TAI) program in suckled cows. In Experiment 1, 853 suckled Nellore cows between 30 and 60 days postpartum were used. Cows were synchronized using an intravaginal device of progesterone plus i.m. injections of 2.0mg of estradiol benzoate (EB). Females received prostaglandin (0.25 mg of sodium cloprostenol), equine chorionic gonadotropin (300IU) at progesterone device removal (eight day after insertion) and 1mg of EB 24 hours afterwards. Cows were timed inseminated 60 to 64 hours after device withdrawal. The ovaries were examined by transrectal ultrasonography (7.5MHz, CTS-3300V, SIUI, China) at TAI. During the exam the largest follicle (LF) was measured and classified as <11mm or e11mm. At TAI cows were randomly assigned into four groups according to the LF diameter present (<11mm or e11mm) and the type of semen used (sexed or non-sexed). There was a tendency (P=0.08) of interaction between type of semen and the LF at TAI on pregnancy per TAI [P/AI; non-sexed e11mm=59.1%; 101/171; non-sexed<11mm=51.0%; 131/257; sexed e11mm=56.8%; 104/183 and sexed <11mm=56.8%; 104/183]. Effects of the type of semen [non-sexed =54.2% (232/428) vs. sexed=45.4% (193/425), P=0.02] and the LF at TAI [e11mm=57.9% (205/354) vs. <11mm=44.1% (220/499), P=0.0001] on the P/AI were observed. In Experiment 2, two TAI synchronization protocols were tested. Suckled Nellore cows (n = 593) from five breeding groups were synchronized using two synchronization protocols (EB24h and EB0h). Cows from the EB24h group were synchronized using the same TAI synchronization protocol previously described in the Experiment 1. However, cows from EB0h receiving the progesterone device during nine days and 1mg of EB at the device withdrawal. Cows from the EB0h and EB24h groups were inseminated using sexed semen on 50-53 and 60-63 hours after device removal, respectively. There was a significant effect of breeding groups (P<0.0001) on the P/AI, however no interaction between breeding groups and synchronization protocol was observed (P=0.39). No effect of synchronization protocol on the P/AI was found (EB24h = 47.1%; 140/297 vs. EB0h = 41.0 %; 121/295; P = 0.25). Therefore, the presence of a larger follicle at TAI was associated with greater P/AI following TAI protocol. The use of sexed semen resulted in lower P/AI than the use of the non-sexed semen. Also, it is possible reduce the number of handlings without affecting the overall efficiency of the synchronization protocol. Acknowledgements: Sexing Technologies; CRV-Lagoa da Serra; Intervet-Shering-Plough and Ouro Fino Saúde Animal.
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OVARIAN RESPONSE AFTER THE USE OF ESTRADIOL OR GNRH FOR SYCHRONIZATION OF OVULATION IN HOLSTEIN DAIRY COWS

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This study evaluated the follicular and luteal dynamics following two type of synchronization of ovulation protocols using GnRH or estrodiol in Holstein dairy cows. A total of 29 females, presenting 249.1 ± 82.3 days in milk and producing 13.4 ± 3.75 kg of milk per day were homogeneously assigned into two treatment groups. Females from GnRH group (n = 15) received GnRH im (150ìg, Fertagyl®, Intervet Schering-Plough) and ear implant containing 3 mg norgestomet (Crestar®, Intervet Schering-Plough) at the beginning of the synchronization protocol. Seven days later, the implant was removed and was administered 0.5 mg cloprostenol im (PGF; Ciosin®, Intervet Schering-Plough). A second administration of GnRH was performed 48 hours after removal of the implant. In the Estradiol group (n = 14), females received at the first day of the treatment the administration of 2 mg estradiol benzoate im (BE; Gonadiol®, Intervet Schering-Plough) with the insertion of the ear implant. Eight days later, the implant was removed and the administration of PGF plus 1 mg of estradiol cyproionate im (SincroCP®, Fine Gold-Animal Health) were performed. Ovarian follicular dynamics were evaluated by ultrasound 24/24 hours during the permanence of the implant and every 12 hours from implant removal to the occurrence of ovulation or until 96 hours after implant withdrawal whichever occurred first. Nine days after implant removal, another ultrasound evaluation was performed to determine the diameter (Ø) of the corpus luteum (CL). The results for the GnRH and Estradiol groups were, respectively: Ovulation after the first treatment [46.7% (7/15) and 21.4% (3/14), P = 0.35], emergence of the new follicular wave (1.4 ± 0.6 and 3.0 ± 0.9 days, P <0.0001), ovulatory follicle diameter (17.4 ± 2.4 and 16.5 ± 4.8 mm, P = 0.74), ovulation rate [100.0% (15/15) and 92.9% (13/14), P = 0.35], interval from implant removal to ovulation (79.2 ± 8.8 and 65.5 ± 11.6 hours, P <0.0001) and CL diameter (26.1± 4.8 and 25.6 ± 5.2 mm, P = 0.61). Therefore, the use of GnRH reduces the interval between treatment and emergence of the new follicular wave compared to the use of BE. Females treated with GnRH present higher interval between implant removal and ovulation than females treated with estradiol cyproionate. The GnRH-based and estradiol-based synchronization of ovulation protocols have similar follicular and luteal responses in lactating Holstein cows.

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Embryo recovery rates obtained from Mangalarga Marchador mares after induced ovulation using different hCG doses

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Human Chorionic Gonadotrophin (hCG) has been widely used in attended equine reproduction as ovulation-inducing, in order to optimize the results in an embryo transfer program. The hCG efficiency for induced ovulation was demonstrated by McCue et al.(2007, JEVS, 27:58-51), but its correlation with embryo recovery has been little investigated. In present study, the influence of different endovenous hCG doses was compared to embryo recovery rate in embryo transfer program. This research was carried out during 2008/2009 and 2009/2010 breeding seasons. Forty-nine Mangalarga Marchador donors between four and 18 years old were used with a total of 224 estrus cycles, after their reproductive tract had been previously examinated through uterine cytology and transrectal ultrassonography. Ovarian activity was examined daily during estrus through transrectal ultrasonoscopy in order to determinate the induced ovulation date and the ovulation (D0). The estrus cycles were distributed in three Groups: Control group with spontaneous ovulations cycles (n=91); group 2, treated cycles with 1000 UI hCG (Chorulon®, Intervet Schering-Plough Animal Health, São Paulo, Brasil) (n=87) and group 3, treated cycles with 1700 UI hCG (n=45). In both treated groups, hCG administration was realized when the dominant follicle reached diameter ø 35 mm and satisfactory uterus edema was observed. Artificial inseminations were done 24 hours after hCG administration, using fertile stallion semen or, if necessary, each 48 hours up to ovulation. Embryos were collected between six and nine days after ovulation. The results were evaluated using χ² Test, with p<0.05. Embryo recovery rates for the groups: control, 2 and 3 were: 56% (51/91), 59.7% (52/87) and 62.2% (28/45), respectively. There was no statistic difference among groups (p>0.05). The results of present study showed that different hCG doses have no interference on embryo recovery rates.

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Profile of released the LH in ovariectomized heifers Nelore treatment with different GnRH analogs

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The objective of the study was to evaluate the profile of released and plasma concentration of LH induced for administration the different GnRH analogs (Sincroforte e Deslorrelina OF) in ovariectomized heifers Nelore (Bos indicus). In this experiment, it was used 12 ovariectomized heifers Nelore, with body weight of 463.8±6.1 Kg, age of 48.0±0.5 months and body condition score above 3 (1 to 5 scale). The animals kept in continuous pasturing at Brachiaria decumbens, supplied "ad libitum" of concentrate, water and salt. The animals were allocated in two different treatments and received 750 µg deslorelin acetate (G-Desio; Deslorrelina OF, Ourofino, Cravinhos, Brazil; n=6) and 10 µg buserelin acetate (G-Buser; Sincroforte®, Ourofino, Cravinhos, Brazil; n=6). After the treatment, blood samples started to be collected for LH profile release evaluation. The samples were collected to each 15 minutes from the moment of treatments to 210 minutes. The analyzed variables were amplitude, duration and value of LH peak, beyond the treatment/peak LH interval. The statistical analysis was accomplished by procedure GLM of Statistical Analysis System (SAS). It was verified statistical difference between the treatments for amplitude of LH peak (G-Desio=18.4±1.6 ng/mL e G-Buser=11.3±1.8 ng/mL; P=0.02), duration of LH peak (G-Desio=177.5±14.2 min e G-Buser=127.5±12.7 min; P=0.03) and average value of LH peak (G-Desio=11.7±1.5 ng/mL e G-Buser=6.9±0.8 ng/mL; P=0.02). However, the moment of LH peak were similar between the treatments (Desio=30.0±5.5 min e Buser=32.5±11.2 min; P=0.54). We conclude that deslorelin promoted greater release of LH in plasma for a longer interval in ovariectomized heifers Nelore. However, both treatments were efficient to release plasma LH.

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THE REPRODUCTIVE EFFICIENCY OF HIGH-PRODUCING DAIRY COWS SUBMITTED TO FTAI

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The aim of the present study was to verify the effect of fixed-time artificial insemination (FTAI) on reproductive efficiency of high-producing dairy cows. Nine hundred eighty five Holstein cows were allocated in one of two groups considering the body condition score, presence or absence of CL and average milk production. The cows of Control Group (G-Control; n=490) that presented CL on D0, received PGF2α (150 µg) and were submitted to estrous detection twice a day for 1 hour and then, they were inseminated 12 hours later concomitantly to the injection of GnRH (100 µg). No cows from G-Control were first inseminated before 57 days in milk (DIM) and those not observed in estrous were evaluated by retal examination 14 days later. The cows submitted to FTAI (G-FTAI; n=495) received a Norgestomet ear implant plus 2 mg of estradiol benzoate. On D8, the implant was removed and 150µg of PGF2α IU of eCG and 1,0 mg of estradiol cypionate were administered. Cows were submitted to FTAI 54h after the implant removal, concurrently with the administration of GnRH. All cows from G-FTAI were inseminated from 57 to 63 DIM. After the FTAI the animals were observed in estrous and the insemination was performed under the same conditions of G-Control. Ultrasonographic pregnancy diagnosis was done 30 and 60 days after AI. Statistical analysis was performed using SAS. Binomial variable were analyzed using the GLIMMIX procedure. There was no difference on conception rate after first [G-Control=21.2% (99/467); G-FTAI=17.8% (88/495); P=0.55], second [G-Control=20.2% (41/136); G-FTAI=21.4% (38/178); P=0.23]. However, the pregnancy loss was high in G-FTAI after the first AI [G-Control=20.8% (26/125); G-FTAI=30.2% (38/127); P=0.04]. There was no difference on pregnancy rate at 150 DIM [G-Control=41.8% (205/490); G-FTAI=41.8% (207/495); P=0.70]. The interval partum to first AI was delayed on G-Control (78.4±1.3 days) compared to G-FTAI (60.6±1.3 days; P=0.001). Also, the interval partum to conception were delayed in Control Group (G-Control: 94.6 ± 1.8 days; G-FTAI: 87.4 ± 1.8 days; P=0.02). These results indicate that the use of FTAI at 60 DIM was efficient to decrease the interval partum/conception. However, the percentage of pregnancy cows at 150 DIM was similar in both groups.
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USE OF MELENGESTROL ACETATE FOR OVARIAN ACTIVITY INDUCTION AND REPRODUCTIVE EFFICIENCY OF NELORE (Bos indicus) HEIFERS

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Nelore heifers (n=380) were allocated in four treatments according to ovarian activity (CL vs. no CL) and to administration or not of melengestrol acetate (MGA®, Pfizer, Brazil) (MGA vs. Control) at the beginning of the breeding season (BS). The experimental design was a factorial 2x2 model. Treatments were: CONTanestro (n=96), CONTciclando (n=95), MGAanestro (n=98), MGAciclando (n=91). Starting on day -16, the heifers received 0.5 mg of melengestrol acetate (MGA)/ head/day, mixed with the mineral supplement. On day -11, 1mg of the estradiol cyponiate (ECP®, Pfizer, Brazil) was administered. MGA was offered daily until day -2. Estrous was observed from day 0 to 10. Between days 20 and 80, all females were submitted to natural service with a bull:cow ratio of 1:20. Induction of cyclicity (presence of CL in anestrous group females), conception rate at artificial insemination and pregnancy rate after BS were evaluated. For reproductive diagnosis, an ultrasonographic (CHISON 500VET, USP Brasil Eletromedicina, São Paulo, Brazil) examination was simultaneously performed at days -17 (only for cyclicity evaluation) to treatram time and day 40 and 110 (for cyclicity, conception and pregnancy rates evaluation). Data were analyzed using the GENMOD procedure of SAS (SAS Institute, Cary, NC, USA). The results of treatments CONTanestro, CONTciclando, MGAanestro and MGAciclando were, respectively: Cyclicity rate at Day 40 (76%,c, 100%,a, 91%b e 100%;P<0.05); Cyclicity rate at Day 110 (81%,b, 100%,a, 94%a e 100%;P<0.05); Conception rate at artificial insemination (19.8%;c; 39.8%ab; 30.3%bc e 44.4%;P<0.05); Pregnancy rate after BS (63.0%;b; 82.8%;a; 67.0%b e 84.9%;P<0.05); Number of days to beginning of BS from conception (32.5±3.0a; 27.1±2.5ab; 25.5±3.1ab; 23.0±2.6b;P<0.05). The MGA increased significantly the cyclicity rates in anestrous heifers. The ovarian cyclicity and MGA administration resulted in a additive effect on the conception rates at artificial insemination, pregnancy rates at BS and earlier establishment of pregnancy. Acknowledgements: Laboratórios PFIZER, USP Brasil Eletromedicina e Fazendas Reunidas Rio Bravo

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RATES EVALUATION OF PREGNANCY, CONCEPTION AND PROLIFICACY WITH THE METHODS USE OF NATURAL MATING AND FIXED-TIME LAPAROSCOPIC INTRAUTERINE ARTIFICIAL INSEMINATION (FTLUIAI) IN SANTA INÊS EWES

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In ovine, several reproductive methods were already described, but at the present time, two methods are used most to the field in technical sheep breeding, the Controlled Natural Mating (CNM) and the Fixed-Time Laparoscopic Intrauterine Artificial Insemination (FTLUIAI). The goal of this study was to evaluate the reproductive rates, in ovine of the Saint Inês breed comparing the methods of CNM (GI) and FTLUIAI (GII). The experiment was led in a property situated in the municipal district of Paraíba do Sul, Rio de Janeiro’s State, during February to September 2009. They were used 68 ewes already given birth, varying from 3 to 5 years old and middleweight of 50 kg, being 40 in the CNM (GI) and 28 in the FTLUIAI (GII), in the synchronized animals it used intravaginais sponges (Progespon®, Syntex) containing 60 mg of acetate of medroxyprogesterona (MAP) during 12 days plus 300UI of eCG (Novormon®, Syntex). The estrous detection was carried out twice daily teaser rams, introduced in the flock soon after the females childbirth (GI) and after the sponge removal (GII). Twice to the day, by morning and in the afternoon, the females were observed and the ones that presented estrus were identified by the presence of ink in the croup region, characterizing the receptivity to the male, being the females of the GI put with the ram to be mating. The semen was collected by artificial vagina of a fertile ram approved in andrological exam and dilution in the Bioxcell® (IMV Technologies) was carried out in order to achieve a final sperm concentration of 150 million spermatozoa per dose, in 0.2 mL of volume, in 0.25 mL straws. The artificial insemination was performed in fixed-time by laparoscopy, between 48 and 50 hours after the sponges’ withdrawal and half insemination dose (0,1 mL) was applied in each uterine horn. The pregnancy diagnosis was performed by ultrasonography exams, with a 5 MHz transducer, 35 days after the CNM and FTLUIAI. Statistical analysis of the evaluated characteristics, it was used the hypothesis test for difference between averages in the Statdisk Software (TRIOLA, 1998). Were obtained for pregnancy rate, conception rate and prolificacy 72,5% (n = 29), 87.5% (n = 35) and 1,24 in the group CNM, and 67,9% (n = 19), 78,6% (n = 22), and 1,21 to the group FTLUIAI, respectively. The differences among rates evaluated in MNC and FTLUIAI methods were not statistically significant (P>0.05), however FTLUIAI’s cost is much more high, turning its economically viable utilization only in elite sheep flocks.
Resumos.

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CYCLICITY AND UTERINE GROWTH EVALUATION OF CROSSBRED BOVINE HEIFERS SUBMITTED TO PUBERTY INDUCTION PROTOCOL

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The objective of this experiment was to evaluate the cyclicality and uterine diameter increase of heifers right after puberty induction treatment with estrogen and progesterone. There were 126 pre-pubertal crossbred heifers, about 24 month of age and 270 Kg of average weight, from Paraíso farm at Pinhal county, São Paulo. Heifers were evaluated by ultrasound exam and uterine horn diameter was measured near uterine body junction. Ovarian structures were also evaluated before and after treatment. Females were randomly allocated in control group (without treatment) and treated group, submitted to a puberty induction protocol. The following protocol was used in treated animals: D0: insertion of 1gr progesterone device previously used for 8 days (PRIMER®, Tecnopec, Brasil); D8: device withdrawal; D9: administration of 1mg of oestradiol benzoate (RIC-BE®, Tecnopec, Brasil). At D20, heifers were evaluated by ultrasound exam to cyclicality examination, to detect corpus luteum in the ovaries and to measure uterine horn diameter. Cyclicality rate and uterine growth measures were analyzed by ANOVA (PROC GLM - SAS). Treatment increased heifers cyclicality when compared with control group: 65,1% (41/63)4 vs. 23,0% (15/63)4 (p<0,001), respectively. There was a discrete significant increase (p=0,002) of uterine horn diameter for treated group: 0,9mm (initial diameter: 16,6mm vs. final diameter: 17,5mm) when compared to control group: 0,3mm (initial diameter: 15,8mm vs. final diameter: 17,2mm). When statistical analyzes were done only in animals with uterine diameter equal or superior than 15mm, uterine growth was more evident in treated animals: 2,0mm (initial diameter: 13,7mm vs. final diameter: 15,8mm) related to control animals: 0,6mm (initial diameter: 13,7mm vs. final diameter: 14,3mm) (p<0,001). The results showed a positive effect of induction puberty treatment with estrogen and progesterone in pre-pubertal crossbred heifers, relative to cyclicality rate and growth stimulus of uterine horn diameter.

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EFFECT OF PRE-TREATMENT WITH BOVINE SOMATOTROPIN RECOMBINANT SUPEROVULATORY RESPONSE AND ON QUALITY OF EMBRYOS OF NELORE DONORS

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One major barrier to the technique of Superovulation followed by collection of donors and embryo transfer, is the variability of animal response to the protocols of SOV Aiming to minimize this effect a study was conducted with the purpose of evaluating the effect of the Recombinant Bovine Somatotropina (rbST) in a protocol of superovulation, from donor Nelore cows on the ovulatory response, quantity and quality of embryonic and the pregnancy rates of recipients of embryos. Six donors used were divided into three experimental groups. The first group received a dose of 500mg of rbST by sub-cutaneous on Day 0 (D0) of the protocol of synchronization of follicular wave (C/rbST-D0), Concomitant placement of the device of intravaginal application of progesterone and of estradiol benzoate; the second group received 2 doses of 500mg of rbST, the first dose 14 days before the start of the protocol of the follicular wave emergence and the other on the start of the protocol (C/rbST-D14-D0) and the third group was the control. For statistical analysis of the characteristics assessed, was used the statistical package Statistical Analysis System (SAS)-version 6 (1996): variables, number of structures, number of viable embryos, embryo viability rate, embryo quality and size of corpus luteum of recipient, werew compared by the use of the PROC GLM, by the use of the using the Student-Newman-Keuls (SNK); the variable rate design had its tables of distribuition and frequency dispersion of the study prepared by PROC FREQ, and so it used the chi-square. The mean number of structures collected, viable embryos and embryo viability rate was not observed difference (P>0.05) between treatments. Mean stage of development and embryo quality did not differ statistically between groups. The pregnancy rates of recipients were: C/rbST-D0=55% (22/40), C/rbST-D14-D0=60% (27/45) and S / rbST=34.38% (11/32). Significant difference between control group and treated group C/rbST-D14-D0. Although there was statistical difference between the control group and treated group C/rbST-D0, there was a trend for increased pregnancy in the treated group. The rbST did not influence the total numbers of recovered structures and viable embryos or in their treatments, as well as the medium of stage of embryo development and quality of embryos recovered. However improved the pregnancy rates of embryos from donors treated with rbST 14 days before the start of the protocol of superovulation and the day of starting treatment, indicating that these embryos have greater potential for survival in the period of deployment.
The aim of the study was to evaluate the effect of different time of PGF injection (D7 vs. D8) in fixed-time artificial insemination protocols on pregnancy rate of high-yielding dairy cows. At the Experiment, 301 Holstein cows with 32.1±0.5 L/Day of milk yield, 156.4±6.2 days in milk, 2.35±0.08 calves and 2.88±0.13 inseminations. In random day of estrous cycle (D0), the cows were evaluated by ultrasonographic examination (UE) to determine the reproductive status. Furthermore, the cows were treated with 2mg of estradiol benzoate (Sincrodiol®, Ourofino, Cravinhos, Brazil) and an intravaginal progesterone device (Sincrogest®, Ourofino, Cravinhos, Brazil). After this moment, the animals were allocated in one of two groups according to presence or absence of CL and cyst on UE and received: 530ig of Cloprostenol (Sincropio®, Ourofino, Cravinhos, Brazil) on D7 - PGF-D7 Group; 530ig of Cloprostenol (Sincropio®) on D8 - PGF-D8 Group. On D8, the animals were injected with 1mg of estradiol cypionate (ECF®, Pfizer, Brazil) and the device was removed. The FTAI was performed 48 hours after de device withdrawal. The pregnancy diagnosis was performed by UE 28 days after FTAI. The statistical analysis was performed by logistic regression using GLIMMIX procedure of SAS. In the statistic model the follow explanatory variables were included: days in Milk, number of calves, number of inseminations, farm, milk yield and management. Effects of treatment, management and interactions were included in the final model. There was no interaction between treatment and management (free stall or pasture). There was no statistical difference between treatments to estrus expression rate [PGF-D7 = 73.15% (109/149) and PGF-D8 = 67.76% (103/152); P=0.15] and pregnancy rate [PGF-D7 = 29.5% (44/149) and PGF-D8 = 27.0% (41/152); P=0.67]. However, it was observed difference on pregnancy rate according to estrus expression [with estrus 32.1% (68/212) and without estrus 19.1% (17/89); P=0.008]. The results indicate that the time of PGF injection in synchronization of ovulation protocols with progesterone device and estradiol do not affect the fertility in high-yielding dairy cows. Furthermore, it was observed increased conception rate in cows that was observed estrus expression.
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FIXED TIME SYNCRONIZATION PROTOCOLS WITH FSHp (FOLLTROPIN-V®) IN EMBRYO RECIPIENTS BOVINE HEIFERS

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After evidence of FSH use to induce follicular growth in Fixed Time Artificial Insemination (FTAI) protocols (Santos et al., Acta Scientiae Veterinariae, 35 supl 3, 1152, 2007), this experiment aimed to test FSH effect in recipients, analyzing transferred/treated rate and pregnancy rate in fixed time embryo transfer (FTET) protocols. Two different experiments were performed to study Folltropin-V® relatively to eCG protocols. The standard synchronization protocol consisted in an insertion of an intravaginal 1gr progesterone device (PRIMER®, Tecnopec, Brasil) and 2mg of oestradiol benzoate (RIC-BE®, Tecnopec, Brasil) administration at D0; at D8, device withdrawal and was injected 112.5µg of d-Cloprostenol (PROLISE®, ARSA, Argentina) plus 1 mg of oestradiol benzoate (RIC-BE®, Tecnopec, Brasil). At this day (D8), 10 or 20 mg of Folltropin-V® (Folltropin-V®, Bioniche, Canada) were administrated comparatively to 400UI of eCG (Novormon®, Syntex S.A., Argentina). At 17° day, embryos were transferred to recipients cows. Data were analyzed by GLIMMIX procedure of SAS. In the first trial, at Embriza Biotechnology, Campo Grande, transferred/treated rate, pregnancy rate of recipients that received an embryo and pregnancy rate of total treated cows were, respectively, 72.6%a (61/84); 49.2%a (30/61) and 35.7%a (30/84) for 10 mg of Folltropin-V® and 87.3%b (69/79); 43.5%a (30/69) and 37.9%a (30/79) for eCG 400UI (Novormon®, Syntex S.A., Argentina). At embryo transfer day, plasmatic progesterone concentration was measured by radioimmunoassay to evaluate luteal production. The results for total cows that received an embryo, pregnant cows and non-pregnant cows were, respectively, 4.3a ng/ml; 4.6a ng/ml and 3.8a ng/ml for Folltropin-V® treated group and 6.2²ng/ml; 5.8a ng/ml and 6.4a ng/ml for eCG group. In the second trial, conducted at Poços de Caldas, 10 and 20 mg of Folltropin-V® were compared to eCG 400 UI. Recipients transferred/treated rate were, respectively, 76.8%a (73/95); 83,0%a (73/88) and 89,5%a (85/95). Pregnancy rate of recipients that received an embryo were 39.7%a (29/73), 39.7%a (29/73), 38.8%a (33/85) and pregnancy rate of total treated cows were 30.5%a (29/95), 32.9%a (29/88) and 34.7%a (33/95) for 10FSH, 20FSH and eCG. Transferred/treated rate was similar between Folltropin-V® 20mg and 400UI eCG, being slightly lower for 10mg dose of Folltropin-V®. However, final pregnancy rates were identical for all groups. These data showed that Folltropin-V® is a viable option to be used as follicular growth inductor for recipients cows in fixed time embryo transfer (FTET) programs.
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OOCYTE RECOVERY RATES AND MORPHOLOGY OBTAINED BY OPU AFTER DIFERENT HORMONAL TREATMENTS IN CATTLE

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The aim of this work was to evaluate the effect of dominant follicle removal (DFR) or estradiol benzoate (EB) and progesterone (P4) treatment, to control follicular wave emergence, and eCG or FSH, to superstimulate follicle growth prior to ovum pick-up (OPU), on oocyte retrieval and quality in beef cows. Brangus and Angus donors (n=12) were superstimulated by four treatments (2 x 2 arrangement of treatments) in a cross-over design. Donors in Group 1 and Group 2 received 2.5 mg EB (Zoovet, Argentina) and 50 mg P4 (Syntex SA, Argentina) intramuscularly (im) on Day 0; whereas donors in Groups 3 and 4 were subjected to DFR on Day 3. All cows received 150 µg D (+) cloprostenol (Ciclar, Zoovet, Argentina) on Day 4, and those in Groups 1 and 3 also received 800 IU eCG (Novormon, Syntex SA); whereas donors in Groups 2 and 4 received 160 mg Folltropin-V (Bioniche Animal Health, Canada) in twice daily equal doses for 2 days (i.e. Days 4 to 5). On Day 7, OPU was performed by ultrasound-guided follicular aspiration and were classified according to the nature of COCs, as described by Chaubal et al. (2006). Data was analyzed by ANOVA. The mean (±SEM) number of follicles aspirated on Day 7, total number of oocytes retrieved and the number of viable oocytes were higher (P<0.05) in cows treated with FSH (18.17±1.12, 9.67±1.01 and 6.79±0.84) than is those treated with eCG (11.21±1.02, 6.33±0.76 and 3.29±0.51). However, no significant effect of follicle wave synchronization treatment (DFR vs EB+P4) was detected in the total number of follicles aspirated (14.88±1.15 vs 14.50±1.41), number of oocytes retrieved (8.25±0.91 vs 7.75±1.00) and number of viable oocytes (5.33±0.79 vs 4.75±0.77).

In conclusion, DFR or EB+P4 are equally efficacious to synchronize a new follicle wave for superstimulation for OPU; however, FSH treatment resulted in more and better oocytes retrieved than eCG.

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COMPARISON BETWEEN PREGNANCY RATES OF BIOPSIED (SEXED) AND INTACT EMBRYOS UNDER FIELD CONDITIONSCONCERNING STAGE AND QUALITY OF EMBRYOS

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After working with embryo transfer under field conditions since 2005, we began to offer the technology of embryo sexing to our clients. One of the benefits of this technology is to decrease number of males calving and, consequently, lessen dystocia - expected to occur 25% more frequently in male's pregnancy than female's, especially in heifers. Also, embryo sexing allows the use of a small number of recipients because only female embryos will be transferred; avoiding the sanitary risk of acquiring more animals. The biopsies were performed by microblade and the embryo sexing determination is done by PCR followed by electrophorese on agarose gel. All biopsied embryos stayed in individual dishes containing holding medium – TQC (Nutricell, Campinas, Brazil) until the results were known. This papers goal is to compare pregnancy rates between intact and biopsied (sexed) embryos in field conditions take into consideration development stage and quality of embryos. All data were collected from intact and biopsied embryos (both transferred) and set them apart in two groups: INTACT(I) AND SEXED(S). In each group two classes were distinguished, based on stage of embryonic development – 1. Morula(M) and 2.Blastocyst(BL) and again, into each class set apart quality of embryos: Grade 1(G1), Grade 2(G2) and Grade 3(G3). The expanded blastocysts were included in the class of Blastocyst (BL)because it were in few number. All embryos transferred (T) and pregnancies (G) were counted during a long period of time.Results were analyzed using Chi-Square test (p<0,05). The results were: MG1: I= 575G/832T(69%) S= 153G/267T (57%); MG2: I= 887G/1496T(59,29%) S= 247G/443T(56%); MG3: I= 401G/912T(43,9%) S= 61G/191 T(32%). 2. BLG1: I= 304G/451T(67%) S= 685G/1121T(61%); BLG2: I= 129G/230T(56%) S= 216G/410T(53%); BLG3: I= 5G/24T (21%) S= 6G/15T(40%). The pregnancy rate for each group based on stage of embryonic development was: M: I= 1863G/3240T(59%) S= 461G/901T(51%) p= 0.069; BL: I = 438G/705T(62%) S= 904G/ 1543T(59%) p= 0.427. The majority of pregnancies 1365G/2444T(55,8%) from sexed embryos were female because only female embryos or embryos without definition of the sex were transferred. There were significant differences in pregnancy rates of sexed and intact Morula G3 (p= 0,043). There were no significant differences in pregnancy rates of M and BL, embryos grade 1 and 2 had the same results. The embryo sexing under field conditions don’t affect the pregnancy rate when embryos grade 1 and 2 are sexed, no matter in which stage of development they are.
There is also the production of toxic substances. The aim of this study was to compare different densities embryo during in vitro drop and the ideal zygote environment. A large density of embryos can produce large quantities of growth factors, however, for cow, causing problems related with in vitro cultivation, as the embryos nutritional needs, density of zygotes per volume

Group-2 (in vitro matured), (1523 in dry, 1491 in the rainy), evaluated after ripening, 25 oocytes were placed in basic medium based on morphology according to Gonçalves et al. (2008; Biotécnicas aplicadas à reprodução animal. Editora Roca, São Paulo: 261-292). Divided into Group-1 (recovered), (1391 in dry, 1340 in the rainy), evaluated immediately after harvest and Group-2 (in vitro matured), (1523 in dry, 1491 in the rainy), evaluated after ripening, 25 oocytes were placed in basic medium maturity (MBM). After collection of oocytes was determined the quality, the enzyme activity of group II caspases with reagent PhiPhiLux-G1D2 and DNA fragmentation (TUNEL) as advocated Paula-Lopes e Hansen (2002; Biochem Biophys Research Com, 295:37-42). After maturation, was given the stage of nuclear maturation, enzymes caspases and DNA fragmentation. Results were analyzed using the variance between groups test, for P<0.05.In the stages of nuclear maturation stages of Germinative Vescicle (3.53±0.11; 4.12±0.22), Germinative Vescicle Break-Down (12.24±1.43; 11.65±1.37), Metaphase I (8.86±0.98; 7.88±1.05) and II (62.4±5.98; 64.4±1±7.6) in dry and rainy seasons respectively, showed no significant difference (P>0.05). There was significant difference (P>0.05) in activity of enzymes and Caspases (G1-9.44%; 8.57%) (GII-11.57%; 8.73%) in DNA fragmentation of oocytes recovered (10.89%; 9.08%) and in vitro matured (11.31%; 9.86%) in dry and rainy seasons respectively. Based on data obtained, we conclude that in the dry and rainy seasons do not influence in vitro nuclear maturation and apoptosis of goat oocytes.

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IN VITRO EMBRYONIC DENSITY CULTURE OF BOVINE EMBRYOS

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In vitro production of commercial embryos exist a large number variation of oocytes recovered in each aspiration for cow, causing problems related with in vitro cultivation, as the embryos nutritional needs, density of zygotes per volume drop and the ideal zygote environment. A large density of embryos can produce large quantities of growth factors, however, there is also the production of toxic substances. The aim of this study was to compare different densities embryo during in vitro culture on the development rate of bovine blastocysts. The aim of this study was to compare different densities embryos during in vitro culture on the development rate of bovine blastocysts. The experiment were repeated three times, using COCS 495 (quantity I and II), from slaughterhouse cows. COCs were matured in TCM-199 Bicarbonate 10%FCS (38.5°C; 5%CO2) for 24h. Fertilization occurred in IVF-TALP environment, in the same terms for 18h. Then, the structures were transferred to SOF culture way, and randomly divided in six groups: G1/35 - zygote/drop of 35ìl (n=30), G1-1 zygote/drop 70ìl (n=30) G5-5 zygotes/drop 70ìl (n=60), G10-10 zygotes/drop70ìl (n=120), G15-15 zygotes/drop 70ìl (n=135) and G20-20 zygotes/drop 70ìl (n=120). The embryos were evaluated during the culture, at day 3 cleavage rate, at day 7 blastocyst rates and on day 9 the hatching rate. For statistical analysis was used logistic regression analysis. The cleavage rate of group G10 was similar to G5 (G10: 92.5%, G5: 86.67; p=0.001) and significantly higher than the other groups (G1/35: 70%; G1/70: 76, 67%; G15: 80.74%; G20: 76.67%; p<0.001). There was no rate blastocyst difference statistical between G5, G10 and G15 (G5: 30%; G10: 42.5%; G15: 29.63%; p=0.001) but G10 was significantly greater than G1/35, G1/70, G20 (G1/35: 20%; G1/70: 26.67; G20: 21.67, p <0.001). On hatching rate, the G10 was similar to group G5 (G5: 83.3% vs. G10: 82.4%, p = 0.001) and significantly higher than the other groups (G1/35: 50%; G1/70: 62.5%; G15: 67.5%; G20: 53.85%, p<0.001), indicates that the increase of density embryo per drop can have a negative effect on the embryo viability implantation, possibly by increasing toxic substances produced by the embryonic metabolism. On the other hand, the isolate embryo development also reduces the chances of become a blastocyst and hatch, suggesting a beneficial effect growth factors from other embryos. In conclusion, the embryonary development in groups 5 to 10 zygotes, with a 14-7ìl volume environment/zygote (G5 and G10, respectively), raises the blastocyst production and hatching rates. Smaller volumes to 5ìl (G15 and G20), decreases the chances of hatching, occur by a bigger liberation of toxic factors and a bigger nutrients competitions, providing a low embryo development. Financial support: Biotechnology center CESUMAR-BIOTEC.
EFFECT OF TRIIODOTHYRONINE ON IN VITRO MATURATION OF BOVINE OOCYTES: ASSESSMENT OF DEVELOPMENT, MORPHOLOGY AND GENE EXPRESSION OF BOVINE EMBRYOS

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The hormone triiodothyronine (T3) has widespread actions in all cells (Moeller et al, 2006, Nuc Recep Sign, 4:1-4). According to SPIKER et al. (2001, Jour Dai Sci, 84:1069-1076) T3 acts directly on granulosa and thecal cells of bovine oocytes and stimulates steroidogenesis, suggesting a possible influence on oocyte maturation. Thus the objective was to evaluate the action of T3 during in vitro maturation (IVM) by evaluating the rate of embryonic development and gene expression. Experiment 1: to determine the best concentration of T3 to be used in IVM, oocytes were selected and randomly distributed between the control group (0 nM T3) and the experimental groups with different concentrations of T3 (25, 50 and 100 nM) in IVM and subsequently fertilized and cultured in vitro, according to Cordeiro et al. (2006, Anim Reprod Sci, 3:376-379). The results were analyzed using the ANOVA test, adopting p<0.05. No difference was observed for rates of cleavage and the total number of cells. The rate of blastocyst in experimental groups were similar to control (38.3±7.3). As the kinetics of development, we observed an increase in the rate of hatching (day 8 of culture) in groups of 50 and 100 nM of T3 compared with controls (62.4±11.7, 53.1±16.3 vs 32.4±5.3, respectively, p<0.05). Furthermore, the highest number of Grade 1 embryos using the concentration of 50nM compared with the others, however, was similar to Control. Experiment 2, blastocysts hatched on the 8th day of culture produced in vivo and embryos produced in vitro from oocytes matured with and without 50 nM T3 (considered better concentration) were analyzed for the expression of OCT-4, GLUT 1 and COX 43 genes. RNA of pools of 10 embryos was extracted using the Trizol ® reagent, and synthesized using the Superscript III First-Strand Synthesis Kit (Invitrogen, Brazil) to obtain cDNA. The samples were analyzed using real time PCR using SYBR Green kit, and the endogenous gene chosen was the H2A. No difference was observed (p<0.05) between groups of embryos for any of the transcripts evaluated. Thus, our results show that the supplementation of IVM medium with 50 nM of T3 stimulated the kinetics of development, however this influence is not related to OCT 4, GLUT 1 and COX 43. Acknowledgements: CNPq, FAPESP and UNOPAR.

In vitro meiotic delay of ovine oocytes using Roscovitine

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The quest for better productive efficiency associated with the genetic progress of herd sheep, stimulated the development of new reproductive biotechnologies. In this context, the in vitro production of ovine embryos (IVP) has become the focus of numerous studies. However, despite of recent scientific advances, there are some barriers to the success of this biotechnology in sheep rearing. The major obstacle to the IVP is the asynchrony between nuclear and cytoplasmic oocyte maturation due to spontaneous meiotic resumption, which occurs with the removal of the oocyte from the follicular environment, independently of some molecular and structural changes necessary for proper oocyte maturation (Gilchrist, 2008; Acta Sci.Vet.,36:257-278). A strategy to solve this problem is to block, temporarily, the nuclear development with the use of pharmacological meiotic inhibitors. Thus, it was evaluated the efficiency of Roscovitine, (a specific inhibitor of maturation promoting factor) at concentration of 100 uM, to inhibit oocyte meiosis. For that, sheep oocytes obtained from slaughterhouses were transported to the Laboratory of Embryo Production of the Department of Animal Reproduction and Veterinary Radiology at Veterinary Hospital- UNESP - Botucatu – SP, in sterile saline solution at 37 °C. After aspiration, COCs were classified as grade 1 and 2 and were submitted to culture in maturation medium (TCM 199 supplemented with fetal calf serum, cysteamine, pyruvate, penicillin, LH and FSH) with ou without (control group) 100 uM of roscovitine, for 24 hours in an incubator at 38.5 °C with 5% of CO2 in air. To evaluate the nuclear maturation, the denuded oocytes stained with Hoechst 33342 were analyzed under fluorescence microscope. The experimental design was completely randomized with two treatments (control group and group treated with roscovitine), 6 replicates with 115 oocytes in each treatment, a total of 300 oocytes evaluated. The results demonstrate that rosocvitine at concentration of 100 uM delay the progression of nuclear maturation in 54% of oocytes, being 18% of oocytes remained in germinial vesicle breakdown and and 36% in metaphase I, while only 24% of oocytes reached the stage of metaphase II.In the control group, 80% of oocytes reached metaphase II, while only 4% and 8% were in metaphase I and germinal vesicle breakdown, respectively. However, the percentage of degenerated oocytes matured in the presence of rosocvitine, was higher than the control group (22% and 8%, respectively). Therefore, it seems that rosocvitine is efficient in delay the progression of nuclear maturation in ovine with great potential to be used in the in vitro production of embryos.
ABSTRACT 120

EVALUATION OF THE SYNERGY EFFECT OF ADDITION OF CAFFEINE AND REDUCTION VOLUME OF MEDIUM FOR IN VITRO FERTILIZATION (IVF) ON CLEAVAGEM RATES AND BLASTOCYSTS PRODUCTION CATTLE

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Despite of the efforts in research teams and the progress made in recent years with in vitro production (IVP), the rate of blastocyst production and embryo quality obtained from a system of in vitro fertilization (IVF) are still far from the desired efficiency and fertility rates observed in vivo. It is estimated that only 40% of oocytes obtained from ovaries from slaughterhouses achieve development to the blastocyst stage. These low rates may be influenced by several factors during the IVP. The Caffeine is a phosphodiesterase inhibitor, a substance that increases the concentration of cyclic AMP during sperm capacitation, modulating adenylate cyclase and the acrosome reaction, leading to sperm capacitation and acrosome reaction, enhancing sperm penetration (BIRTH, 2003). This study evaluated the effect of caffeine supplementation and medium volume reduction through fertilization of 355 bovine oocytes from ovaries of cattle from slaughterhouses of Campos dos Goytacazes, RJ, Brazil. The oocytes were matured and fertilized in vitro in four experimental groups (CONTROL, G1, G2 and G3). The control group was fertilized in 100ìL of medium fertilization talp-fert (IVF medium), the group 1 (G1) used 100 µl of IVF medium with caffeine, in group 2 (G2) the reduction of medium volume (from 100 µL to 40 µL) was evaluated, and the oocytes were fertilized in 40ìl of medium from IVF without caffeine, and in group 3 (G3) we evaluated the reduction of fertilization medium and the addition of caffeine 2M. The in vitro cultured was carried out in groups of 20 zygotes in 100 µL of synthetic oviduct fluid (SOF) at a temperature of 38,5 ºC and atmosphere of 5% CO₂ in air for 7 days. Data were analysed using ANOVA and comparison of means by Tukey test at a 5% level. Cleavage rates were 91,00±15,16, 89,20±8,26, 87,60±10,09 e 91,00±13,71 respectively for control, G1, G2 and G3 groups. Blastocyst rates for experimantal groups were: control (19,60±9,91), G1 (21,20± 8,76), G2 (11,40±8,32) e G3 (13,40±11,08) and no difference among treatments were shown. As a conclusion, the addition of caffeine in fertilization medium and the reduction of the drop volume does not affect cleavage and blastocyst production rates in bovine embryos.

ABSTRACT 121

EMBRYO RECIPIENT MARES HAVE BIGGEST GESTATIONAL LOSS WHEN THEIR FOLLICULAR FASE IS SHORT (1 TO 3 DAYS) OR LONG (7 OR MORE DAYS), BUT THERE IS NO INFLUENCE OF THE SIZE OF THEIR PREOVULATORY FOLLICLE

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Equine embryo transfer (ET) fertility rates today reaches more than 75% at 15 days, because of the great developments in technology since its first description in 1970, with various factors described as influencing the maintenance of pregnancy rates in recipients (McKinnon & Squires, 2007; Embryo Transfer and Related Technologies. In: Samper et al. Current Therapy in Equine Reproduction, pp.319-34), but there is no mention in the literature on characteristics of estrus of the recipient as one of these factors. We evaluated the pregnancy rates at 15 days and the rates of embryonic loss between 15 and 60 days in TE of Mangalarga Marchador mares. The follicular diameter of recipients was evaluated daily by ultrasonography from 30mm to ovulation. Pregnancy diagnoses were performed at 15, 30, 45 and 60 days. To evaluate the effects of the length of the follicular phase of recipients, 230 ETs were divided into groups, 1-3 (1-3d, n=92), 4-6 (4-6, n=98), and seven or more days (>7d, n=40). To evaluate the effects of the preovulatory follicle size of the recipient, 240 ETs were divided into groups: greater than or equal to 30 and less than 40mm (f30-40, n=66) greater than or equal to 40 and less than 50mm (f40-50, n=116) greater than or equal to 50mm (f50, n=58). The groups were compared by Chi-square and Fisher’s Exact test (P<0.05). With regard to the duration of the follicular phase, the pregnancy rates at 15 days were similar (1-3d - 79.4%; 4-6d - 85.7%; >7d - 82.5%) (P>0.05) and the rate of pregnancy loss from 15 to 60 days was lower (P<0.05) in 4-6d (7.1%) than in 1-3d (24.7%) and >7d (30.3%), the two latter similar (P>0.05). With regard to follicular size, pregnancy rates at 15 days were similar (f30-40 - 86.4%; f40-50 - 79.3%; f50 - 82.8%) (P>0.05) as well as the rates of pregnancy loss between 15 and 60 days (f30-40 - 12.3%; f40-50 - 15.2%; f50 - 6.3%) (P>0.05). The reasons for the higher rate of embryonic loss between 15 and 60 in a 3d and >7d groups were not identified, and this fact should be investigated in the future, but it is concluded that the duration of the follicle period may be a factor in choice of recipient mares.
ABSTRACT 122

REduced OOCYTE MITOCHONDRIAL DNA MAY COMPROMISE IN VITRO EMBRYO PRODUCTION OF REPEATE-BREEDER HOLSTEIN COWS DURING SUMMER HEAT STRESS

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This study was conducted on 2 dairy farms during summer (S) and winter (W) 2009. Heifers (H), peak lactation (PL) and repeat-breeder (RB) cows started a protocol to synchronize follicular wave emergence: 2mg estradiol benzoate (Sincrodiol®), OuroFino, Brazil + 50mg P4 (OuroFino, Brazil) + 150ig D-cloprostenol (Sincrodiol®), OuroFino, Brazil IM + norgestomet ear implant (Crestar®, Intervet, Brazil) on D0; implant removal and OPU on D5. Respiration rate (RR), rectal (RT) and cutaneous temperature (CT) were recorded on D0. Part of the retrieved oocytes went through IVF (sperm from 1 Holstein bull), the remainder kept at -80°C without cumulus cells. Analyses were done with PROC GLIMMIX of SAS. Heifers were 16.8±0.3 months old; PL and RB cows had 110±3.8 vs 425±17.5 DIM, 34.3±0.7 vs 23.2±0.9 kg milk/day, and 0.7±0.1 vs 7.1±0.3 services; mean±SE). H, PL and RB had similar RR during W, but cows had higher RR in the S [W vs S: H=40.59±0.14 vs 44.78±0.13, PL=43.74±1.49 vs 278.6±2.25, RB=43.10±1.23 vs 73.83±1.41; P<0.0001]. Heifers kept similar RT in W and S (38.65±0.07 vs 38.61±0.06, P>0.05), while PL and RB had enhanced RT in H (PL=39.21±0.07 vs 39.76±0.12, RB=38.81±0.07 vs 39.51±0.11; P<0.01). The CT was lower in H (31.37±0.14) than cows [PL=32.73±0.22, RB=32.40±0.22; P<0.01] and in W (31.09±0.09) than S (33.10±0.17; P<0.01). At IVF, cleavage rate was similar among categories [H=51.7% (194/375), PL=37.9% (148/390), RB=41.9% (279/666); P<0.18] and periods [W=42.3% (414/979), S=45.8% (207/452); P=0.45]. However, blastocyst rate (D7) was affected by category and period [W: H=30.3% (74/244) vs PL=22.0% (42/191) vs RB=22.5% (93/413); S: H=23.3% (35/150) vs PL=14.6% (279/191) vs RB=7.9% (14/177); P<0.01]. Hatching blastocyst rate and blastocyst cell number (D8) were greater in W (23%, 60 ± 261 and 231±7) than S (6.5%; 7107; P=0.03 and 253±12; P=0.01). Also, blastocyst cell number was greater in H (253±12) than PL (203±10) and RB cows (207±8; P<0.01). RB blastocysts had higher nuclear fragmentation rate during S (3.85%) than H (1.82%) and PL (2.48%), and this rate was also greater than that in RB during W (2.15%). Finally, mitochondrial DNA (mtDNA) was reduced by >4 folds (P<0.01) in oocytes from RB than H and PL during S, however this difference was not observed during W. In conclusion, heat stress negatively affects IVF in Holstein cattle, regardless the category. However this effect is more pronounced in RB cows than H and PL during the S when blastocyst rate and fragmentation (nuclear and mitochondrial) are considered. These findings suggest that the worse reproductive performance that has been described for RB cows are related to some injury in their oocyte quality. This hypothesis is supported by the reduced amounts of mtDNA found in RB oocytes.

ABSTRACT 123

THE EFFECT OF DIFFERENT HORMONAL TREATMENTS FOR OVARIAN ESTIMULATION ON OOCYTE PRODUCTION AND IN VITRO MATURATION IN CANINDÉ GOATS

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The Canindé breed was naturalized in Northeastern Brazil and is characterized by its high rusticity. Due to the risk of being extinct, the use of reproductive biotechnologies may greatly increase the number of individuals of this breed. The aim of this study was to evaluate the effect of different hormonal treatments for ovarian stimulation on the quantity and quality of cumulus-oocyte complexes (COCs) recovered by laparoscopy and their subsequent in vitro maturation rate (IVM). Eighteen Canindé goats received intravaginal sponge impregnated with 60 mg of medroxyprogesterone acetate (Progespon, Syntex, Buenos Aires, Argentina) for 11 days and 50 lg d-cloprostenol (Ciosin, Schering Plough, São Paulo, Brazil) i.m. at eighth day of prostegagen treatment. Goats were allocated into three groups and received: i) five doses (FD) of pFSH (120 mg, Folltropin-V, Bioniche, Ontario, Canada) i.m. in 12 h intervals from the eight day; ii) three doses (TD) of pFSH (120 mg) also from day eight, in 38.61±0.06 h intervals and iii) single dose (SD) of pFSH (70 mg) associated to eCG (200 IU; Novormon, Syntex, Buenos Aires, Argentina) i.m., administered 36 h before sponge removal. It was performed three hormonal treatment/oocyte recovery sessions and for each of them, six goats were allocated to a different hormonal treatment. Oocyte recovery was performed by laparoscopy at the same moment of sponge removal and submitted to IVF in 5% CO2 and 38.5°C for 24 h. Data were submitted to ANOVA and compared by Tukey or Qui-square test, according to the case. All tests were performed using STATSOFT 7.0 software and the results were described as mean ± SEM. The mean for punctured follicles and COCs recovered was 15.1 ± 0.7 and 11.3 ± 0.8, respectively, resulting in a total recovery rate of 74.5%. There were no differences (P>0.05) among treatments for the number of punctured follicles and COCs recovered.

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The Canindé breed was naturalized in Northeastern Brazil and is characterized by its high rusticity. Due to the risk of being extinct, the use of reproductive biotechnologies may greatly increase the number of individuals of this breed. The aim of this study was to evaluate the effect of different hormonal treatments for ovarian stimulation on the quantity and quality of cumulus-oocyte complexes (COCs) recovered by laparoscopy and their subsequent in vitro maturation rate (IVM). Eighteen Canindé goats received intravaginal sponge impregnated with 60 mg of medroxyprogesterone acetate (Progespon, Syntex, Buenos Aires, Argentina) for 11 days and 50 lg d-cloprostenol (Ciosin, Schering Plough, São Paulo, Brazil) i.m. at eighth day of prostegagen treatment. Goats were allocated into three groups and received: i) five doses (FD) of pFSH (120 mg, Folltropin-V, Bioniche, Ontario, Canada) i.m. in 12 h intervals from the eight day; ii) three doses (TD) of pFSH (120 mg) also from day eight, in 24 h intervals and iii) single dose (SD) of pFSH (70 mg) associated to eCG (200 IU; Novormon, Syntex, Buenos Aires, Argentina) i.m., administered 36 h before sponge removal. It was performed three hormonal treatment/oocyte recovery sessions and for each of them, six goats were allocated to a different hormonal treatment. Oocyte recovery was performed by laparoscopy at the same moment of sponge removal and submitted to IVF in 5% CO2 and 38.5°C for 24 h. Data were submitted to ANOVA and compared by Tukey or Qui-square test, according to the case. All tests were performed using STATSOFT 7.0 software and the results were described as mean ± SEM. The mean for punctured follicles and COCs recovered was 15.1 ± 0.7 and 11.3 ± 0.8, respectively, resulting in a total recovery rate of 74.5%. There were no differences (P>0.05) among treatments for the number of punctured follicles and COCs recovered. The average number of obtained COCs was similar (P>0.05) for FD (12.4 ± 1.0) and SD (10.8 ± 1.0). However, lower (P<0.05) recovery and maturation rates were obtained in TD (67.9%; 32.1%) when compared to FD (84.1%; 49.1%) or SD (72.4%; 46.2%). Therefore, TD treatment was less efficient on oocyte production and further IVM when compared to the other ones. Although the SD treatment produced a similar response to the FD, the former has the advantage of being more practical thus recommended for ovarian stimulation, and it may even be used in conservation programs for the Canindé breed. Financial support: CNPq and FUNCAP.
ABSTRACT 124

DETECTION OF Leptospira interrogans serovar Grippotyphosa IN BOVINE OOCYTES AFTER MATURATION PERIOD AND SUBSEQUENT TREATMENT OF HEALTH CONTROL ESTABLISHED BY THE INTERNATIONAL EMBRYOS TECHNOLOGY SOCIETY (IETS)

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Treatment of embryos with trypsin or antibiotics in alternating washes with culture medium, established by IETS, is intended to remove or inactivate infectious agents that may interfere with the final product. There are several questions about the interaction of the cumulus cells/oocyte and/or embryo with the infectious agent, such structures may return a vector for disease transmission. Therefore, responses to questions about the possible penetration and adherence of microorganisms in the cumulus-oocyte complex (COC) and the zona pellucida, in its various stages, are of great concern. The aim of the present study was to detect the pathogen in oocytes exposed experimentally during the period of in vitro maturation to Leptospira interrogans serovar Grippotyphosa after treatment with trypsin/antibiotic standardized by IETS. The oocytes were obtained from slaughterhouse ovaries, selected and separated in two groups: control (n=480) and exposed to the pathogen (n=540) at 30ìL concentration of 4.7 x 10^5 bacteria/mL and brought to 5% of CO₂ emissions, relative humidity of 90% at 37°C for 24 hours. After this period, the oocytes were treated in accordance with rules established by IETS, involved in 2% agarose and maintained in 2.5% glutaraldehyde at 4°C to be prepared and cut with ultramicrotome, collected on nickel screens for ultrastructural analysis of oocytes. The paintings were examined in a transmission electron microscope Philips EM208 (Holand). Similar structures were observed at the leptospira (25.5%), the zona pellucida, penetrating the ooplasm of the oocyte previously treated, without morphological changes in cumulus cells and zona pellucida, which may suggest that the efficacy of the IETS, becomes compromised, for this pathogen. Leptospirosis is a disease that affects beef cattle, the practice of a in vitro embryo production (IVP) biotech growing fast, the analysis of results obtained in the present study demonstrates that norms of quality control in IVP bovine embryos should be reassessed and updated.

ABSTRACT 125

IN VITRO MATURATION OF OOCYTES FROM IMMATURE RABBITS

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Most of embryology studies in rabbits applies oocytes in vivo maturation. However, the synchronization of estrus and multiple ovulation limit the use of animals consumption. Rabbits have been one of the most used models in scientific research, due to easy handling, elasticity, a more transparent cytoplasm and the capable to differentiate somatic cell nuclei of various species including giant pandas, chickens, monkeys, cats and humans, supporting the development of interspecies nuclear transfer until the blastocyst stage and even in early pregnancy (WEN et al., 2005, Journal of Experimental Zoology, 303:689-697). Undoubtedly maturation is one of the most important stages of the IVP, which will influence the quantity and quality of embryos to be produced (FREITAS et al., 2003, Acta Scientiae Veterinariae, 31:380-381). This study aimed to establish a protocol for in vitro maturation of oocytes from immature rabbits, allowing the development of embryology studies, as it occurs in cattle. Oocytes were collected from ovaries of immature rabbits at 3-4 months of age intended for human consumption, of mixed breeds. Oocytes were acquired through the technique of slicing and maturedin vitro in TCM 199, with different concentrations of hormones: 0.5 ìg/mL of FSH, 50 ìg/mL of LH and 1 ìg/mL of estradiol (B) 1 ìg/mL for FSH, LH and Estradiol (Y), 10 ìg/mL of FSH, 10 ìg/mL of LH and 1ìg/mL of Estradiol (T) and control (CO), with 10% FCS and 0.2% pyruvate and no hormones supplementation. We used four different times of maturation, 15, 18, 22 and 30 hours in an incubation at 38°C and 5% CO₂. After maturation the oocytes were fixed in paraformaldehyde 3% and stained by Hoechst 33342 to observe the stages of Germinal Vesicle, Metaphase I, Metaphase II and Spontaneous activation at electron microscopy. The averages were analyzed by ANOVA and compared by the Tukey-Kramer HSD, for P <0.05. There was no significant difference between the media supplemented with hormones Y = 23% (121/523), T = 23% (133/575) and B = 22% (129/577), but was effect of hormonal supplementation observed by the significant difference between control and other treatments, CO = (20%; 107/531).No effect of maturation lenght used were observed. The best rate of Metaphase II was observed as soon as 15 hours (25.7%; 185/720). Although the rates of maturation were considered low compared with other species, possibly due to the immature status of females, one can observe the positive effect of hormonal supplementation.
ABSTRACT 126

Replacement of Girolando herd with F1 embryo produced in vitro with oocytes from GYr and Holstein cows using sexed semen

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Many researches have been developed to more efficient results in in vitro production (IVP) of bovine embryos. However, there are still many questions and few data on rates of embryonic death, higher birth weight and incidence of dystocia, mainly due to F1 embryos Girolando because of more concentration of studies in European and Zebu purebred. The objective of this study was to evaluate the feasibility of sexed semen in IVP of F1 (½ Holstein and ½ Gyr) bovine embryos in order to the herd replacement, the pregnancy rates obtained with the F1 embryos, to verify the ease of delivery of the recipients, the sex of the calf and survival time of 60 days. The experiment was conducted in commercial herds in the south region of the state of Rio de Janeiro, during the period of 2007 and 2008. Eight-one donor Gyr and 108 Holstein, all with regular estrous cycles, were selected and punctured. The embryos were produced in a commercial laboratory and transferred on day 7 to the cows of the own herds, used as recipients. The diagnoses of pregnancy and fetal sexing were performed 60 days after the transfers by palpation per rectum and transrectal ultrasonography. Statistical analysis were done using chi-square test. The pregnancy rate with F1 embryos produced with sexed semen was 41.9% and the mortality rate within 60 days of 9.7%, allowing the rate of annual replacement on 34%. Difficulties in receiving delivery were not observed, indicating no occurrence of the large offspring syndrome in F1 product. The weight of the calves was between 35 to 40 kg, with no congenital abnormalities in calves born, and we observed 90.2% of females. We conclude that it is feasible to standardize the Girolando herd using sexed semen in IVP of F1 embryos.

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PREGNANCY AND EMBRYONIC LOSS RATES FROM RECIPIENT MARES TREATED WITH A NEW LONG-ACTING PROGESTERONE FORMULATION AND ALTERNATIVE PROTOCOLS

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At the beginning and end of the breeding season, the percentage of recipients showing normal estrous cycles is low, making synchronization less likely. Long-acting progestosterone compounds containing 150mg/mL of progesterone have been weekly administered to acyclic mares, so they can be used as recipients. The objective of this study was to evaluate pregnancy and embryonic loss rates from acyclic recipients treated with a new long-acting progestosterone formulation, produced in our laboratory, containing 300mg/mL of progesterone (P4LA300) and to determine whether alternative treatment protocols can be used in cyclic recipients. Recipients were classified according to their reproductive status. G1: Acyclic mares (n=193) showing endometrial edema 2 days after treatment with 5mg (I.M.) of estradiol benzoate (Estrogin®, Farmavet, São Paulo, Brazil) were injected with 10mL (I.M) of P4LA300. Embryos were transferred 4 to 10 days after progesterone, when 5mL (I.M.) of P4LA300 were administered. Pregnant recipients received 10mL (I.M.) of P4LA300 every 2 weeks until fetuses reached 110 days. G2: Cyclic mares (n=28) showing a follicle e>35 mm and endometrial edema were treated with 2.500UI (I.V.) of hCG (Vetecor®, Hertape Calier, Minas Gerais, Brazil) and 10mL (I.M.) of P4LA300. Embryos were transferred 4 days after treatment and progesterone injections, as for G1, were discontinued if pregnant recipients were ovulated at the day of transfer. G3: Diestrous mares (n=48) at D5 to D14, showing endometrial edema 2 days after I.M.treatment with 5mg of estradiol benzoate (Estrogin®, Farmavet, São Paulo, Brazil) were injected with 10mL (I.M) of P4LA300. Embryos were transferred 4 to 10 days after progesterone injection, when 5mL (I.M.) of P4LA300 were administered. Pregnant recipients were treated as G1. G4: Cyclic mares (n=267) receiving embryos between D4 and D8. Pregnancy at 15 days and embryonic loss rates at 60 days were evaluated through Fisher's exact tests, being similar between groups, of, respectively, 71.50% (138/193) and 8.70% (12/138) in G1; 67.86% (19/28)and 5.26% (1/19) in G2; 56.82% (25/44) and 8.0% (2/25) in G3 and 67.42% (180/267) and 10% (18/190)in G4. 71.43% (20/28) of G2 recipients were ovulated at the day of transfer and 75% (15/20) became pregnant. From the remaining that did not ovulate (28.57%; 8/28), 50% (4/8) were pregnant and one suffered embryonic loss.P4LA300 treatment, every 2 weeks, was effective in maintaining pregnancy of acyclic and cyclic recipients, reducing overall injections and allowing transfers until the 10th day post-injection. This is the first study that demonstrates the use of progesterone-treated cyclic recipients, in estrous or following an artificially-interrupted diestrous.
IN VITRO PRODUCTION OF EMBRYOS FROM Bos indicus AND Bos taurus COWS SUPPLEMENTED WITH TWO ENERGY DIETARY LEVELS

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The nutrition affects reproduction features of donor oocytes from Bos indicus and Bos taurus breeds. Then, this study aimed to compare data from in vitro embryo production of cumulus oocyte complexes (COC’s) recovered by ovum pick up (OPU) from Gir and Holstein cows supplemented with two levels of diet energy. Eight cows of each breed were randomly distributed in a 2x2 factorial design: 1) low energy Gir (LG) and 2) low energy Holstein (LH) – 04 cows of each group received 100% of maintenance diet; 3) high energy Gir (HG) and 4) high energy Holstein (HH) – 04 cows of each group received 170% of maintenance diet. At first, all donors were subjected to an adaptation period for 3 weeks (maintenance diet). Afterwards, they were directed to their respective treatment for 46 days before OPUs. The four OPU sessions were conducted using Aquila Pro ultrasound equipment (Esaote Pie Medical, USA) and occurred each 14 days. Before each OPU session follicular waves were synchronized with norgestomet (Crestar, Intervet, Netherlands) for 5 days and estradiol benzoate (Sincrodiol, Ouro Fino, Brazil). The COC’s were recovered from 2-8mm follicles and after they were matured for 22-24h in medium TCM 199 (Invitrogen, USA) added with antibiotics, FSH (Sigma, St. Louis, USA), LH (Sigma) and fetal calf serum (FCS; Invitrogen). Fertilization occurred after semen process by Percoll gradient, following gametes co-incubation for 18-20h. After this, the presumptive zygotes were cultured for 8 days in medium SOFaaci supplemented with 5% FCS. The statistical analysis of data were performed using X2 test (P<0,05). There were no significant differences on cleavage rates (D3) comparing LG and HG (mean=66,84%) neither between HG and HH (mean=35%). However, mean blastocyst rate (D8) for the 3 first sessions on Gir breed there was a tendency to higher production from HG than LG, while in the last session occurred the contrary: 36,58%a x 22,37%a; 53,13%a x 30,39%b; 29,58%a x 20%a e 19,7%b x 37,04%a; HG x LG, respectively. The Holstein breed, in relation to Gir, behaved in an opposite manner: 3,92%a x 10,81%a; 5,75%a x 10,09%a; 5,32%b x 14,14%a e 13,13%a x 6,73%a; HH x LH, respectively. These results suggest that Bos indicus and Bos taurus respond totally different to energetic diet, in terms of competence of in vitro embryo production. We also observed that until days 75 and 89 of the treatment (between 3rd and 4th OPU session) high levels of energy intake could be beneficial for Holstein donors, in opposite to Gir cows. Acknowledgments: Epamig and Fazenda Calciolândia. Financial Support: CNPq/FAPESP/Fapemig/Embrapa

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EPIDEMIOLOGICAL INQUIRY WITH NESTED-PCR FOR DETECTION OF BABESIA EQUI IN A PROGRAM OF EMBRYO TRANSFER

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Horses' health is vitally important to this Embryo Transfer Program, any infectious diseases, particularly babesiosis, can compromise the health of the whole squad and result in early embryonic loss and low pregnancy rates (LOUSINNO et al., 2006, Acta Science Veterinariae, 34:39-49). Babesiosis has been cited as a major equine parasite by direct prejudice, such as performance losses and embryonic mortality, as well as indirect prejudice like export prevention (FRIEDHOFF et al., 1990, Inter. J. Parasit., 20: 525-535,). The Nested-PCR Technique has demonstrated a sensitivity of more than 100 times the technique of direct microscopy, detecting levels of parasitemia of 10-8to 10-9, with high specificity, lower cost and faster time to babesiosis diagnosis (FIGUEROA et al., 1993, Vet. Parasit, 50: 69-81). Blood samples were collected in two stables in the city of Seropédica and an equine reproduction center in the city of Itaguai- RJ with the objective of diagnosing, by Nested-PCR positive animals for B. equi. In order to analyse the material, 4 ml of blood were collected in Vacumtainer tubes with EDTA by puncturing of the jugular veins in 28 donors and 54 recipients of embryos Mangalarga Marchador breed is asymptomatic for babesiosis. After the tests in laboratory, 100% of the mares were positive for B. equi, demonstrating the epidemiological importance of this hematozoa, which can influence in recovery rates (62,7%) and embryo fixation (63,6%) in Mangalarga Marchador breed mares (SANTOS et al., 2008, Acta Science Veterinariae, 36:633). Acknowledges to Animal Parasitology Department- UFRRJ.
EFFECTS OF NITRIC OXIDE AND CYCLIC GMP PATHWAY ON BOVINE OOCYTE MEIOTIC RESUMPTION: PRELIMINARY RESULTS

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Nitric oxide (NO) acts through the guanylate cyclase (GC) signaling pathway, stimulating the production of cyclic guanosine monophosphate (cGMP), that in turn is capable of influencing the levels of another nucleotide, the cyclic adenosine monophosphate (cAMP). The second messenger cAMP is an important constituent of the gonadotrophins signaling pathway in the control of the meiotic cell cycle of oocytes. The objective of the present study was to investigate the involvement of cGMP in the meiosis. The involvement of NO in the in vitro maturation (IVM) of bovine oocytes and its effect on the cAMP pathway. Cumulus oocyte complexes (COCs) were aspirated from 2 to 8 mm follicles and submitted to IVM in semi-defined medium (TCM-199 with 0.04% BSA) for 9h (38.5°C and 5% CO2 in air) and were assessed for germinal vesicle breakdown (GVBD) by acetic-orcein (1%) staining. GVBD rates were analyzed by ANOVA and a level of 5% significance was considered. Increasing concentrations of a NO donor (0, 10−9, 10−8 and 10−7M SNAP) were added to the maturation medium to determine the effect of the increase of NO on the capacity of the oocytes to resume meiosis. Control and 10−9M e 10−8M SNAP presented an average of 70% GVBD (p<0.05) and the highest NO concentration (10−7M) presented only 36% GVBD (p<0.05), suggesting that low NO levels do not affect oocyte maturation, but higher levels cause a retardation on the maturation process. Coincidental effects occurred when a GC stimulator (5,10 and 50mM ProtoporphyrinIX) and a cGMP analog (1, 2 and 4mM 8-BrGMPc) cause an average 20% decrease (p<0.05) in oocyte meiotic resumption compared to control oocytes (about 70% GVBD). The cGMP and cAMP levels were measured with immature COCs (0h), and with 1 and 3h of IVM, treated with 10−7M of SNAP and/or increased with 200mM of O2D (inhibiting of the GC). None treatment interfered (p>0.05) in the concentrations of cGMP, 6.2 pmol/COC in the immature group and about 3.5 pmol/COC after 1h and 3h of IVM. The cAMP levels were also not altered, average of 44fmol/COC with 0h, after 1h and 28.5fmol/COC after 3h of IVM. In conclusion, the negative effect that the excess of NO causes in the meiotic resumption (retardation of the disruption of the GV), suggests the effectiveness of the pathway of cGMP, compared to the similar effect when using the stimulator and the analog of cGMP. However, the cGMP and cAMP levels, were not influenced by the NO donor, indicating another signalling pathway of the NO or that the increase of the levels of the evaluated nucleotides did not occur in the first 3h of IVM. New measurements of cGMP and cAMP in the IVM must be carried out to confirm or to discard the involvement of cGMP in the action of the NO in the meiosis.

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Induction of apoptosis in bovine oocytes subjected to heat shock during germinal vesicle and in vitro maturation periods

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The acquisition of oocyte competence and preimplantation embryonic development can be interrupted by alterations in the reproductive tract microenvironment. This phenomenon has been well characterized in animals exposed to heat stress. The susceptibility of bovine oocytes to elevated temperature can be observed during the germinal vesicle (GV) stage as well as oocyte maturation (Hansen, P.J. 2004. Anim Reprod Sci 82-83: 349-360). The development of in vitro models using GV oocytes is challenging since removal of oocytes from antral follicles induces spontaneous progression to metaphase II stage (Pincus e Enzmann, 1935. J Exp Med, 62: 665-675). Therefore it is necessary the use of meiotic inhibitors such as roscovitine (ROSC). The objective of the current study was to evaluate the induction of apoptosis in bovine oocytes subjected to heat shock during GV and in vitro maturation (IVM) periods. Slaughterhouse cumulus-oocyte complexes (COCs) were subjected to the following treatments: IVM-Control (38.5°C for 24 h, n=32), IVM-Heat Shock (41°C for 14 h followed by 38.5°C for 10 h, n=47), GV-Control (38.5°C for 14 h in the presence of 50µM ROSC followed by 38.5°C for 24 h without inhibitor, n=18) and GV-Heat Shock (41°C for 14 h in the presence of 50µM ROSC followed by 38.5°C for 24 h without inhibitor, n=25). There were two additional control groups to determine the percentage of GV oocytes at the time of collection (0 hour, n=63) and after 14 hours in the presence of ROSC (n=40). The COCs were mechanically denuded by repeated pipeting and subjected to TUNEL assay associated Hoechst 33342 (5µg/ml) to determine DNA fragmentation characteristic of apoptosis. Preliminary data were analyzed by least-squares analysis of variance using the General Linear Models procedure of SAS. Preliminary results indicated a statistical tendency (Temperature; P= 0.07) suggesting that exposure of bovine GV oocytes to elevated temperature increased the percentage of TUNEL-labeled oocytes from 11.1 ± 0.23% to 23.5 ± 0.23% in GV-Control and GV-Heat Shock groups, respectively. Similarly, exposure of oocytes to elevated temperature during IVM increased the percentage of TUNEL-positive oocytes from 0.0 ± 0.23% to 46 ± 0.23% in the IVM-Control and IVM-Heat Shock groups, respectively. Based on this data it is possible to suggest that exposure of GV and maturing bovine oocytes to heat shock activates the apoptotic cascade resulting in DNA fragmentation.
THE INFLUENCE OF TIMING OF EMBRYO TRANSFER ON PREGNANCY RATE OF BOVINE EMBRYOS PRODUCED IN VITRO

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The in vitro production of bovine embryos is a tool that facilitates the rapid multiplication of genetically superior animals. However, several factors influence their results. The timing of embryo transfer, can often vary, mainly due to the distance between the laboratory and where the recipients are housed. There seems to have a relationship between the time for embryo transfer and pregnancy rates. The objective was to evaluate the influence of time of embryo transfer on pregnancy rates of in vitro-produced bovine embryos transferred to recipients. Cumulus-oocyte complexes of Nelore donors raised in Bahia state were obtained by ultrasound-guided follicular aspiration. They began the process of maturation in cryotubes containing medium itself at 38ºC, saturated humidity and 5% CO2 in air and were subsequently sent to a commercial laboratory in São Paulo. The time spent in transport was approximately 12 hours. In the laboratory ended up the stage of maturation and followed the steps of fertilization and culture in vitro. Seven days after fertilization, the embryos produced were sent to the state of Bahia in cryotubes with specific culture medium at 37ºC, saturated humidity and an atmosphere of 5% CO2 and were transferred to recipients. The embryos produced (n = 893) were randomized into three groups according to the time of embryo transfer: TE-M (n = 440), embryos transferred during the morning (06:00 to 11: 59), TE-A (n = 330), embryos transferred during the afternoon (12:00 to 17:59) and TE-N (n = 120), embryos transferred during the night (18:00 to 24:00), and all remained in cryotubes until near the time of embryo transfer; when were transferred to specific medium, measured and packaged in straws of 0.25 mL. The pregnancy diagnosis was performed by ultrasound examination (Aloka SSD 500, Aloka, Japan) 30 days after fertilization and the results were processed and analyzed by chi-square (SAS, 1996). Pregnancy rates were similar among embryos transferred in the morning and afternoon (TE-M: 35.9% vs. TE-A: 40.8%; P = 0.162) and the same happened between afternoon and night (TE-A: 40.8% vs. TE-N:50%; P = 0.083), however, there were differences between pregnancy rates in the morning and night (TE-M: 35.9% vs. TE-N: 50.0%; P = 0.005). The results showed that there is an influence of the time of embryo transfer in the pregnancy rate, and the embryos transferred at night (TE-N) had better rates. One explanation for these findings would be the greatest time available for embryo growth during the period of transfer.

POST-HATCHING DEVELOPMENT OF IN VIVO AND IN VITRO PRODUCED BOVINE EMBRYO: MORPHOLOGICAL AND MOLECULAR CHARACTERISTICS

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Post-hatching embryos culture system (PHD) allows monitoring the development of in vitro embryos, produced by different assisted-reproduction techniques, for a long period. Aiming to characterize the embryos that are able to develop in this system, the present study compared the size and quality of day (D) 14 in vivo and in vitro produced embryos, cultured in vivo or in the PHD system. Three groups were used: G1 (totally in vitro until D14), G2 (in vivo until D7/in vitro until D14) and G3 (totally in vivo until D14). On G1, embryos were produced using oocytes obtained from slaughterhouse ovaries. The in vivo D7 and D14 embryos were obtained by uterine flushing of superovulated cows, being part of them recovered on D7 and then submitted to PHD system (G2), and the others recovered on D14 (G3). The G1 and G2 embryos were cultured in the PHD system described by Brandão et al., 2004, where the embryos were placed in agarose gel tunnels. Biopsies were removed of D14 embryos for gender determination and the rest was stored for gene expression evaluation (qPCR). After gender verification, five pools of embryos (n=3) were formed for G1 and G3, which were used to evaluate the expression of PLCB8, KRT8, CD9, GLUT-1, GLUT-3, G6PD, PGK-1, HSF1, MnSOD, HSP70 and INFT genes. The G2 was not used for this assessment because only three embryos developed in the system. For embryos sizes and gene expression analyzes t test or Mann-Whitney test were used, and for sex ratio the c². The level of significance was P<0.05. To compare G1 and G2, a total of 119 in vitro and 101 in vivo D7 embryos was placed in the PHD system. Of those, a greater number of in vitro embryos (n=11, 37%) developed until D14 compared to the in vivo embryos (n=5, 17%). Although a few number on in vivo embryos have developed in the PHD system, no difference was observed in size of D14 embryos from both groups G1: 1.83±0.33 mm vs G2: 1.88±0.46 mm. In other experiment, to compare G1 and G3 embryos, 21 in vitro and 27 in vivo D14 embryos were produced. The mean size of in vivo embryo (10.29±1.83 mm) was greater than the in vitro (2.68±0.33 mm). The percentage of males (n=17/21, 81%) in G1 was greater than in G2 (n=12/27, 44%). Only HSP70 gene presented more mRNA relative abundance in embryos of G3. This study showed that even though in vitro embryos cultured in PHD system were smaller than the in vivo embryos, of the 11 genes analyzed, only one was differentially expressed between the groups. Therefore, it can be suggested that PHD system can be used for a more reliable evaluation of in vitro embryo’s quality.

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OVULATORY RESPONSE EVALUATION IN MANGALARGA MARCHADOR MARES AT TWO DIFFERENT HCG DOSES

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Ovulation-inducing are widely used on equine embryo transfer programs to increase reproductive efficiency. Among the more used ovulation induction drug, the human Chorionic Gonadotrophin (hCG) has some notorious roles. The principal purpose of using this drug is to increase the synchrony between de coupling or artificial insemination and ovulation. Several researches have been conducted attaining ovulation within 48 hours using alternative hCG doses from 1500 to 5000 UI. Lesser doses would reduce expenses for veterinarians if the efficiency of low hCG doses were corroborative. The aim of this study is to compare the efficacy of two endovenous hCG doses (1000UI and 1750UI) so as to induce ovulation within 48 hours after its administration. About 92 mangalarga marchador mares (recipient and donors) were used during 2008/2009 and 2009/2010 breeding seasons, adding up to 210 estrus cycles. Mares were between four and 23 years old and body weight between 335 to 460 Kg (1Kg = 2.2 lbs), healthy and reproductively active. Ovarian activities were exanimed by transrectal ultrassonography, every 24 hours, starting on estrus and thus every other day during this time to determine the induction ovulation moments and the ovulation days (D0). Ovulation induction were done using hCG (Chorulon®, Intervet Schering-Plough Animal Health, São Paulo, Brasil) when the major follicle reached a diameter ≥35mm and uterus edemus as concerning estrus period. The results were available by x² Test with p<0.05. To evaluate the ovulatory response in relation to 1750 UI hCG dose, 86 estrus cycles were used , from this, 87,21% of the mares ovulated in 48 hours (75/86); 4,65% within 48 and 96 hours (4/86) and 8,14% didn't ovulate or showed haemorrhagic follicle (7/86). With regard to 1000 UI hCG dose, it were used 124 estrus cycles, of which 89,52% mares ovulated in 48 hours (111/124); 4,84% from 48 to 96 hours (6/124) and 5,65% didn't ovulate or showed hemorrhagic follicle. Both hCG doses displayed similar effect (p>0.05), with the great majority of mares ovulating in 48 hours. These results showed that 1000 UI hCG dose can be used as assurance ovulation inductor for Mangalarga Marchador cycling mares. Acknowledgment to Intervet Schering-Plough Animal Health.

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EFFECT OF FOLLICULAR SYNCHRONIZATION AND FSH ESTIMULATION ON RESULTS OF OVUN PICK UP

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Differences in the oocytes and embryos production using the technique of in vitro fertilization (IVF) has been observed in different breeds of cattle. Moreover, there are wide variations among the donors of the same breed. Profile differences in development and variations in the follicular population between Bos taurus and Bos indicus cattle are the main causes of variation in the results. Synchronization in follicular development and atresia reduction in the early stages of development can reduce these variations. The aim of this study was to compare the yield of oocytes and embryos from donor Bos taurus and Bos indicus cattle, using or not a hormonal protocol for synchronization of the follicular wave and reduction of atresia. It were used 193 donors, 109 Bos indicus divided into two groups: G1Z (n = 56) without any protocol before the aspiration and G2Z (n = 53) : the donors received progesterone (norgestomed) implant (Crestar Intervet Schering-Plough), 0.530 mg cloprostenol (Ciosin Intervet Schering-Plough) and 2mg of estradiol benzoate (Intervet Schering-Plough Gonadiol) 5 days prior to the aspiration and the day before aspiration 100mg of FSH (Pluset-Hertape Calier) divided into two doses 12-hour intervals. 84 donors Bos taurus divided into two groups: G1T (n = 42) without treatment and G2T (n = 42): same treatment for G2Z. All OPU were performed by same technician. The total number of oocytes recovered per donor were 13.45 ± 5.67 21.11 ± 7.62 8.06 ± 5.81 and 15.42 for groups G1Z, G2Z, G1T and G2T, respectively. The average number of viable oocytes was 10.56 ± 4.49, 17.66 ± 5.88, 5.69 ± 4.28 and 12.34 for groups G1Z, G2Z, G1T and G2T, respectively. The results show that the zebu cows (Bos indicus) produce more total and viable oocytes (P <0.05, Tukey test) than Bos taurus, considering the same treatment. The protocol was effective in increasing the number of total and viable oocytes in Bos taurus females. In Bos indicus females used protocol leads only an increase in the number of viable oocytes. It is concluded that the used protocol leads to an increase in the number of viable oocytes in the technique of OPU.
New experiments are in progress to confirm these effects of the FSH in the follicular population of females submitted to follicular aspiration. In random day of the estrous cycle (D0), all the animals received 150µg of PGF2α (cloprostenol, Prolise®, Arsa S.R.L, Argentine) and was performed aspiration of follicles larger than 5mm of diameter with the finality of synchronizing the emergence of a new surge of follicular growth. In the Day 2, the donors were placed in random in two experimental groups. In the control group (n=4), the follicular aspiration guided by ultrasound scan was carried out five days after the beginning of the treatment (D5). In the group M.P-FSH (n=4), the animals received a polymeric array (D2) containing 30 mg of FSH-p (Folltropin-V, Bioniche, Canada) by subcutaneous way and the follicular aspiration was carried out in the Day 5 of the treatment. For preparation of the polymeric array was used Pluronic F127® (Sigma-Aldrich Chemical Co, USA) and PVA (Sigma-Aldrich Chemical Co, USA) diluted in PBS, being carried out subsequently to the addition of the FSH, the adapted polymeric array was cooled at 4°C for 12 hours up to the use. The cows were subjected daily to evaluation of the follicular population through ultrasound scan to check the functionality of the polymeric array and growth rate of the follicles. The statistical analysis was carried in a completely randomized design, with the aid of computer program SAEG. WE used confidence interval considering the significance level of 5%. There was no statistical difference (P>0.05) in any of the evaluated variables. The average number of visible follicles for the group 1 was of 25.1±7.1 and for the group 2 of 23.3±9.3, the average number of recovered oocytes for both experimental groups (G1 and G2) were of 9.3±2.7 and 8.5±3.5, respectively and average number of viable oocytes for group 1 was of 7.2±2.8 and for group 2 was of 5.0±2.4. There was also no significant difference for the G1 and G2 in the number of follicles with diameter > 5 mm and follicles > 5 mm. Our preliminary results showed that protocols of hormonal stimulation with FSH-p incorporated in a polymeric array, had a response in the follicular growth, allowing an average number of visible follicles, average number of follicles with diameter > 5 mm and follicles > 5 mm. Our preliminary results showed that protocols of hormonal stimulation with FSH-p incorporated in a polymeric array, had a response in the follicular growth, allowing an average number of follicles, average number of visible follicles and average number of viable oocytes similar the group of animals without hormonal stimulation. New experiments are in progress to confirm these effects of the FSH in the follicular population of females submitted to follicular aspiration. Acknowledgements: APTA Regional Extremo Oeste.

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**EFFECT OF THE HORMONAL STIMULATION WITH FSH CONVEYED IN A POLIMERIC ARRAY ON THE FOLLICULAR POPULATION AND VIABLE OOCYTES OF NELORE COWS SUBMITTED AT OPU – PRELIMINARY RESULTS**

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This work aimed to evaluate the effect of the hormonal stimulation using a polymeric array controlled release of FSH on the response of follicular growth and quality of recovered oocytes of Nelore cows submitted to a program of OPU. It was used 8 cows of the Nelore breed according to age, weight, score of physical condition and reproductive historical. The donors were submitted to two repetitions, with interval of 10 days between each session of follicular aspiration. In random day of the estrous cycle (D0), all the animals received 150µg of PGF2α (cloprostenol, Prolise®, Arsa S.R.L, Argentine) and was performed aspiration of follicles larger than 5mm of diameter with the finality of synchronizing the emergence of a new surge of follicular growth. In the Day 2, the donors were placed in random in two experimental groups. In the control group (n=4), the follicular aspiration guided by ultrasound scan was carried out five days after the beginning of the treatment (D5). In the group M.P-FSH (n=4), the animals received a polymeric array (D2) containing 30 mg of FSH-p (Folltropin-V, Bioniche, Canada) by subcutaneous way and the follicular aspiration was carried out in the Day 5 of the treatment. For preparation of the polymeric array was used Pluronic F127® (Sigma-Aldrich Chemical Co, USA) and PVA (Sigma-Aldrich Chemical Co, USA) diluted in PBS, being carried out subsequently to the addition of the FSH, the adapted polymeric array was cooled at 4°C for 12 hours up to the use. The cows were subjected daily to evaluation of the follicular population through ultrasound scan to check the functionality of the polymeric array and growth rate of the follicles. The statistical analysis was carried in a completely randomized design, with the aid of computer program SAEG. WE used confidence interval considering the significance level of 5%. There was no statistical difference (P>0.05) in any of the evaluated variables. The average number of visible follicles for the group 1 was of 25.1±7.1 and for the group 2 of 23.3±9.3, the average number of recovered oocytes for both experimental groups (G1 and G2) were of 9.3±2.7 and 8.5±3.5, respectively and average number of viable oocytes for group 1 was of 7.2±2.8 and for group 2 was of 5.0±2.4. There was also no significant difference for the G1 and G2 in the number of follicles with diameter > 5 mm and follicles > 5 mm. Our preliminary results showed that protocols of hormonal stimulation with FSH-p incorporated in a polymeric array, had a response in the follicular growth, allowing an average number of visible follicles, average number of recovered oocytes and average number of viable oocytes similar the group of animals without hormonal stimulation. New experiments are in progress to confirm these effects of the FSH in the follicular population of females submitted to follicular aspiration. Acknowledgements: APTA Regional Extremo Oeste.

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**FACTORS THAT INFLUENCE OOCYTE QUALITY AND NUMBER OF IN VITRO-PRODUCED EMBRYOS IN CATTLE**

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The aim of this experiment was to study and correlate the effects of donor's breed and season on the number of oocytes collected by follicular aspiration (OPU), oocyte quality and in vitro embryo production (IVP). The work was carried out in 12 months, from January to December, 2008. The herd consisted of Bos taurus (Holstein and Angus) and Bos indicus cows (Brahman, Gir and Nelore) with body condition score between 3 to 3.5 (scale 1-5); in a total of 167 animals and 300 OPU (one or two sessions/cow). The dry season was considered the period from May to October and the wet season between the months of January and April, and November to December. Sessions of OPU were performed once a month, with beginning at five days after administration of estradiol benzoate, progesterone auricular device and cloprostenol, aimed at synchronization of follicular wave. Were recovered cumulus-oocyte complexes (COCs) from follicles with diameters between 3 and 8mm. Subsequently, the COCs were classified and those with scores of 1 to 3 (in a scale of 1 to 4) were submitted to in vitro fertilization. Grade 1 IVP embryos (excellent quality) were transferred eight days after OPU. For this, 990 previously synchronized crossbred heifers, weighing more than 350 kg, were used as recipients. Pregnancy diagnosis was performed between 28 and 35 days of pregnancy, by ultrasonography. To study the effects of donor's breed and seasons (wet/dry) on the number of oocytes retrieved, oocyte quality and number of IVP embryos, the nonparametric Kruskal-Wallis test was used, to 5% probability. In this experiment, Bos taurus donors presented similar numbers of viable oocytes during the wet season (7.59 ± 0.64) or during the dry season (9.23 ± 0.86; P> 0.05). However, the number of viable oocytes collected from Bos taurus donors during wet season was smaller (P <0.05) than for Bos indicus at any time of year (14.89 ± 1.13 in the wet season and 12.86 ± 0.19 in the dry season). Regarding the number of in vitro-produced embryos, Bos taurus donors produced less embryos during the wet season compared to Bos indicus (1.65 ± 0.28 versus 4.85 ± 0.56, P<0.05) but, in dry season, the embryo production was similar (Bos taurus 2.62 ± 0.37 versus Bos indicus 3.92±0.46, P>0.05). Probably the animals B. taurus were influenced by heat stress during the wet season, which influenced the number of oocytes and in vitro-produced embryos. Pregnancy rates were not significantly different, regardless of season (average 47.6%; P>0.05). In conclusion, Bos indicus cows were superior in number and quality of collected oocytes, and also in number of in vitro-produced embryos compared to Bos taurus, during the wet season.
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Research of the virus of ibr and bvd in embryos treated with trypsin deriving from naturally infected donors

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The aim of this study was to identify the presence of IBR and BVD virus in embryos deriving from naturally infected cows. In vivo embryos were produced from serum positive cows (n=8) as determined by the serum neutralization technique. The animals were previously evaluated via transrectal ultrasonography and underwent a superovulation protocol. A progesterone implant (CIDR® - Pfizer – Saúde Animal, São Paulo, Brazil) was used during eight days. At the moment of the CIDR insertion, 2mg of estradiol benzoate were given IM. From days four to eight, a FSH superovulation protocol (220mg of Folltropin® - Tecnopec, São Paulo, Brazil) was used in decreasing doses. Subsequently, the animals were artificially inseminated and embryos were flushed seven days later. The recovered structures (n=76) were evaluated with a stereo-microscopic and classified as viable (n=27), non-viable embryos (n=14) or not fecundated oocytes (n=35). All the structures were frozen in ethylene glycol and underwent a PCR according to Cortez at al. (1999; Arq. Bras. Med. Vet. Zootec., 58:1226-1228) for the search of BHV-1 and BVDv. Before thawing, the recovered embryos, viable and non-viable, were treated with trypsin 0.25%, according to Stringfellow et al. (1990; Theriogenology, 34:427-433). Samples from flushing media and from drops 8, 9 and 10 of the trypsin washing media were also evaluated by PCR. BHV-1 and BVDv virus were not detected in any of the tested samples. Preliminary data of the absence of IBR and BVD virus in embryos produced from naturally positive cows indicate the possibility of the use of these animals as embryo donors. Acknowledgments: FAPEMIG, CNPq

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EVALUATION OF QUANTITY, QUALITY AND OF THE IN VITRO MATURATION OF OVINE OOCYTES OF SANTA INÊS BREED SUBMITTED TO SUCCESSIVE FOLLICLE ASPIRATION SESSIONS

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The small ruminants have been a good model to development of domestic animals reproductive biotechnologies. The in vitro production (IVP) can potentiate the programs of genetic improvement. However there are still many factors related to this technique to be study. The IVP success requires an efficient and effective technique to obtain oocytes, and the best results in ovines are from laparoscopic follicle aspiration. The aim of this study was to evaluate if successive follicle aspiration sessions interfere in quantity, quality and in vitro maturation of ovine oocytes. Five follicle aspiration sessions, with seven days of interval, were carried out in six Santa Inês sheep. The estrous cycle of these animals were synchronized by using a short protocol (intravaginal device of 60mg of medroxyprogesterone acetate during 6 days; on D5 animals received 37,5µg of D-cloprostenol and 300UI of eCG, IM) and after synchronization sheep received, before 36 hours of follicle aspiration, 80mg of FSHp and 300 UI of eCG. The procedure of aspiration was done by videolaparoscopy. The oocytes were evaluate according to cytoplasm homogeneity and the number of cumulus cell layers, as describe by Hewitt and England (1997; Journal of Reproduction and Fertility, 51:83-91). The maturation medium was TCM-199, supplemented with FSH, hCG, estradiol, cysteamine, sodium pyruvate, antibiotic and fetal calf serum, in which medium the oocytes remained for 24 hours in an incubator at 39ºC with 5% CO2 in air and saturated humidity. In each session, oocytes recovered were evaluated by quantity, quality and nuclear and cytoplasmatic rates. All data were analyzed by one way ANOVA and Tukey test. The number of oocytes recovered was 5±2,517 with no variation among the weeks (p>0,05); there were significant results in quality of oocytes (p<0,001): grade I 2±1,180, grade II 2± 1,130, grade III 1± 0,8307 and degenerated oocytes 0± 0,3742. There was no difference in nuclear and cytoplasmatic maturation among the weeks (p>0,05), and 50% of oocytes were able to maturation, as a total per animal 3±1,291, and in nuclear maturation 1±1,129 matured oocytes, 0±0,9335 immature and 1±1,250 degenerated or not identified(p>0,05) and in cytoplasmatic maturation 1±1,057 matured oocytes, 0±0,9016 immature e 1±1,286 degenerated or no identified. The results showed that there were no alteration in oocyte quality, quantity and maturation of Santa Inês breed submitted up to five follicle aspiration sessions.
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APOPTOSIS IN BOVINE EMBRYOS PRODUCED WITH OOCYTES MATURED IN SERUM-FREE MEDIUM UNDER LOW OXYGEN TENSION


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In vitro maturation (IVM) is a critical step for in vitro embryo production (IVP) and several factors can influence its efficiency, such as protein supplementation and atmospheric tension. The current study aimed to investigate the effect of serum and oxygen tension (O2) during in vitro maturation (IVM) on incidence of apoptosis in bovine blastocysts. Immature COCs were distributed randomly in the following IVM groups: G1 (10% estrus cow serum [ECS] with 20% O2); G2 (0.1% polyvinyl alcohol [PVA] with 20% O2), G3 (10% ECS with 5% O2) and G4 (0.1% PVA with 5% O2). Basal maturation medium was TCM199 (Invitrogen, California, USA) and O2 tension was 5%. Maturated oocytes were subjected to in vitro fertilization in 100-µl drops of Fert-TALP supplemented with heparin and 2x10⁶ spermatozoa/mL for 21 h in a humidified atmosphere of 5% CO2 and 38.8 ºC in air. Presumptive zygotes were denuded by vortexing in 0.1% hyaluronidase solution and cultured in CR2aa medium (Wilkinson et al. 1996; Theriogenology, 45:41:49) with 2.5% fetal calf serum (FCS) (Nutricell, Campinas, SP, Brazil) under 5% CO2, 5% O2, 90% N2 at 38.5 ºC. Blastocysts at eight day post-fertilization from G1 (n=22), G2 (n=18), G3 (n=19) and G4 (n=18) were fixed and permeabilized for TUNEL assay (DeadEnd™Fluorimetric TUNEL System-PROMEGA), according to the manufacturer instructions. Total cell number, apoptotic cell number and apoptotic cell index (calculated by dividing the apoptotic cell number by total cell number) were analyzed by analysis of variance and mean compared by Student Newman Keuls. Significance was estimated at the level of P<0.05. The total cell number were not affected (P>0.05) when the ECS (G3: 112.73±2.87) was replaced by PVA (G4: 111.11±2.67) under 5% O2, whereas higher (P<0.05) total cell number were found with ECS (G1: 116.90±2.60) compared to PVA (G2: 85.77±2.49), both under 20% O2. Blastocysts from G4 showed lower (P<0.05) number of apoptotic cells (10.72±1.25) than those from G1 (20.95±1.29), G2 (19.50±1.42) and G3 (21.73±1.29), and lower (P<0.05) apoptosis index (G4: 0.09±0.01) than blastocysts from other groups (G1: 0.18±0.01; G2: 0.22±0.01 and G3: 0.19±0.01). In conclusion maturation with PVA and 5% O2 provides an in vitro maturation that results in blastocysts with low apoptosis index. Financial support: Fapemig.

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EVALUATION OF MORPHOLOGY CHANGES DURING THE DEVELOPMENT OF BOVINE EMBRYOS FERTILIZED IN VITRO WITH SEMEN EXPERIMENTALLY CONTAMINATED WITH ESCHERICHIA COLI SHIGA TOXIN-PRODUCING STX2 (STEC)

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Although increasingly used, assisted reproduction techniques still need researchs to asses the health risks of oocytes, embryos and sperm, because embryonic and fetal mortality have a major impact on the profitability of any livestock system. The aim of this study was to evaluated by optical microscopy and electron on transmission, changes in morphology during the development of bovine embryos, fertilized with semen experimentally contaminated with Escherichia coli producing the Shiga toxin stx2. Oocytes were aspirated from ovaries of slaughtered cows and ones with the intact zona pellucida were selected and matured. After 20 hours, the oocytes were divided into control group (n=418), fertilized with semen control and infected group (n=415), fertilized with sperm exposed to 200 UFC E. coli shiga toxin-producing stx2. Each semen was treated by the technique of discontinuous Percoll gradient, and the sperm concentration was adjusted to approximately 100 thousand sperm for each oocyte. After the period of fertilization, the embryos were evaluated for their morphology by optical microscopy and electron on transmission. The oocytes fertilized with contaminated semen (52.8%; n=219/415) showed cytoplasmic shrinkage, blastomere division failures, asymmetry of blastomeres, granular ooplasm with dark brown color, formation of vacuoles, degeneration and disruption of the zona pellucida. The thin sections of the same group showed granular cells and vacuolated, with structures similar to E. coli, that have been confirmed by transmission electron microscopy (70.3%; n=294/418). The presence of E. coli shiga toxin-producing stx2 cause morphological changes during the development embryo. Good hygiene of the prepuce of the bull and materials used during the collection, with the appropriate disinfectants, are extremely important, and the use of antibiotics effective for the dilution of semen, to ensure the descontamination, since the procedure used during IVF (Percoll) was not effective.
TRANSPORT ENVIRONMENT EMBRYO AND TRANSPORT TEMPERATURE IN BOVINES BLATOCYSTS PRODUCED IN VITRO

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It is known that IVP embryos have poor quality, are vulnerable to heat and oxidative. Besides, they are constantly submitted to long distance transporting, producing low conception rates. The aim of this study is to compare the supporting way of embryos transport and his development in high and low temperature in embryos production in vitro. Was used 823 COCs (quality I and II), from slaughterhouses cows ovaries. The COCs were matured in TCM-199Bicarbonate 10%SFB (38,5ºC, 5%CO2) for 24h. The fecundation occurs in TALP – FIV way, for 18h. After, the structures were transferred for a SOF way (5%O2, 38,5ºC) for 7days. At day 7, 366 qualitys embryos I and II (IETS), were divided randomly to form experimental groups. Experiment (Exp1): blastocysts were filled into vanes and kept for 12h in carrier embryos at 36ºC using the supporting way as independent variable according to the following groups: GMan - embryo maintenance way without FBS and amino acids (AAs; n=115) and GHSOF—embryo way of growth “Synthetic Oviduct Fluid” (HSOF) with FCS and AAs (n=105), both ways buffered in HEPES. Exp.2: blastocysts were filled with HSOF way, with vanes and sustained in the transportation for 12 hours, using temperature as independent variable according to the following groups: G36- 36ºC (n=65) and G38- 38ºC (n=81). After transportation, in both experiments, the embryos were evaluated and classified as viable and nonviable, and recultured in the same conditions mentioned before. On day 10 were estimated hatching rates and degeneration. Statistical analysis consisted in logistic regression. Exp.1 the rate of blastocyst viability after 12h of transport was higher in the growth way (GHSOF: 91.43%, Gman: 75.65%, p <0.001). The chance of hatching in the growth way was 5 times higher than the supporting way (GHSOF: 72.38%, Gman: 34.78%, p <0.001). Exp. 2 the rate of blastocyst viable post-transport was the same in both temperatures (G36: 92.31%, G38: 82.72%, p> 0.09). The rate of hatched blastocyst was 5 times higher than the supporting way (GHSOF: 72.38%, Gman: 34.78%, p <0.001). The chance of hatching in the growth was 3.3 times greater than 38ºC. The immediately hatching after 12h transportation was minimal, so, was not possible to apply the method of logistic regression in any experiment. The use of growth transport way in the presence of FCS and amino acids reduces the degeneration of embryos post-transport by increasing the viability and hatching. Embryos transported at 36ºC for 12h, increase their viability and hatching, by decreasing their metabolism and consequently the stress caused by transport conditions.

COMPARISON OF INITIAL PREGNANCY RATE BETWEEN HEIFERS AND COWS AS RECIPIENTS OF IN VITRO PRODUCED EMBRYOS

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The born of health animals is one the most important data to validate reproductive biotechnologies. Considering that major gestational losses occur until 30 first days of pregnancy, the diagnostics soon after this phase is a great indicator in the context of in vitro production (IVP) of embryos experiments. However, it is necessary to consider the recipients or surrogate mothers conditions among the factors that more influence this parameter. Then, this study aimed to compare the use of heifers and lactation cows, both crossbred, with milk ability and body score varying between 2,7 to 3,8 (scale 1 to 5), as IVP embryos recipients, evaluating the pregnancy rate between 30 to 45 days. The embryos were produced from cumulus oocyte complexes (COC's) recovered by ovum pick up (OPU) from Gir breed cows. These COC’s, after in vitro maturation, followed to fertilization using female sex-sorted semen of Gir or Holstein bulls processed by Percoll gradient. Next, the presumptive zygotes were cultured in SOFaaci medium for 7 to 8 days and those reached blastocyst stage were transferred to both experimental groups: G1) crossbred heifers Gir x Holstein, mean age of 36 months, with blood degree varying 1/2 to 5/8 (European/Zebu); and G2) crossbred cows, age between 61 to 102 months-old, in lactation, with blood degree similar to heifers. The embryos transfers were done at maximum ±2 days of asynchron between the ages of embryo and recipient uterus. Moreover, the non-surgical technique was elect to transfer the blastocysts. Single transfers were carried out in 151 female recipients, which 88 on G1 and 63 on G2. Ultrasonographic exams between 30 to 45 days of gestation showed that there were 31 (35.2%) and 9 (14,2%) pregnant females, in G1 and G2, respectively. The statistical analysis by chi-square (P<0.05) demonstrated that there were no differences between pregnancy rate considering the father's breed, Gir or Holstein. However, there were significant differences on the use of heifers embryonic programs which use well managed heifers would be favored in relation to other ones that utilize lactating milk cows as recipient for IVP embryos. Hence, it is important to emphasize that the use of latter needs to be conscious about their low efficiency in terms of establishment of this kind of pregnancy. Acknowledgment: Epamig e Fazenda Calciolândia Financial support: Embrapa/Fapemig/CNPq/FAPESP
EVALUATION OF COCONUT WATER CHEMISTRY IN NATURA (Cocos nucifera) IN DIFFERENT STAGES OF MATURITY OF THE FRUIT IN COMPARISON WITH THE MEDIUM 199

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Due to its physicochemical characteristics the coconut water has been tested as a medium of maintaining cell culture and with great perspectives within the reproductive biotechnologies. Therefore, the objective was to evaluate the chemical characteristics of in natura coconut water at different stages of maturation of the fruit (3, 4 and 5 months), in comparison to Medium 199. Were collected a total of 72 fruits (24 with 3, 24 with 4, and 24 to 5 months). Chemical analysis was performed at the Laboratory of Beverages and Vinegars - LANAGRO-PARÁ according to internal protocol. Statistical analysis was performed by ANOVA (Tukey post-test, with significance level of 5%). With respect to the osmolarity (mOsm/L) there was a significant difference (p<0.05) between the Groups 3 (273±8.2) and 5 months (335±24.6) in relation to 4 months (290±8.6) and Medium 199 (290). The values of soluble solids (3.7±0.17; 4.29±0.21; 4.97±0.26 and 1.4 ±Brix), density (1.0129±0.0006; 1.0151±0.0009; 1.0173±0.0013 and 0.905 g/mL), total solids (3.6074±0.08; 4.1964±0.24; 0.253±4.6389 and 1.4336 g/100 ml), protein (0.2557±0.0138, 0.4699±0.05, 0.7966±0.0045 and 0.853 mg/mL) and glucose (11.7±0.8, 15.8±0.5, 19.9±0.3 and 4.3 ±g/100 ml) in Groups 3, 4, and 5 months and Medium 199, respectively, were statistically different (p<0.05). The analysis of sodium (mg/100 mL) revealed differences (p<0.05) in Group 3 months (34.09±4.36) groups compared to Groups 4 months (0193±30.11), 5 months (30.16±1.752) and Medium 199 (28.64). In relation to potassium (mg/100mL), significant differences (p<0.05) in Group 4 months (132.33±2.32) compared to Group 3 (177.81±12.23) and 5 months (156.37±25.0284) and all in compared to Medium 199 (34.66). In contrast, calcium and magnesium (mg/100mL) concentration were different (p<0.05) among all experimental groups (3 months: 17.07±2.76 and 1.57±0.20; 4 months: 12.1±1.73 and 1.04±0.01; 5 months: 7.72±0.32 and 3.86±0.20, and Medium 199: 3.55 and 2.15, respectively). With respect to iron (mg/100mL) water-coconut in Group of 5 months (0.0036±0.0007, p<0.05) showed much higher compared to 3 (0.0013±0.0001) and 4 months (0.0014±0.0002). No iron was detected in the sample of Medium 199. Regarding chloride (mg/mL), no change (p>0.05) in concentration between the experimental groups analyzed. Therefore, the coconut water in natura shows considerable variations in the concentrations of the constituents analyzed, especially when compared to Medium 199, which can difficult its standardization as a medium of cell culture.Acknowledgments: FAPESPA, CNPQ e Laboratório de Bebidas e Vinagres – LANAGRO-PARÁ.

EFFECT OF SYNCHRONIZATION AT THE BEGINNING OF FOLLICULAR WAVE EMERGENCE ON OPU-IVP OF NELORE (Bos indicus) AND BRANGUS (Bos taurus x Bos indicus)

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The aim of this study was to determine the effect of synchronization of follicular wave emergence in Nelore (Experiment 1) and in Brangus (Experiment 2). In each experiment 12 multiparous were divided into four treatments (n = 3 per treatment): control (CONT - OPU on random day of the estrous cycle), OPU 1 day after wave emergence (D1), OPU 1 day after wave emergence associated with bST (D1 + bST) or OPU 1 day after wave emergence associated with eCG (D1 + eCG). The beginning of the follicular wave was synchronized with a combination of estradiol benzoate (2 mg; Gonadiol®) plus intravaginal progesterone device (DIB®), which was maintained for 5 days in all animals. At the same time, a dose of PGF2αi(Preloban®) was also administered. In Nelore (Experiment 1) and in Brangus (Experiment 2) a higher number of total oocytes, viable and poor oocytes was found in group D1 + eCG (19.7±2.9) and D1 + eCG (19.1±1.7, 13.3±1.1 and 5.8±0.8, respectively), than in the D1 (12.5±0.6, 7.8±0.8 and 4.8±0.7, 5.8±0.9) and blastocyst rate (38.4, 40.5, 39.5 and 52.3). Conversely, in Brangus (Experiment 2) a higher number of total oocytes, viable and poor oocytes was found in group D1 + eCG (19.1a±1.7, 13.3a±1.1 and 5.8a±0.8, respectively), than in the D1 (12.5b±0.6, 7.8b±0.8 and 4.8b±0.7). D1 + bST (12.2bc±1.8, 8.8b±1.4 and 3.4bc±0.7) and CONT (8.9c±1.3, 6.4b±1.1 and 2.5c±0.7). There was no difference between groups D1, D1 + eCG, D1 + bST and CONT, respectively, for the number of viable oocytes (14.8±2.2, 16.5±3.1, 11.8±1.4 and 13.6±2.6), the percentage of viable oocytes (75.4, 79.2, 80.7 and 84.5), the number of poor quality oocytes (4.8±1.1, 4.3±1.4, 2.8±0.8 and 2.5±0.7), number of blastocysts (5.3±0.8, 6.0±1.0, 4.8±0.8 and 5.8±0.9) and blastocyst rate (38.4, 40.5, 39.5 and 52.3). However, blastocyst rate was favoured in D1 group compared to D1+eCG, D1+bST and CONT (30.0b, 32.0b and 27.7b, respectively). In conclusion, synchronization of wave emergence contributed to the increase in total oocytes in Nelore and Brangus, and in the last breed, an increase in the number of viable oocytes was also found. However, no increase in the number of blastocysts was produced.
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EFFECT OF DIFFERENT ENERGY LEVELS DIET ON OOCYTE QUANTITY AND QUALITY OF NON LACTATING COWS (Bos indicus AND Bos taurus) SUBMITTED TO OVUM PICK-UP


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It was evaluated the effect of different energy levels on oocyte quantity and quality of non lactating Bos indicus and Bos Taurus cows. At this study, 28 dry cows (14 Bos indicus - Gir and 14 Bos taurus - Holstein) were allocated according the breed in two different diets, maintenance (M) and high energy (1.7M). The donors were maintained in Tie stall system and the food were given two times/day (8:00h a.m. and p.m.). The animals were submitted to a 21 days adaptation period, when they received the M diet. After this period, the experimental groups were submitted to eight (8) 14 days apart ovum pick-up (OPU) sessions. To OPU sessions, on D0, the donors were synchronized with 2mg of estradiol benzoate (Gonadiol®, Intervet-Schering-Plough, Brazil) and a Norgestomet ear implant(Crestar®, Intervet, Boxmeer, Holand). On D5, the OPU were performed. After the OPU, the collected materials were taken to lab to evaluate the quality and quantity. The dependents variables of the normal distribution were analyzed for repeated measures used the PROCMIXED and binomial variables for PROC GLIMMIX of the SAS. There was no interaction between Bos indicus and Bos taurus cows or diet level (M e 1.7M). It was not observed difference on oocyte quality and quantity according to diets. However, it was observed difference on this variable according to studied species. Bos indicus cows showed more quantity of recovery structures by OPU and better oocytary quality than Bos taurus donors [recovery Oocytes - Gir 23.4 ±1.6 and Holstein 14.9 ±0.9 (P=0.003), recovery rate - Gir 91.2% (2604/2856) and Holstein 61.1% (1633/2673; P=0.001), grade A oocyte - Gir 5.33±0.48 and Holstein 1.6±0.18 (P=0.001), grade B oocyte - Gir 9.83±0.67 and Holstein 5.16±0.39 (P=0.001) and apoptosis rate (TUNEL) - Gir 16.6% (21/117) and Holstein 40.6% (34/82; P= 0.004)]. We can conclude that the increase in dietary energy do not promote reduction on oocyte quality and quantity obtained by OPU. However, Bos indicus cows showed bigger quality and quantity of oocytes than Bos taurus.

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SEXED IN VITRO EMBRYOS USED ON COMMERCIAL HERDS IN THE BREEDING SEASON


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The in vitro production of embryos (IVP) has been restricted only to elite animals. Our objective is to demonstrate the IVP as a viable possibility for multiplication of commercial herds during the breeding season, with the advantage of using sexed semen. At the city Curionópolis - Para, on a farm with breeding season already established, Gir (n = 492) and Tabapuã donors (n = 121) were submitted to OPU (2 to 4 times / donor), and 13 635 oocytes were obtained from Gir (average = 27.7) and 4698 from Tabapuã ( average = 38.6). All oocytes were designated to in vitro fertilization with sexed semen for female (Holstein bull for Gir oocytes) or male (Tabapuã bull for oocytes of the same breed). Tabapuã recipients (n = 3636) received all fresh embryos after a timed embryo transfer protocol, consisting of intravaginal progesterone device at day zero (D0) plus 2 mg estradiol benzoate IM. On the eighth day (D8) the implant of progesterone was removed and animals were injected with 0.15 mg D-cloprostenol (PGF) IM, 300 IU of eCG IM and 1 mg of ECP (estradiol cypionate). On day 17 (D17) all recipients presenting a CL received one embryo non-surgically. From Gir oocytes with Holstein semen 2205 embryos were transferred, resulting in 876 (39.7%) pregnancies. For Tabapuã, 813 embryos were transferred, with 353 (43.4%) pregnancies. In a period of only 60 days at the beginning of the breeding season, we transferred a total of 3018 embryos, which resulted in 1229 pregnancies (40.7%). Although the pregnancy rate of 40% is slightly below of the 50% expected after an artificial insemination, we emphasize some important aspects for using in vitro sexed embryos compared to A.I in the breeding season. First, the great advantage of determining the sex of products, with males to meat (Tabapuã) or females for milk (Girolanda). Also, it is possible to choose the best cows as oocyte donors. We expect a consistent genetic improvement in a short period of time. With the possibility of embryo transfer at the beginning of the breeding season, we can consider this strategy of using in vitro embryos in vitro as an alternative, or a complement to the TAI.
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GENE EXPRESSION OF IGF I AND II, ITS RECEPTORS (IGFR-I AND IGFR-II), ITS BINDING PROTEINS (IGFBP 2 AND 4) AND PAPP-A ON IMMATURE BOVINE OOCYTES (BOS INDICUS VS BOS TAURUS)

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There is evidence that IGF system can be involved in the oocyte competence and consequently in the embryo development. To better understand possible differences between Bos taurus and Bos indicus in in vitro embryo development, the present work aimed to assess the expression of IGF ligands (IGF-1 and 2), type 1 and 2 IGFR receptors (IGFR1 and 2), IGFBP-binding proteins 2 and 4 (IGFBP-2 and 4) and type A pregnancy associated plasmatic protein (PAPP-A) mRNAs in bovine immature oocytes from Bos taurus and Bos indicus. Nellore and Holstein cows were submitted to OPU (ovum pick-up) and the oocytes from Nellore as compared to Holstein cows. The gene expression of target genes was measured by real-time RT-PCR with oligo-dT in the RT and bovine-specific primers in the PCR. Expression of cyclophilin A (CYC-A) was used as internal control. The means of mRNA levels of target genes between the breeds were compared using t test and Man-Whitney test when the data had or not normal distribution, respectively. The means values of mRNA expression of IGF-1, IGF-2, IGFR receptors (1 and 2) and IGFBP 2 and 4 were higher in Holstein (0.96±0.21, 0.74±0.27, 1.08±0.04, 1.19±0.5, 1.21±0.23, 0.53±0.15, respectively) compared with Nellore (0.48±0.10, 0.07±0.02, 0.06±0.02, 0.06±0.01, 0.03±0.15, respectively; p<0.01). Nevertheless, mRNA expression of PAPP-A was much higher in Nellore (28.10±18.96) than Holstein (1.32±0.17; p<0.05). These results suggests that high expression of PAPP-A and low expression of IGFBP-2 and 4 could allow a more efficient degradation of IGFBPs and increase the IGF bioavailability in the oocytes from Nellore as compared to Holstein cows.

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EFFECT OF HEAT STRESS ON EXPRESSION OF SOME GENES RELATED WITH COMPETENCE AND IMPLANTATION IN NELLORE BOVINE IVP EMBRYOS

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Several factors affect early embryonic development in cattle, among them heat stress. These factors can contribute to high early embryonic loss, probably altering gene expression. Studies using microarray profiled genome-wide RNA expression for in vitro-produced blastocysts, comparing embryos resulting in calf delivery or no pregnancy, and they identified genes with potential roles in pregnancy and embryo competence. The aim of the present work was to compare the expression of some genes (PLAC8, HSF1, COX-2 and CDX-2) related with embryo competence and embryonic implantation between in vitro produced embryos from Nellore breed (Bos indicus), submitted or not to heat stress. Oocytes from Nellore cows were aspirated by OPU and matured for 22h (TCM 199 with bicarbonate, supplemented with 10% FCS, 2µL/ml pyruvate, 75µg/mL amicacin, 20µg/mL FSH and 2U/mL hCG) at 38,5°C, 5% CO2 in air. The fertilization (D0) was performed with semen from Nellore bulls. After 12h fertilization period, in TL Stock medium supplemented with 6mg/mL BSA, 2mL/ml pyruvate, 75mg/mL amicacin, 11mg/mL heparin and 44 mL/mL PHE solution, presumptive zygotes were denuded and randomly divided in two groups: non-stressed and stressed. The culture medium was SOFaaci supplemented with sodium pyruvate (0,2%), 5mg/mL BSA and 5% FCS. Embryo cultur was performed at 38,5°C, 90% N2, 5% CO2 and 5% O2. In D7, pools of 5 blastocysts (non-stressed n=9; stressed n=7) were submitted to total RNA extraction (RNeasy, Qiagen). The gene expression of target genes was measured by real-time RT-PCR with oligo-dT in the RT and bovine-specific primers in the PCR. Expression of cyclophilin A (CYC-A) was used as internal control. The means of mRNA levels of target genes between the groups were compared using t test. The PLAC8 mRNA levels were higher in non-stressed blastocysts in comparison with stressed group. The HSF1 and CDX2 expression on non-stressed blastocysts in comparison with stressed group. The HSF1 and CDX2 mRNA was only detectable in non-stressed embryos. COX2 mRNA levels did not differ between groups. The higher levels of PLAC8 and the CDX2 expression on non-stressed embryos indicate a better competence of embryos not submitted to heat stress. Furthermore, the absence of HSF1 mRNA in the stressed embryos does not reflect the lacking of biological activity of this protein. In conclusion, the data indicate that heat stress alters the gene expression pattern of in vitro-produced embryo in Nellore breed.
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EVALUATION OF THE BOVINE RECOMBINANT SOMATOTROPIN EFFECT ON IN VITRO FERTILIZATION TECHNIQUE IN SANTA INÊS EWES

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The in vitro fertilization technique has been highlighted as an important tool in sheep production. The recombinant bovine somatotropin has been identified as a substance that helps reproduction technologies used in various species of ruminants. The aim of this study was to evaluate the possible effect of recombinant bovine somatotropin on in vitro fertilization technique in Santa Inês ewes. It was used 19 ewes embryo donors of the Santa Ines breed and 23 receptors, also of Santa Ines breed, created in Atibaia- SP, Brazil. The donorsunderwent to the following superovulation protocol: D0 - Progespon® (Intervet Schering-Plough); D8 e D9 – two Pluset® (Hertape Calier) and one Preloban® (Intervet Schering-Plough) aplications and were aspirated at D10. At D5, nine donors received 250 mg of Boostin® (Intervet Schering-Plough) subcutaneous (treated group - TG) and ten ewes received placebo in equal volume (control group - CG). A total of 240 ovaries follicles were observed in the ewes donors, of which 167 oocytes were aspirated, resulting in 71 embryos with a conversion rate of 42.5%. No significant statistic difference was observed between the treated group and the control group by the student t (TG= 8,78; CG=8,80), although the average number of follicles by animal (TG=13,67; CG=11,70), the average number of embryos by animal (TG=4,22; CG=3,30) and conversion rate by animal (TG=58,39%; CG=44,26%) favored the treated animals with somatotropin. At D15, twenty three embryos were implanted in synchronized recipients and generated 10 pregnancies, and the pregnancy rate observed was 43,47%. Six embryos were from the control group that resulted in four pregnancies, and 17 were from the treated group that resulted in six pregnancies. The remaining embryos were vitrified. The results allowed to conclude that in vitro fertilization technique is an important technique in the sheep production development and that researches are necessary to confirm the tendency of results with the use of the bovine recombinant somatotropin. Acknowledgments: Intervet/Schering-Plough and Morro Verde Ranch.

ABSTRACT 151

USE OF THE L-ARGININE IN SPERM CAPACITATION AND IN VITRO FERTILIZATION OF BOVINE OOCYTES

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Works with L-arginine in the sperm capacitation in mammals do not surpass its effect to the in vitro fertilization (IVF). Moreover, in the in vitro embryos production (IVP) in bovines is observed that the race (Bos taurus X Bos indicus) affects the success of the technique. The aim of this work was to evaluate the effect of the use of L-arginine in the sperm capacitation and IVF on the in vitro embryonic development, using one bull Bos taurus and one Bos indicus semen. In experiment 1, the spermatozoa were incubed without oocytes for different times (1, 2 and 3 hours) in IVF medium according PARRISH et al. (1998, Biology of Reproduction, 38: 1171-1180) supplemented with L-arginine and acrosome reaction rate was evaluated according to BENDAHMANE et al. (2002, Archives of Biochemistry and Biophysics, 404: 38-47). For experiment 2, bovine oocytes had been in vitro matured (IVM) for 18h. In both experiments 5 repetitions had been carried. The L-arginine was added during the IVF and its effect was evaluated through the IVF rate (18 hpi), cleavage and blastocyst rates (2º and 7º day of culture, respectively), blastocyst cell number (7º day of culture) and the Gries colorimetric method was applied to measurement of the NO3- /NO2- produced during the IVF. For statistical analysis, ANOVA test were used, with 5% of significance level. The results had shown that L-arginine (1 mM), when added during sperm capacitation for two hours, increased the acrosome reaction rate as compared to Bos taurus control group (31,1±2,78 versus 23,4±2,65). The addition of L-arginine (50 mM) to IVF medium (experiment 2), in both Bos taurus and Bos indicus spermatozoa, decreased cleavage rate (78,7±2,17 versus 65,7±9,32; 72,7±3,36 versus 45±7,12; respectively) and blastocyst rate (39,4±3,78 versus 15,2±6,12; 39,4±4,39 versus 16±8,54; respectively) as compared to control group. These results provide that L-arginine increased the acrosome reaction rate in Bos taurus, however reduced the cleavage and blastocyst rates in both sub-races, but had not influenced the embryo quality (blastocyst cell number). Acknowledgments: CNPQ, Universidade Norte do Paraná, Laboratório de Parasitologia da UFPA.
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RECOVERED INDEX AND QUALITY OF OOCYTES FROM NELORE CROSBREED AND BUFFALOS COWS FROM OVARIES COLLECTED AT SLAUGHTERHOUSE

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The objective of this work was to know the incidence of the number and quality of aspirates oocytes of 29 ovaries of bovine cows and 30 buffaloes, with corporal score from 3 a 5, collected from slaughterhouse and to evaluate the index of nuclear maturation after culture in vitro. The CC0 was washed in alcohol 70° GL and saline solution 0,9 % with penicillin 100/ml/ml, after transferred to thermal bottle contend solution saline phosphate (PBS) being carried at Laboratory of Genetics and Biotechnology of the State University of Maranhão. The follicles that presented diameter between 2 and 6 mm, had been punched, using syringes (10 ml) and needles (25 x 8), deposited in Petri dish 25x 10 mm with MTC-119 (Sigma M-5017) e Heps (Sigma H 0763) and classified in quality (QI, QII, QIII, QIV and QV), agreement the species and score corporal. The CC0 with three or more compact layer of homogeneous cytoplasm, were mature in vitro with MTC-199 (Sigma M-5017) medium supplemented with 10 % of bovine foetal serum (BFS), 10UI of eCG). The oocytes were cultivated by period of 20 hours in CO2 incubator at 5 % and temperature of 39 °C in air. After maturation, samples of the oocytes were fixed with ethanol and acetic acid (3:1) by 20-24 hours and stained using Acetic orcein 2 %, and observed by inverse optic microscopic (100x) for evaluation of maturation. The results were evaluation using SAS statistical program. The present average of follicles in the ovaries was significantly superior (19,1±4,9) in vacas bovina of that in Buffaloes (8,6±4,4) of exactly corporal score. The average index of oocytes gotten of the cows with corporal score 4 was superior (20.1±3,8) that the cows with corporal score of 3 and 5 (16,0±4,0) and (13,0±2,3). The cows of score 5 had presented percentile greater of oocytes of QI 44,2%, in relation of score 3 and 4 (29,2 and 21,0 %). The average indices of oocytes gotten of the buffalos of Corporal score 3 and 4 had been 6,80±4,2 and 13,2±2,3 oocytes/ovarian. The buffalos of group 3 had presented percentile greater of oocytes of QI (24, 8%) in relation the buffalos of score 4 (18, 8%). Was observed that 72,8% of the oocytes bovine and 68,2 % buffaloes with expansion of cells of cumulus oophorus were in stage of metaphase II at 20- 24 hours of culture. Could be concluded that the average of follicles bovine ovaries is superior in relation of buffalos ovaries, as well as the corporal score had influence in the quality of the oocytes in both species, the rate of maturation there was not different (P>0.05) when was culture by 20 hours. REFERENCIAS: OHASHI, O. M. Cinética da maturação nuclear in vitro de oócitos bubalinos. Brazilian Journal of Veterinary Research Science. v.39, nº. 5. São Paulo, 2002.

ABSTRACT 153

FOLLICULAR DEVELOPMENT AND OOCYTE RECOVERY EVALUATION IN SANTA INÊS EWES SUBMITTED TO SEQUENTIAL FOLLICULAR ASPIRATIONS

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Folicular aspiration to in vitro embryo production is the major tool for in vivo oocyte obtaining in ovine. However, it's necessary to know, if this technique is harmful to the ovarian parenchyma and decreases follicular production. Thus, the aim of the present study was to evaluate oocyte recovery in ewes submitted to ovary stimulation for successive follicular aspirations. Six Santa Inês ewes had the estrus synchronized with vaginal sponges, with 60 mg of MAP (Day 0) for 6 days. On day 5, 35.850g of D-Cloprostenol and 300IU of eCG were administrated intramuscularly. Afterwards, ewes were stimulated with 80 mg of FSHp and 300IU of eCG in a single injection, 36 hours before the procedure. Aspirations were performed by three portal sites videolaparosopy, using a 16G catheter associated to simple lumen aspiration system. Each animal was submitted to nine ovarian punctures with seven-day interval between procedures. One-way ANOVA (parametric and non-parametric) was carried out; comparing data between each session by Tukey test. The number of follicles observed, aspirated and oocytes recovered was 14±2,8, 12±2,9 e 5±2,5, respectively. The recovery rate was 41.66% (aspirated follicles/recovered oocyte). No statistical difference was noticed between nine sessions (P >0.05). In conclusion, nine weeks of superovulation and aspiration procedures did not interfere in follicle production and development, and oocyte recovery in Santa Inês ewes.
**ABSTRACT 154**

**EMBRYO PRODUCTION WITH CANOLA SEED IN THE DIET OF GOATS**

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The increasing concentration of fat in the diet, with values above 3% of dry matter, allows a significant increase in reproductive performance of goats. It is known that the seeds of canola are rich in unsaturated fatty acids, which act on the synthesis and maintenance of cellular membranes and act as precursors of hormones that are involved in biochemical functions essential to reproduction. This experiment was conducted to determine the effect of dietary supplementation with canola seeds in the production and quality of goat embryos. Twelve crossbred goats, weighing 36 kg ± 5.5 were randomly divided into two groups: treated group (canola seeds) and the control group (without canola seeds). We used the cross-over statistical linear general model, by GENMOD SAS, with six replicates per treatment. The animals were fed diets with the same crude protein (CP) and total digestible nutrients (TDN). The goats were overstimulated with FSH and submitted to non-surgical embryos collection. The animals in the untreated group produced 96 structures, an average of 7.83 ± 0.10 structures, and 2.17 ± 0.20, while the treated animals produced 56, 4.58 ± 0.14 structures (p > 0.01). Although the untreated group showed 4, 08 ± 0.14 degenerated embryos while the group treated with canola showed 1.00 ± 0.29 degenerated structures (p < 0.01). The result show increase in the viability in the treatment group. The addition of canola seeds does not increase the embryo production in goats, but promotes a smaller amount of degenerated embryos.

**ABSTRACT 155**

**ULTRA-STRUCTURE OF IN VITRO PRODUCED BOVINE EMBRYOS CULTURED WITH DIFFERENT CONCENTRATIONS OF FORSKOLIN**

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Over the past decades, several studies aimed to establish a satisfactory medium composition to provide in vitro development of bovine embryos. However, in vitro produced (IVP) embryos still present lower response to cryopreservation. In the literature, the reduced cryotolerance of IVP embryos is associated with the high lipid content present in their cytoplasm, and this feature is particularly evident in embryos cultured in presence of fetal calf serum (FCS). Several drugs have been used to produce embryos with lower lipid contents and consequently with greater resistance to cryopreservation. Among those, the use of forskolin, an adenylate cyclase regulator, has been reported to induce an increase in pig embryo lipolysis. Therefore, the present experiment studied the ultra structure of bovine embryo produced in the presence of three concentrations of forskolin (5, 10 and 20 μM), in comparison with embryos produced with 10% FCS and with no FCS. Cumulus oocyte complexes (COCs) were matured in TCM 199 and fertilized with frozen bull semen from a single batch, selected through a Percoll gradient. Part of the presumptive zygotes were cultured in SOF with 1% non-essential amino acids and 1% essential amino acids supplemented with 0.6% BSA (SOFaa), and the other part, in SOFaa supplemented with 10% FCS. Embryos were kept in a humidified atmosphere with 5% CO₂, 5% O₂ and 90% N₂ at 38.5°C. On day 6, embryos were divided into 4 groups: Control – embryos cultured without FCS and without forskolin; Control FCS – embryos cultured in the presence of FCS but without forskolin; F5 – same medium as control FCS but with the addition of 5 μM forskolin; F10 – same medium as control FCS with 20 μM forskolin. Transmission electron microscopy was performed in 6 embryos from each group. The ultra structural analysis showed similar features in embryos from all studied groups. However, embryos cultured in absence of FCS presented lesser and smaller lipid droplets. However, embryos cultured without FCS also presented more cellular debris in the perivitellin space and in the blastocoelic, indicating blastomers degeneration. Independently of the concentration used, the addition of forskolin did not induce any structural changes in the studied embryos. In fact, the use of forskolin was not able to reduce lipid droplets in serum produced embryos. The results indicate that the presence FCS induces an increase in IVP embryo lipid content, and this effect is not reduced by the presence of forskolin. On the other hand, the absence of FCS reduced embryo quality demonstrated by the increase in cellular debris observed. Acknowledgement: FAPESP and CNPq.
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PERCOLL VERSUS MINI OPTIPREP: GRADIENTE EFFECT ON DEVELOPMENTAL RATES AND SEX RATIO FROM IVP BOVINE EMBRYOS

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The objective this study was to compare the use of two different density gradients for sperm selection, Percoll and OptiPrep (Sigma, Aldrich, St. Louis, USA), on embryonic developmental rates and sex ratio of in vitro produced bovine embryos. Cumulus-oocyte complexes (COCs) were aspirated from ovaries collected from slaughterhouse cows, selected and matured for 22 to 24h in TCM 199 25 mM hepes added with FSH/LH, piruvate, FBS and antibiotics. For sperm selection, gradients were prepared in different concentrations, Percoll (P) (90 and 45%, with total volume of 4mL) and Mini OptiPrep (OP) (30, 28 and 26%, with total volume of 1.2mL), from the dilution of these in Sp-TALP medium. In the moment of IVF, the semen was thawed and added on the gradients. The Percoll was centrifuged for 20min at 700xg and the OP for 15min at 900xg, followed by a second centrifugation for 5min at 700xg. The COCs were randomly divided among treatments, P (n=524) and OP (n=375), inseminated and maintained for 18h in IVF medium. Soon after, they were transferred to culture medium (SOFaa with 5% FBS) and incubated for 8 days, at 39°C and 5% CO2. The day of insemination was considered D-0. Cleavage and developmental rates were observed at D-2, D7 and D8, respectively. Embryos of each treatment (P n= 39; OP n=21) were harvested at D8 of culture and prepared for sexing by PCR. A chi-square analysis with significance level of 5% was performed to compare cleavage, developmental rates, sex ratio among treatments and to expected ratio of 50%. No difference were observed (P>0,05) for cleavage (71% P versus 67% OP), developmental rates at D7 (30% P versus 29% OP), developmental rates at D8 (25% P e 30% OP) and global rates (blastocysts/inseminated) at D8 (18% P e 20% OP). The sex ratio observed was similar among treatments (19M:19F P and 11M:09F OP). These results indicated no influence from both treatments and therefore mini OptiPrep is an alternative useful for IVP bovine systems.

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EFFICIENCY OF REPRODUCTIVE TECHNOLOGIES APPLIED TO THE FLAMENGA BREED FOR GENETIC CONSERVATION (PRELIMINARY DATA)

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The Flamenga cattle breed was originated in France, being introduced in Brazil in 1945. Flamenga is a double purpose breed known to render meat and milk of excellent quality. Such features, associated with the good adaptation to the altiplane, favored the establishment of the Flamenga breed in the mountain region of the Santa Catarina State. However, the introduction of more specialized breeds in recent decades caused a gradual loss of interest in the Flamenga breed over the years, resulting in a drastic reduction in the herd size, with only about fifty animals still remaining, all located at the Epagri Experimental Station, in Lages, SC. The high risk of loss imposed on this small genetic pool, along with its importance for biodiversity, justifies the need for conservation of the Flamenga breed. Among other reproductive biotechnologies that could be readily applied for such purpose, the Ovum Pick Up (OPU), used for oocyte retrieval, and superovulation (SOV) for the production of embryos, stand out. However, data regarding the oocyte retrieval efficiency by OPU or the embryo yield by SOV for the Flamenga breed are still lacking. The aim of this study was to evaluate the recovery rate of viable oocytes by OPU and the embryo production efficiency following SOV in the Flamenga breed, comparing with Holstein control counterparts. Eight multiparous females from both breeds were subjected to 17 OPU sessions, at weekly intervals, and to three SOV protocols, in the embryo production efficiency following SOV in the Flamenga breed, comparing with Holstein control counterparts. Eight multiparous females from both breeds were subjected to 17 OPU sessions, at weekly intervals, and to three SOV protocols, in the embryo production efficiency following SOV in the Flamenga breed, comparing with Holstein control counterparts. Eight multiparous females from both breeds were subjected to 17 OPU sessions, at weekly intervals, and to three SOV protocols, in the embryo production efficiency following SOV in the Flamenga breed, comparing with Holstein control counterparts.

No breed differences were observed in embryo yield after SOV, with 9.9 ± 5.8 and 5.0 ± 2.7 viable embryos recovered from Flamengas and Holsteins. A higher number of viable COCs was obtained from Flamenga females than from Holstein cows, demonstrating the breed´s potential for in vitro embryo production for the genetic conservation of the Flamenga breed.
Embriologia, Biologia do Desenvolvimento e Fisiopatologia da Reprodução

Embryology, Developmental Biology and Physiology of Reproduction
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BOVINE EMBRYO PRODUCTION BY IN VITRO FERTILIZATION OR SOMATIC CELL NUCLEAR TRANSFER USING ALTERNATIVE SUBMARINE INCUBATION SYSTEM

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The standardization of the procedures for cattle embryo production by in vitro fertilization (IVF) or somatic cell nuclear transfer (SCNT) allows for educational institutions the generation of research and the training of students according to market demands. However, in many cases structural limitations hinder the access of research groups to such technologies. To universalize the access of IVF and SCNT biotechnologies, this study aimed to determine the viability of a simplified submarine incubation system in comparison to a conventional incubator (39ºC and 5% CO₂ in air). The submarine incubation system (SIS) was made using a thermal box equipped with a digital temperature control thermostat (Aquaterm 8, FullGauge) and a water circulation pump (Vigo AR A100). In this system, water temperature was maintained in 38.8ºC (±0.1ºC) and the atmosphere stabilization of media and incubation was performed using aluminized plastic bags, filled with a gas mixture of 90% N₂, 5% CO₂ and 5% O₂. To IVF embryo production, 509 and 679 oocytes were incubated in conventional incubator and SIS, respectively. Efficiency criteria was based on the blastocyst rate on day 7 of culture (chi-square test, where P = 0.05). No statistical difference was found on blastocyst rates between 15.3% (78/509) and 16.6% (113/679) in conventional incubator (control) and SIS, respectively. Efficiency criteria was based on the blastocyst rate on day 7 of culture (chi-square test, where P = 0.05). No statistical difference was found on blastocyst rates between 15.3% (78/509) and 16.6% (113/679) in conventional incubator (control) and SIS, respectively. Using only the alternative SIS, metaphase II oocytes (in vitro matured) were either electrically activated to develop parthenogenetically (with n = 53, or without zona pellucida n = 48), or used as recipient cytoplasts in SCNT (n = 50) process, by handmade cloning. Cleavage and blastocyst rates were respectively 79.2% and 37.7% for zona enclosed parthenotes, 75.0% and 31.3% for zona free parthenotes, and 80.0% and 20.0% for SCNT. Based on the results, the proposed submarine incubation device allows the obtaining of similar blastocyst rates as does the conventional incubation system. Additionally, the obtaining of SCNT derived embryos reinforces the stability of the proposed incubation system. The proposed device may be considered a suitable alternative to implement the cattle in vitro embryo production biotechnologies. Key Words: Culture system, IVF, SCNT, embryos, incubator.

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EVALUATION OF BODY WEIGHT IN SYRIAN HAMSTER (Mesocricetus auratus) FEMALES AFTER OVARIECTOMY

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Estrogens, in addition to its role in reproductive functions, also exert influence on the energy balance and weight gain. Accelerated weight gain associated with greater food intake after ovariectomy (OV) was reported in rats and in several other species like rhesus monkeys and also in women after menopause. In a previous work, we observed that in hamsters, ninety days after ovariectomy, no difference was observed between the concentrations of fecal estrogens metabolites in females after OV and intact female (IN). Moreover, in this work an increase in aromatase gene expression, an enzyme catalyzing the synthesis of estrogens in various tissues of these females, was observed. Based on these data, the hypothesis of this study was that female Syrian hamsters (Mesocricetus auratus) OV are able to produce non-ovarian estrogens which act in energy metabolism and fat deposition, with consequent weight gain. The aim of this study was to evaluate the body weight of female Syrian hamsters OV and IN. To this, 11 adult females, from the FMVZ-USP vivarium were divided randomly into two groups: OV (n=6) and IN (n=5). The mean body weight was similar in both groups (IN: 105.0 ± 3.5 g; OV: 109.0 ± 9.5, p = 0.7). From the second month after OV, females were weighed monthly. The average weight gains were assessed by ANOVA and the mean weight by the Mann-Whitney test. Females from both groups were still growing and gained weight during the two months following the surgical procedure (OV: 54.4 ± 11.0 g; IN: 39.5 ± 7.4 g, p=0.03), resulting in different weight in both groups. Over the months it was reversed and IN females gained weight during the 4th and 5th months while the weight of OV females started to stabilize and then decrease (5th month: OV: -6.5 ± 5.4 g; IN 0, 38 ± 3.1 g, p=0.02). At the 6th month the OV females continued losing weight (OV: -1.68 ± 2.87 g; IN: -0.64 ± 8.3 g, p=0.805). At the 7th month there was an inversion, IN lost weight and OV stabilized (OV: 0.1 ± 9.1 g; IN: -5.5 ± 6.7 g, p=0.329). At the end of the 7th month there was no difference between the average weights of the two groups (OV: 162.9 ± 17.5 g; IN: 164.9 ± 14.3 g, p=0.2). These results, taken together with equal concentrations of fecal estrogen metabolites in females IN and OV and the increase in aromatase gene expression in some tissues of OV female, suggest an increased synthesis of estrogens in non-ovarian tissues in response to ovariectomy, which would exert compensatory effects similar to those exerted by ovarian estrogens in the female IN on feeding behavior and fat deposition.
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EFFECT OF THE OOCYTE AS A PROMOTING FACTOR IN IVM AND IVC IN CATTLE


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In vitro maturation of oocytes, fertilization and development of blastocysts has been achieved in several species, however the rate of blastocyst development is still limited. According Thompson et al. (2007; Reprod. Fert. And Dev., 19: 43 – 52) this efficiency is limited by the oocyte competence. To improve these results, some research groups have studied the interaction among factors secreted by oocytes and oocyte maturation. The aim of our experiments was to determine the cleavage and blastocyst rates, from two procedures of addition of denuded oocytes (DOS) to IVM droplets medium. The Cumul/oocyte complexes (COC) were recovered by aspiration of 2 to 8 mm follicles using a 18 G needle. Oocytes with a compact Cumulus cells (CC) and homogeneous cytoplasm were selected for maturation and denudation. The COCs were matured in 50 mL drops in TCM199 medium (supplemented with 26 mM NaHCO3, 0.2mM sodium pyruvate, 50 µg gentamicin, 0.5µg de FSHb/mL (Folltropin V, Vetrepharm), 0.03UI hCG/mL (Profasi, Serono) and 10% FCS), under mineral oil at 38.5°C in an humidified atmosphere of 5% CO2 in air. Denuded oocytes were generated by removing CCs from COCs by repeated passage of the oocytes through a firepolished glass pipette in TCM/Holding199 medium (M-2250) supplemented with 2,361 mM NaHCO3 (S-5761), 0.2mM sodium pyruvate(P-2256), 50 µg gentamicin and 0.4% BSA. The COCs remained 9 hours in IVM when they were placed in co-culture, respecting the density of 0.5 DO/µL. COCs were randomly allocated into 2 treatment groups during IVM: group 1, COCs were placed into maturation medium droplets with the were the DOS; group 2, DOS received the COCs into their maturation medium droplet. All groups, experimental and control, remained into IVM medium for 24 hours. Fertilization was performed with frozen-thawed semen, with selected spermatozoa by swim-up, using an insemination dose of 1X106 sperm/mL. After insemination (DI), the oocytes were incubated with sperm for 20 hours into the same atmospheric conditions of IVM. The presumptives zigos were in vitro cultured (IVC) in SCFm supplemented with 1.5 mM glucose (G-6152) and remained into 80 µL droplets under mineral oil at 38.5°C in an humidified atmosphere 5% O2, 5% CO2 e 90% N2. Cleavage rates were evaluated in D1 while the blastocyst rates were evaluated in D7 after insemination. Three replications were realized. The results were analyzed applying the chi-square, for P <0.05. Cleavage rate was higher in G2 82.22% (37/45) when compared to G1 73.07% (38/52) and the GC 68.54% (85/124). However, the rate of development to the blastocyst stage was similar in both experimental groups, G1 32.69% (17/52) and G2 26.66% (12/45), moreover G1 was higher than GC 25.00% (31/124). The tested procedures to put together the COCs and the DOS during MIV were similar to promote further embryo in vitro development to the blastocyst stage.

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OVARIAN FUNCTION AND CIRCULATING HORMONES IN NONLACTATING NELORE VERSUS HOLSTEIN COWS

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The ovarian function and circulating concentrations of reproductive and metabolic hormones were compared between Bos taurus and Bos indicus cows under similar environmental and nutritional conditions. Nelore (n=12) and Holstein (n=12) nonlactating cows were kept in individual stalls, receiving a maintenance diet according to NRC (2000). The BCS and BW were kept at 3.1±0.1 and 2.8±0.2 (scale 1 to 5) and 508±17 and 575±20 kg for Nelore and Holstein, respectively throughout the experiment. After 21 days of adaptation, estrus was synchronized and the cows were monitored daily by ovarian ultrasonography using a 7.5 MHz linear transducer for an interovulatory interval. During the first follicular wave, ultrasonography was performed twice a day. Blood samples were collected once a day for hormone assays. Results were analyzed using the Mixed procedure of SAS (P<0.05) and are presented as least squares means ± SE. It was observed that 80% of Nelore cows and 60% of Holstein cows had three follicular waves during the estrous cycle. All the others cows presented two waves. Moreover, the average length of the estrus cycle was 23 days (range 21 to 26) and it was similar between Nelore and Holsteins. Substantial differences in the number of antral follicles were observed between Nelore and Holstein cows. At wave emergence, the number of 2 to 5 mm follicles present in the ovaries was 42.7±5.9 (ranging from 25 to 100) in Nelore and 19.7±3.2 (ranging from 5 to 40) in Holstein cows. Follicle deviation occurred, on average, 2.3 days after ovulation, independent of breed, when the diameter of the greatest follicle reached 7.0±0.2 and 8.9±0.4 mm in Nelore and Holstein cows, respectively. The maximum diameter of the ovariolyte follicle (15.7±0.3 vs 13.4±0.3 mm) as well as the CL volume (7611±512 vs 4917±548 mm3) were greater in Bos taurus than in Bos indicus. However, plasma progesterone concentrations on D7 (2.8±0.16 vs 2.0±0.04 ng/mL) and on D14 (4.6±0.40 vs 4.1±0.18 ng/mL) of the estrous cycle and the preovulatory estradiol peak (12.7±0.98 vs 7.7±0.67 pg/mL), as well as pre feeding plasma insulin concentrations (9.9±1.51 vs. 3.0±0.70 ìIU/mL, P<0.01) were higher in Zebu than in European females. Furthermore, pre feeding plasma insulin concentrations were higher in the follicular phase as compared to the luteal phase in Nelore (11.9±2.05 vs 8.3±1.47 µIU/mL) as well as in Holstein (4.2±1.05 vs 2.2±0.64 µIU/mL) cows. Therefore, it was shown that even under similar environmental and nutritional conditions, there are substantial differences in ovarian function and circulating hormones between Bos indicus and Bos taurus cattle.
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STUDY OF GABAERGIC SYSTEM ROLE ON GH SECRETION IN NELLORE HEIFERS

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The aim of this study was to investigate the growth hormone (GH) secretion regulation mediated by GABA in prepuberal Nellore heifers. Eight Nelore heifers (Bos taurus indicus) with 14 months of age were divided in two groups: treated group (n = 5), received GABA antagonist (picrotoxin, Sigma-Aldrich CO., St. Louis, MO, U.S., 0.18 mg / kg, IV) and control group (n = 3) received saline 0.9% (IV). GH concentration was evaluated in plasma samples collected 1 h before and every 15 min for 10 h after drug or saline infusion. Hormonal quantification was performed by radioimmunoassay and peaks, secretion area, peak secretion frequency and amplitude, were identified by GrafPad Prism Comparison between groups was performed using analysis of variance (ANOVA, Instat), data were transformed into log to meet the variances homogeneity. There was no difference (p = 0.2601) of GH mean concentration in the post-treatment period between the treated group (5.53 ± 2.90 ng/mL) and control (3.67 ± 1.17 ng/mL). When GH total area secretion average (P = 0.1470; 1446.68 ± 600.86 ng / mL x min, 870.86 ± 410.70 ng / mL x min) number of peaks (p = 0.5353, 2.80 ± 0.84, 3.33 ± 1.16), the largest peak area (p = 0.0696; 353.46 ± 176.24 ng/mL x min, 144.50 ± 101.65 ng/mL x min) and time needed to stimulate the highest peak (p = 0.9141; 336.00 ± 217.27 min, 230.00 ± 191.12 min), were compared between groups there were no significant difference. There was significant difference on peak maximum amplitude (p = 0.0198; 16.82 ± 7.87 ng/mL; 6.03 ± 1.93 ng/mL) and total area of peaks (p = 0.0478; 643.78 ±283.02 (ng/mL)x min; 272.40 ±216.02(ng/mL)x min) between the treated group and control group. Therefore, it was possible to conclude that reducing GABA action with picrotoxin (0.18 mg/kg, IV) in heifers at 14 months of age increased maximum amplitude and total area of peak GH secretion, and that the effect of GABA on GH secretion, may be related to variation in the type of receptor activated, age or dose of picrotoxin used. Acknowledgments: FAPESP # 2008/56680-1.

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EFFECT OF SEASONS ON CELLULAR CHANGES OF OOCYTES AND EMBRYOS OF SHEEP


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This study aimed to determine the effect of seasons on nuclear maturation in vitro and inductions of cell death by apoptosis in sheep oocytes as well as the effect on the ability of develop from oocytes in vitro sheep embryos. The ewes ovaries during the dry season (October to March) and rainy season (April-September) were collected in a slaughterhouse and transported to the Laboratory of Biotechnical Reproduction of UFRPE. The cumulus oophorus complexes (COCs) were collected by the technique of “slicing” of the follicles from 2 to 6 mm in diameter and selected based on morphology. In this stage were performed 12 repetitions, where the COCs were subjected to maturation, and in vitro fertilization. The percentage of cleaved oocytes was determined on day 3 (D-3) and the oocytes that developed to the stages of 8-16 cells (D-4), morula (D-5) and blastocyst (D-8) after fertilization. The blastocysts quality was assessed with the pigment DAPI and the determination of blastomeres positive for apoptosis and DNA fragmentation by TUNEL test. For statistical analysis was performed the compare variances, F test for variances to the 5% significance level (P <0.05). Then, a t test to compare means at significance level 5%. It was found significant difference (P <0.05) during D-3 and D-4 fertilization stages. No significant difference (P> 0.05) was observed on morula and blastocyst in vitro maturation phases and DNA fragmentation (TUNEL). In the stages of nuclear maturation stages of germinal vesicle, metaphase I, telophase I and metaphase II showed no significant difference (P> 0.05). Significant difference (P <0.05) in enzyme activity of caspases in vitro matured oocytes. The DNA fragmentation was not significantly different (P> 0.05). Under the conditions observed in this study, the results indicate that there was no effect of season on in vitro nuclear maturation and apoptosis of oocytes as well as on the development of oocytes and in vitro production of sheep embryos.
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INTRA-CITOPLASMATIC SPERM INJECTION (ICSI) OPTIMIZING THE IN VITRO EMBRYO PRODUCTION IN BOVINE EMBRYOS

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The in vitro production (IVP) of embryos is influenced by the oocytes and semen quality. However, the use of oocytes with low quality and inadequate semen is frequently imposed, being necessary to find new strategies to minimize such negative effects. This study evaluated the sperm penetrating capacity of two bulls, by the technique of sub-zonal sperm injection(SÜZI; experiment 1), and compared the embryos IVP from the two bulls by IVF (experiment2) and ICSI (experiment 3), using oocytes of quality 1 and 2 (good) or 3 and 4 (regular). On experiment 2 and 3 it has been evaluated the blastocyst rate (day 7). Data from all the experiments were submitted to the chi-square test, with 5% of significance. The oocytes where recovered from slaughterhouse ovaries, selected and distributed among the experimental groups, based on the oocyte quality. The IVP steps were performed according to Vieira et al. (2007, Anim Reprod Sci, 99:377-383), being the in vitro culture after D2 performed in lung air atmosphere. On experiment 1, each regular oocyte was microinjected with sperm from one of two bulls, and penetration rate observed 3h after the injection. The penetration rate from bull 1 (29.6%; 34/115) was lower than the bull 2 (53.8%; 64/119). On experiment 2 (5 replications) oocytes were allocated into 4 groups: On group G1 (n = 96) it was done IVF with bull 1 and good oocyte. On G2 (n = 173), IVF with bull 1 and regular oocytes. On G3 (n = 140), IVF with bull 2 and good oocytes. On G4 (n = 152), IVF with bull 2 and regular oocytes. The highest blastocyst rate was obtained on G3 (25.7%), being also similar to G1 (19.9%). The groups G2 (12.7%) and G4 (9.2%) were similar between them, and also similar to G1. When the oocyte quality was not considered, there was no observed difference in the blastocyst rate with this oocytes (23.3%) in comparison to regular oocytes (11.1%). On experiment 3 (3 replications), both the bulls were used for ICSI with good or regular oocytes, according to the following experimental groups: group G1 (n = 30) ICSI bull 1, good oocytes; group G2 (n = 35) ICSI bull 1, regular oocytes; group G3 (n = 30) ICSI bull 2, good oocytes and group G4 (n = 32) ICSI bull 2 and regular oocytes. When ICSI was employed, no bull or oocyte quality effect was observed, being blastocyst rates 26.7% for G1, 22.9% for G2, 30.0% for G3 and 21.0% for G4. The results allow to conclude that the oocyte quality exerts high influence on embryo production by IVF, and that ICSI is an adequate alternative to overcoming the limitations imposed by low quality oocytes. Key words: ICSI, fertilization, oocyte quality.

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EFFECTS OF ADDING RUMEN-PROTECTED FAT (MEGALAC® AND MEGALAC-E®) ON SERUM CONCENTRATION OF PROGESTERONE AND INSULIN OF DAIRY COWS

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Fat supplementation is associated with an increase in energy balance (Staples, 1998, J. Dairy Sci, 81:856-871), which can result in a bigger CI, with a higher progesterone (P4) production (Vasconcelos, 2001, Theriogenology, 56:307-14). (Castaneda, 2007, J. Dairy Sci, 90:4253-4264) showed that greater concentrations of CLA related to greater P4. Another proposed mechanism would be the decrease the capacity of P4 clearance the by liver, by increase fat acids in the diets (Sangsritavong, 2002, J. Dairy Sci, 85:2831-2842). Two experiments were conducted to evaluate the effects of adding rumen-protected fat with different concentrations of linoleic acid (LA) on serum concentrations of progesterone (P4) and insulin of dairy cows. In Exp. 1, non-lactating, non-pregnant, ovariecctomized Gir x Holstein cows were stratified by Body Weight (BW) in a Latin square 3 × 3 (3 different concentrations of linoleic acid for serum concentrations of progesterone (P4) and insulin of dairy cows. In Exp. 1, non-lactating, non-pregnant, ovariecctomized Gir x Holstein cows were stratified by Body Weight (BW) in a Latin square 3 × 3 (3 experimental periods of 7 d each) to receive 1 of 3 dietary treatments: 4.5 kg/d of concentrate (Control, CO); CO + 0.220 kg/d of finely corn + 0.220 kg/d of inert substance (Control, CO); CO + 0.220 kg/d of Megalac® (MG) or 0.220 kg/d of Megalac-E® (ME) for 14 d. Blood samples were taken similarly to Exp 1. Dietary treatments did not affect (P = 0.47; SEM = 1.45) insulin among dietary treatments. Cows having insulin > 11.14 ng/mL (median) had lesser (P < 0.01) P4 than those with insulin < 11.14 ng/mL (1.25 vs 1.29 ± 0.52 ng/mL). In Exp. 2, 28 multiparous cows and 17 heifers (pregnants e nonlactating) were stratified by BW, Body Conditional Score (BCS) and pregnant days to receive a corn silage-based diet added with 0.500 kg/d of finely corn + 0.220 kg/d of inert substance (Control, CO), 0.220 kg/d of Megalac® (MG) or 0.220 kg/d of Megalac-E® (ME) for 14 d. Blood samples were taken similarly to Exp 1. Dietary treatments did not affect (P = 0.65; SEM = 0.20) concentrations of P4 of multiparous cows. However, ME heifers had greater (P = 0.03) concentrations of P4 than those fed CO (7.58 vs. 6.43 ± 0.33 ng/mL), but similar (P = 0.77) P4 compared with MG heifers (7.58 vs. 7.43 ± 0.33 ng/mL). In conclusion, on Exp. 1 diets with greater linoleic acid no resulted in greater P4, no being observed effect on decrease in P4 clearance. On Exp. 2 adding ME resulted in greater P4 in heifers, no observed the same effect in multiparous.
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SPERM PRE-CAPACITATION AND IN-VITRO FERTILIZATION PERIOD INFLUENCES THE PORCINE EMBRYO PRODUCTION

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The porcine embryo in vitro production (IVP) has low efficiency when compared to other species, such as bovine and ovine. This fact might be due to the inconstancy of results, as well as a lack of consensus about which methodology is better in each step. Then, the aim of this study was to evaluate different pre-capacitation and in vitro fertilization periods, in the porcine IVP. A total of 754 gilts oocytes classified as quality 1 and 2, derived from slaughterhouse ovaries were in vitro matured (IVM) for 44h in TCM-199. Oocytes were allocated into 4 treatments, according to different pre-capacitation and IVF periods: G1 (n=189) 1h of pre-capacitation and 3h of IVF; G2 (n=185) 1h of pre-capacitation and 6h of IVF; G3 (n=195) 2h of pre-capacitation and 3h of IVF; G4 (n=185) 2h of pre-capacitation and 6h of IVF. Pre-capacitation was performed in mTBM without caffeine, in an incubator at 38.5°C, and IVF performed in mTBMMert, added of 2mM of caffeine. The IVM and IVF were also performed in incubator at 38.5°C, 5% CO2 and 95% of humidity in air. The in vitro culture (IVC) was performed in NCSU-23 medium, with 90% N2, 5% CO2 and 5% O2 atmospheric. Embryo development was evaluated on morulae production on day 7 basis, and data analyzed by the chi-square test with 5% of significance level. Morulae rate on G3 (18.5%) was higher than G1 (9.0%). Groups G2 (13.5%) and G4 (15.1%) were similar, and did not differ from the other groups. When only the pre-capacitation period was considered, independently of the IVF period, pre-capacitation for 1 h (G1+G2) resulted in significantly lower morulae production (11.2%) in comparison to the pre-capacitation period of 2 h (G3+G4, 16.8%). Nonetheless, when only the IVF period was considered, no difference was showed in morulae yield with 3 h (13.7%, G1+G3), in comparison to 6 h of IVF (14.3%, G2+G4). Data allow to deduce that a longer sperm pre-capacitation period ensure higher morulae yield, that is, however, dependent on the IVF period. New studies should be conducted to optimize each step of the porcine IVP. Key words: oocyte, porcine, capacitation, IVP, fertilization.

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BOVINE AND SWINE FOLLICULAR FLUID LIPID PROFILE BY MASS SPECTROMETRY: NEW INSIGHTS INTO FOLLICULAR MICROENVIRONMENT

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The study of the composition of the physiological follicular environment is important for the improvement of in vitro maturation (IVM) and in vitro production (IVP) of embryos in domestic species. Bovine and swine embryos are known to have different needs, so IVF systems are peculiar for each species. The aim of the present work was to characterize the lipid composition of bovine and swine follicular fluid (FF) in a fast and broad manner by means of the mass spectrometry technique (MS). The contents of small (< 6mm) and large (> 6mm) follicles were aspirated separately from bovine and swine ovaries collected at a commercial abattoir. The lipid extract was obtained by the Bligh & Dyer extraction protocol using 300µL FF. For analysis, each sample of lipid extract was diluted in 1.0 mL methanol with 0.1% formic acid. The samples were sodiated (addition of 2 µL saturated NaCl solution) and introduced by a syringe-pump at a 5µL/min flux. A mass spectrometer (Bruker HCT Ultra) equipped with an electrospray source (ESI) was used and operated in positive mode; a range of 100-1200m/z was used. Protonated and sodiated lipid species were detected and corresponded to lipids of phosphatic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingomyelin (SM) and triacylglycerol (TAG) classes, saturated and with one to four unsaturations. Large bovine follicles and small bovine and swine follicles displayed a lipid profile with more intense ions below 700 m/z. Large swine follicles presented more intense ions atm/z values corresponding to phospholipids of 34, 36 and 38 carbons, saturated or presenting up to 4 unsaturations, as well as the presence of TAG (such as 853.7 and 881.7m/z). The attribution of the lipid species will be confirmed by fragmentation experiments (MS/MS). In conclusion, obtaining the lipid profile by MS provides fast (direct injection of lipid extract) information on the structural lipid composition of FF. The structural characterization of the lipid species present and the analysis in negative mode will contribute decisively to the establishment of IVM conditions which are more adequate according to the ideal lipid environment for bovine and swine oocytes. Financial support: FAPESP and CNPq.

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MORPHOLOGIC AND MORPHOMETRIC ASPECTS OF THE CERVIX OF MONGREL GOATS NATIVE FROM THE STATE OF PIAUÍ

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Caprine breeding in the Northeast region of Brazil is primarily of mongrel animals and responds for 93.8% of the Brazilian herd. The result of artificial transcervical insemination (ATCI) in goats depends on semen deposition site and requires anatomical cervix understanding. This study aimed at studying cervical morphology and morphometry of 30 mongrel goats native from the state of Piauí, collected in slaughterhouses from Teresina – PI. Length and width were measured with gauged caliper square (CALIER®) and diameter was measured with a suture cotton thread. Fragments of 0.5 cm were obtained from cranial, medial and caudal portions and cervical rings from three cervix, immediately after slaughter. Fragments were put in 10% formaldehyde solution, were buffered and submitted to histological routine and dying with HE. In average, cervix measures 5.25 cm length, 1.47 cm width and 4.82 cm diameter. It has 7.57 tapered rings with minimum and maximum values of 5 and 9 rings, respectively; the first is located close to the external uterine ostium and the last forms a protrusion in the body of uterus. So, 3.3% of goats presented with five rings, 20% with six rings, 16.7% with seven rings, 36.7% with eight rings and 23.3% with nine rings. Average internal ring length is 0.66 cm for the first, 0.79 cm for the second, 0.75 cm for the third, 0.78 cm for the fourth, 0.74 cm for the fifth, 0.73 cm for the sixth, 0.79 cm for the seventh, 0.75 cm for the eighth and 0.79 cm for the ninth. Histologically, cervix has the mucosa with irregular projections to the lumen forming ring plicae, ciliated pseudo-stratified epithelium and mucous-secreting cells, and a lamina propria made of connective tissue. Submucosa has a large amount of mucous glands made up of mucous acinus and two thick muscle layers: one internal and circular with transversal fiber bundles at longitudinal cross-section and the other external and longitudinal with longitudinal fiber bundles and the serous layer made up of simple pavimentous epithelial tissue anchored on dense connective tissue with abundant blood vessels. Results allow concluding that the cervix of mongrel goats has morphometric differences as compared to other breeds, suggesting the presence of adaptive morphologic changes, the understanding of which may contribute to the development of transcervical AI programs and commercial equipment.

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USE OF PHYTOHEMAGLUTININ-L ON CHIMERISM BY AGGREGATION OF IN VITRO PRODUCED BOVINE EMBRYOS

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One of the techniques used to obtain embryonic chimeras is aggregation, which can be performed with whole or demi-embryos, in different stages of the development, produced by in vivo or in vitro systems and in different wells. However, its efficiency tends to be reduced when advanced stages (e.g., morulae and blastocysts) are used. The aim of this work was to evaluate the effect of the treatment with an agglutinating agent (phytohemaglutinin-L; PHA) in the percentage of chimeras produced with IVF bovine embryos. Bovine ovaries (from abattoir) were used to obtain 445 cumulus-oocyte-complexes (COC; quality I and II). COCs were matured in drops of 90µL of TCM-199 Bicarbonate supplemented with 10% of FCS and incubated in vitro for 22 to 24h. The fertilization occurred in TALP-IVF medium, and the COCs were maintained in the incubator for 18h.

After fertilization, the presumptive zygotes were transferred to SOF medium to in vitro culture. Incubation conditions were 38.5°C and 5% CO2 in air. The aggregation was performed between two whole (zona free) 8 to 16 cells stage embryos in the presence of PHA (G4, n=29) or absence of PHA (G2, n=34) and between demi-morula and demi-blastocyst with (G3, n=28) or without PHA (G4, n=29). The embryos were treated with PHA in a concentration of 500µg/mL for 3 min. The percentages of aggregation among the four experimental groups and the main effects were analyzed by Chi-square or Fisher’s exact test and significance was considered when P<0.05. The rate of embryo aggregation was higher in the group G1 than G2 (75.0 and 50.0%, respectively; P=0.045). The rate of aggregation of demi-embryos was similar, regardless the use (G3, 39.3%) or no use of PHA (G4, 20.7%; P=0.16). The presence of PHA in G3 increased the rate of aggregation on those demi-embryos to similar rate observed for control group G2 (39.3 and 50.0%, respectively; P=0.45), however, the presence of PHA also significantly increased the aggregation rate of the whole embryos pre-compaction (G1) when compared to G3 (75.0 and 39.3%, respectively; P<0.01). The use of PHA resulted in higher rates of aggregation (58.3%) than non-use (36.5%; P=0.03), while the embryonic stage of development pre-compaction (G1+G2) produced a higher rate of aggregation (62.1%) than post-compaction demi-embryos (G3+G4, 29.8%; P<0.001). We could infer a positive effect of PHA on the aggregation rate of bovine IVP embryos, regardless the stage of embryonic development and its integrity. Financial support: FAPESP, Brazil.
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EMBRYONIC LOSS (BETWEEN 30 AND 60 DAYS) FOLLOWED TO ARTIFICIAL INSEMINATION OR EMBRYO TRANSFER IN HIGH PRODUCTION FRIESIAN DAIRY CATTLE

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This retrospective study evaluated the embryonic loss, between 30 and 60 days of pregnancy, in high production Holstein-friesian cows, submitted to artificial insemination (AI) or embryo transfer (ET) during the different weather seasons of the year. The data was collected in the Santa Rita Agrindus S/A dairy farm, and contains 5040 AI and 2109 ET in high production cows (29.4 ±0.6 kg/day) during the year of 2004 through 2008. The cows were submitted to detection of estrus (EO) following AI 12h after the EO or ET 7 days after EO. The in vivo embryos were produced by multiple ovulation technique, and the produced embryos were transferred fresh or frozen-thawed. All the embryo donors were high production cows of the same herd. The pregnancy diagnosis was performed by ultrasonography, at day 30 and 60 of pregnancy. Statistical analysis was performed by the GLIMMIX procedure of the SAS statistical program, using logistical regression. No interaction (P= 0.55) between the procedure (AI or ET) and the season (summer or winter) was found. However, Holstein cows submitted to ET [20.5% (432/2109)] have more embryonic loss than that cows submitted to AI [(17.3% (870/5040)); P= 0.001]. Also, females submitted to AI or ET during the summer-spring season have more embryonic loss than during the winter-autumn season [20.3% (593/2916) vs. 16.8% (709/4234); P= 0.002]. There was no difference [AI (P= 0.62) and ET (P= 0.55)] between repeat breeder cows (e≥ 4 services) and cows with < 4 services, on the embryonic loss [AI – 17.9% (198/1107) for repeat breeders and 17.1% (67/346) for < 4 services; ET – 20.7% (365/1763) for repeat breeders and 19.4% (67/346) for < 4 services]. In conclusion, embryonic loss between 30 and 60 days of pregnancy is higher in dairy cows receiving an embryo (ET) 7 days after estrus detection. Also, embryonic loss is increased during months of the year with higher humidity and temperature (summer-spring). Furthermore, repeat breeder cows presented similar embryonic losses compared to cows with < 4 services. Acknowledgements: Santa Rita Farm.

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EVALUATION OF BVDV EFFECT IN CUMULUS CELL EXPANSION OF BOVINE OOCYTES DURING IVM

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Bovine viral diarrhea virus (BVDV) is broadly distributed among the world's cattle population. Fluids, gametes and somatic cells from infected animals are likely contaminated with the virus (Gard et al., 2007 Theriogenology 434-442). The aim of the present study was to evaluate the BVDV effect in cumulus cells expansion of bovine oocytes during in vitro maturation (IVM). Bovine ovaries were collected immediately after slaughter at a commercial abattoir, and washed with modified phosphate-buffered saline (PBSm). Cumuli–oocyte complexes (COCs) were aspirated from ovarian follicles 2–8 mm in diameter. Selected oocytes surrounded by multilayers of compact follicular cells were split in two groups, control (C) and experimentally infected during maturation time (V). The V-group received 10 µL of BVDV suspension (NADL, titre 104–105 TCID50/mL) and the C group 10 µL of culture media (DMEM). Both groups were matured in 100 µL of in vitro maturation (IVM) medium, with approximately 15 oocytes per drop, at 38.5°C and 5%CO2 in air with 100% humidity for 24 h. After IVM COCs maximum (dmax) and minimum (dmin) diameter were measured (100X) using an ocular micrometr in an inverted microscope, for cumulus cells (CC) expansion evaluation (Carl Zeiss, Axiovert 135, Germany) and their area was estimated (COC area mm² = pxdminxdmax/(dmin) diameter were measured (100X) using an ocular micrometrum in an inverted microscope, for cumulus cells (CC) expansion evaluation (Carl Zeiss, Axiovert 135, Germany) and their area was estimated (COC area mm² = pxdminxdmax/4x10⁶). Afterwards, the COCs have their CC mechanically removed to assess maturation rate (tIVM) by polar body extrusion (45X). The CC removed were cultured (37.5°C, 5%CO2) for citopathic effect (CPE) observation as an infection positive control of CC cells by BVDV. The data were analyzed by comparison of the mean area (µ) of C and V groups, using a hipothesis test (t=2.84), our data suggest that both groups showed different CC expansion patterns, beeing that C group displayed a more homogeneus (s² 0.19) expansion pattern than V group (s² 0.35), despite the lower µ value (µC=0.32 mm²; µV=0.36 mm²). Maturation rates for C and V groups were 68.32% (69/101) and 48.35% (44/91), respectively. The observed CPE after 48 h culture confirmed BVDV infection in CCs. Thus, our findings suggested that the BVDV presence during IVM infected CC and this affects their expansion pattern, which may be related to the observed reduced maturation rate.
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ADDITION OF LYOPHILIZED EQUINE SEMINAL PLASMA ON SEMEN EXTENDER REDUCES THE MOTILITY DEGRADATION RATE OF SHEEP FROZEN SEMEN

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The low pregnancy rates usually obtained in sheep after cervical AI with frozen semen are commonly linked to the increased sperm sensitivity to cryopreservation in this species. As an alternative, the addition of ram seminal plasma (PS) to thawed semen was proven effective in protecting and reversing much of the cellular damage caused by the cryopreservation process. However, the use of ram PS is limited by the reduced volume of the ram’s ejaculate and by sanitary constraints. The aim of this study was to evaluate the effect of the addition of lyophilized equine seminal plasma (PSEL) to the semen extender for ram semen freezing. The equine PS obtained from a pool of ejaculates from five fertile stallions was lyophilized and evaluated for its protein content. Four pools of ejaculates from six Dorper rams were diluted in a 1:1 ratio with Tris-Yolk-Glycerol medium (TG), according to the following treatments: (Control) TG with no PSEL; (PSEL-300) TG+PSEL at 300 µg/mL; (PSEL-600) TG+PSEL at 600 µg/mL; and (PSEL-1200) TG+PSEL at 1200 µg/mL. After freezing the semen batches, three straws from each treatment were thawed and submitted to TTR for 6 h, with assessment by the CASA system equipped with the software Sperm Class Analyser® (SCA®, Microptic SL, Barcelona, Spain) at times 0, 2, 4, and 6 h for the following parameters: a) Total Motility (MT), b) Progressive Motility (MP), c) sperm viability by supra-vital staining with Eosin-Nigrosine (EN), and d) plasma membrane integrity by the hyposmotic swelling test (HOST). The degradation rate of total motility (TDMT) was calculated by the formula TDMT=(initial motility-end motility) x100/initial motility. Data were analyzed by GLM (Minitab, State College, USA), for P<0.05. The MP in the group PSEL-600 was higher than in the control group (P<0.05) at the end of TTR. All parameters deteriorated after 2 h of incubation, being different between times 2, 4 and 6 h (P<0.05). During the TTR, the MT (45.8%, 36.2%, 26.1%, 18.2%) and MP (16.5%, 14.6%, 11.1%, 4.8%) in the PSEL-600 group remained higher than the MT (49.8%, 28.2%, 14.6%, 8.7%) and MP (16.8%, 8.6%, 3.9%, 0.7%) in the control group. This was reflected in a smaller TDMT in the group PSEL-600 (59.2%) than in the control group (84.5%), with no differences seen in groups PSEL-300 (73.5%) and PSEL-1200 (67.3%). The improvement in the sperm motility parameters during the TTR evaluations by the addition of 600 µg/mL PSEL to the extender, also observed by the reduction in TDMT, suggests a potential beneficial effect of PSEL that ultimately increased the sperm cell viability to cryopreservation.

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SODIC CLOPROSTENOL AT DIFFERENT POSTPARTUM PERIODS IN DAIRY COWS

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Reproductive activity in postpartum is dependent on uterine involution and resumption of ovarian cyclicity. As the delayed involution may affect ovarian activity, stimulation of uterine contractility through the use of analogs of prostaglandin F2α (PGF2α) can improve the reproductive performance of dairy cows. This study aimed to determine the best time to perform the treatment with PGF2α in the postpartum period. Postpartum Holstein cows (eutocic), housed in three properties (Minas Gerais, Brazil) were randomly assigned to three treatments: placebo (n = 124), two doses (1 and 4 days postpartum) of 0.530 mg D + L-cloprostenol (Ciosin® - Intervet Schering Plough - Brazil) and PGF2α - third week (n = 125), two doses (14 and 17 days postpartum) of 0.530 mg of D + L - Cloprostenol. The variables were: interval from calving to first estrus, days open and number of services per conception (Tukey test, P<0.05). The average number of days between calving and first estrus was 53.58 ± 15.56a, 37.12 ± 17.05b and 42.34 ± 18.31c (P<0.05); the mean number of days open was 149.34 ± 26.54a, 123.38 ± 28.8b and 135.23 ± 30.12c (P<0.05) and the average number of services per conception was 3.32 ± 1.03, 2.98 ± 0.97, 3.15 ± 1.10 (P>0.05) in the placebo groups; PGF2α - first week and PGF2α - third week, respectively. Although no significant differences in the number of services per conception, the results of the interval from calving to first estrus and days open support the relationship between uterine involution and resumption of postpartum ovarian activity with the use of PGF2α. Based on the results it is concluded that the application of PGF2α in the first week postpartum reduces the period from calving to first estrus and days open.
QUANTIFICATION OF TRANSCRIPTS IN OOCYTES EXPOSED TO BMP4 DURING IN VITRO MATURATION

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Bone Morphogenetic Proteins (BMPs) are implicated in many aspects of follicular growth and generation of a competent matured oocyte in many species. In bovine, BMP4 is expressed in theca cells. Its receptors are found in granulose cells as well as in the oocyte itself. Noggin is a potent inhibitor that binds BMP4 directly avoiding interaction with receptors and is critical for proper regulation of BMP signaling. In this work, we have studied the effects of BMP4 and Noggin on mRNA expression of several genes during in vitro maturation of bovine oocytes. COCs were aspirated from abattoir ovaries and randomly assigned to one of the maturation treatments: TCM containing 0.6 % BSA, 2 mM FSH, 2 mM cysteamine, 1% penicillin-streptomycin and 1% sodium pyruvate or supplemented with 100 ng/ml of BMP4 or Noggin in a 5% CO2 humidified atmosphere at 39°C. After 24 h, Cumulus cells were removed with Hyaluronidase by agitation in vortex. To compare the relative quantity of ZAR1, GDF9, BCl2, Bax, Mater and Hsp70 transcripts, matured oocytes were selected by the presence of first polar body and kept in RNALater®(Ambion, CA, USA) at -20°C until RNA extraction. Total RNA extraction was performed using Rneasy Microkit (Qiagen, Valencia, EUA) protocol. Reverse transcriptions were done with total RNA from 10 oocytes per replicate, 3 replicates per group, using Superscript™ III (Invitrogen, Carlsbad, EUA) first strand synthesis kit. Relative Quantification was performed in triplicate using Real time PCR. PCR Reactions were performed using a mixture of iTaqTM SYBRGreen Supermix with ROX (Bio-rad, Hercules, EYUA), cDNA and gene specific primers. Expression of the GAPDH and B Actin genes were used as endogenous references. Calculations of relative quantification were performed by the comparative Ct method, analyzed with the REST 2008 program version 2.0.7 (Pfaffl et al. 2002. Nucleic Acid Res 30:26-36). No differences in the quantity of analyzed transcripts were found between groups. So we conclude that our maturation treatments did not affect directly de novo synthesis or degradation of mRNA of genes related to oocyte quality (GDF9), Apoptosis (Bax, Bcl2), Oocyte developmental competence (Zar1, Mater) and stress (Hsp70). Future studies are needed to determine effects of BMP4 and Noggin on expression of other genes.

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EFFECTS OF BODY WEIGHT LOSS ON SERUM PROGESTERONE CONCENTRATIONS OF NON-LACTATING DAIRY COWS

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Negative energy balance is associated with reduced reproductive performance of postpartum lactating dairy cows (Lucy, 2001, J Dairy Sci, 84:1277-1293). Substantial quantities of progesterone (P4) can be stored in adipose tissues of cows, and released into the bloodstream if these tissues are mobilized (Hamidukwanda et al., 1996, Anim Reprod Sci, 43:15–23). However, elevated P4 before first postpartum ovulation can impair GnRH secretion and thus prevent the LH peak, potentially leading to failed ovulation and formation of ovarian cysts (Hatler et al., 2003, Biol Reprod, 69:218-223). The objective of this study was to evaluate serum concentrations of nonesterified fatty acids (NEFA), cortisol, insulin, and progesterone (P4) of dairy cows maintaining or mobilizing body weight (BW). Eleven non-lactating, non-pregnant, and ovariectomized Gir x Holstein cows were stratified by BW and body condition score (BCS), and randomly assigned to one of two treatments on d -7 of the study: 1) BW loss (6 cows; LOSS) and 2) BW maintenance (5 cows; MAINT). Treatments were achieved through a grazing schedule utilizing 3 pastures (A, B and C). From d -7 to 1 of the study, all cows were maintained in pasture A (12 kg of DM/cow/day). From d 2 to 30, LOSS were maintained in pasture B (less than 1.0 kg of DM/cow/day), whereas MAINT were maintained in pasture C (12 kg of DM/cow/day). However, from d 3 to 30, cows from both treatments were re-grouped daily into pasture A from 0600 to 1200 h to allow LOSS cows to consume, on average, 4.5 kg/d of forage DM. From d-66 to 3, all cows were inserted with an intravaginal drug releasing device containing 1.9 g of P4 to promote P4 uptake by adipose tissues, which was replaced every 14 d and removed on d 3. Cow BW and BCS were assessed on d 0 and 30. Blood samples were collected daily, from d 0 to 30, at 0600 and 1200 h, and harvested for serum. The GLM procedure was utilized to determine effects of treatment and collection hour on correlation coefficients. Changes in BW and BCS were greater in LOSS compared to MAINT (-0.9S vs. -0.07 kg of BW/d, SEM = 0.216; -0.30 vs. 0.00 of BCS change, SEM = 0.092). Within samples collected at 0600 h, serum NEFA concentrations were greater in LOSS compared to MAINT after d 14. Serum P4 concentrations were greater in LOSS compared to MAINT from d 16 to 24. Serum cortisol concentrations were greater for LOSS compared to MAINT, but only on d 6, 28, and 29. In conclusion, data from this study indicate that BW loss increases circulating concentrations of P4 in non-lactating ovariectomized dairy cows, and this outcome might be mainly attributed to fat mobilization and consequent release of P4 stored in adipose tissues.
The in vitro chimeras development from aggregation of different embryonic cell masses, producing just one individual with two or more cell lines is a technique that can be used to improve mammalian clone production. Thus, this experiment was designed to evaluate the efficiency of Mus domesticus domesticus embryo chimeras production by in vitro culture (IVC) using the Well-of-the-Well (WOW) modified (Feltrin et al., 2006; Reprod Fertil Develop, 18:126) system. Mice females, strain CF1 Swiss Albino were induced to superovulation by intraperitoneal application of 10IU eCG and 46 hours later 10IU of hCG, and finally mated with fertile males. Forty-eight hours after observation of vaginal plugs the donors were sacrificed and the embryo collection was performed by flushing oviducts with PBSm plus 0.4% BSA solution, under stereomicroscope (15X). Four, 8- and 16-cells embryos were evaluated and classified according to morphology and divided aleatory into one control group and two experimental groups. The embryos of experimental groups were first exposed to PBSm supplemented with 26UI/mL of pronase, to remove the zona pellucida (ZP), and then transferred to KOSM medium supplemented with 0.4% BSA under mineral oil, in a humid gaseous atmosphere of 5% CO2 in air at 37°C during 72h for IVC. Group 1 (G1): embryos pairs (n=35) without ZP were cultured in microdroplets of 10μL of medium; Group 2 (G2): embryos pairs (n=34) without ZP were cultured in WOW modified system, which manually produced microwells, using four wells plates containing 500μL of medium; Control group (CG): embryos (average of 10 per replication) with intact zona pellucida were cultivated in drops of 100μL of medium. The results of three replicates were analyzed by ÷2 test (P <0.05). The rates of cellular masses aggregation shows a significant difference between G1=57% (20/35) and G2=82% (28/34). The embryo development rates to the blastocyst stage differ significantly among the three groups: G1=40% (14/35), G2=68% (23/34) and GC=94% (30/34). The WOW modified embryo culture system is more efficient to produce mice in vitro chimeras than the microdroplets system.

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The influence of high or low feed intake on plasma insulin and progesterone concentrations was evaluated in 24 prepubertal 5 months old F1 Dorper x Santa Inês ewes weighing 25 kg. After 15 days of adaptation, the animals were blocked by initial body weight (BW) and randomly allocated to two groups. The same experimental diet was provided to both groups, but the feed intake was different. The Ad libitum Group (Ad Group) had a feed intake average of 3.8% of BW and the Restriction Group (R Group) of 2% of BW, during 4 weeks. At the end of 4 weeks, all animals received an i.m. injection of 10 mg prostaglandin F2α (PGF2α, Lutalyse, Pfizer, Brazil) and the insertion of an intravaginal progesterone-releasing device (0.35 g of P4, CIDR, Pfizer, Brazil), on Day 0. The treatment with 10 mg PGF2α was repeated on Day 6. On Day 8, blood samples were collected by jugular venipuncture at -0.5, 2, 4 and 6 hours in relation to feed offer (0 hour). Blood samples were centrifuged and plasma was stored at -20ºC for determination of insulin and P4 plasma concentrations, by radioimmunoassay. Data were analyzed using generalized linear models and results are presented as least squares means ± standard error. At the end of the 4 week-period, ewes from the Ad Group weighed 35.2 kg and of the R Group weighed 29.0 kg. Plasma insulin concentrations in the Ad Group were higher than in the R Group (13.5±2.8 vs. 2.8±0.4 μIU/mL, respectively; Pd<0.003) and this difference was observed in all evaluated periods, i.e. -0.5, 2, 4 and 6 hours (10.2±1.6 vs. 2.0±0.3; 12.6±2.7 vs. 2.9±0.4; 15.8±5.1 vs. 2.4±0.5 and 15.5±3.5 vs. 3.8±0.7 μIU/mL; Pd<0.02). Regarding plasma P4, the R Group had a higher concentration of this hormone than the Ad Group (4.4±0.3 vs. 2.5±0.2 ng/mL, respectively; Pd<0.0001). There was no interaction between treatments and periods of blood sampling for both variables - insulin and P4 (P>0.64 and P>0.10, respectively). The P4 concentrations at -0.5, 2, 4 and 6 hours were also higher in the R Group (4.8±0.3 vs. 2.7±0.25; 4.2±0.3 vs. 2.5±0.25; 4.1±0.3 vs. 2.3±0.25 and 4.5±0.3 vs. 2.4±0.25 ng/mL; Pd<0.0001). We concluded that the higher plasma insulin concentrations in the Ad Group were in consequence of a high feed intake. However, P4 concentrations in ewes with high feed intake were dramatically lower, possibly due to the higher hepatic blood flow, increasing the metabolism of this steroid hormone. Acknowledgments: To Pfizer Animal Health for the hormones.
in pregnant mares luteolysis must be blocked. The PGF$_2\alpha$ has low diffusion through the plasma membrane, wherein the pregnancy, whose affinity for PGT is similar to affinity for PGF$_2\alpha$ ACKNOWLEDGEMENTS FAPESP – process n° 07/51359-8 and the number of nuclei in the blastocysts was also reduced; hence blastocysts showed BCL2 increased expression and few TUNEL increase in mtTFA expression and a recovery in mitochondrial DNA amount at 72 hpa. At the same time, there were increases in BCL$_e$ e PI3KCaanti-apoptotic factors. Development to the blastocyst stage decreased compared to centrifuged group and the number of nuclei in the blastocysts was also reduced; hence blastocysts showed BCL$_e$ increased expression and few TUNEL positive cells. MECFtransfer from supplemented group, surprisingly, had no effect in mitochondrial DNA amount nor had significant differences on the evaluated characteristics at the 1-cell stage. These embryos, indeed, showed a decrease in development to the blastocyst stage and a reduced nuclei number compared to controls. Increase Ømm was also observed and confirmed by COXI and mtTFA expression. At this moment, there was an increase in BAX/BCL$_e$ proportion and in the number of TUNEL positive cells. These changes in the supplemented group suggest that mitochondria from MECFmay have led to oxidative stress due to an increase in ROS amount. The absence of the nucleus and of a translation machinery may have led to organelles impairment, which justifies the maintence of mtDNA amount in the depleted group. Depletion model was developed to study the variation of organelles number during embryogenesis. The supplementation model may represent an opportunity to study organelles behavior without the control of the nucleus and other cytoplasmatic extracts, prior to or at the time of reconstruction.

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GENE EXPRESSION OF THE PROSTAGLANDIN TRANSPORTER PROTEIN (PGT) IN ENDOMETRIUM OF CYCLIC AND PREGNANT MARE


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In cyclic mares, luteolysis occurs between the 14th and 16th days after ovulation, due to endometrial PGF$_2\alpha$ However, in pregnant mares luteolysis must be blocked. The PGT has low diffusion through the plasma membrane, wherein the Prostaglandin Transporter Protein (PGT) is needed to carry out the transport from producer cell until the target cell. This experiment aimed to identify the RNAm of PGT in endometrium of cyclic and pregnant mare. One estrous cycle of 11 mares (5 to 12 years old) was examined by transrectal palpation and ultrasound examination. When a follicle with diameter $> 35$mm and endometrial edema were detected, 2,500UI of hCG (Vetecor®, Hertape Calier, Spain) were given, via I.V. Six mares were not inseminated and only the time of ovulation was recorded. Endometrial biopsies were performed when pre-ovulatory follicles (diameter $> 35$mm) and endometrial edema were detected (EST; n=6), 7 (M. DIEST; n=6) and 14 days (DIEST; n=6) after ovulation. Five mares were inseminated with semen of known fertility after the detection of ovulation. The pregnant mares were submitted to endometrial biopsy on the 14th day of gestation (GEST; n=4). Total RNA was submitted to amplification by one step real-time polymerase chain reaction (RT-PCR), in which the primers were deduced from equine PGT sequence (XM_001917260.1), available in Genebank. A primer for equine RNAr 18S was used as housekeeping gene. The experimental design was completely randomized and the treatments were EST, M.DIEST, DIEST and GEST. After homogeneity of variances and normality of residues verification, the abundance relative average (± standard of error mean), obtained by 2$^-\Delta\Delta$CT method, was submitted to variance analysis and the averages separate for the LSD test (P=0.05). It was found largest RNAm relative expression to PGT in GEST and M. DIEST endometria (2.29±0.84 and 2.03±0.45, respectively). In the DIEST was found similar value (1.78±0.25) to previous and to EST (1.04±0.12), which presented the lowest value compared with the previous. The lowest relative value of RNAm for equine PGT found in endometrium of mares in estrous, compared to diestrous is compatible with the physiological importance of PGF$_2\alpha$ in the respective phases of the estrous cycle of mares. The largest value found in endometrium of pregnant mares may be due to another PGT function, the transport of PGE$_2$, an important hormone for embryonic mobility and maternal recognition of pregnancy, whose affinity for PGT is similar to affinity for PGF$_2\alpha$. ACKNOWLEDGEMENTS FAPESP – process n° 07/51359-8 and n°08/55845-7

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IMPACT OF THE SURGICAL EMBRYO TRANSFER ON THE NATURAL CONCEPTION OF MURINE RECIPIENTS

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Nowadays, several areas of research have used mice as animal model. Ethical and economic aspects pressure to reduce both suffering and animal utilization. The aim, in this work, was to evaluate the impact of surgical embryo transfer on the subsequent natural fertility (pregnancy and prolificacy) of murine recipients. It was utilized females (Swiss Webster, SW), with two to six months old, weighing approximately 60g and allocated randomly in control (C) or experimental (E, n = 10/group). Females of the group E were submitted to surgical embryo transfer procedure (according to Nagy, A. Manipulating the Mouse Embryo, A laboratory manual. CSHL Press. 3rd ed. 2003. p.268-71), but not pregnant. Females of the Group C (n=10; same strain, age, weight, and housing of those of Group S) were not submitted to surgical procedure. Sixty days after sham surgery, both groups were allocated to mating with males SW during a week. To avoid individual male influence, each male was designed to mate two females (one of each group). After the mating period, male was kept apart from females and females (groups control and E) were kept together until the birth. The groups were compared based on the percentage of pregnant females, the number of pups born per female and the period between male removal and parturition (MRP, in days).

There was no significant difference between percentage of pregnant females in Group control (90%, n=10) and Group S (70%, n=10; p=0.582, exact Fisher’s test). The pups were born per female (mean±sd) were 9.1±4.88 and 9.7±3.55, respectively for groups control (n=8) and E (n=7, p=0.796, t-Student test). The period MRP was 15.4±1.67 and 17.0±1.73 days, respectively for groups control (n=9) and S (n=7, p=0.09, t-Student test). Although not significant, the p value of 0.09 (MRP) could suggest a trend - which only could be accepted after further experiments - to increase the days to conceive after surgical procedures. If confirmed, this deleterious surgical effect on the time to conceive should be evaluated with regard to type and location. In a similar experiment Kolbe et al. (2010, Transgenic Research 19:335) reported the feasibility of repeated use of recipients as a method of reducing animal surgery since the transfer did not affect the fertility of the recipients. Based on these results, we conclude there were no significant effects of surgical embryo transfer procedure on the fertility parameters (% of pregnancy, amount of pups per female and time to conceive after male removal) that might be an impediment to a reutilization – in experiments that require fertility maintenance - of murine recipients. Financial support: FAPESP, Brazil.

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THE ROLE OF CUMULUS-OOCYTE COMPLEX QUALITY AND INSULIN-LIKE GROWTH FACTOR-I ON DEVELOPMENTAL COMPETENCE OF BOVINE OOCYTES EXPOSED TO HEAT SHOCK

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Alterations in the reproductive tract microenvironment compromise oocyte growth and maturation as well as preimplantation embryonic development. Exposure of bovine oocytes to heat stress reduces embryonic development and pregnancy rates (Hansen, P.J. 2004. Anim Reprod Sci 82-83: 349-360). These events are regulated by a variety of growth factors and dynamic communication between the oocyte and its cumulus cells (CC). The objective of the current study was to evaluate the modulatory effects of cumulus-oocyte complexes (COCs) quality and insulin-like growth factor-I (IGF-I) on developmental competence of bovine oocytes exposed to heat shock during in vitro maturation (IVM). In this study high (≥ 3 layers of compact cumulus cells and homogeneous cytoplasm) and low-grade COCs (< 3 layers of less compact cumulus cells and irregular cytoplasm) were exposed to Control (38.5°C for 22 h) or Heat Shock (41°C for 12 h followed by 38.5°C for 10 h) treatments in the presence of 0 or 100 ng/ml IGF-I during IVM. These COCs were subjected to in vitro fertilization and culture. Cleavage and blastocyst rates were evaluated on days 3 and 9 post fertilization, respectively. This experiment was replicated 5 times using 1082 COCs. Data were analyzed by least-squares analysis of variance using the General Linear Models procedure of SAS. Cleavage and blastocyst rates were affected by COCs quality (P< 0.05) and temperature (P< 0.0001). However, there was no effect of IGF-I or statistical interaction. In high quality COCs cleavage rate was 86.2% for 38.5°C versus 30% for 41°C in the absence of IGF-I and 63.4% for 38.5°C versus 21.3% for 41°C in the presence of IGF-I. In low quality COCs cleavage rate was 57.2% for 38.5°C versus 5.8% for 41°C in the absence of IGF-I and 61.4% for 38.5°C versus 9.5% for 41°C in the presence of IGF-I. Similarly, in high quality COCs blastocyst rate was 22.3% for 38.5°C versus 3.1% for 41°C in the absence of IGF-I and 35% for 38.5°C versus 1.5% for 41°C in the presence of IGF-I. In low quality COCs blastocyst rate was 6.7% for 38.5°C versus 9% for 41°C in the absence of IGF-I and 20% for 38.5°C versus 16% for 41°C in the presence of IGF-I. There was a tendency for IGF-I to stimulate embryonic development in low quality COCs exposed to Control and Heat Shock. In conclusion, high and low quality COCs are compromised by heat shock regardless of IGF-I supplementation.
CONTROL OF Neospora caninum INFECTION AND ITS IMPACT IN THE REPRODUCTIVE PERFORMANCE IN DAIRY COWS

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Neospora caninum is an intracellular protozoan that infects domestic and wild canids, ruminants and other species (DUBEY, 2003. J. Parasitol. 89:S42-56). Outbreaks of Neospora-associated abortion in herds can be caused by point source infection by the parasite or by reactivation of the parasite in chronically infected cows (LOPEZ-GATIUS et al., 2004, Theriogenology 62:606-613). The aim of this study was to verify the presence of antibodies to Neospora caninum and yearly fluctuation of serological positive cows during a three years period in a dairy herd in the state of Rio Grande do Sul, as a health measure to reduce abortion rates. Serological examination was performed by indirect immunofluorescence in Holstein cows 2 to 12 y.o. (x=4 y.o.), from prelactation to the 8th lactation (x=2 lactations) and with an average milk production of 26 L/ day. Artificial insemination was performed with beef bull semen in seropositive cows and in repeated abortion cows. Cows with two or more abortions were eliminated of the herd after the first year. The number of cows submitted annually to serological examination during the study was 243, 226 and 239 for the first, second and third year, respectively. The percentage of seropositive cows in each year was 38.0, 22.0 and 15.0%. The abortion rate in the first, second an third year was 36.0, 19.0 and 9.0%. Identification of Neospora caninum by PCR was performed in five of seven fetuses aborted. The incidence of abortions in seropositive cows was 83.0, 62.5 and 70.0%, respectively in 2007, 2008 and 2009. The incidence of seropositive cows with a history of abortion that repeated abortion was 61.0, 45.0 and 28.5%, respectively, in 2007, 2008 and 2009. Abortions in seropositive cows represented 86.9, 74.0 and 87.5% of total abortions, respectively, for each year. The number of inseminations per pregnancy decreased linearly in the herd during the three years (2.7, 2.2 and 1.9). The decrease in the number of seropositive animals was due to elimination of seropositive cows with two or more abortions from the second year of observation. This study demonstrates the need for annual serological testing for Neospora caninum, as well as the tentative agent detection in aborted fetuses by PCR in herds with higher rates of abortion. Insemination of seropositive dairy cows with beef bull semen and eliminating cows with history of repeated abortions induces the drastic drop of seropositive cows of a herd. This measure also reduces the number of Neospora caninum seropositive daughters born in the herd and prevents vertical transmission of the agent and its dissemination through aborted fetuses and placenta as a source of infection for definitive hosts. The actions adopted during this study contributed to improve reproductive performance in the herd.

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INFLUENCE OF EXERCISE ON EMBRYO TRANSFER OF TRAINNING AND PERFORMING QUARTER HORSE ATHLETIC MARES

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It is usual in Brazil and other countries, to use as embryo donors, Jumping and Quarter Horse mares that are training and competing in racing and barrel shows. Recent work showed that exercise was detrimental to embryo quality and recovery in mares, which disagrees with field observations from several ET Centers from Brazil. The primary goal of this study was to compare embryo recovery rates and pregnancy rates between athletes and breeding non-athletes Quarter Horse mares in a tropical climate. We used 39 barrel racing mares in a training regimen including exercise 4 times a week for 60 to 90 minutes. Thirty-one of the 39 mares participated, at least twice a month, on high performance competitions. Other 135 non training breeding mares were used as control donors. During the period of training, the ambient temperature ranged from 31 to 36 °C and the average humidity from 70% to 90%. In both groups, after the detection by ultrasound of a 35 mm follicle, ovulation was induced with 1.0 mg of deslorelin acetate (IM) and insemination was performed 24 latter with cooled or fresh semen. Embryos were collected on day 8, and donor mares were treated with prostaglandin (Lutalyse, 5 mg, i.m). To assess the mean variation of body temperature that occurs with exercise, rectal temperature was evaluated from 8 randomly selected athletic embryo donor mares. Temperature was measured just before the exercise and every 15 minutes after the training was finished, until the temperature returned to the pre exercise temperature. Temperature was checked in a moment of the day where the ambient temperature ranged from 31 to 35 °C. The embryo recovery and pregnancy rates were analyzed using Fisher’s Exact Test. A total of 138 embryo collections were performed in athletic donor mares and 657 collections were performed in breeding non training mares with a total of 105 (76%) and 466 (71%) embryos collected (p>0.05), respectively. No differences (p>0.05) were observed on the pregnancy rates at day 15 (78 % x 79 %) and day 40 (69% and 70%) between the training and breeding non training donor mares. The body temperature immediately before the training was in average 37.7° C, ranging from 37.1 to 38.6 ° C. Just after training the body temperature increased in average to 39.4° C and the respiratory rate from 14.5 to 35.3 breaths per minute. The results of the present study showed that under appropriated training, exercise does not have negative influence on efficiency of embryo transfer in athlete Quarter Horse mares. The reasons for the disagreement between our results and a recent publication must be related with the physical conditioning of studied mares, as well as the level of training that these animals are submitted.
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REESTABLISHMENT OF FIRST POSTPARTUM LH SURGE WITH GNRH OR ESTRADIOL BENZOATE (EB) ADMINISTRATION, IN NELLORE COWS WITH OR WITHOUT NUTRITIONAL SUPPLEMENTATION

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The presence of calf, body condition score, number of births (multiparous vs primiparous) and breed are factors that influence the duration of post-partum anoestrus in beef cows. The objective of the present study was to evaluate, during early post-partum, the time of the reestablishment of LH stocks, measured by the hypothalamic-pituitary axis responsiveness to exogenous administration of GnRH or EB. Primiparous lactating Nellore cows (Bois indicus, n=38) were randomly allocated into four groups, according to the hormonal treatment: EB Group (1mg EB, i.m., Estrogin®, Farmavet, Sao Paulo, Brazil; n=11), GnRH Group (100μg, licerelina, i.m. Gestran Plus®, ARSA S.L.R., Buenos Aires, Argentina; n=9), EB-SUP (n=9) and GnRH-SUP (n=9) groups received the same treatments above specified and were supplemented with a balanced diet, based on cotton meal and ground corn. The drugs were administered from seven days post-partum (∓4 days), at intervals of 7 days, until 140 days postpartum or the occurrence of the first ovulation that was observed by weekly ultrasonography (US, Aloka 900, Tokyo, Japan, transrectal probe 7.5 MHz). Blood samples were collected just before and 4 h (GnRH groups) or 18 h (EB groups) after hormones administration in order to determine LH concentrations by RIA. The data was analysed by regression analysis (PROC PHREG and Fisher test). The occurrence of LH surge until 140 days postpartum was significantly less frequent in EB group (2/11 cows) when compared to the other groups [EB-SUP (6/9); GnRH (7/9), and GnRH-SUP (7/9), p<0.01]. Excluding EB group, since only 2 cows had increase on LH concentrations, LH surge was observed earlier (days postpartum ± SEM) in animals supplemented (GnRH-SUP: 30.4±0.9) as compared to non supplemented (GnRH, 41.6±1.3, p<0.09), and in cows that were treated with GnRH (GnRH-SUP: 30.4±0.9) vs EB-SUP (73.5±0.9, p<0.001). It is concluded that nutritional supplementation anticipates the first post partum LH surge induced by GnRH or EB. Additionally, EB administration did not induce LH surge in most Nelore cows from EB group, possibly due to the sensitivity of the hypothalamus to negative feedback of estrogens, inhibiting the pre-ovulatory surge.

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HORMONE DOSAGE AND ULTRASONOGRAPHY AS DIAGNOSIS OF TUMOR GRANULOSA CELLS (TCG) IN FRENCH SADDLE MARE

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The aim of this study was evaluate the efficacy of hormone dosage and ultrasonography as a diagnosis method of granulosa cell tumor (GCT) located in the left ovary of a French Saddle mare 18 years old. Although the low incidence of ovarian neoplasms in horses, the granulosa cell tumor (GCT) is the most frequent type observed, which represents 2.5% of all tumors occurred in the species. This is a benign tumor that causes inactivity in the contralateral ovary. The mare presents clinical signs of male behavior, deep anoestrus and persistent estrus (WILSON, D. A., 1989, Journal of American Veterinary Medical Association, V. 194, n. 5, p. 681-2). A French Saddle mare has a history of not embryo recovering after successive inseminations and shows male behavior. This mare underwent rectal examination and ultrasonography. The left ovary increased in size with a follicle reaching up to 95 mm in diameter. The contralateral ovary was small, consistent, without follicles, indicating lack of cyclical activity. The hormonal assay was performed by radioimmunoassay methodology. The hormonal protocol used for the diagnosis of GCT was divided into three stages: the first blood serum sample was collected at baseline, with no hormonal treatment. The hormones evaluated were total estrogens: 0.1 pg / mL - Indicative Values: anestrus (not pregnant): <50 pg / mL gestation (over 110 days) > 150 pg / mL; progesterone: 0.08 ng / mL - Indicative Values: anestrus, Estrus: <1.0 ng / mL Diestro, Gestation: 4-10 ng / mL; testosterone: 482.9 pg / mL - Indicative Values: Stallion: 500-2000 pg / mL. Gelding: <50 pg / mL Female: <50 pg / mL. The second blood serum sample was collected 1 hour after the intravenous administration of 10,000 UI of HCG. The values of total estrogens were 0.1 pg / mL and testosterone 527.5 pg / mL. The third sample was taken two hours after the intravenous administration of 10,000 UI of HCG. The values of total estrogens were 0.1 pg / mL and testosterone 497.6 pg / mL. Testosterone levels above 100 pg / mL are conclusive for diagnosis of GCT (McCUE, P. M., 1998, Review of ovarian abnormalities in the mare, p. 125-33). The ultrasonography, the rectal examination, the masculine behavior, the reproductive history and the hormone levels are strong evidences of a granulose cell tumor, therefore the ovariecotmy surgery was indicated as treatment. Keywords: mare, ovary, granulosa cell tumor.
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INFLUENCE OF FOLLICLE STIMULATING HORMONE ON THE NITRIC OXIDE PATHWAY IN BOVINE CUMULUS-OOCYTE COMPLEXES

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The present study aimed to evaluate the effects of recombinant human FSH (rhFSH) on in vitro maturation (IVM), nitric oxide (NO) production and cyclic GMP and AMP (cGMP and cAMP) levels, which can be affected by NO and gonadotrophins, in bovine oocytes. Oocytes were aspirated from slaughterhouse ovaries and divided in two groups: cumulus-oocyte complexes (COC) and denuded oocytes (DO, by mechanical removal of cumulus cells). Both groups were cultured submitted to IVM for 24h in TCM-199 with or without rhFSH (0.05IU/mL), pyruvate (0.2mM), gentamycin (10µg/mL) and 0.1% polyvinylalcohol (PVA) in 5% CO2in air and at 38.5°C. The oocytes were then evaluated for maturation rate (determination of meiosis stage by lactomid staining) and nitrate (subproduct of nitric oxide production) concentration in IVM medium by the Griess method. For the determination of cGMP and cAMP levels, COC were analyzed at 0, 1 and 3h IVM, supplemented or not with rhFSH. The measurements were performed using an enzymatic immunoassay kit. The first experiment was developed with COC and DO to define the optimal rhFSH concentration for meiosis stimulation to metaphase II (MII). The concentrations 0; 0.005; 0.05; 0.5 and 5IU/mL resulted, respectively for COC and DO, in 14.3 and 63%, 62 and 64.6%, 80.6 and 89%, 74 and 74.2%, 56.6 and 72.3% MII rates. For both groups, 0.05IU/mL rhFSH showed the highest MII rates (p<0.05) and was used for the next experiments. Indirect measure of NO levels (nitrate) was not different for COC and DO matured with or without rhFSH (27.6 to 58.8µg/mL, p<0.05). cGMP levels for COC were 0.19nmol/mL at 0h and increased to 0.27nmol/mL (p<0.05) after 1h IVM in the presence of rhFSH. In the absence of the hormone, the cGMP levels also increased as after 1h IVM (0.24nmol/mL), but levels were similar to those observed at 0h and at 1h in the group cultured with FSHrh (p<0.05). After 3h IVM, cGMP levels returned to levels similar to those at 0h in both groups (0.19nmol/mL with rhFSH and 0.17nmol/mL without rhFSH). CAMP levels at 0h were 0.44pmol/mL and after 1h IVM without rhFSH there was no variation (0.3pmol/mL, p=0.05). However, in the presence of rhFSH CAMP levels increased (7.6pmol/mL, p<0.05). After 3h IVM, there was again an increase in CAMP levels (p<0.05), but in the absence of rhFSH this increase was inferior to that observed in the presence of the hormone (0.76pmol/mL and 7.8pmol/mL, with and without rhFSH, respectively, p<0.05). Thus, we can conclude that under the conditions of this study, rhFSH stimulates meiosis progression in COC and DO and influences CAMP and cGMP levels in early IVM, but without effects on NO production. Therefore, rhFSH stimulates maturation and cyclic nucleotides, without interference of NO.

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EFFECT OF DIET, WITH EXCESS OF MICRONUTRIENTS, ON THE EMBRYO VIABILITY RATE IN MICE

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The availability in excess of micronutrients, such as vitamins and minerals present in the diet, may interfere on reproduction of most animals, including a lower embryonic viability. This pilot study aimed to evaluate the influence of micronutrients, contained in the ration given to the animals, in weight and embryonic viability, since that information is scarce on the literature. A total of 12 mice C57/BL6/EGFP were used, ageing from 2 to 3 months and allocated to four experimental groups (n=3/group). Before mice superstimulation, there was a preliminary period of adaptation (21 d) with ad libitum ration containing excess of micronutrients (NA; Labina, Purina®; Brazil – non autoclaved; G1 and G2) or recommended normal ration (A; Labina ration, autoclaved according to product recommendation; G3 and G4). The animals were superovulated according to Mancini et al. (2008;Trans Res,17:1015) on D22 (eCG) and hCG treatment on D24. After hCG administration (13:00 h) the females were put to mating with fertile males (n=2), of the same strain, with previous adaptation by 21 d with NA or A ration, respectively to NA - 3/6 - and A - 4/6 - males). Among the groups, there were no significant differences to weight on D22 and D29, vaginal plug rate or embryo viability rate (viable/total structures of 5/13, 0/3, 5/5 and 11/18, respectively for G1, G2, G3 and G4). However, the most discrepant values of vaginal plug rate were observed on G1 and G2 (100 and 0%, respectively). The vaginal plug presence was a non significant predictive factor (P=0.089) to viable embryos recovery (58.3 and 0%, respectively to females with and without plug detection). The males produced an embryo viability rates of 31.3 and 69.6%, respectively to NA and A (P=0.025), suggesting a potential deleterious effect to excess of micronutrients in some steps (e.g., libido, mating, etc) responsible for embryonic viability. Financial support of FAPESP, Brazil.
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EFFECT OF HEAT STRESS DURING MATURATION OF OOCYTES AND IN VITRO PRODUCTION OF EMBRYOS IN GOATS AND SHEEP


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In the Northeast region of Brazil, the environmental conditions compromise the reproductive efficiency of domestic animals with economic interest, resulting in economic losses in the region. The oocyte and embryo are the most affected with the negative effects induced by thermal stress. This study aimed to determine the effect of heat stress during maturation of oocytes and in vitro production of embryos in goats and sheep. Ovaries were collected in a slaughterhouse and transported to the Laboratory of Biotechnological Reproduction of UFRPE. The cumulus-oocyte-complexes (COCs) were collected by the technique of “slicing” of the follicles from 2 to 6 mm in diameter and selected based on morphological classification and placed on the basic stages. In 10 replicates the COCs were submitted to the caloric heat stress at 41°C for 0 (the thermoneutral 39°C), 3, 6, 12 and 24 hours of maturation in vitro. The percentage of oocytes was determined in the maturation, fertilization, cleaved (D-3), stage of 8-16 cells (D-4), morulae (D-5), blastocyst (D-8) after fertilization and blastocyst positive for apoptosis by TUNEL assay. The statistical analysis was performed to compare variances and F test meant for variances with significance level of 5% (P<0.05). Then, the t test was performed to compare means at significance level 5%. Significant difference (P<0.05) was observed at all periods of maturation with thermal stress in both species. In goats, there was significant difference (P<0.05) in fertilization 0h (42%), 3h (25.4%), 6h (17.7%), 12h (11.9%), 18h (7.9%) and 24 (3.4%). On D-3 was different at 0h (28.1%) and 3h (23.6%), 6h (14.1%), 12h (6.8%), 18h (4.7%) and 24 (1.8%). On D-4 0h (26.9%), 3h (22.5%), 6h (12.1%), 12h (4.8%), 18h (2.6%) and 24 (1.5%) as the D-5 0h (20.3%), 3h (15.9%), 6h (8.8%), 12h (3.0%), 18h (1.6%) and 24 (1.0%) significant differences were observed between the different exposure times. The D-8 was not significantly different (P>0.05) between the periods of 3 vs 6 to 18 vs 24 h and the blastocyst TUNEL positive at 0 vs 3, 3 vs 6, 12 vs 18 and 18 vs 24 h of heat stress. In sheep, significant difference (P<0.05) was observed between the different exposure times in maturation, 0h (75.8%), 3h (50.0%), 6h (35.4%), 12h (12.4%), 18h (9.9%) and 24h (4.6%). Significant difference (P<0.05) was not observed in fertilization, D-3, D-4, D-5 and D-8 between the periods of 18 vs 24 h and the blastocysts TUNEL positive at 0 vs 3, 3 vs 6, 12 vs 18 and 18 vs 24 h of heat stress. Under the conditions observed in this study, the results allows to conclude that a minimum of 3 hours exposure to heat stress at 41°C during in vitro maturation is sufficient to cause significant deleterious effects on embryonic development and in their level of apoptotic cells.

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LUTEAL VOLUME AND PROGESTERONE SERUM CONCENTRATION IN SANTA INES SHEEP SYNCHRONIZED WITH GnRH IN A LONG OR A SHORT PROTOCOL

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This study had the objective to evaluate the number of CLs, the volume of the luteal mass and progesterone serum concentration in different protocols for estrus synchronization in Santa Ines sheep. Forty one sheep were subjected to four protocols for estrus synchronization and induction of ovulation: Long+GnRH (n = 11) where the vaginal sponge containing 60 mg of medroxyprogesterone (MAP) were maintained for 12 days, administration of 300 IU of eCG on day 12 and 25 ìg of GnRH (gonadorelin acetate) 27 hours after sponge removal; Long (n = 10) stayed with the sponge for 12 days and 300 IU of eCG was administered in its removal; Short+GnRH (n = 10) had sponge staying for 7 days, associated with administration of 37.5 ìg D-cloprostenol on the fifth day, 300 IU of eCG on the seventh day and 25 ìg of GnRH 27 hours after sponge removal; Short (n = 10) the sponge stayed for seven days, on the fifth day was administered 37.5 ìg of D-cloprostenol and at sponge removal was administered 300 IU of eCG. Twelve days after sponge removal blood was collected, and the serum was frozen at -20°C for later determination of progesterone concentration by radioimmunoassay. In the same day laparoscopic and ultrasonographic evaluation was performed to count CLs and for measuring the volume of the CLs. The volume of the luteal mass was calculated by summing the volume of each CL. For statistical analysis were used ANOVA and Duncan’s test with significance level of 5% (P<0.05). Then, the t test was performed to compare means at significance level 5%. Significant difference (P<0.05) was observed at each period of treatment. Differences between treatments (P> 0.05) were not observed in the serum progesterone concentrations (5.2±5.7 ng/ml, 4.1±2.9 ng/ml, 5.7±2.4 ng/ml in Long+GnRH, Long, Short+GnRH and Short protocols respectively) showed no differences between treatments (P> 0.05). Despite the Long+GnRH protocol had a higher number of CLs, it showed no higher volume of the luteal mass and no higher progesterone concentration. By presenting the luteal volume and progesterone levels similar to other treatments, what is important to achieve a good pregnancy rate, the Long+GnRH protocol showed up as an alternative to increase ovulation rates in sheep TAI programs.

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The time in which a calving dam should be assisted may vary considerably, depending on the level of parturition difficulty. Prolonged parturition may lead to neonatal asphyxia and unfavorable consequences to the dam. However, premature obstetrical assistance is known to cause neonatal problems and retained placenta. The aims of the present study were to compare neonatal vitality and the incidence of retained placenta in Hostein dairy cows submitted to obstetrical assistance or presenting spontaneous calving and to compare the pulmonary status of calves born under distinct obstetrical conditions. Holstein cows were grouped into Spontaneous (SPO-G; n=7) and Assistance Group (ASS-G; n=5). Spontaneous calving was considered a normal parturition with no obstetrical intervention and cows of the ASS Group were submitted to fetal extraction but still within eutocia. Also, calves were grouped into Eutocia (EUT-G; n=5) and Dystocia Group (DYST-G; n=5) defined, respectively, as the time passing for calf delivery below 2h or between 2 and 5hs. Calves were subjected to clinical assessment and arterial gas analysis at birth, 2 and 4hs after calving. Chest radiography was performed at birth. Placenta retention was diagnosed within 12 hours after fetal expulsion. Values were compared by ANOVA and Tukey Test with p<0.05. No statistical differences were verified between SPO-G and ASS-G regarding acid-base status. Regardless of the obstetrical intervention, all calves presented hypoxemia during the initial 4hs. On the other hand, normal blood pH and bicarbonate were verified, indicating no metabolic disorders and adequate tissue oxygenation. Hence, the high oxygen consumption at tissue level leaded to a decrease in blood oxygen. No retained placenta was observed. DYST-G calves presented moderate to severe pulmonary radiographic changes, such as little to no definition of cardiac silhouette and main bronchi consistent with fluid content. Only DYST-G calves showed hypoxia at birth, but presented full recovery after 2h. Prolonged calving associated with placenta detachment lead to fetal hypcapnia which may trigger inspiration and fulfilling of the expanded lung with liquid, hindering oxygen absorption. Hence, dystocia promoted neonatal asphyxia, however, oxygen saturation significantly increased after 2hs. In conclusion, a calving period of less than 2hs can favor neonatal adaptation and obstetrical intervention does not cause neonatal injury or predisposes to placenta retention, therefore, proved to be a safe assistance at this time point. Prolonged calving interferes with fetal oxygen supply and pulmonary gas exchange, extending the period by which calves overcome hypoxia.

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**EFFECTS OF INTRAVENOUS GLUCOSE INFUSION AND NUTRITIONAL BALANCE ON SERUM CONCENTRATION OF NEFA, GLUCOSE, INSULIN, AND PROGESTERONE IN NON-LACTATING DAIRY COWS**

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The objective of this study was to evaluate serum concentrations of nonesterified fatty acids (NEFA), glucose, insulin, and progesterone (P4) in non-lactating dairy cows according to nutritional balance and glucose infusion. Ten non-lactating, ovariectomized Gir × Holstein cows were stratified by Body weight (BW) and body condition score (BCS) on d -28, andrandomly assigned to: 1) negative nutrient balance (NB) and 2) positive nutrient balance (PB). From d -28 to d 0, cows were allocated according to nutritional treatment into 2 low-quality pastures with reduced forage availability. However, PB cows received, on average, 3 kg/cow/d (as-fed) of a concentrate. All cows were inserted with an intravaginal P4 releasing device on d -14, which remained in cows until the end of the study. On d 0, cows within nutritional treatment were randomly assigned to receive, in a crossover design containing 2 periods of 24 h each: 1) intravenous glucose infusion (0.5 g/kg of BW; GLUC), or 2) intravenous saline infusion (SAL). At the beginning of each period, all cows were fasted for 12 h. Blood samples were collected at -12 and -11.5 (beginning of fasting), and at -0.5, 0, 0.5, 1, 2, 3, 4, 5, and 6 h. Following the last blood collection of period 1, cows returned to respective pastures and received (PB) or not (NB) concentrate. Data were analyzed using the MIXED procedure of SAS (SAS Inst., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Changes in BCS and BW were greater in NB cows compared to PB cows (-0.60 ± 0.25 kg for BW, respectively; -22.4 and 1.2 ± 6.58 kg for BW, respectively). At the beginning of each period, all cows were fasted for 12 h. Blood samples were collected at -12 and -11.5 (beginning of fasting), and at -0.5, 0, 0.5, 1, 2, 3, 4, 5, and 6 h. Following the last blood collection of period 1, cows returned to respective pastures and received (PB) or not (NB) concentrate. Data were analyzed using the MIXED procedure of SAS (SAS Inst., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Changes in BCS and BW were greater in NB cows compared to PB cows (-0.60 ± 0.25 kg for BW, respectively; -22.4 and 1.2 ± 6.58 kg for BW, respectively). Cows receiving GLUC had greater glucose concentrations from 0.5 to 3 h relative to infusion compared to SAL cows. Insulin concentrations were greater in PB cows assigned to GLUC compared to SAL cohorts at 0.5 and 3 h following infusion, indicating a biphasic increase in circulating insulin, whereas NB cows assigned to GLUC had greater insulin concentrations compared to SAL cohorts at 0.5, 1, 2, and 3 h, indicating a single-phase increase in circulating insulin. P4 concentrations were greater in PB cows assigned to GLUC at 2, 3, and 4 h following infusion compared to SAL cohorts, whereas no infusion treatment effects were detected for progesterone in NB cows. In conclusion, the effects of glucose infusion on serum concentrations of insulin and P4in non-lactating dairy cows were dependent on cow nutritional status.
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STANDARDIZATION OF IMMUNOHISTOCHEMICAL TECHNIQUE FOR ESTROFEN ALPHA AND BETA RECEPTOR DETECTION IN BITCH CERVIX AND ENDOMETRIUM

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The aim of the present study was to validate the immunohistochemical technique for the detection of estrogen alfa and beta receptors in bitch cervix and endometrium. Samples collection was performed after ovariohysterectomy. These were fixed in 10% buffered formalin for 24h, and then kept in alcohol 70% of inclusion in paraffin. After inclusion, 4mm thick histological slices were made and placed on slides previously treated with Poly-Lisine (Poly-L-lisine®–Sigma Chemical Co-P8920–USA). Initially deparaffinization and hydration were performed. For estrogen receptor alpha detection the antigen recovery were performed with 10mM – pH 6.0 citrate solution in a Pascal pressure chamber (Dako, USA) for 30 minutes; and for estrogen beta detection was also employed the 10mM – pH 6.0 citrate solution, but with incubation in microwave for 15 minutes (3 cycles of 5 minutes). After heating, slides were allowed to cool for 20 minutes and the washed 10 times in distilled water. Endogenous peroxidase activity was quenched with 3% peroxidase solution for 20 minutes followed by 10 baths in distilled water, incubation with 3% milk solution for 1 hour at room temperature for blocking, washed 10 times in distilled water and Tris buffered solution 10mM (pH 7.4, Trizma Base®–Sigma Chemical Co–USA). Then the incubation with the primary antibody was performed. For estrogen receptor alpha in a 1:50 dilution (monoclonal antibody, clone 1D5, Dako, USA) and for estrogen receptor beta a 1:100 dilution (monoclonal antibody, clone PPG5/10, Dako, USA) in a humidified chamber for 18 hour at 4°C. Then, slides were washed in Tris buffered solution and incubated with the secondary antibody (Advance, Dako, USA). Each tissue section was washed in Tris buffered solution and DAB chromogen (3,3´-diaminobenzidina- Liquid DAB Cromogen®–DakoCytomation–USA) was added as a chromogen staining substrate for 5 minutes. Reaction was stopped by rinsing in Tris buffered solution. Tissue sections were counterstained with Mayer’s hematoxylin for 30 seconds and then washed in tap water for 10 minutes. Then, the slides were dehydrated and preserved using Permount (Fisher Scientific–cód.UN1294,USA) mounting medium. For positive control it was employed bovine endometrium previously tested (Martin, I., Reprod. Dom. Anim. 43, 415-21, 2008). For negative controls, another section was incubated with mouse immunoglobulin (N-Universal Negative Control Mouse, Dako, USA) instead of primary antibody. Cells presenting hormonal receptors of estrogen alpha and beta, after the treatment, have their nuclei stained showing the efficiency of these technique in bitches.
Clonagem, transgênese e células-tronco

Cloning, transgenesis and stem cells
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HANDMADE CLONING IN GOATS

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Animal cloning technology has been the focus of interest by many research groups around the world. Despite the effort, cloning continues to be inefficient, becoming even less effective when applied to other species rather than cattle. More recently, alternative cloning methods, such as the handmade cloning (HMC), have simplified the process, also decreasing the time and cost for training of personnel and for embryo production per se. The aim of this study was to adapt the handmade cloning procedure to the goat, based on our procedures in cattle (Ribiero et al., 2009, Cloning Stem Cells, 11:377-386), for the production of transgenic cloned embryos. Goat cumulus-oocyte complexes (COCs) from slaughterhouse ovaries were in vitro-matured in holding medium supplemented with 10 µg/mL EGF, 5 µg/mL FSH, 10 µg/mL LH, 1 µg/mL 17β-estradiol and 100 µM cysteamine, for 20 h at 38.5°C, 5% CO₂ and high humidity. Subsequent to cumulus cells removal and polar body (PB) selection, matured oocytes were briefly exposed to 0.25% protease for zona pellucida removal, followed by a rinse in pure fetal calf serum (FCS) and multiple washes in HM + 10% FCS. Zona-free oocytes were hand-bisected in 2.5 µg/mL cytochalasin B and screened under UV light for selection of enucleated hemi-cytoplasts. Then, two hemi-cytoplasts briefly exposed to phytohemoagglutinin were adhered to a single fibroblast cell from primary culture cells between the 2nd and 4th passage and at high confluence (>95%), established from a skin biopsy collected from an adult animal. Reconstructed structures were electrofused by two 1.1 kV/cm DC pulses for 5 s (~26 h post-fusion), after a brief exposure to a 7.0 V pre-fusion AC pulse. Fused structures were activated in ionomycin/6-DMAF followed by in vitro culture in the WOW system in SOFaa + 2% FCS + 0.3% BSA, at 38.5°C, 5% CO₂, 5% O₂ and 90% N₂, for seven days. The maturation rate attained after 3 replications, based on PB selection, was 84.6% (549/649), with the fusion rate for reconstructed structures reaching 83.2% (139/167). Cleavage rate was 85.4% (105/123), with 17.1% of embryos developing to a transferable stage (8 compact morulae and 13 blastocysts/105) on Day 7 of development. Such preliminary results are similar to those reported by others using standard cloning procedures. In conclusion, the HMC procedure appears to be an effective alternative for the production of transgenic cloned embryos in goats. Further studies are underway for the production of live born offspring.

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EFFECT OF MITOMYCIN C ON VIABILITY AND CELL CYCLE OF BOVINE ADULT FIBROBLASTS AFTER DIFFERENT EXPOSURE TIMES


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Currently, the derivation of embryonic bovine cells (CTE-like) is a great challenge, because the culture protocols used are very divergent and inefficient. One way to improve this methodology would be to use species-specific feeder cells. Mitomycin C (MMC) is an antiproliferative agent, which has inhibitory action in the S e G2/M of cell cycle but may cause cytotoxicity. The effect of MMC on bovine fibroblasts derived from adult animals (FBA) is still little known. Thus, the aim of this study was to establish a protocol for mitotic inactivation of FBA, with intention to apply it in the derivation CTE-like. Adult bovine fibroblasts was cultured in DMEM medium supplemented with 10% fetal calf serum and incubated at 38,5°C, 5% CO₂ and 95% of humidity. After reaching 60% of confluence, the cells were treated with MMC (10 µg/ml) at different exposure times: 0h (control), 2h, 3h, 4h e 5h. Subsequently, the fibroblasts were analyzed for flow cytometry for evaluation of the cell cycle phases (G1/G0, S, G2/M) and cell viability. For cell cycle analysis, fibroblasts were fixed (ethanol 70%) for 4 ° C for 2.5 h. After the cells were treated to RNAse A (100 ng/mL) for 10 min, and they were stained with propidium iodide (50 µg/mL) for 5 min before reading. Cellstained with propidium iodide (50 viability was evaluated in non-fixed cells stained with PI (50 µg/mL) for 30 min at 37°C. Three repetitions with three replicate each one were performed. Statistical analysis was performed by ANOVA and means compared by Student Newman Keus. In the results presented in the cell cycle it can be observed an increase of the peaks of the phases S and G2/M and a decrease of G1 in all exposure times to MMC compared to control. No had difference (p>0.05) in cell proportion in S phase between exposure times 2 (30.6 % ± 3.05%), 3 (30.85 ± 3.41%), 4 (29.79 ± 4.81%) h. The proportion of cells in G1/G0 phase in the 2 (42.42 ± 3.29%), 3 (44.82 ± 4.24%) h did not differ (p>0.05), and 3h was similar (p>0.05) to 4h (47.38 ± 5.39%). The time 5h had higher (p<0.05) proportion of cells in G1/ G0 (51.34 ± 3.67%). However, 0 time 2h had higher amounts of cells concentrated in S and G2 (22.90±2.59%) compared with other exposure times, while the time 5h showed lower (p<0.05) proportion of cells in S (22.576 ± 3.21%). A lower ratio (p<0.05) of viable cells was observed in the times 2 (89.67±3.78%), 3 (89.87±2.07%), 4 (88.28±3.82%), 5 (87.12±2.52%) h compared of control (93.59±2.12%), but despite that there was no difference (p=0.05) between treatments. Through the results, it can be concluded that MMC inhibits the proliferation of fibroblasts and the time 2 h of exposure was most effective because it keep cells viable. This information is being used to improve the efficiency of the protocol for mitotic inactivation of FBA, with intention to apply it in the derivation CTE-like.
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GFP EXPRESSION IN TROPHOBLAST CELLS OF TRANSGENIC CLONED BOVINE PLACENTA

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The migratory ability of trophoblast giant binucleated cells (TGC) towards to maternal tissues, during pregnancy, has been previously described in many species including the bovine. After migration these cells fuse with maternal epithelial cells to deliver fetal proteins to maternal placenta side. TGCs are reported to express specific proteins, such as, placental lactogens and pregnancy associated glycoproteins. In cloned bovine placenta an increase of TGCs number accompanied of many other placental abnormalities was reported. Herein, we used the transgenic GFP cloned embryo model to evaluate the migration of fetal cells in the bovine placenta. Samples of placentomes, membranes and intercaruncular endometrium from pregnant and nonpregnant uterine horns were obtained from GFP transgenic cloned embryo pregnancies at 60 (n=3) and 90 (n=3) days. Samples were preserved in 4% buffered PFA and paraffin-embedded for immunohistochemistry (IHC) analysis or snap-frozen in liquid nitrogen for western blotting (WB), Real time PCR and fluorescence microscopy. Through this model we expected to observe the migration of TGCs to maternal tissues. However no positive cells for GFP were observed in maternal side. The IHC analysis showed a negative GFP labeling in the TGCs whereas a positive throughout the trophoblastic cells in all analyzed samples. In WB analysis, as expected GFP was present in placentome and placental membranes. We also observed GFP presence in the intercaruncular endometrial samples from both uterine horns. The absence of expression of GFP in TGCs suggests that a GFP gene silencing in response to the highly specific protein synthetic profile. Presence of GFP in intercaruncular endometrium of both uterine horn tissues may be generated by the high GFP solubility or by other mechanisms allowing permeability of the uterus to fetal protein during pregnancy in the cow. Funded by FAPESP.

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PRODUCTION OF NUCLEAR-TRANSFERRED BOVINE EMBRYOS WITH OOCYTES MATURATED IN VIVO

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Oocyte cytoplasm plays important role on nuclear transfer with somatic cells. The current study evaluated the competence of in vivo matured oocytes obtained by OPU from superstimulated donor cows for production of nuclear-transferred embryos. Two experimental groups were established: G1 – in vitro-matured oocytes collected by OPU from non-superstimulated donors (n=8 OPU), and G2 – oocytes collected by OPU from donors (n=8 OPU) superstimulated with FSH (Folltropin, Bioniche, Ontario, Canada) and in vivo maturation induced by a GnRH analogue (Gestran, ARSA, Buenos Aires, Argentina). Donors of G2 received a norgestomet ear implant (Crestar, Intervet, Boxmeer, Holanda) followed by i.m. injection of 5 mg estradiol valerate and 3 mg norgestomet (day 0=D0). On D4, donors received 180 mg FSH divided in eight decreasing doses. On D6 afternoon and D7 morning 500µg sodium cloprostenol (Ciosin, Intervet) was injected i.m. On D7 the ear implant was withdrawn and 50 µg Gestran was injected i.m 12h after. OPU was performed 18h after Gestran injection. Oocytes of G1 group were matured in vitro for 17-18h and G2 oocytes were taken to enucleation. Matured oocytes were denuded and exposed to Hoechst 33342 (Sigma, St Louis, USA) and cytochalasin (Sigma) before enucleation. Embryos of G1 (n=52) and G2 (n=28) were reconstructed with somatic cells from adult Gyr cow, fused with double electric pulse of 2.4 kV/cm for 30 µsec and activated with ionomycin (Sigma) followed by 6-DMAP (Sigma). Embryos were culture in CR2aa supplemented with 2.5% fetal calf serum (Nutricell, Campinas, Brazil) under 5% CO2, 5%O2 and 90% N2. Cleavage and blastocyst rate were evaluated at 72h and 168h after activation. Data of oocyte total number and viable oocyte number/donor were evaluated by analysis of variance and rates of viable oocytes, fusion, cleavage and blastocyst were analyzed by Chi-square. There was no difference (P>0.05) on oocyte total number (16.25±3.2 and 13.75±3.9) and viable oocyte number (12.7±2.8 and 7.75±3.6) between G1 and G2, but viable oocyte rate was greater (P<0.05) for G1 (78.46%) than for G2 (56.36%). A greater expansion of cumulus cells was observed in G2, making the searching and identification of cumulus-oocyte complexes more difficult. No difference (P>0.05) on fusion (G1=69.2% and G2=57.1%), cleavage (G1=77.7% and G2=73.3%) and blastocyst (G1=25.0% and G2= 20.0%) rates was found. Induction of in vivo maturation by GnRH after superstimulation with FSH produces oocytes with similar competence to in vitro-matured oocyte in generating nuclear-transferred embryos; nevertheless, it impairs identification and decreases proportion of viable oocytes available for enucleation. Financial support: CNPq and Fapemig.
Embryonic stem cells (ESC) have been used in attempts to obtain specific tissues or even individuals. Those cells are pluripotent, allowing the differentiation of cell types from three germ layers. The establishment of a stable lineage of ESC is a valuable tool, however there are refractory strains of mice to ESC derivation and/or generation of chimeric animals (e.g., C57BL/6). Supplementation of culture medium with FCS, in the ESC derivation, may influence the potentiality to derivation and/or use of these strains in tetraploid complementation assays (Sato et al. 2009; Tsukuba Research Institute 47:414-22). Thus, its replacement was carried out using knockout serum replacement (KSR®; Gibco, Carlsbad, CA) to minimize the deleterious action of serum (Wang, H. et al. 2007; Inst. of Biotech. Mar.;23(3):269-72). The obtention, characterization of plurioty and perpetuating of these strains, is a important model for 37. Moreover, in species with economic interest, ESCs were obtained from female lineage C57BL/6EGFP aged between 21 and 30 d, weighting approximately 35g and superstimulated according Mancini et al. (2008; Transg. Res., 17: 1015). The animals were placed to mating - fertile males of same strain – and each male had access to a single female. The copulation was confirmed by plug vaginal. Embryo recovery was performed on days 3.5 to 4.0 dpc in order to obtain expanded (BX) and/or hatched blastocysts (BE). Zona pellucida was removed from BX embryos with the aid of Pronase solution and the whole embryos (n=8) were deposited on 4 well dishes (Nunc, Thermo Fisher Scientific, Rochester, NY) pretreated with pig skin gelatin 0.1%, under FPM in DMEM medium (Gibco, Carlsbad, CA) supplemented with 7.5% FCS (Orpion, Andradina, BR) and 7.5% KSR®(Gibco), α-mercaptoetanol 10mM, 1mM sodium pyruvate, 2mM L-glutamine and 83.4 mg/mL amikacin (Biochimica Institute, RJ, BR) for 24 h. After this period, the medium was replaced by DMEM supplemented with 15% KSR. The colonies began to grow between 3 and 6 days after culture of embryos. Once established the colony, it was picked and placed into new plates containing MFP(murine fibroblast primary) every 48 to 72 h. After 14 d, the derivation was confirmed (proof of pluripotency) by immunefluorescence for Oct3/4, Sox2 and Nanog, besides karyotyping for ploidy detection. The reaction was positive for all tested markers, in addition to the detection of the endogenous fluorescence of EGFP protein itself (C57BL/6EGFP origin). It was concluded that the derivation of ESC, originated from refractory strain as C57BL/6EGFP is feasible, although with a success rate of 12.5%. Unless stated, all reagents are from Sigma, St Louis, MO, USA. Fellowships and grants from FAPESP, Brazil.

**ABSTRACT 197**

**USE OF MEDIUM SUPPLEMENTED WITH FCS AND KNOCKOUT SERUM REPLACEMENT® FOR DERIVATION OF MOUSE EMBRYONIC STEM CELL FROM C57BL/6/EGFP**


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**ABSTRACT 198**

**ISOLATION AND CULTURE OF AMNIOTIC MEMBRANE MESENCHYMAL STEM CELLS OF CANINE FOETUS**


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Multipotent mesenchymal stem cells derived from the developing foetus are presents in amniotic membrane. These cells act in hematopoiesis and can be differentiated into mesenchymal tissues as osteogenic, adipogenic, and chondrogenic cells lines. Recently, the interest in this cell type has grown exponentially as an alternative to embryonic stem cells because of the rapid growth, good plasticity, and regeneration of damaged tissues and organs. It could be used as a therapy. The aims of this study were the isolation, culture and characterization of mesenchymal stem cells derived from amniotic membrane of canine's foetus. Samples of amniotic membrane were collected from mongrel's dogs' foetuses during cesarean section in term pregnancies. The amniotic membrane fragments were washed in PBS, and placed in a collagenase solution (Sigma) at 37 °C for 40 minutes. The suspension was centrifuged, the pellet was resuspended in culture medium DMEM (Invitrogen) with 20% Fetal Bovine Serum, supplemented with antibiotics and antimycotic and centrifuged again. The pellet was resuspended in 5 mL of medium, placed in a 25cm² flasks and cultured in 5% CO₂ incubator at 37.5°C. The medium was refreshed every 48 hours, and then twice a week. After trypsinization for the third passage, cells were resuspended in culture medium DMEM (Invitrogen) with 20% Fetal Bovine Serum, supplemented with antibiotics and antimycotic and centrifuged again. The medium was refreshed every 48 hours, and then twice a week. After trypsinization for the third passage, cells were resuspended in culture medium DMEM (Invitrogen) with 20% Fetal Bovine Serum, supplemented with antibiotics and antimycotic and centrifuged again. The culture was centrifuged again. The cells were placed on slides, fixed for Vimentin (marker for mesenchymal stem cells) and Cytokeratin (marker for cells of epithelial origin) immunocytochemistry evaluation. Negative controls were performed for both markers. Mesenchymal stem cells derived from canine amniotic membrane are easy to obtain and isolate. In 24 hours cells has adhered to the bottom of the flask and fibroblastic-like morphology was observed in 48 hours. The fibroblastic-like cells derived from amniotic membrane were positive for Vimentin and were negative for Cytokeratin. The analysis of immunohistochemistry positive for vimentin and negative for Cytokeratin demonstrated the characterization of undifferentiated mesenchymal cells. Tests to demonstrate the presence of multi-potential markers, studies of cellular ultrastructure by electronic microscopy, cytogetic tests to assess the presence of karyotype and osteogenic, condrogenic and adipogenic in vitro induction, are being made.
Microinjection into the male pro-nucleus, retroviral factors and transfected stem cells was being used to produce transgenic animals. All results judged inefficient as trial/success, expensive and involve complex techniques. Sperm Mediated Gene Transfer (SMGT) is studied since 1989 as a technique capable of solving these problems. Bracket in 1971 show the mammalian sperm’s ability to incorporate DNA through membranes. Lavitrano et al. (Cell, 1989) proved possible to make: the addition of exogenous DNA fractions to epididymal sperm in mouse, his subsequent transfer to the egg during the fertilization process, the transgene expression in a proportion of animals born normally and its transfer to subsequent generations. Also these authors prove that the ejaculated sperm was resistant to this process because of a glycoprotein in seminal plasma. Removing this obstacle, it is possible that the exogenous DNA will be incorporating to ejaculated spermatozoa (SPZ) preserving fertility potential. Subsequently, several researchers reported transgenic animals achieved by this method. In swine ejaculated SPZ capacity to incorporate exogenous DNA is correlated positively with best zootechnical parameters of semen quality. Our goal is develop transgenic sheep expressing heterologous proteins in milk using SMGT. As a preliminary step, we propose to obtain through IVF and AI, founders who have expressed a fluorescent plasmid (pEGFP-N1-Clontech) in cells of the adult organism. Six rams of Milschaff breed, after a health assessment and a clinical examination, were used to donate semen. In successive extractions four parameters were evaluated: libido, volume, density and vitality. Libido and vitality in subjective scale, from 0 to 5. Objective scales were used to volume and concentration (cc and SPZ/mm3). A full amount of 60 attempts produce a total of 48 semen collections. Libido: range amplitude from zero to five. Average 2.95. Volume average was 0.73 cc. rank 0-5 cc. SPZ concentration showed a great extent, among rams and between samples of the same ram. Standard, 7 x 10^6 sperm per mm^3. Range 0.163 x 10^7 to 9.2 x 10^7 SPZ per mm^3. Vitality: motility, vigor and strength showed smaller amplitude. Range 3-5. Average 4.38. Libido, ejaculate volume and concentration were highly variable between rams and between samples. Vitality was less variable. Last parameter showed more relevance at the time of judge the tolerance of semen to the co-incubation with fluorescent plasmid. Results in our trial are consistent with data presented by researchers cited for swine. Only 2 of 6 rams tested (33.3%) were selected at the moment of deciding their co-incubation with exogenous ADN.

**ABSTRACT 199**

**SPERM MEDIATED GENE TRANSFER IN SHEEP: SELECTING RAMS AS SEMEN DONORS**

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ARTIFICIAL ACTIVATION OF EQUINE AND BOVINE OOCYTES USING IONOMYCIN ASSOCIATED WITH 6 DMAP IN TWO CALCIUM CONCENTRATIONS

**ABSTRACT 200**

**ARTIFICIAL ACTIVATION OF EQUINE AND BOVINE OOCYTES USING IONOMYCIN ASSOCIATED WITH 6 DMAP IN TWO CALCIUM CONCENTRATIONS**

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Periodic and transient calcium (Ca2+) elevations and intracellular MPF activity blocking allow oocyte activation, essential event in TN and ICSI protocols. This study evaluated the artificial activation using ionomycin + 6 DMAP in equine and bovine oocytes with two Ca2+ concentrations. Equine compact or expanded oocytes and bovine oocytes were matured (IVM) in 5% CO2 in air at 38.5 ° C for 30 and 24 h, respectively. After the period of IVM, the oocytes (50 per group) were used in oocyte activation protocols in three replicates. Groups 1Bov, 1EqEx and 1EqComp- Incubated in the H-MEM + 10% FCS + 5ìM ionomycin for 8 min, washed in H-MEM + 10% FCS and incubated in H-MEM + 10% FCS + 2mM 6 DMAP + 5mg/ml cytochalasin B for 4 h. Groups 2Bov, 2EqEx and 2EqComp- Incubated in same conditions + 0.84 mg / ml CaCl2, washed in H-MEM + FCS and incubated in same conditions + 0.42 mg / ml CaCl2. After the activation period the oocytes were cultured for 18 to 20 h. The nuclear decondensation evaluation was performed in fluorescence microscopy with Hoescht 33342. The statistical differences was analyzed with Fisher exact test (0.05). According to Choi et al. (2001, Reprod, 122:177-183), the equine oocytes have a low rate of spontaneous parthenogenetic activation (1.42%), and this may be related to the inefficiency of artificial activation (Hinrichs et al., 2006, Reprod, 131: 1063 -1072). Apparently MII compact oocytes has reduced ability to respond of activation stimuli (Hinrichs et al., 1995, Biol Reprod, 1: 319-324), however, in this work were not obtained significant differences in activation rates after the protocol (1EqComp 26.67%; 2EqComp 37.78%, p = 0.367). When expanded oocytes were evaluated, we observed a positive numerical effect on combination ionomycin + 6 DMAP, independent of calcium concentrations (1EqEx 44.74%; 2EqEx 59.46%, p = 0.296). For Galli et al. (2007, An Reprod Sci, 98: 39-55), the pronucleus formation after artificial activation of equine oocytes using ionomycin + 6 DMAP was 60%, corroborating the results of this experiment. In bovine oocytes the treatment was effective in inducing oocyte activation (1Bov 40.82%; 2Bov 48.89%, p = 0.562), corroborating with the 40-70% obtained by Presicce and Yang (1994, Mol Reprod Develop, 37: 61 - 68). In this study, no significant differences were obtained between artificial activation groups in relation to calcium concentration. Thus, a more specific study of each step of the assisted reproduction techniques becomes increasingly necessary. Acknowledgement: FAPESP (04/00822-1).
RECONSTRUCTION OF BOVINE CLONED EMBRYOS BY handmade cloning (HMC) WITH 70% OR 100% TOTAL CYTOPLASMIC VOLUME

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In the HMC procedure, the reconstruction of each cloned embryo with 100% of cytoplasmic volume requires two cytoplasts from two distinct oocytes, obtained by manual bisection of each oocyte. For this, the half containing the metaphase plate (MII) is eliminated, whereas the enucleated half is used for embryo reconstruction. The procedure also allows the splitting of oocytes in unequal parts, using the 70% of the cytoplasm as a cytoplast and the nucleated 30% for other scientific purposes, such as for biochemical and molecular analyses. As embryo culture in HMC is performed individually in the WOW system, it is possible to perform a “cytoplasmic biopsy” in the oocyte for molecular analysis that can be matched to each embryo in culture, allowing the relationship between the cellular profile of the oocyte biopsy with the subsequent phenotype and cellular profile of the developing embryo. The aim of this study was to compare the in vitro development of embryos obtained by HMC, reconstructed with the cytoplasms from two oocytes (50% +50%) or from one oocyte (70%). Embryos were produced by HMC according to Ribeiro et al. (2009, Stem Cells Cloning, 11:377-385). For the production of 70% volume embryos (Group 70%), each oocyte was split into an enucleated part with 70% of the volume (used for embryo reconstruction) and another nucleated with 30% (stored individually for molecular analysis). In group 100%, each oocyte was splitted in half, with each embryo reconstructed with two enucleated 50% volume cytoplasts. Embryos were cultured individually for 7 days in the WOW system. Cleavage and blastocyst rates were evaluated by the χ² test, for p<0.05. No significant differences were seen in cleavage rates between groups 70% (59.9%, 200/334) and 100% (61.1%, 96/157). However, development to blastocyst was higher in group 100% than in group 70% (23.6%; 37/157 vs. 12.6%; 42/334, respectively). Nevertheless, when the number of oocytes used for embryo reconstruction was taken into account (two oocytes for each reconstructed embryo in group 100% and one oocyte for each reconstructed embryo in group 70%), no differences were detected between groups in the overall efficiency for embryo production (12.6% vs. 11.8%, respectively). Therefore, the use of an oocyte for the reconstruction of each individual embryo by HMC, in a per oocyte basis, did not affect the in vitro development to the blastocyst stage, thus enabling the use of this strategy for assessing the intrinsic ability of each oocyte to sustain embryonic development during pre-implantation stages.

PRODUCTION OF CLONE EQUINE PREGNANCIES USING DIFFERENT IN VITRO CULTURE MEDIA

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The aim of our study was to evaluate two culture media (group I: SOF, group II: DMEM/F12) during the first three days of embryo development. Ovaries of slaughtered mares were collected during the breeding season (Argentina, Southern hemisphere). COCs were recovered by a combination of scraping and washing of all visible follicles with a syringe filled with DMEM supplemented with 1 mM sodium pyruvate and 15 IU mL-1 heparin. The medium of maturation was TC1-199 supplemented with 10% fetal bovine serum (FBS), 1 L mL-1 insulin-transferrin-selenium, 1 mM sodium pyruvate, 100 mM cysteamine, and 0.1 mg mL-1 of FSH at 39°C in a humidified atmosphere of 5% CO2 in air. The cumulus were removed by a trypsin treatment and vortexing in hyaluronidase (1 mg mL-1). Cloning and fusion procedures were performed following the zona-free technique described by Lagutina et al. (2007 Theriogenology 67, 90–98). The activation process was 8.7 mM ionomycin in H-TALP for 4 min followed by 4 h culture in 1 mM 6-DMAP and 10 mg mL-1 cycloheximide in SOF. Embryos were cultured in well of well (WOW) system in two different culture media: SOF (group I) or DMEM/F12 with 5%FBS and antibiotics (group II). The medium of both groups were replaced on Day 3 with DMEM/F12 with 10% FBS. Cleavage was assessed 48 h after activation; the rate of blastocyst formation was recorded at Day 7 and Day 8. Blastocysts were evaluate and classified in three grades according to their morphology. Some of these embryos were placed in 0.5 mL equilibrated SOF in a straw transported in a shipping container warmed to 38.2°C, and shipped 3h before transfer to mares. Transfers were performed transcervically to 5-8 days after ovulation. Mares were synchronized by induction of ovulation using hCG (OVUCIN®). Pregnancies were evaluated by transrectal ultrasonography starting 7 days after transfer. Results were compared using Fisher test (P < 0.05). Cleavage rates did not differ statistically between groups (I: 63/66, 94.45%; II: 61/67, 91.04%). No significant differences were found in blastocyst development; however, we observed (I: 6/63, 9.09%; II: 12/61, 17.91%). In group I, 6 blastocysts were transferred to 4 mares obtaining one vesicle that was lost at day 30. In group II, 5 blastocysts were transferred to 3 recipients obtaining two vesicles. Only one of them was lost. In conclusion, despite the fact that both culture media were able to produce in vitro cloned embryo, a tendency to a higher blastocyst rate and an advance pregnancy were obtained when the culture medium was DMEM/F12.
EFFECT OF HEAT SHOCK AND DMSO IN THE INTEGRITY AND MOTILITY PATTERNS OF SPERM CELLS UNDERGOING MURINE SMGT

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Sperm-mediated gene transfer (SMGT) enables the production of transgenic animals by exploiting the ability of sperm cells to bind and internalize DNA. However, even though the sperm membrane has an intrinsic mechanism of DNA internalization through plasma membrane (PM) receptors, it still acts as a barrier to SMGT. This study aimed to evaluate sperm membranes and acrosome integrity, mitochondrial potential and motility profile of C57Black6 mice after dimethylsulfoxide (DMSO)-SMGT at 4°C. Thus, sperm from caudate epididymides and vas deferens were incubated with circular PCX-EGFP membranes and acrosome integrity; mitochondrial potential and motility profile of C57Black6 mice after dimethylsulfoxide (DMSO)-SMGT at 4°C. Also, sperm from testicular ducts were incubated with circular PCX-EGFP membranes and acrosome integrity; mitochondrial potential and motility profile of C57Black6 mice after dimethylsulfoxide (DMSO)-SMGT at 4°C. The integrity of PM, acrosome and mitochondrial potential were assessed by flow cytometry (Guava EasyCytemini) with fluorescent probes PI, FITC-PSA and JC-1, respectively (n=10,000 cells/group). For this, 5x10⁶ cells were stained with 3μL PI (0.5mg in PBS), 50μL FITC-PSA (10μg in PBS) and 2μL JC-1 (153μM in DMSO). The motility patterns were analyzed by computer-assisted semen analysis (CASA) (IVOS-OX, Hamilton). The results were verified by interaction analysis checking the variables temperature, presence of DMSO and presence of plasmid DNA no interaction among the three variables. The test of equality of variances and Student's t-test were performed, comparing each variable individually, adopting 5% of significance level. Of all the categories analyzed (motility, progressive motility (PROG), VAP, VSL, VCL, ALH, BCF, STF, LIN, area, intact acrosome and sperm membrane integrity (AIIM)), damaged membrane intact acrosome (AIIM), intact acrosome damaged membrane (AIIM), membrane and acrosome damaged (ADML) and low mitochondrial potential (LP)), the variables that showed differences were DMSO and the presence of plasmid DNA (p<0.05); categories PROG, VSL and area for variable DMSO and VCL, LIN and STF for the variable DNA. This suggests that heat shock does not affect significantly the motility pattern or the sperm integrity in DMSO-SMGT. Both the presence of DMSO or DNA affect the motility patterns, but only the DMSO affects plasma membrane and acrosome integrity.

IN VIVO EMBRYO PRODUCTION IN A FOUNDER TRANSGENIC GOAT FOR hG-CSF

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In order to multiply the founders obtained simple and efficient techniques such as in vivo embryo production should be employed. In 2008, our group obtained the birth of two transgenic animals (1 male and 1 female) for the human Granulocyte Colony Stimulating Factor (hG-CSF). Thus, this study aimed to evaluate the superovulation response of female founder for posterior collection and embryo transfer. Two Canindé goats, one transgenic and one non-transgenic (control) were used as embryo donors and eight undefined breed goats as recipients. Two sessions for in vivo embryo production were performed. All females were submitted to estrous synchronization using intravaginal sponges with 60 mg MAP (Progespon, Syntex, Argentina) for 10 days and 75 μg d cloprostenol on the eighth day (Prolise, Syntex, Argentina) i.m. Donors received 120 mg pFSH (Folltropin, Bioniche, Canada) i.m., at a 12h interval, starting 48h before sponge removal for three days. Recipients received on the eighth day 400 IU eCG (Novormon, Buenos Aires, Argentina). In the second session flunixin-meglumine (1.1 mg/kg; Flumedin, Jofadel, Brazil) was used starting 72h after sponge removal for four days, with a 12h interval. Donor fertilization was performed at estrous onset and 24h after, using two non-transgenic bucks. Embryo collection was performed by laparotomy seven days post-estrus and structures were evaluated by a stereomicroscope. Pregnancy was diagnosed by ultrasound 30, 45, 60 and 90 days after post-estrus. In the first superovulation session, donors showed estrus at 24h after sponge removal and both were responsive to superovulatory treatment (e.g. ovaries/females), with a total of 10 (transgenic) and 12 (non-transgenic) corpora lutea (CLs). However, the female transgenic presented CL premature regression that derailed embryo recovery. In the second session, donors showed estrus at 24h after progestagen treatment and responded to superovulation, with 13 (transgenic) and 12 (non-transgenic) CLs. In both sessions all recipients showed estrus and ovulated. Embryo recovery was 69.2% (9/13) in the female transgenic and 100% (21/21) in the non-transgenic. Non-transgenic female embryos were vitrificated and those from transgenic transferred to five recipients. On day 30, the pregnancy rate was 80% and dropped to 60% at 45 days after estrus. From the three pregnant recipients, two showed twin pregnancy, for a total of five fetuses. The founder transgenic female obtained in our laboratory may be used in programs of in vivo embryo production in an attempt to accelerate and increase its descendants.
In the nuclear transfer technique, the ability of the oocyte remodel the cell's nucleus and result in embryo development is influenced by the cell types. The formation of a somatic cells bank is an important strategy, and involves a cryopreservation process. Considering the importance of bovine cloning, this work had the following objectives: a-evaluate the possibility of isolation and culture of umbilical cord cells, adipocytes cells and fibroblasts from bovine fetuses b-test the effect of two cryopreservation solutions containing DMSO or DMF to protect the cells, c-determine if the trypsin blue dye is better than the fluorescent probes (carboxyfluorescein acetate and propidium iodide) for identify the viability of frozen cells. Bovine tissues were collected at the slaughterhouse, cut into small pieces, placed in petri dishes, covered with 3 mL of Dulbecco's Modified Eagle Medium (DMEM) and incubated in an incubator at 38.5 °C and with CO25%. After 7 days, biopsies were retrieved and the medium changed. Passed over seven days, the cells were treated with trypsin and placed in culture bottles until reach confluence. For cryopreservation, cells were distributed in two cryoprotectant solutions: T1-DMEM with Fetal Bovine Serum (FBS) 10% and DMSO 10%, T2-DMEM with FBS 10% and DMF 5%. Then, the cell solutions were placed in 0.25 ml straws, kept at -80°C for 24 hours and stored in liquid nitrogen. The rate of cell viability after thawing was assessed with trypsin blue dye and carboxyfluorescein acetate-propidium iodide. In all tissues studied was possible to isolate cells with the same growth pattern in DMEM. The medium with cryoprotectant DMSO preserved 85.50 ± 4.95%, 37.5 ± 10.6% and 82 ± 8.48, of fetal fibroblasts, umbilical cord cells and adipocytes cells, respectively. Already the cryoprotectant medium with DMF preserved 54 ± 5.65%, 28.5 ± 21.92% and 56 ± 1.41%, these same cells, respectively. The medium with DMSO was significantly (P=0.0096) more efficient than the medium with DMF to preserve the viability of different cell types. There was no statistical difference between the trypsin blue and carboxyfluorescein acetate-propidium iodide to detect cell integrity. Thus, we conclude that is possible to isolate and cryopreserve fetal fibroblasts, umbilical cord cells and adipocytes cells to compose a cryobank with potential use in nuclear transfer technique. The cryopreservation medium with DMSO is indicated to cryopreserve the cell types studied and cell viability can be efficiently evaluated with trypsin blue or carboxyfluorescein acetate and propidium iodide.
DNA FRAGMENTATION AND OXIDATIVE STRESS IN HEAT SHOCK DMSO SPERM MEDIATED GENE TRANSFER

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Sperm-mediated gene transfer (SMGT) uses sperm cells as vectors for transgenesis. However, damage to this cell during transfection is poorly evaluated. Thus, this study aimed to analyze the susceptibility to fragmentation of genomic DNA and oxidative stress of sperm subjected to dimethylsulfoxide (DMSO)-SMGT and heat shock, a technique that increases 20% of SMGT efficiency (SHEN et al., 2006; Mol Reprod Dev, 73:589-594). Thereby, sperm from caudate epididymides and vas deferens of oxidative stress of sperm subjected to dimethylsulfoxide(DMSO)-SMGT and heat shock, a technique that increases 20% of SMGT were obtained from umbilical artery catheterization five minutes after birth, with values for pCO2, pO2, TCO2, pH, HCO3 and BE of cm to 2.13 cm from Days 60 to 132 day. Renal ultrasound images suggested pielectasia and hydronephrosis. Blood gas data were obtained by thiobarbituric acid reactive substances assay (TBARS), in which 1.5x10^6 spermatozoa were treated with 100µl ferrous sulphate(4mM) and 100µl sodium ascorbate(20mM). After 90min incubation at 37°C, was added cold trichloroacetic acid 10% in a 1:2 ratio for each sample and then centrifuged at 18,000xg for 10min. Supernatant was chilled to 0°C to stop reaction. The lipid peroxidation product (malodialdeide - MDA) was measured by spectrophotometry. Statistical analyses showed interaction among variables temperature, presence of DMSO and plasmid DNA. The test of equality of variances and Student's t test were performed, comparing each variable individually, adopting a 5% significance level. The 3 variables temperature, DMSO and DNA showed no significant differences in any category (p>0.05). However, the presence of plasmid DNA, albeit not significant, increased production of MDA (p=0.0910). Thus, the challenge of SMGT to sperm cell with DMSO and heat shock did not alter the susceptibility to DNA fragmentation or oxidative stress. However, incubation with exogenous DNA suggests an increased oxidative stress in murine spermatozoa.

GESTATIONAL AND NEONATAL FINDINGS IN CLONED SHEEP OBTAINED BY SCNT

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The low viability of the newborn due to placental and fetal abnormalities are some of the problems in animal cloning, specially in sheep. Aiming to know how, embryonic and fetal development of sheep clones derived from SCNT were monitored by ultrasound (U.S.), associating them with the main changes observed in neonates. Oocytes collected by oviduct flushing of hormonally synchronized donor ewes were used as nucleus recipients of fibroblast cells from an adult Santa Ines sheep. Two to four reconstructed embryos were transferred to the female, to 15 recipients. Only one pregnancy has reached term. The monitoring of heart rate (BPM) and measures of the diameter of the umbilical cord insertion into the abdomen were performed by transabdominal ultrasound (Aloka 500, Pie Medical). A gradual reduction of the 214HR to 152HR was seen from Days 60 to 132, decreasing to 146HR on Days 144-147 of pregnancy. For the umbilical cord, changes were observed from 1.12 cm to 2.13 cm from Days 60 to 132 day. Renal ultrasound images suggested plicetasia and hydrenephrosis. Blood gas data was obtained from umbilical artery catheterization five minutes after birth, with values for pCO2, pO2, TCO2, pH, HCO3 and BE of 62.8 mmHg, 7 mmHg 36 mmol/L, 7.332, 33.2 mmol/L and 7 mmol/L, respectively. Although the lamb did not undergo acidosis and neonatal asphyxia, the increased value for pCO2 and the extremely low pO2 level demonstrated the animal’s difficulty in removing CO2 and uptaking oxygen in the first moments of extrauterine life. A cardiac arrhythmia and severe dyspnea during the first 10 min of life was followed by bradycardia and cardiopulmonary arrest 30 min after birth. The main changes visualized by U.S. and confirmed at birth were larger diameter of the umbilical cord (2.2 cm) and fetal gigantism (5.950 kg) compared with the parameters of the breed, and kidney abnormalities. At necropy, incomplete expansion of the lungs, severe pulmonary congestion and edema, serum-bloody ascites, and severe pelvic diatasis and edema of the kidneys with congestion and hemorraghic foci were highlighted. No anatomical abnormalities of the heart or liver were detectable. A histopathologically pulmonary congestion was observed, with consequent thickening of the alveolar wall and hemorrhagic foci. Also, the analysis detected renal medullary tubular dilatation and degeneration, congestion and intense intratubular hemorrhage, poor disclosure of the cortical and glomeruli. A total of 60 cotyledons were visible in the fetal membranes, which is 10% lower than the number cited for the species, for singleton pregnancies. Renal changes may have interfered in the establishment of cardio-respiratory function after birth, with no expansion of the lungs and a likely systemic hypertension or uremic syndrome, resulting in swelling and pulmonary congestion, and death of the newborn. The monitoring of fetal development by U.S. can be a valuable tool for the identification of changes and to predict the chances for survival of cloned animals, making cloning sheep perhaps a less difficult challenge to overcome.

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EFFICIENT TRANSGENE EXPRESSION IN IVF AND PARTHENOGENETIC BOVINE EMBRYOS BY INTRACYTOPLASMIC INJECTION OF DNA-LIPOSOME COMPLEXES

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Transgenic animals constitute an important tool with many biotechnological applications. Although there have been advances in this field, we propose a novel method that may greatly increase its application. This new technique consists of the intracytoplasmic injection of liposomes, in bovine oocytes and zygotes, to introduce exogenous DNA. For this, bovine oocytes were matured in TCM-199, containing 2mM glutamine, 10%FBS, 10ug/ml FSH, 0.3mM pyruvate, 100mM cysteamine and 2% antibiotic, for 22h. In-vitro fertilization (IVF) was done by co-incubation for 5 h with thawed spermatozoa in a final concentration of 12-15x10^6/ml in BO medium. Parthenogenetic activation was done with 5µM ionomycin for 4 min and 1.9mM 6-DMAP in SOF for 3h. Before or after IVF or parthenogenetic activation, oocytes and presumptive zygotes were injected, throw micromanipulation, with a DNA-liposome/PVP mixture (final DNA concentration 0.5µg/ml). The plasmid used was pCX-EGFP, which contains an enhanced green fluorescent protein gene (egfp). Fertilized and activated oocytes were cultured in SOF with 2.5% FBS in a humidified atmosphere of 5% O₂, 5% CO₂ and 90% N₂ in air at 39ºC. Cleavage and EGFP expression were evaluated on day 3, and blastocysts on day 7 in in-vitro culture. These treatments were analyzed by Fisher test (P<0.05). In the first experiment, we evaluated embryo development and EGFP expression of IVF embryos, after injection of DNA-liposome complexes into pre-fertilized oocytes and presumptive zygotes (16 and 24 h post-fertilization). The percentages of EGFP-positive cleaved embryos for the pre-fertilization group was 6.3% (4/27) and for the 16 h and 24 h post fertilization were 68.4% (n=52/76) and 30.1% (21/68), respectively. In our conditions, control groups injected with the plasmid alone, were always negative. The highest EGFP-positive blastocyst rate was obtained from the 16 h post-fertilization group 52.2% (12/23). In the 24 h post-fertilization group, 31.6% (6/19) of the blastocysts were EGFP-positive, and none of the pre-fertilization group expressed the transgene. Additionally, the presence of the transgene was confirmed by PCR analysis in all the blastocysts analyzed, that were previously injected. In the second experiment, we examined the development and EGFP expression of parthenogenetic embryos after the injection of egfp-liposome complexes into pre-activated oocytes, and 3 h and 11 h post-activated oocytes. The evaluation of these embryos after 3 days of culture showed that the group with the highest EGFP-expression rate was the one injected 3 h post-activation (48.4%, 15/31). The pre-activation and 11 h post-activation groups had lower percentages of EGFP-expression, 14.3% (2/14) and 13% (3/23), respectively. On day 7 of culture, 20% of the blastocysts (1/5) from the pre-activation group were EGFP-positive, and in the 3 h post-activation group 60% (6/10) expressed the transgene, whereas, in the 11 h post-activation group all the blastocysts were EGFP-negative. In addition, no differences in the percentages of blastocyst development were found between injected embryos and IVF or parthenogenetic controls. In summary, this study reports the efficient, reproducible and fast production of IVF and parthenogenetic embryos expressing EGFP, by intracytoplasmic injection of liposomes to introduce foreign DNA.
Biotecnologias Suporte: Criopreservação e Criobiologia, Diagnóstico por Imagens, Biologia Molecular e “Ômicas”

Supportive Biotechnologies: Cryopreservation and Cryobiology, Image Analysis and Diagnosis, Molecular Biology and “Omics”
The aim of the present study was to in vitro evaluate the "S" phase of the cellular division using the immunocitochemistry technique BrdU in equine embryos vitrified using different solutions. A total of 16 equine embryos (<300µm) were divided in 4 groups: G1(n=4)– commercial kit produced by Bioniche™; G2(n=4) e G3(n=4)– solutions prepared in our laboratory with glycerol and dimethylformamide or glycerol and ethylene glycol, respectively; G4(n=4)– fresh embryos used as control. The vitrified embryos were warmed and stabilized in TQC holding™ solution for 5min, after this they were incubated in PBS solution with 1mM of BrdU for 1h at 39°C, then they were washed in 3 baths in PBS with FBS (0.05%) of 5min each. Immediately after the last bath each embryo was fixed in a solution containing 2.5% of paraformaldehyde. Then they were assigned into batches containing at least one embryo of each studied group. The cellular membrane permeabilization was performed with a solution 0.15% of Triton X-100 with 10% FBS during 20min at 38°C. After this, the embryos were washed in 3 baths in PBS with FBS (0.05%) during 5min each, then incubated for 1h in mouse monoclonal IgG anti-BrdU (Sigma, B2531) diluted in PBS with BSA (0.05%) at 1:500 dilution. After another bath of 15min the embryo was placed in goat polyclonal immunoglobulin anti-mouse IgG labeled with FITS. After all this: incubated with 1mM of BrdU, fixed, permeabilized and stained (Ag-Ac), the embryo was exposed to 0.5µg/ml DAPI during 5min at 38°C, to a general staining of nuclei. Then they were incubated with 1mM of BrdU, fixed, permeabilized and stained (Ag-Ac), the embryo was exposed to 0.5µg/ml DAPI during 5min at 38°C, to a general staining of nuclei. Then they were incubated with 1mM of BrdU, fixed, permeabilized and stained (Ag-Ac), the embryo was exposed to 0.5µg/ml DAPI during 5min at 38°C, to a general staining of nuclei. Then they were incubated with 1mM of BrdU, fixed, permeabilized and stained (Ag-Ac), the embryo was exposed to 0.5µg/ml DAPI during 5min at 38°C, to a general staining of nuclei.

The embryos were observed and the cells were counted at the fluorescence microscope using distinct filters. Were performed a repetition of each cell counting, and the mean value were analyzed using ANOVA at 5% of significance. The result of each studied group. The cellular membrane permeabilization was performed with a solution 0.15% of Triton X-100 with 10% FBS during 20min at 38°C. After this, the embryos were washed in 3 baths in PBS with FBS (0.05%) during 5min each, then incubated for 1h in mouse monoclonal IgG anti-BrdU (Sigma, B2531) diluted in PBS with BSA (0.05%) at 1:500 dilution. After another bath of 15min the embryo was placed in goat polyclonal immunoglobulin anti-mouse IgG labeled with FITS. After all this: incubated with 1mM of BrdU, fixed, permeabilized and stained (Ag-Ac), the embryo was exposed to 0.5µg/ml DAPI during 5min at 38°C, to a general staining of nuclei. Then they were incubated with 1mM of BrdU, fixed, permeabilized and stained (Ag-Ac), the embryo was exposed to 0.5µg/ml DAPI during 5min at 38°C, to a general staining of nuclei. Then they were incubated with 1mM of BrdU, fixed, permeabilized and stained (Ag-Ac), the embryo was exposed to 0.5µg/ml DAPI during 5min at 38°C, to a general staining of nuclei. Then they were incubated with 1mM of BrdU, fixed, permeabilized and stained (Ag-Ac), the embryo was exposed to 0.5µg/ml DAPI during 5min at 38°C, to a general staining of nuclei.

The objective of this study was to identify the damage caused by cryopreservation, assessing the ultrastructural viability of sheep embryos undergoing classic freezing and vitrification in OPS (Open Pulled Straw). Embryos (N = 186) obtained from superovulated Santa Ines sheep were randomly divided into three groups: control, dimethylsulfoxide (DMSO) and dimethylformamide (DF). Embryos in the classic method group were frozen using automatic freezing. The embryos of the second group were placed in a DMSO solution containing 10% EG and 10% DMSO and then transferred to a vitrification solution with 20% EG and 20% DMSO + 0.5M sucrose. The embryos of the third group were placed in a balanced solution containing 10% EG and 10% DMSO and then transferred to a vitrification solution with 20% EG and 20% DF + 0.5M sucrose. Embryos of all three groups were maintained in liquid nitrogen until experimental procedures. Straws containing embryos were thawed at room temperature for ten seconds, immersed in water at 37 °C for twenty seconds and washed with maintaining medium. For transmission electron microscopy embryos were processed as laboratory routine and observed on FEI Morgagni 268D microscope. According analysis of in vitro survival vitrified embryos with DF had higher rates of survival (53.33%) than embryos vitrified with DMSO (26.66%) and frozen by the traditional method (33.33%). The ultrastructural study also showed that DF vitrified embryos had a greater preservation of cells although a great vacuolation, mitochondria showed preserved cristae, the Golgi lamella were well organized and the mitochondrial matrix showed normal aspect. Embryos from DMSO group showed mitochondrial cristae destruction, microvilli disruption, large vacuoles and cytoplasm disordering. Embryos from the classic freezing method presented mitochondrial swelling and cytoplasm extraction. The most important results observed were the loss of junctions between the cells and nucleus with blebs. It can be concluded that the vitrification solution containing DF is an effective method for cryopreservation of goat embryos produced in vivo. Keywords: Cryopreservation, vitrification, embryos, cryoprotectants, electron microscopy.
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USE OF DILUENT FOR ETHYLNE GLYCOL SEMEN CRYOPRESERVATION OF SHEEP

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Glycerol has been the cryoprotectant used in for freezing in various species, but in sheep sperm has a toxic effect and induces or accelerates the acrosome reaction (Slavik, 1987, Journal of Reproduction and Fertility, 79, 99-103). However, the ethylene glycol has been attracting interest for ram semen cryopreservation due to its toxicity and high permeability to plasma membrane. This study aims to compare the effect of ethylen glycol and glycerol as cryoprotectants for freezing ram semen, analyzing its effect on: total motility (TM) and progressive (PM), vigor (V) and membrane functionality after thawing. Seven collections were made with an artificial vagina. The analysis of seminal parameters were measured before and after freezing semen. The motility was expressed as percentage, the vigor was rated 0-5, where 0 meant absence of movement and 5 meant fast movement of spermatozoa. The frozen semen was performed in two steps. The semen was diluted in fraction A (FA: TRIS-yolk). Then the samples were placed inside a beaker of 200 mL with water to 30 °C. Then it was added to a styrofoam box containing 500 mL of water and two ice packs for 30 minutes at 10 °C. Then it was added to fraction B (FA + cryoprotectant) (1:1) containing 14% glycerol or 6% ethylene glycol. Were added two more ice packs in the box styrofoam, for 30 minutes, until the temperature reached 5 °C. The semen was packaged in 0.5 mL straws, and placed 6 cm from the level of liquid nitrogen (N2L), for 15 min. Were immersed and stored in N2L in cryogenic cylinder. The straws were thawed in a water bath at 37 °C for 30 seconds and evaluated for TM, PM and V. The hyposmotic test was performed in semen in natura and after thawing of semen which, counting 200 cells, where linear tail represented intact cells and rolled up tail injured cells. We performed staining with PSA, propidium iodide and JC-1, counting 200 spermatozoa in a fluorescence microscope. The results were evaluated with the computer application SAEG, submitted to variance analysis and means compared by the TUKKEY test 5%. The semen freezing with glycerol or ethylene glycol did not present significant difference for TM (65.8 % and 51.0 %), PM (40.1 % and 32.1 %), V (3.4 and 3.7) and mitochondrial activity (36.2 % and 32.7 %), respectively. However samples freezing with ethylene glycol had presented greater percentage of intact cells (74.8% and 66.3%) and minor percentage of acrosoma reacted (16.9 % and 29.9 %) that the samples freezing in glycerol, respectively. The results show that both the extender preserve the physical parameters of the spermatozoa, however ethylene glycol preserved better its functional characteristics, what it can contribute to reduce premature the acrosome reaction of ram frozen semen.

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TECHNICAL REPORT: CONCEPTION RATE OF IN VITRO PRODUCED BOVINE EMBRYOS AFTER VITRIFICATION BY CRYOTOP METHOD

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Cryopreservation of in vitro produced (IVP) bovine embryos is a key step to improve the commercialization of genetic material. IVP embryos differ considerably from those produced in vivo, providing physical and morpho-physiological characteristics that make them more sensitive to cryoinjury (VAJTA 2000, Anim Reprod Sci, 60:357-364). Furthermore, IVP embryos from Zebu cattle seem to be even more sensitive to traditional slow freezing. However, satisfactory conception rates have been obtained when these embryos are vitrified. Transfer of IVP vitrified embryos from Gir (Girolando) and Simbrasil donors, performed by this team, showed that conception rates was 61.1% (n = 36) and 50.0% (n = 10) at 30 days, respectively. Based on these results, the aim of this report was to evaluate the conception rates of IVP fresh or vitrified embryos from Nellore cows, on the same commercial farm. The experiment was conducted at Eldorado’s farm, Itapetininga, SP, Brazil. After in vitro fertilization using conventional semen, embryos were vitrified at the expanded blastocyst stage (Bx) by Cryotop method with cryoprotectants solutions of Ethylene Glycol (EG) + Dimethyl sulfoxide (DMSO). The conception rates of 480 IVP fresh embryos were 49.4 and 40.8% at 30 and 60 days, respectively. The IVP vitrified embryos showed 47.7% (n=44) of conception. These results, combined with the other reports with the same technique, have established that vitrification is an applicable alternative to improve the viability of IVP cryopreserved embryos, possibly by preventing the intracellular ice formation and by promoting less damage to embryonic cells. Acknowledgements: Golin Group.
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CAPRINE OVARIAN TISSUE CRYOPRESERVATION USING ETHYLENE GLYCOL, PROPANEDIOL OR DIMETHYL SULFOXIDE


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One of the main factors for the success of cryopreservation is the choice of the type, as well as the association or not of intra-and extracellular cryoprotective agents, or other supplement as fetal calf serum (FCS). Thus, the objectives of this study were to evaluate the addition of FCS and sucrose (SAC) to the freezing solution for goat ovarian tissue and to compare the efficacy of three intracellular cryoprotectants (ethylene glycol - EG, propanediol - PROH and dimethyl sulfoxide - DMSO). For this purpose, five pairs of ovaries of adult mixed-breed goats were collected at a local slaughterhouse and transported to the laboratory in Minimum Essential Medium (MEM). At the laboratory, each pair of ovaries was divided into 13 fragments of approximately 9 mm³. One fragment was randomly selected as the fresh control and it was fixed for histological analysis (HA). The remaining fragments were exposed to 1.8 mL of freezing solution consisting of MEM plus 1.0 M EG (5 min), PROH (5 min) or DMSO (10 min), supplemented or not with 10% FCS and/or 0.1 M SAC. After exposure, samples were transferred to a programmable freezer pre-cooled to 20 °C and cooled until -70°C. Finally, the samples were stored in liquid nitrogen. After seven days, the fragments were thawed and the cryoprotectant were removed by three washes in decreasing concentrations of sucrose (0.5 M, 0.25 M and 0 M). The fragments were fixed and destined to HA to evaluate follicular morphology. The percentage of morphologically normal follicles from fresh control and after cryopreservation was subjected to ANOVA followed by Dunnett and SNK tests (P < 0.05). Follicular morphology showed no significant difference in the percentage of normal follicles among different cryoprotectants. Similar results were observed when freezing medium was supplemented with FCS and/or SAC. However, only solutions containing DMSO alone (69.3%) or plus 10% FCS (70.0%) were able to maintain follicular morphology similar to control (85.5%). Based on these results, we recommend the use of 1.0 M DMSO with or without 10% FCS for caprine ovarian tissue cryopreservation.

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VITRIFICATION OF Mus domesticus domesticus EMBRYOS LOADED IN QUARTZ MICROCAPILLARIES OF TWO DIFFERENT DIAMETERS


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One major goal of vitrification techniques is reduce the toxicity effect of the cryoprotectant solutions on embryo viability. Reduce the volume of these solutions and use high-speed vitrification curves are approaches to achieve suitable embryo survival rates (HE et al., Cryobiology, v.56 p.232-232, 2008). The aim of this experiment was to determine survival rates after vitrification of murine blastocysts loaded in quartz microcapillaries (QCM) of two different diameters. Mus domesticus domesticus blastocysts were collected on day 4 of pregnancy, morphologically selected and aleatory divided into four groups. Group 1 (control)- embryos transferred immediately after collection into 100 µl droplets of KSOM medium for culture; Group 2 – embryos loaded into QCM of 0.1 mm diameter; Group 3 – embryos loaded into QCM of 0.2 mm diameter; Group 4 – embryos were placed into culture medium droplets after vitrification of groups 2 and 3 embryos. Groups 2 and 3 selected embryos were exposed for one minute at a dehydration solution containing PBSm, supplemented with 0.5% PVA, 10% DMSO, 10% ethylene glycol (EG). After they were exposed for 30 seconds to vitrification solution containing PBSm, supplemented with 0.5% PVA, 20% DMSO and 20% EG. Then these embryos were loaded into quartz micropipettes of 0.1 or 0.2 mm of diameter. The vitrified embryos were rewarmed into PBS supplemented with 0.4% BSA and sucrose (0.25 M) at 37°C for 5min to remove the cryoprotectant. The blastocysts were finally placed into KSOM 100µl medium droplets for in vitro culture and evaluated 72h later. The hatching rates were: Group 1 – 91% (21/24), Group 2 – 54% (13/24), Group 3 – 62% (18/29) Group 4 – 88% (38/34). The results shows that there was no significant difference among the blastocysts hatching rates of experimental groups 1 and 2. The tested QCM diameters allows similar embryo survival rates after vitrification.
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EVALUATION OF THE CYTOSKELETON, PATTERN OF MITOCHONDRIAL ACTIVITY AND ULTRASTRUCTURE OF FROZEN OR VITRIFIED SHEEP EMBRYOS

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Cryopreservation of sheep embryos has been widespread, but their survival rate and pregnancy have shown great variation. The aim of the present work was to evaluate in vivo produced ovine embryos after slow freezing or vitrification, by determining the pattern of mitochondrial activity, cytoskeleton integrity and ultrastructural characteristics. Embryos were collected from superovulated ewes, classified and selected for cryopreservation (IETS). Embryos subjected to slow freezing (n = 22) were exposed to 0.75 Methylene glycol (EG) for 10 minutes, 1.5M EG for 10 minutes, packed in 0.25mL straws and subjected to programmable freezing (-1 °C/min, seeding at -7 °C, 0.3 °C/min to -35 °C) followed by immersion in liquid nitrogen. Vitrified embryos (n = 24) were placed in 10% EG + 10% DMSO solution for 1 minute and 30 seconds, then in 20% EG + 20% DMSO solution with 0.5M sucrose for 30 seconds, packed in OPS and plunged into liquid nitrogen. The cryopreserved embryos were warmed, in vitro cultured for 1 hour, reclassified and subdivided for evaluation. The cryopreserved embryos showed variable degree of disorganization of the cytoskeleton, and expanded blastocysts were vitrified points of discontinuity. The absence of labeling of mitochondrial activity after rewarming was a common finding in both groups, but these organelles were mostly normal ultrastructure. Areas free of cytoplasmic organelles, the Golgi complex from the nucleus and decreased frequency of intercellular junctions specialized ultrastructural features were consistent with the disorganization of the cytoskeleton in both groups. In general, cryopreserved embryos showed ultrastructural features similar to fresh embryos of the same quality, however, the vitrified had higher frequency of large vesicles digestion.

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EFFECTS OF SMALL RUMINANT OVARIAN TISSUE CRYOPRESERVATION PROCEDURE ON THE FOLLICULAR MORPHOLOGY AND STROMAL CELLS DENSITY

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The aim of this study was to evaluate the effects of exposure to ethylene glycol (EG) and cryopreservation of caprine and ovine ovarian cortical fragments on the morphology of primordial follicles and stromal cell density. Caprine and ovine ovarian fragments were exposed to 1.0 or 1.5 MEG for 5, 10 or 20 min, either followed or not by slow cooling. Non-treated fragments were immediately fixed and called as control. Follicular morphology and stromal cell density of controls (goat and sheep) and exposed or cryopreserved fragments were evaluated by histological analysis. Primordial follicles were classified as morphologically normal or atretic based on the integrity of the oocyte, granulosa cells and basement membrane. Stromal cell density was calculated in controls from both species, as well as after exposure and cryopreservation. Results expressed as mean ± SEM were submitted to ANOVA, according to a 2´3´2 factorial arrangement and differences were considered statistically significant when P<0.05. In both species, exposure and cryopreservation of caprine and ovine ovarian tissue significantly decreased the percentage of normal primordial follicles when compared to controls (P<0.05), 78.50±4.88% and 84.45±3.70% in goat and sheep, respectively. After exposure, there was no significant effect of time on the follicular morphology in both species (P>0.05). The effect of concentration only was observed in goats, in which 1.5 M EG significantly reduced the percentage of normal primordial follicles (P<0.05). After cryopreservation, there was no effect of time exposure or cryoprotectant concentration (P>0.05). When compared to exposure, cryopreservation significantly decreased the percentages of normal primordial follicles in goats and sheep (P<0.05), except after exposure of caprine ovarian tissue to 1.5 M EG (P>0.05). The exposure significantly reduced stroma cell density when compared to controls (P<0.05), 10.92±0.36 and 10.88±0.85 in goat and sheep, respectively, except when caprine and ovine tissues were exposed to both concentrations for 5 min (P>0.05). When compared to exposure, cryopreservation significantly decreased the stroma cell density in both species (P<0.05). In conclusion, lower exposure time and/or concentrations of EG can be applied successfully to caprine and ovine ovarian tissue cryopreservation, being an important step towards improvements in follicular development by in vitro culture or transplantation after cryopreservation.
The ability to reversibly inhibit such meiotic resumption has been reported and is a potentially useful method for studying developmental competence acquisition in oocytes as well as in some cases allowing flexibility in an IVF system where oocytes are collected from distant locations or on different days. The effects of such treatment on the somatic compartment of cumulus-oocyte complexes (COCs), however, remain largely unknown. Considering the importance of cumulus cells function for the proper development and maturation of oocytes, the aim of the present study was to determine the effect of temporary inhibition of meiotic resumption using the cyclin-dependent kinase inhibitor butyrolactone I (BLI) on gene expression in bovine cumulus cells. Immature bovine COCs were recovered from the ovaries of slaughtered heifers at a commercial abattoir and assigned to 1 of 4 groups: (1) Control immature COCs were collected either immediately or (2) after in vitro maturation (IVM) for 24 h in TCM199 containing 10 ng/ml EGF and 10% (v/v) fetal calf serum, (3) Inhibited COCs collected either 24 h after incubation in the presence of 100 µM BLI in TCM199 with 3 mg/ml BSA or (4) after meiotic inhibition for 24 h followed by in vitro maturation. All cultures were carried out at 38.5ºC under 5% CO2 in air and maximum humidity. For mRNA relative abundance analysis, cumulus cells were removed from pools of 10 denuded oocytes and were snap frozen in liquid nitrogen and stored at -80ºC until use. A total of 14 transcripts related to oocyte competence or cumulus function were evaluated in cumulus cells by quantitative real time PCR. Transcript abundance data were analyzed using the relative standard curve method. The expression level of these genes was normalized to the average levels of the endogenous reference genes (PPIA and H2AZ) in immature cumulus cells.

In conclusion, the changes occurring in transcript abundance in cumulus cells during maturation in vitro were mostly unaffected following inhibition of meiotic resumption prior to IVM and subsequent release from inhibition and maturation. However, the only gene affected (BMPRII) is an important receptor for cumulus cells functions driven by the oocyte. The consequences of such effect remain to be elucidated.

FOLLICULAR FLUID BASED SOLUTION FOR VITRIFICATION OF BOVINE IMMATURE OOCYTES

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The production of embryos derived from immature cryopreserved oocytes still presents low efficiency, being the cryoprotectant toxicity as well as the employed solutions formulation factors to be considered. In a previous study we observed that follicular fluid is adequate for maintaining bovine oocytes for up to 6 hours, so that it might be an important alternative medium to vitrification solution. The aim of this study was to evaluate vitrification of immature bovine oocytes in 50 or 100% follicular fluid-based (FF) solution. Viability criteria used were oocyte nuclear maturation and sperm penetration (experiment 1; n = 593), as well as cleavage and blastocyst rates (experiment 2; n = 394). Quality 1 and 2 oocytes derived from slaughterhouse oocytes were allocated into the 4 experimental groups: control group (CG); Vitr-Hepes group (vitrification in TCM-Hepes); Vitr-Hepes+FF (vitrification in 50% TCM-Hepes + 50% FF) and group Vitr-FF (vitrification in FF). Vitrification and re-warming was performed according to Vieira et al. (2007, Cryobiology 45:91-94), in glass micropipettes. Oocytes were then submitted to in vitro maturation, fertilization and culture (IVM, IVF, IVC) according to Vieira et al. (2007, Anim Reprod Sci, 99:377-383). Data were analyzed through the chi-square test with significance level of 5%. On experiment 1, nuclear maturation and sperm penetration rates were evaluated at 19 h IVF. On CG nuclear maturation (86.8%) and sperm penetration (82.6%) rates were higher than both groups that used a TCM-Hepes based vitrification solution, Vitr-Hepes (66.7%; 55.8%) and Vitr-Hepes+FF (75.8%; 65.8%). The group Vitr-FF (78.0%; 73.2%) was similar to groups CG and Vitr-Hepes+FF. Polyspermy rates on vitrified groups were similar among each other, being 5.4% on group Vitr-Hepes, 4.9% on Vitr-FF, and 5.8% on Vitr-Hepes+FF. All these groups were significantly higher than the CG that did not present polyspermy. On experiment 2, cleavage rate on CG (66.6%) was similar to Vitr-Hepes (55.4%) but higher than Vitr-FF (45.9%) and Vitr-Hepes+FF (51.9%). Moreover, the groups Vitr-Hepes, Vitr-Hepes+FF and Vitr-FF did not show significant difference among each other. Regarding the blastocyst rates, CG (29.0%) was higher than the other groups, that showed no difference among each other Vitr-Hepes (8.7%), Vitr-Hepes+FF (10.6%) and Vitr-FF (14.3%). The results show that FF-based vitrification solution is a suitable alternative for immature bovine oocytes cryopreservation, providing maturation and sperm penetration rates higher than TCM-Hepes-based vitrification solution. Key-words – Cryopreservation, immature oocyte, maturation, sperm penetration.
FREEZE-DRYING TOMCAT SPERM

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As an alternative method to preserve spermatozoa, many groups have been using the lyophilization technique. Attempts to freeze-dry spermatozoa are not new. The objective of the present study was to test whether Human Tubal Fluid (HTF) and/or Synthetic Oviductal Fluid (SOF) are able to protect tomatc sperm cell nucleus and thus be used as extenders for the freeze-drying process. Each Sperm-rich fraction from the pool of 5 ejaculates collected using an artificial vagina was divided into two aliquots. Aliquots were then added with either HTF or SOF having a final concentration of 2x10^5 sperm/mL. Aliquots were then put into 2.0mL Cryogenic Vials, being 0.5mL the maximum volume per vial in order to aid the freeze-drying process. Vials were kept at 4°C for 60 minutes, then kept in N2 vapor for 8 minutes, and finally plunged into N2. Samples were then taken to the freeze-drying machine in order to obtain a stable product and remove the liquid fraction. With a view to analyzing DNA integrity it had been used acridine orange, and to analyze membrane integrity it had been used the association of 5,5’,6,6’-tetramethyl-1,3’,3”-tetraethylbenzimidazolyl carbocyanine iodide (JC-1), Pisum sativum agglutinin conjugated with fluorescein isothiocyanate (FITC-PSA), and propidium iodide. It was used the PROC MIXED (SAS, Cary, NC) in order to perform the statistical analysis. Both media (SOF and HTF) showed that they may be used as freeze-drying extenders since they both somehow protected the sperm cells’ DNA despite the fact that they both showed extensive cell membrane and acrosome damages, when analyzed via epifluorescence microscopy. SOF showed better DNA integrity (84.2%) when compared to HTF (79.2%). P<0.0001 However, SOF was considered of better performance due to the fact that sperm cells were easier to isolate for the intracytoplasmic sperm injection (ICSI) procedure when samples were reconstituted. Samples that used HTF as media for both freeze-drying procedure and reconstitution required the use of Vortex followed by the use of Pasteur pipettes because sperm cells were strongly bonded. Bearing in mind the fact that both media would provide a defined extender for freeze-drying, the SOF medium needed less effort to be handled if compared to the HTF because sperm cells were easier to separate from one another. Cells that were immersed in HTF needed extra care since they required vortexing and pipetting to separate spermatozoa from one another. Thus it can be concluded that these media may be of good use during the freeze-drying process. This research was supported by CAPES.

FREEZE-DRYING TOMCAT SPERM

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The importance of mice as an animal model for research promoted the emergence of many strains, with important characteristics that need to be maintained. Embryo cryopreservation is the most suitable way for this, however until this moment there is not an effective methodology for this specie. This study aimed to evaluate three methods for the cryopreservation of mouse embryos. Five hundred sixty five embryos were obtained from 42 F1 females (Balb C male x C57 Black female) 6 to 8 weeks old, superovulated with 10IU of eCG and 10IU hCG 46 hours later. Females presenting vaginal plug in the next morning (positive for mating) were kept apart and collected on day 3, by uterine horns flushing with DPBS + 20% estrous mare serum (EMS) with the aid of a syringe and a needle. Recovered embryos at the morula stage were randomly allocated in the 4 experimental groups: Fresh control embryos (CG), vitrification in glass micropipette (MP), vitrification in 0.25mL straws (VS) and ultra-rapid freezing (UR). For vitrification embryos were exposed for 60 seconds to 10% ethylene glycol (EG) + 10% propylene glycol (PROP), followed by exposure for 25 seconds to 20%EG + 20% PROP. During this interval embryos were loaded according to the experimental group (MP or VP) and immersed in liquid nitrogen (N2). Re-warming was performed with decreasing sucrose (SUC) solutions (0.26M and 0.16M) for 5 min each. In UR group, embryos were exposed for 5 min in 3.0M glycerol + 0.5M SAC in D-PBS + 20% EMS, loaded in straws and maintained 2cm above the N2, for 1 min, being then immersed. Thawing was performed in water bath at 37°C, for 20 sec, followed by exposure for 5 min in 0.5M SUC solution. The subsequent in vitro culture was in D-PBS + 20% EMS for 72 h, in cell culture incubator with 5% CO2 at 39°C. The first evaluation, within 24 h of culture, considered blastocyst stage as viability criteria. The second, within 72 h considered the hatching rate. Data were analyzed using the chi-square test, with significance level of 5%. The GMP group (n = 137) presented the highest blastocyst rate among the treatments (95.6%) as well as the highest hatching rate (94.9%), similar to the GC (97.8% and 93.4%). The UR group (n = 124) presented 91.9% of blastocyst, statistically different from VP, even though lower than GC. The VP group (n = 121) had a significantly lower (78.5%) blastocyst rate in comparison with the other treatments. However, the hatching rate (76.0%) was higher than the UR (61.3%). Data allow to conclude that vitrification in glass micropipettes is the most suitable method of cryopreservation for mice morulae, allowing similar viability to that obtained with fresh embryos. Key Words: cryopreservation, freezing, Mus musculus, embryos.
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CALF PRODUCTION BY ARTIFICIAL INSEMINATION WITH SPERMATOZOA OBTAINED FROM EPIDIDYMIDES REFRIGERATED AT 5°C FOR LONG PERIODS AFTER DEATH

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The viability study of spermatozoa recovered postmortem animal is important to determine the maximum period to extract viable cells of the epididymides. In this work bovine testicles were collected in abattoir, transported to the laboratory and stored at 5 °C for different periods (0h, 24h, 48h e 72h). The spermatozoa were retrieved from each epididymides, evaluated and diluted in tris-egg yolk-glycerol 7% medium and cryopreserved in liquid nitrogen. The morphological and functional characteristics of the spermatozoa were analyzed in vitro and in vivo. The data were submitted to analyze of variance One Way Anova and t test with 5% of significance. Pathologies of sperm immaturity, motility decreased after 72 h of epididymides refrigeration and after thaw sperm were observed. The membrane and acrosome integrity were only affected in G48 h and G72 h groups after cryopreservation. However, the sperm capacity of fertilization post-cryopreservation was sufficient to promote two pregnancies and birth of healthy calves from G24 h and G72 h groups. These results indicated that recovery and cryopreservation of chilled epididymal sperm from dead animals is a viable option to preserve male gametes to compose a germplasm bank.

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CRYOTOLERANCE OF MORULAE AND BLASTOCYSTS PRODUCED IN VIVO IN BOS INDICUS

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The aim of this study was to evaluate the cryotolerance of morulae and blastocysts produced in vivo in Sindhi and Nellore (Bos indicus) donors. In Experiment 1, 24 lactating and non-lactating Sindhi donors were superovulated with 100 mg porcine FSH with protocols in which the last two FSH treatments were replaced or not by 300 IU eCG. Embryos were collected 7 days after ovulation induction and embryo development and quality degree were accessed according to Ahmad et al. (1995; Biol Reprod, 52:1129-1135). Two thirds of the embryos were cryopreserved, by conventional freezing or vitrified using a new vitrification method (Vitri-ingá®, INGÁMED, Perobal, PR, Brasil), similar to the Cryotop method. After that, embryos were thawed/warmed and transferred to synchronized recipients, simultaneously to fresh embryos. In Experiment 2, 31 Nellore cows were superovulated with 133 mg porcine FSH and two thirds of the embryos were cryopreserved and transferred similarly to Experiment 1. Results were analyzed using generalized linear models and are presented as least squares means ± standard error. In Experiments 1 and 2, fresh embryos had a higher conception rate at Day 30 than those vitrified and frozen (54.8±7.4, 17.7±7.3 and 19.5±6.8%, respectively; Pd<0.0013) in Sindhi donors (n=231 embryos) and (46.0±6.1, 31.2±5.4 and 28.1±5.3%, respectively; Pd=0.04) in Nellore donors (n=297 embryos). There was no difference between the conception rates of morulae and blastocysts at 30 days (27.8±5.6 and 28.2±8.3%, respectively; P>0.90) and at 60 days (27.1±5.1 and 28.4±7.9%, respectively; P>0.90) in Sindhi donors. In Nellore donors, developmental stage also seemed to not have influenced conception rates at 30 days (39.1±4.8 and 30.5±4.9% for morulae and blastocysts, respectively; P>0.17) or at 60 days (30.6±4.6 and 23.9±4.3%; P>0.24). Finally, there was no effect of embryo quality (Grade 1 versus 2) in conception rates of fresh, vitrified and frozen embryos at 30 or 60 days. We concluded that fresh embryos had higher viability than the cryopreserved ones in Zebu breeds and different stages of embryonic development as well as the cryopreservation methods had similar cryotolerance in Bos indicus embryos.
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FREEZABILITY OF SEMEN OBTAINED FROM EQUINE EPIDIDY MAL'S BODY


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Sudden death or any other event that makes semen collection or mating impossible may prematurely terminate a stallion's reproductive life. Freezing epididymal sperm is an alternative to preserve the genetic value of the stallion. Freezing samples obtained from epididymal's body can enhance the chances of obtaining an offspring using techniques such as ICSI. The aim of the present study was to verify the influence of motility-enhancing media on the freezability of stallion sperm obtained from epididymal's body. Ten stallions from different breeds were castrated and sperm were immediately harvested from the epididymis. The samples from the corpus were split into 3 parts and diluted 1:1 with the following extenders: Botu-Semen™ (BS); Talp + caffeine (Sperm-Talp) and Talp + Heparin + penicillamine, hypotaurine and epinephrine (PHE) (Fert-Talp), incubated at 25°C for 15 min, then were centrifuged at 600 x g for 10 min, the supernatant discharged, and the pellet resuspended using Botu-Crio™. For freezing, the semen was packed into 0.5mL straws and maintained at 5°C for 20 min, followed by 20 min at 6 cm above liquid nitrogen before immersion. After thawing at 46°C/20” the samples were analyzed by CASA (HTM – IVOS 12).

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CYTOPLASMIC DISTRIBUTION AND MITOCHONDRIAL DNA QUANTIFICATION IN BOVINE OOCCYTES TREATED WITH BUTYROLACTONE I

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The accumulation of mitochondria during oogenesis is essential for providing energy resources for early development. Aiming to improve the developmental competence of bovine oocytes during meiotic block, this study evaluated the effects of butyro lactone I (Bi-I) diluted in mSOF medium on the oocyte nuclear and cytoplasmic maturation (measured by mitochondrial distribution), and copy number of mitochondrial DNA (mtDNA). Oocytes were matured (pre-IVM) during 24h with 100 µM Bi-I diluted in modified SOF medium (mSOF; 108 mM NaCl, 7.2 mM KCl, 0.5 mM MgCl₂, 2.5 mM NaHCO₃, 1.7 mM CaCl₂·H₂O, 0.5 mM glucose, 0.33 mM sodium pyruvate, 3 mM sodium lactate, 2 mg/ml BSA, 150 mg/ml gentamicin and 0.01% phenol red) with 1.4 mM glycine, 0.4 mM alanine, 1 mM glutamine, 1% nonessential amino acids and 2% essential amino acids. After pre-IVM, oocytes were matured (IVM) in mSOF with 0.8% BSA and hormones during 24h (control group: C 24h) or during 20h (oocytes pre-IVM 24h + MIV 20h). Cultures were carried out at 38.5°C in 5% CO₂ in humidified air, in 100 ìl droplets of medium. After pre-IVM and IVM, oocytes were stained with 10 ig/ml mitotracker green to assess the cytoplasmatic distribution of mitochondria (peripheral = immature; dispersed = mature) and with 10 ig/ml Hoechst 33342 to evaluate the nuclear maturation (n=768). A group of oocytes was analysed immediately after aspiration from the follicle (control 0h). mtDNA analysis of individual oocytes were performed according to Soto & Smith (Mol Reprod Dev 76:637-46, 2009; n=33). Comparisons of means were evaluated by ANOVA and Tukey's test (P<0.05). After pre-IVM, it was observed that 95.5% (C 0h) and 57.9% (Bi-I) of oocytes was in germinal vesicle stage (GV - immature; P<0.05). After IVM, 76.0% (C 24h) and 77.1% (Bi-I; P<0.05) oocytes reached metaphase II (MII). Oocytes showed immature distribution of mitochondria in 94.5% (C 0h) and 71.2% (Bi-I) after pre-IVM (P<0.05), and mature at 49.6% (C 24h) and 61.1% (Bi-I) after IVM (P<0.05). Since the results indicated that some oocytes had escaped from the meiosis blockage by Bi-I during the prematuration in mSOF medium, only oocytes without polar body (PB) after pre-IVM were selected for mtDNA analysis. We found 34.1×10⁴ (C 0h) and 52.5×10⁴ (Bi-I; P<0.05) mtDNA copy number per oocyte after pre-IVM, and 39.7×10⁴ (C 24h) and 49.5×10⁴ (Bi-I; P<0.05) after IVM. These preliminary results indicate that pre-IVM of bovine oocytes with Bi-I in mSOF medium negatively affects meiotic arrest. Nonetheless, mtDNA numbers increased in oocytes that underwent pre-IVM with Bi-I, suggesting that meiosis block improves oocyte quality.

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OVULATION INDUCTION OF FOLLICLES WITH DIFFERENT DIAMETER USING DESLORELIN IN MARES

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The equine industry is developing every day; Brazil has an equine herd of international level of quality, with that comes looking for new biotechnology of reproduction. The aim of the present study was to evaluate the efficacy of the GnRH analogue Deslorelin to induce ovulation of follicles with 33 to 35mm in diameter (Group 1, G1) and with 36 to 38mm in diameter (Group 2, G2), using as control mares (Group 3, G3) non-induced ovulation follicles. Eight quarter horse mares with age ranging from 4 to 10 years and weight with 350 to 450Kg were assigned one time in each group. The mares were housed in outdoor area feed with grass hay, mineral salt and water ad libitum. The 3 group were: - G1: (n=08) mares with detected follicle size of 33 to 35mm received 1mg IM dose of deslorelin acetate in controlled release vehicle; - G2: (n=08) mares with detected follicle size of 36 to 38mm received 1mg IM dose of deslorelin acetate in controlled release vehicle; - G3: (n=08) control mares, with no administration of agents to induce ovulation. The ultrasonographic evaluation were performed every 24h till the detection of a follicle with a size to be designed to one of the 3 groups, when applied the deslorelin the mares were evaluated every 6h till the ovulation detection. In order to compare the mean diameter of the dominant follicles among the different groups were used the analyze of variance for repeated measure (ANOVA RM), followed by the Student-Newman-Keuls test when necessary at 5% of significance. All mares treated with the deslorelin ovulated, at the non induced control group (G3) the mares had take 67.625±1.996a hours to ovulate from follicles with 35.625±0.694 in diameter (mean±SD). At G1 and G2 the time to ovulation were, respectively (mean±SD): 44.875±3.079a e 45.500±2.915a hours (p<0.05). The presented results permit to conclude that the deslorelin acetate in controlled release vehicle was efficient to hasten the ovulation time with a single shot of 1mg IM. This action can facilitate the breed management, reducing the number of services per embryo gestation, as well the number of reproductive exams the veterinary needs to perform, facilitating the AI program, specially with frozen and cooled semen. Key words: ovulation, deslorelin acetate, mare.

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OOPLASM TRANSFER INCREASES VIABILITY OF LOW QUALITY VITRIFIED OOCYTES

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The ooplasm transfer (OT), although controversy for human proposes, represents a potential alternative for solving cases of pregnancy losses due to oocyte lack of competency in animals of economic interest. This study evaluated the OT (10-15% of cytoplasmic volume) from quality 1-2 maturated (MII) bovine oocytes to either fresh or vitrified quality 3-4 MII oocytes. Oocytes recovered from slaughterhouse ovaries were allocated according to quality criteria, as 1-2 and 3-4, and in vitro matured (IVM) for 20 hours in TCM-199. After IVM a sample of quality 3-4 oocytes were vitrified (Vieira et al., 2007; Cryobiology 45:91-94) in glass micropipettes, being then re-warmed and allocated into one of each experimental group, as follows: group G1 (quality 1-2 oocytes, n = 140); group G2 (quality 3-4 oocytes, n = 168); group G3 (quality 3-4 oocytes + OT, n = 157); group G4 (quality 3-4 vitrified oocytes, n = 159); group G5 (quality 3-4 vitrified oocytes + OT, n = 155). The OT was performed by micromanipulation, being the ooplasm from a donor oocyte totally aspirated, and 10 to 15% transferred to each recipient oocyte. For the vitrified oocytes, the OT was done 2 to 3 h after re-warming, with the in vitro fertilization being at the same time for all experimental groups. Cleavage (day 2) and blastocyst (day 7) rates were analyzed by the chi-square test, with 5% of significance level, based on the number of oocytes initially IVM. Cleavage for G1 (67.1%) was similar to G2 (69.0%), being both higher than G3 (55.4%), G4 (47.8%) and G5 (43.9%). Also, there was significant difference between G3 and G5. With respect to the blastocyst rates, the group G1 (25.7%) was higher than the groups G2 (10.1%), G3 (13.4%) and G5 (9.7%), being them similar among each other, and all superior to G4 (3.1%). Data showed allow us to infer that the OT from quality 1-2 oocytes to quality 3-4 oocytes increases the blastocyst rate. With fresh oocytes, even of quality 3-4, the OT from quality 1-2 did not affect the blastocyst rate. Moreover, the OT performed to quality 3-4 vitrified oocytes, provides similar viability to that obtained by fresh oocytes of the same quality. We concluded that the transfer of 10 to 15% ooplasm from a good quality oocyte might be used as a strategy to increase the blastocyst yield when oocytes of low quality are vitrified. Key-words: Cytoplasm donation, micromanipulation, vitrification, oocyte quality.
The bovine immature oocytes cryopreservation is an important tool for the storage of female gametes, allowing a variety of future genetic crosses and avoiding the risk of equivoces on crossing direction. It also provides the preservation of extinction threatened breeds. However, cryopreserved oocytes result in a low embryo production rate, being the hardening of zona pellucida one possible reason for such low rates. This may be overcome through the intra-cytoplasmatic sperm injection (ICSI). This study compared the use of in vitro fertilization (IVF) and ICSI on embryo production using either fresh or vitrified bovine immature oocytes. Cumulus-oophorus complexes (COCs) classified as quality 1 and 2 were recovered from slaughterhouse ovaries, and used as fresh control or vitrified and re-warmed according to Vieira et al. (2007). Cleavage rate of G1 (65.6%) was higher than G2 (50.0%), G3 (48.3%) and G4 (46.9%). On day 7, blastocyst rates were similar between G1 (26.4%) and G2 (28.9%), being both higher than G3 (4.9%) and G4 (11.9%). However, on G4, where ICSI was used, blastocyst rate was statistically higher than G3. The embryo rate on day 9 displayed the same behavior (G1: 32.0%, G2: 29.7%, G3:7.7% and G4: 15.6%). Results show that cryopreservation negatively affects embryo production, and that ICSI at least partially overcome such problems, increasing embryo production rates for vitrified bovine immature oocytes. Key words: ICSI, fertilization, cryopreservation, zona pellucida.

The manipulation of culture media and using drugs to modify embryo metabolism have been used to produce embryos with greater resistance to cryopreservation. The present experiment aimed to induce lipolysis, using forskolin (Sigma-Aldrich, St. Louis), an adenosil cyclase activator, in bovine embryos produced in vitro. Nelore cow cumulus oocyte complexes were matured in TCM 199 and fertilized with frozen Nelore semen. Presumptive zygotes were cultured in SOFaas+BSA under 2 concentrations of FCS (2.5% and 0% - from day 1 to day 7). On day 6, embryos were divided into 4 groups: FCS (2.5% FCS without forskolin), %FCS (without FCS and forskolin), FCS+F (2.5% FCS plus 10ìM forskolin ) and %FCS+F (without FCS but with 10ìM of forskolin). Embryo cleavage was recorded on day 3, and blastocyst production on day 7. In vitro produced embryos were compared with embryos obtained in vivo from Nelore cows. Embryo quality was estimated by the index of damaged cells observed after the staining with propidium iodite and Hoechst 33342. Embryos were vitrified on day 7. In vitro produced embryos were compared with embryos obtained in vivo from Nelore cows. Embryo quality was estimated by the index of damaged cells observed after the staining with propidium iodite and Hoechst 33342. Embryos were vitrified on day 7. In vitro produced embryos were compared with embryos obtained in vivo from Nelore cows. Embryo quality was estimated by the index of damaged cells observed after the staining with propidium iodite and Hoechst 33342. Embryos were vitrified on day 7. Embryos were vitrified on day 7, using the protocol developed by Campos-Chillón et al. (2006). Cleavage (day 2) and blastocyst (day 7 and 9) rates were analyzed by the chi-square test with 5% of significance level, based on the number of oocytes submitted to IVM. Cleavage rate of G1 (65.6%) was higher than G2 (50.0%), G3 (48.3%) and G4 (46.9%). On day 7, blastocyst rates were similar between G1 (26.4%) and G2 (28.9%), being both higher than G3 (4.9%) and G4 (11.9%). However, on G4, where ICSI was used, blastocyst rate was statistically higher than G3. The embryo rate on day 9 displayed the same behavior (G1: 32.0%, G2: 29.7%, G3:7.7% and G4: 15.6%). Results show that cryopreservation negatively affects embryo production, and that ICSI at least partially overcome such problems, increasing embryo production rates for vitrified bovine immature oocytes. Key words: ICSI, fertilization, cryopreservation, zona pellucida.

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IN VITRO SURVIVAL OF VITRIFIED IVP Bos taurus EMBRYOS CULTERED IN PRESENCE OF GROWTH HORMONE

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The higher cryosensitivity of IVP embryos are one technical limitation to widely use of IVP in bovine industry. The objective of this study was to evaluate the in vitro survival after vitrification of IVP bovine bos taurus embryos cultured with or without addition of growth hormone (GH). Cumulus oocyte complexes (COC) were recovered from slaughterhouse-derived ovaries (Angus Red crosses) and by ovum pick-up (OPU) from Jersey donors. After IVM and IVF, the presumptive zygotes were transferred to culture dish containing 400mL droplets SOFaa medium with or without addition of 100ng/mL growth hormone under a layer of mineral oil at 39°C in an atmosphere of 5% CO2. Blastocyst graded as I and II were recorded 8 days after IVF and vitrified in OPS as described by Vatja et al. (Mol Reprod Dev 51, p. 5-58, 1998). Briefly, vitrification procedure was performed following 2 steps: blastocysts were placed in vitrification solution 1 (VS1; 7,5% DMSO, 7,5% ethylene glycol in TCM 199 supplemented with 20% FCS) for 3 min; blastocysts were then transferred to vitrification solution 2 (VS2; 16,5% DMSO, 16,5% ethylene glycol in TCM 199 supplemented with 20% FCS and 0.3 M sucrose) for 40 seconds and loaded in OPS before immersion in liquid nitrogen. After warming at 39ºC embryos were placed in decreasing concentration of sucrose solutions (0.6 and 0.3M 5 min each step) and then transferred to culture dish containing 100mL droplets SOFaa conditioned medium. Embryo survival was evaluated by in vitro culture during 24 (re-expansion) and 48 h (hatched). Ac2 analysis was performed to compare survival rates after vitrification among different culture medium. The results showed similar embryo survival rates in IVP cross breed Angus Red at 24 h of culture (82% control versus 71% GH group), but different at 48 h of culture (79% control versus 60% GH group, P<0,05). In IVP Jersey embryos was observed a tendency (P<0.2) to better developmental rates in control group 24 and 48 h of culture (75% control versus 57% GH group and 69% control versus 50% GH group). We concluded that GH addition to SOFaa medium didn’t improve in vitro embryo survival rates of vitrified Angus Red cross breed or Jersey IVP embryos.

CRYOPRESERVATION OF CELLS IN STRAWS FOR BOVINE NUCLEAR TRANSFER PURPOSES

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Generally cells are cryopreserved in criotubes and kept in LN2. Herein we tested a freezing protocol for cells adapted from embryos and semen applying straws. The logic for this strategy is to allow an easier ranging of the straws in conventional containers already adopted for semen and embryos. This study was carried out with the objective of organizing a simple protocol that allows criopreserving cells in straws and further ranging in conventional containers. Overall skin biopsies of 8 animals (bovine) were collected and processed in order to produce fibroblast cell culture. Biopsies were washed in PBS, with antibiotics and processed to small pieces and explants cultured in [Iscove's Modified Dulbecco's Medium (IMDM) + 20% FCS + antibiotic] until the appearance of the firsts colonies. Thereafter the explants were removed and cells were cultured with 10% of FCS until confluence. Confluent cells were treated with tripsin at 30ºC for 5 min and centrifuged. The cellular extract was ressuspended on freezing medium [IMDM + 20% FCS + 10% dimethyl sulfoxide (DMSO) and amikacin] and separated in 3 different groups. In the treatment 1 group (T1) the straws were cooled gradually (-1ºC/min) until -32°C. The treatment 2 consisted in the same strategy but with a small interval at -6ºC to perform the seeding. The treatment 2 consisted in the same strategy but with a small interval at -6°C to perform the seeding. The control group was cryopreserved in criotubes applying a freezer container and exposed to a -80ºC for 4 hours. After this protocols all cells were stored in LN2, for 5 days. For the viability test all cells were evaluated in terms of ability of establishing cell cultures and in terms of survival rates applying the FACS of cells stained with Hoeschst 33342 and PI for 5 min. Cultured cells were then submitted to karyotype analysis. Data was evaluated by ANOVA with P<0,05. All freezing treatments resulted in a high survival rate (T1 76,45; T2 72,34; T3 71,5%). Karyotype analyses were also similar and showed a normal number of chromosomes. However a high rate of cellular death was observed in 3 out of 8 lineages showing more than 45% of dead cells after thawing (70,9; 45,3; 54,9%). The other lineages showed less than 10,26% of dead cells. Together this data allows us to conclude for the viability of the freezing process applying straws.
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EQUINE EMBRYO LIPID FINGERPRINTING BY MASS SPECTROMETRY


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Matrix-assisted laser desorption-ionization mass spectrometry (MALDI-MS) allows the lipid profile study of individual mammal embryos. The data collection is rapid, highly sensitive, can tolerate some level of impurities, and easy to interpret. The aim of this study was report the lipid profile obtained from a single equine embryo by MALDI-TOF. The mare was submitted to rectal ultrasound evaluation and ovulation was induced (hCG 2500 ui, Vetecor®) upon detection of a 35mm follicle. Fresh semen (500 x 10⁶ viable sperm) was used for AI and embryo was collected using Lactate Ringer solution, nine days after ovulation, and transported in 50/50 (v/v) methanol/phosphate buffer solution at 4ºC to the laboratory. MALDI-MS spectra were acquired in the positive ion mode using MALDI Synapt HDMS mass spectrometer (Waters, Manchester, UK) m/z 700-950 range. The sample was coated with 1.2 µL matrix of 2,5-dihydroxybenzoic acid (DHB) 1.0 ml/L in methanol. Due to the equine embryo volume, it was possible to divide it and get two mass spectra, which were identical. Spectra processing was performed using the MassLynx 4.0 software (Waters, Manchester, UK). It was observed the presence protonated and sodiated species of sphingomyelins (SM), phosphatidylcholines (PC) and triacylglycerols (TAG). The most intense ions assigned by comparison with data obtained from bovine embryos were m/z 723.5 [PC (34:1) and loss of N(CH₃)₃]+, 725.5 [SM (16:0) + Na]+, 778.6 [PC (36:1) + Na]+, 780.6 [PC (34:2) + Na]+ or [PC (36:5) + H]+, 782.6 [PC (34:4) + H]+ or [PC (34:1) + Na]+, 788.6 [PC (36:1) + H]+, 806.6 [PC (38:6) + H]+ or [PC (36:3) + Na]+, 808.6 [PC (38:5) + H]+ or [PC (36:2) + Na]+, 810.6 [PC (38:4) + H]+ or [PC (36:1) + Na]+, 907.7 [TAG (54:3) + Na]+ and 909.7 [TAG (54:2) + Na]+. Regarding the lipid profile by MALDI-TOF previously reported for oocytes and embryos of several species (Ferreira et al., 2010, J Lipid Res, v.51, p.1218-1227), it were detected similar lipid species, but with different relative intensities. Because of the single equine embryo volume and MALDI-MS technique sensitivity, we intend to observe if there will be differences between the lipid profile of the inner cell mass and trophoblast in the future. The analysis of a greater number of embryos as well as different development periods and MS/MS experiments will contribute to building a database of lipid profile that allows a better understanding of the lipid profile physiology in equine embryos and the meaning of differences among other mammalian embryos. Financial Support: FAPESP – Brazil.

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TECHNICAL REPORT: PREGNANCY RATES EVALUATION AFTER LARGE SCALE TRANSFER OF IN VITRO PRODUCED AND VITRIFIED BOVINE EMBRYOS FROM DIVERSE BREEDS


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There is a growing commercial interest in Brazil for the cryopreservation of in vitro produced (IVP) bovine embryos, since this technique allows commercializing and transporting of bovine superior genetic inside the country and abroad. We present herein the results obtained during one year of routine bovine embryo IVP with the use of vitrification of expanded blastocysts. Vitrification was the cryopreservation technique chosen by this company after several field experiments. Vitrification protocol included the use of Cryotop with ethylene glycol (EG) and dimethyl sulfoxide (DMS) as cryoprotectants. Results were obtained at 26 routines (at different days or farms) during the period of one year. A number of 845 embryos were produced, which 41% were from Zebu breed, 2% from taurine breeds, and 57% crossbreed of Zebu and taurine (Girolando). From the transfer of these embryos, 152 pregnancies were obtained from vitrified Zebu embryos (42% pregnancy rate), seven pregnancies from vitrified taurine embryos (41% pregnancy rate), and 175 pregnancies from Girolando embryos (35%). Our laboratory results for pregnancy after fresh transfer of 34,000 embryos in the same period was 43%. These results show that nowadays it is possible to obtain satisfactory results of pregnancy rates from cryopreserved IVP Zebu and taurine embryos, compared to IVP embryos of fresh transfer.
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VITRIFICATION OF EMBRYOS OF SANTA INÊS EWES BREED WITH ETHYLENE GLYCOL AND DIMETHYLFORMAMIDE

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The ovine embryo cryopreservation is still a challenge once pregnancy rates after embryo transfer are inconsistent. Besides, literature on ovine embryo cryopreservation of national breeds is limited. The aim of the present study was to evaluate the postcryopreservation viability of Santa Inês breed embryos using as cryoprotectants ethylene glycol (EG) and dimethylformamide (DMF). The last has not yet been used with embryos in this species. Embryo recovery was performed by hemi laparoscopy six days after heat detection, when they were assigned to one of the following treatments: (T1) Conventional freezing with 1.5M EG (n=37), (T2) Vitrification with EG (n=34), and (T3) Vitrification with DMF (n=33). In the conventional method embryos were frozen in a computer controlled machine (rate of 1°C/min from 20°C up to -7°C, seeding at -7°C, rate of 0.3 /min up to -35°C and dropped in liquid nitrogen). In the vitrification protocol embryos were equilibrated in a solution containing 80% of maintenance medium (MM) + 20% EG (T2) or 20% MM + 20% of DMF (T3) for 5 minutes. After that, they were exposed to a vitrification solution containing 40% EG + 16% Ficoll + 10% Sucrose (T2) or 40% DMF + 16% Ficoll + 10% Sucrose (T3). The embryos were loaded in Open Pulled Straw “OPS” and after 20 seconds dropped in liquid nitrogen. Embryo thawing procedure in the conventional method was performed at 35°C for 30 seconds. The vitrified embryos were warmed at room temperature in air for 3 minutes. All embryos were rehydrated in decreasing concentrations of sucrose and cultured for 48 hours in SOF medium at 38.5°C, in incubator containing 5% of CO2 in air and saturated humidity. After 24 hours of culture, the re-expansion rate of T1, T2 and T3 was of 54% (20/37), 47% (16/34), 36.6% (12/33) and after 48 hours, the hatching rate was of 40.5% (15/37), 35.3% (12/34), 15.5% (5/33), respectively. The hatching rate of T3 was significantly lower than T1 and T2 (p <0.05). The rate of embryonic zona pellucida fracture from T1, T2 and T3 was of 24.3% (9/37), 2.9% (1/34), 21.2% (7/33), respectively, which was lower in T2 (p <0.05) compared to the other treatments. DMF was not efficient in vitrification of ovine embryos. Although vitrified embryos in EG (T2) showed a lower incidence of zona pellucida damage, their hatching rate was similar to that observed in the conventional freezing method (T1). Therefore, it is possible that both methods (T1 and T2) were suitable for embryos cryopreservation of Santa Inês breed ewes. However, more studies evaluating the pregnancy rates after transfer of these embryos should be accomplished.