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Abnormalities in bovine conceptus development during the embryonic phase after *In Vitro* Fertilization (IVF) and cloning by nuclear transfer (NT)

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ABSTRACT

***Background*:** Embryonic mortality is a major cause of reproductive failure in cattle, resulting in a lot of problems to the industry. Recently, many techniques have been used in the production of genetically modified animals mainly related to improve the animal production. The possibility to genetically manipulate living organisms through the addition or inactivation of genes has revolutionized the understanding of biological and molecular mechanisms. Herein, we showed data about what is known about the abnormalities in bovine conceptus using IVF and NT techniques.

***Review*:** The establishment of pregnancy results from the interaction between the trophoblast and maternal tissues. Embryonic/fetal loss occurs throughout pregnancy in cattle; however, it is concentrated mainly in the first 42 days after breeding. Recently data have showed that approximately 50% of cloned bovine embryos do not establish the gestation after the transference of the blastocyst. In addition, placental abnormalities occur in high levels in cloned animals during the initial and in the end of gestation. Low viability of cloned embryos is mainly expressed by the reduction in the rate of deployment, the increased rate of perinatal mortality and fetal, and the various anomalies observed in newborn animals. Among the pregnancy complications from placental transfer of manipulated embryos (IVF and NT) there are changes in the morphology of the placentome, in the region of the fetal-maternal contact, increase of allantoic or amniotic fluid, vascularity, lower number of cotyledons, and increased of the interplacentomal area "Offspring Syndrome". In addition, the placenta, fetal membranes and umbilical cord from cloned animals have rudimentary development. According to Wells *et al*., the overall efficiency of cloning in bovine is limited to 5-6%. In IVF pregnancies the placentome percentage, fetal villi, density and volume of binucleated cells is reduced. However, the volume of blood vessels in increased maternal wattles, believing there is a compensatory mechanism in the vascular network of the placentae.

***Conclusion*:** Many technological innovations could help to obtain a better quality production and significant improvements in animal breeding such as those brought about by nuclear transfer and *in vitro* fertilization. New studies on the dynamics of development in cattle embryology derived from nuclear transfer techniques and *in vitro* fertilization are necessary, focusing on different systems in order to find greater success in the artificial producing and selection of interest characteristics.

Keywords: Bovine, *In Vitro* Fertilization, Nuclear transfer, Cotyledonary Placenta.

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I. INTRODUCTION

Embryonic mortality is a major cause of reproductive failure in cattle, resulting in delayed pregnancy, fewer calves born, reduced in milk production, and slower genetic progress, with a concomitant financial loss to the industry. Recently, many techniques have been used in the production of genetically modified animals mainly related to improve the animal production.

The possibility to genetically manipulate living organisms through the addition or inactivation of genes has revolutionized the understanding of biological and molecular mechanisms [3].

A number of groups of scientists have been worked with this theme in order to establish new protocols and techniques which result in an artificial embryo production and to understanding the normal development of the bovine conceptus.

II. PREGNANCY PROGRESS AND RELATED PROBLEMS

The establishment of pregnancy results from the interaction between the trophoblast and maternal tissues. Furthermore, numerous internal and external factors can affect follicular development: oocyte quality, the endocrine factors, the receptivity of the uterus, the capacity of the embryo to signal its presence and determine whether or not pregnancy will be successfully established and maintained.

Embryonic/fetal loss occurs throughout pregnancy in cattle; however, it is concentrated mainly in the first 42 days after breeding. Recently data have showed that approximately 50% of cloned bovine embryos not establish the gestation after the transference of the blastocyst inside the receptor mother [4]. In addition, placental abnormalities occur in high levels in cloned animals during the initial and in the end of gestation [9,18].

The pregnancy loss in cloned cattle occur in the first six months affecting approximately 30% of the clones that grow until delivery, and low viability of cloned embryos is mainly expressed by the reduction in the rate of deployment, the increased rate of perinatal mortality and fetal, and the various anomalies observed in newborn animals [2].

Among the pregnancy complications from placental transfer of manipulated embryos (IVF and NT) there are changes in the morphology of the placentome, in the region of the fetal-maternal contact, hydrallantois (increase of allantoic fluid) hidroâmnio (increased amniotic fluid), vascularity, lower number of cotyledons, and increased of the interplacentomal area "macrosomic Calf Syndrome" (offspring syndrome), which treats the more recent phenomenon associated with embryos produced by IVF and somatic cell nuclear transfer (SCNT).

Hill *et al.* [7], Hill *et al.* [8] and Batchelder *et al*. [1] report that placentae from cloned animals have rudimentary development containing cuboidal trophoblastic epithelium, reduced vascularity and small area with some parts cotyledonal bleeding. Other results showed that the third trimester of gestation of cloned fetuses are characterized by a high incidence of delayed development and placental insufficiency, which are not associated with chromosomal abnormalities [14].

Hashizume *et al.* [6], Numabe *et al.* [13], Miglino *et al*. [11] and Hoffert-Goeres *et al*. [10] argue that the high incidence of placental edema and hydrallantois hampering development of lymphatic vessels, extra-embryonic circulation, or that the permeability of blood vessels are altered in many pregnancies resulting from the cloning process.
As for the clones, the mortality rates of pre-and postnatal period are significantly higher compared with controls. Wakayama *et al.* [15] and Yanagimachi [17] found high rates of embryo implantation (57 - 71%) but low fetal rates (5-16%) and very low in development to term (2 - 3% or less) after the transfer using adult somatic cell nuclear. By using embryonic cells to high incidence of abortion is the 40th day of gestation, accompanied by poor development of the specimens [9]. Edwards *et al.* [4] confirms the findings of Hill *et al.* [9] reporting that during the 30 to 60 days of pregnant the embryo mortality can occur in 50 to 100% of pregnancies. Wells *et al.* [16] that complements the overall efficiency of cloning in bovine is limited to 5-6% at most.

Miglino *et al.* [11] describe the umbilical cord and edematous fetal membranes with placentome fusion, which result in an increased size and decreased number of placentomes. Increased number of functional small

cotyledons or accessories (<1 cm) were present at the maternal surface of fetal membranes, extensive extravasation of blood into the cotyledons and regions interplacentomais were also observed.

The bovine clone showed an increased umbilical cord, characterized by an increase in the size of the allantoic duct and blood vessel walls, as a result of excessive tissue growth and edema.

Farin and Farin [5] and Miles *et al.* [12] described that the percentage of placentome in the surface area is reduced in the IVF group, besides the fetal villi, density and volume of binucleated cells that are reduced in these cotyledons. However, the volume of blood vessels in increased maternal wattles, believing there is a compensatory mechanism in the vascular network of the placentae of bovine embryos from IVF.

III. PERSPECTIVES

The technological innovations available today open new perspectives for animal breeding and selection of characters of interest that can be apply in different areas of science.

New studies on the dynamics of development in cattle embryology derived from nuclear transfer techniques and *in vitro* fertilization are necessary, focusing on different systems in order to find greater success in the artificial producing and selection of interest characteristics.

IV. CONCLUSIONS

The technological innovations could be help to obtain a better quality production and significant improvements in animal breeding such as those brought about by nuclear transfer and in vitro fertilization.

REFERENCES

1 Batchelder, C. A. bertoline, M. Manson, J. B. Moyer, A. L. Hoffert, K. A. Petkov, S. G. Famula, T. R. Angelos, J. George, L. W. & Anderson G. B. 2007. Perinatal physiology in cloned and normal calves: physical and clinical characteristics. *Cloning and Stem Cells*. 9(1): 63-81.

2 Bordingnon V. 2003. Clonagem animal por transferência nuclear: Avanços e desafios. *Acta Scientiae Veterinarie*. 31 (Suppl 1): 64-73.

3 Bressan, F. F. Miranda, M. S. Perecin, F. De Bem, T. H. Pereira, F. T. Russo-Carbolante, E. M. Alves, D. Strauss, B. Bajgelman, M. Krieger, J. E. Binelli, M. & Meirelles F. V. 2011. Improved production of genetically modified fetuses with homogeneous transgene expression after transgene integration site analysis and recloning in cattle. *Cellular Reprogramming*. 13: 29-36.

4 Edwards, J. L. Schrick, F. N. Mccracken, M. D. Van Amstel, S. R. Hopkins, F. M, Welborn, M. G. & Davies C. J. 2003. Cloning adult farm animals: a review of the possibilities and problems associated with somatic cell nuclear transfer. *American Journal of Reproductive Immunology*. 50(2): 113-123.

5 Farin, P. W. & Farin C. E. 1995. Transfer of bovine embryos produced *in vivo* or *in vitro*: survival and fetal development. *Biology of Reproduction*. 52(3): 676-682.

6 Hashizume, K. Ishiwata, H. Kizaki, K. Yamada, O. Takahashi, T. Imai, K. Patel, O. V. Akagi, S. Hirasawa, A. Tsujimoto, G. Todoroki, J. & Izaike Y. 2002. Implantation and placental development in somatic cell clone recipient cows. *Cloning and Stem Cells.* 4(3): 197-209.

7 Hill, J. R. Burghardt, R. C. Jones, K. Long, C. R. Looney, C. R. Shin, T. Spencer, T. E. Thompson, J. A. Winger, Q. A. & Westhusin M. E. 2000. Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. *Biology of Reproduction.* 63(6): 1787-1794.

8 Hill, J. R. Edwards, J. F. Sawyer, N. Blackwell, C. & Cibelli J. B. 2001. Placental anomalies in a viable cloned calf. *Cloning.* 3(2): 83-88.

9 Hill, J. R. Rousell, A. J. Cibelli, J. B. Edwards, J. F. Hooper, N. L. Miller, M. W. Thompson, J. A. Looney, C. R. Westhusin, M. E. Robl, J. M. & Stice S. L. 1999. Clinical and pathlogic features of cloned transgenic calves and fetus (13 case studies) *Theriogenology*. 51(8): 1451-1465.

10 Hoffert-Goeres, K. A. Batchelder, C. A. Bertolini, M. Moyer, A.L. Famula, T. R. & Anderson G. B. 2007. Angiogenesis in day-30 bovine pregnancies derived from nuclear transfer. *Cloning Stem Cells.* 9(4): 595-607.

11 Miglino, M. A. Pereira, F. T. V. Visintin, J. A. Garcia, J. M. Meirelle, F. V. Rumpf, R. Ambrosio, C. E. Papa, P. C. Santos, T. C. Carvalho, A. F. Leiser, R. & Carter A. M. 2007. Placentation in cloned cattle: structure and microvascular architecture. *Theriogenology.* 68(4): 604-607.

12 Miles, J. R. Farin, C. E. Rodrigues, K. F. Alexander, J. E. & Farin P. W. 2004. Angiogenesis and morphometry of bovine placentas in late gestation from embryos produced *in vivo* or *in vitro*. *Biology of Reproduction.* 71(6): 1919-1926.

13 Numabe, T. Oikawa, T. Kikuchi, T. & Horiuchi T. 2000. Birth weight and birth rate of heavy calves conceived by transfer of in vitro or in vivo produced bovine embryos. *Animal Reproduction Science.* 64(1): 13-20.

14 Sousa, P. A. de King, T. Harkness, L. Young, L. E. Walker, S. K. & Wilmut I. 2001. Evaluation of gestational deficiencies in cloned sheep fetuses and placentae. *Biology of Reproduction.* 65(1): 23-30.

15 Wakayama, T. Perry, A. C. Zuccotti, M. Johnson, K. R. & Yanagimachi R. 1998. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature.* 349(6691): 369-374.

16 Wells, D. Misica, P. & Tervit H. 1999. Production of cloned calves following nuclear transfer with cultured adult mural granulose cells. *Biology of Reproduction.* 60: 735-745.

17 Yanagimachi R. 2002. Cloning: experience from the mouse and other animals. *Molecular and Cell Endocrinology.* 187(1-2): 241-248.

18 Young, L. E. Sinclair, K. D. & Wilmut I. 1998. Large offspring syndrome in cattle and sheep. *Journal of Reproduction and Fertility.* 3(3): 155-163.