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Fixed time Artificial Insemination and Embryo Transfer Programs in Brazil

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ABSTRACT

Background: Currently, fixed-time protocols for either artificial insemination or embryo transfer can be routinely applied in the reproductive programs on commercial farms. The control of the follicular wave emergency and the induction of ovulation on a pre determined time without the need for heat detection facilitate the application of such biotechnologies on a large scale basis increasing the reproductive and productive efficiency. This article will discuss the development of reproductive programs that became practical to apply on Brazilian commercial farms as well as the factors that affect its efficiency.

Review: Nowadays, Brazil is the world leader on bovine meat market and also in commercial application of biotechnology, such as fixed-time artificial insemination (FTAI) and fixed-time embryo transfer (TETF). In Brazil, there are several hormones commercially available that can be used for manipulation of follicular wave dynamics and induction of ovulation. The evolution of such protocols were also driven towards the decrease on the amount of time that the animals should came to the curral for hormones treatments in order to make it practical to be use on a large scale basis. Among the commercial established synchronization protocols, one of the most commonly used is with the insertion of a progesterone (P4) device associated with an injection of Estradiol Benzoate (EB) at the beginning of the synchronization protocol to induce an emergence of the new follicular wave. Eight days later, the P4 device is removed associated with the intramuscular administration of prostaglandin, equine chorionic gonadotropin (eCG) and Estradiol Cipionate (ECP). In spite of satisfactory follicular manipulation and precisely synchronization of the time of ovulation there are several factors that can affect the efficiency of FTAI or TETF programs. The FTAI pregnancy rate was influenced by the farm as well as by the body condition scores that the cows presented at the beginning of the synchronization protocol. Other important factors that alter the programs results were the bull used and personal performance during artificial insemination. In general, treatments used for FTET are very similar to those applied for FTAI. In the FTET protocols, the main objective is to increase follicular growth and the diameter of the dominant follicle in order to increase P4 concentrations of the subsequent cycle. Some of the strategies used during the evolution of FTET protocol were to superstimulate the growing follicles of the induced wave after the injection of EB at the beginning of synchronization protocol with eCG or to decrease P4 blood concentration increasing LH pulse frequency promoting an increase on the growth of the dominant follicle during synchronization treatment by advancing the PGF treatment. The final result is to have a large pre ovulatory follicle or a pool of follicles in order to produce a single large or multiples functional CL at the time of embryo transfer. The FTET pregnancy rate was influenced by the diameter of the single CL; by recipient superovulation response by the time of year being lower during the months of autumn and winter in relation to the months of spring and summer. Besides, pregnancy rate at 30 days was also affected by the age of the embryo. Additionally, the correct application of cited biotechnologies enhances reproductive efficiency of livestock bringing sustainable and economic return, increasing the viability of the activity.

***Conclusion*:** Therefore, the standardization of the procedures is necessary for the commercial application of FTAI and FTET in Brazil being fundamental for obtaining expressive results, so that Brazil could also export such technologies for countries under the same management production system.

Keywords: cattle, estrus synchronization, embryo, artificial insemination, biotechnology.

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I. INTRODUCTION

The animal protein production system is under constant pressure by the great demand for these products in a growing world market. Particularly in the case of beef, the decrease of available areas implies the need for greater production efficiency and therefore the use of reproductive biotechnology is the tool to achieve these goals.

Nowadays, Brazil is the world leader on bovine meat market, in addition the country find itself in a prominent place in scientific development and commercial application of biotechnology, such as fixed-time artificial insemination (FTAI) and *in vitro* embryo production (IVP). Once these techniques are inserted into the herd reproductive management, it resulted on an increased of reproductive efficiency and a genetic improvement on its livestock, projecting the country as one of the largest beef producer and meat supplier for the coming years.

The dimensions and the national productive system feature require the development of programs for FTAI and FTET that can be practical and easy to apply on a large scale basis decreasing the amount of hormonal treatments and cattle handling required for such protocols.

The objective of this article will be discuss the main factors that can affect the FTAI and FTET programs, as well as the scientific lines of research commercially applied in Brazil.

II. THE EVOLUTION OF THE FTAI PROTOCOLS IN BRAZIL

In Brazil, there are several hormones commercially available that can be used for manipulation of follicular wave dynamics and induction of ovulation enabling large-scale employment of FTAI programs. The protocols are well established and without doubt one of the most commonly used is with the insertion of a device containing progesterone (P4) associated with an injection of 2 mg of Estradiol Benzoate (EB) at the beginning of the synchronization protocol (D 0) to induce a new wave of follicular growth. Eight days later (D 8) along with the withdrawal of the device, an injection of prostaglandin (PGF) (to ensure luteolysis) and 400 IU of the hormone equine chorionic gonadotropin (eCG) is performed along with 0.5 mg of Estradiol Cipionate for an induction of ovulation [1,4]. The benefits of the application of eCG at P4 device removal made it possible to obtain an average pregnancy rate around 50% [2,8] that drove the implementation of this biotechnology throughout the national herds.

III. FACTORS THAT AFFECT THE EFFICIENCY OF FTAI PROGRAMS

In spite of satisfactory follicular manipulation and precisely synchronization of the time of ovulation there are several factors that can affect the efficiency of FTAI programs. In a retrospective field study performed with information originated from Firmasa and Geraembryo, companies specialized in applied biotechnology; an average of 32.000 FTAI was analyzed in order to identify the factors affecting its efficiency when applied on a large scale. The FTAI pregnancy rate was influenced by the farm ($P < 0.01$; Figure 1) as well as by the body condition scores that the cows presented at the beginning of the synchronization protocol ($P < 0.01$; Figure 2). Other important factors that alter the programs results were the bull used (Figure 3) and personal performance during artificial insemination (Figures 4 and 5). An interesting point was that only 50% of the bulls achieved results above 50% of pregnancy rate. These results corroborate with those observed by other authors [8]. The knowledge of the factors that affect commercial FTAI programs in Brazil has fundamental importance for increasing reproductive and productive efficiency of the national herd. Currently, the FTAI was inserted into the routine management of commercial properties serving as example for other countries that rely on productive systems similar to those used in Brazil.

IV. EVOLUTION OF FTET PROTOCOLS IN BRAZIL

In general, treatments used for FTET are very similar to those applied for FTAI. In the FTET protocols, the main objective is to increase follicular

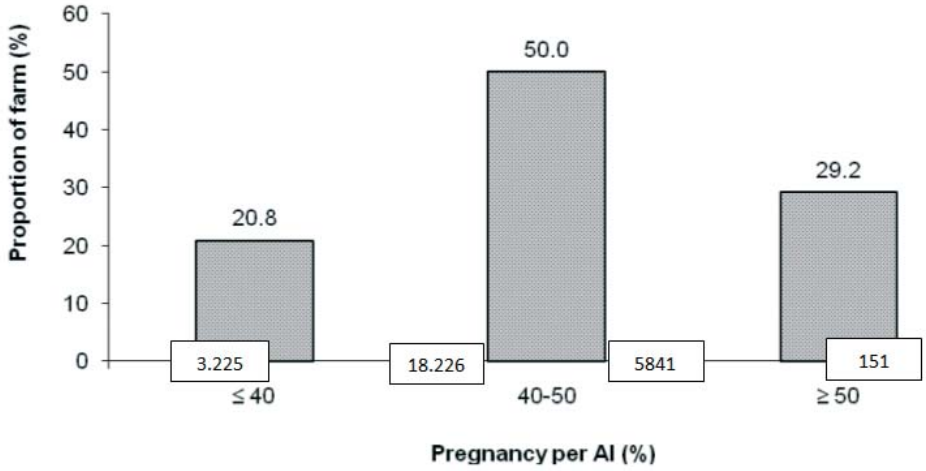

Figure 1. Effect of farm on the pregnancy per fixed time artificial insemination (FTAI; n=32.213 FTAIs performed on 24 commercial farms). Proportion of farms that achieved low (= 40 %; n= 5), average (entre 40 a 50 %; n= 12) or high (= 50 %; n= 7) pregnancy results after FTAI.

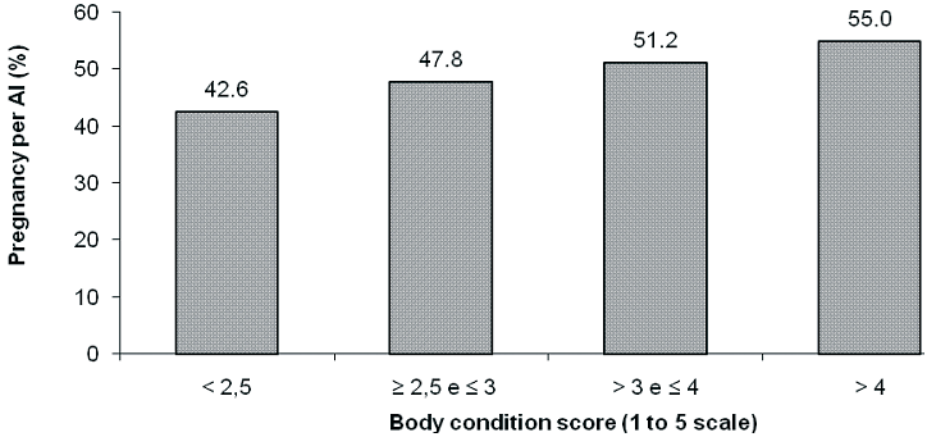

Figure 2. Effect of body condition score (1 to 5 scale) at the first day of the synchronization protocol on the pregnancy per fixed time artificial insemination (FTAI; n=27.443 FTAIs).

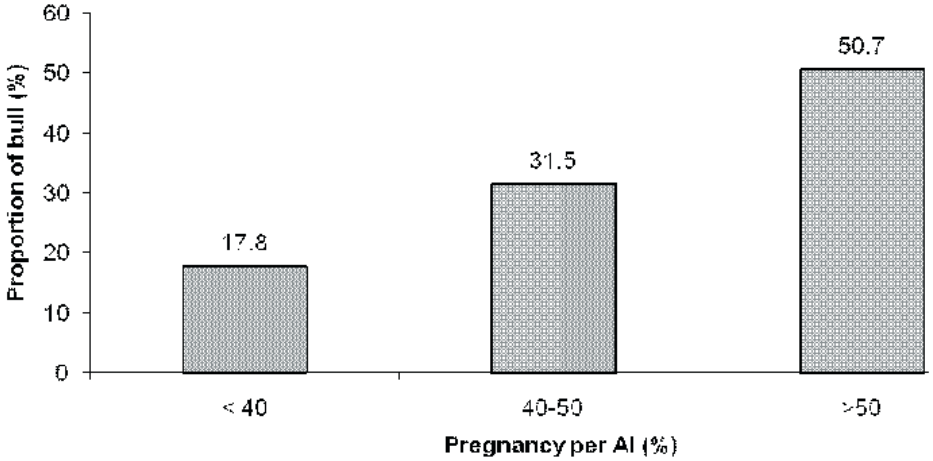

Figure 3. Effect of bull on the pregnancy per fixed time artificial insemination (FTAI; n=32.213 FTAIs performed on 24 commercial farms). Proportion of bull that promoted low (= 40 %; n= 13), average (entre 40 a 50 %; n= 23) or high (= 50 %; n= 37) pregnancy results after FTAI.

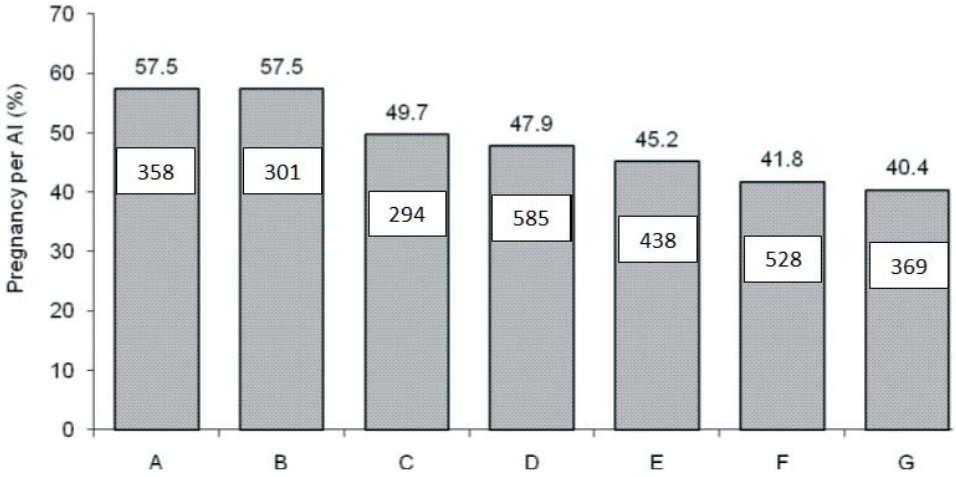

Figure 4. Pregnancy per fixed time artificial insemination (FTAI) according the AI technician (n=2.873 FTAI performed on a single commercial farm).

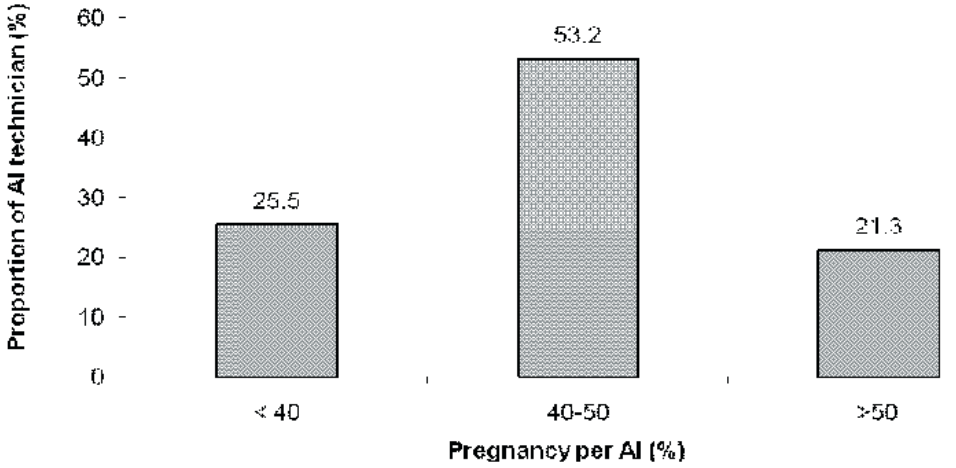

Figure 5. Effect of AI technician on the pregnancy per fixed time artificial insemination (FTAI; n=31.906 FTAIs performed by 47 different AI technicians). Proportion of AI technician that achieved low (= 40 %; n= 12), average (entre 40 a 50 %; n= 25) or high (= 50 %; n= 10) pregnancy per AI.

growth and the diameter of the dominant follicle in order to increase P4 concentrations of the subsequent cycle.

Firstly, studies were done in order to compare the effect of PGF treatment on day 5 of the synchronization protocol with the traditional luteolytic treatment at the time of P4 device removal. The intention of advancing the PGF treatment is to decrease P4 blood concentration increasing LH pulse frequency promoting an increase on the growth of the dominant follicle during synchronization treatment [4].

Another strategy used to improve the results of the FTET was the inclusion of hormone equine chorionic gonadotrophin (eCG) on day 5 of the protocol [3]. In an initial study carried out in Argentina with crossbred recipients [4], eCG treatment increased the corpus luteum (CL) diameter when compared to control group (18.5 ± 17.7 ± 0.4 vs 0.4 mm, respectively, $P < 0.05$), as well as the conception rate [76/132 (57.6) v. 53/127 (41.7%), respectively, $P < 0.05$]. Although previous studies demonstrate the effectiveness of the protocol on achieving high rates of utilization and satisfactory conception rates, the recipients had to be brought to the curral at least five times (four times for drugs administration and one for embryo transfer). In this sense, with the aim of reducing the number of managements required to achieve the FTET, studies were conducted to simplify synchronization protocol [1].

In a first moment, it was studied the effect of the change of time of administration of eCG and PGF usually done on day 5 to day 8, coinciding with the removal of the P4 device in order to try to avoid the treatment on day 5. In addition, it was further investigated the effect of different doses of eCG (400 IU 500 IU and 600 IU) on pregnancy rate on *Bos indicus x Bos taurus* recipients which received an *in vitro* embryo produced [7]. The results showed that the dose of eCG does not interfere with the effectiveness of the Protocol. However, administration of eCG on day 8 of the Protocol has reduced the pregnancy rate when compared to administration on day 5. Similar results were reported by Nasser et al. [5], who demonstrated that recipients receiving eCG on day 5 achieved higher pregnancy rates than those treated at day 8 of the protocol [(47.0% (71/151) and 40.7% (61/150), respectively].

It was also demonstrated by Nasser et al. [5] that the administration of 2 mg EB without 50 mg of progesterone at the beginning of synchronization protocol did not reduce the FTET protocol efficiency. Treatment with BE + P4 presented the same pregnancy rate than the treatments with only EB [(45.3% (68/150) to EB and 42.4% (64/151) EB + P4], eliminating the need for an application of injectable progesterone at the beginning of synchronization protocols for FTET.

These studies have demonstrated that changing the day of administration of eCG (5^{th} to 8^{th} after the beginning of the synchronization protocol) had a negative influence on the results of FTET Protocol. This decrease can be related to the suppression of follicular growth between days 5 and 8. Since the majority of recipients used in the experiment have a CL at the moment of P4 device insertion, the delay in the administration of PGF for day 8 of the protocol may have exposed the recipients to high circulating concentrations of P4 (CL more P4 device), which may have reduced the LH pulse frequency [9]. This factor, associated with the absence of eCG treatment to stimulate follicular growth at the beginning of the follicular wave, compromised the dominant follicle growth.

An alternative to reduce P4 concentrations during synchronization treatment with intravaginal P4 device without increasing the number cattle of management is to administrate PGF at the moment of P4 device insertion (D0). In a study using *Bos indicus x Bos taurus* cross heifers, heifers receive a half dose of PGF at the P4 device insertion and another half dose at P4 device removal or a single dose of PGF on P4 device removal [6]. All recipients received 1 mg EC and 400 IU of eCG on P4 device removal (Day 8). Recipients treated with PGF on day 0 and 8 have larger diameter of the dominant follicle (14.7 ± 0.4 vs. 12.6 ± 0.5 mm, $P < 0.05$) and the CL on the day of embryo transfer (19.3 ± 0.5 vs. 18.1 ± 0.4 mm) and presented higher pregnancy rate [50.5% (50/99) vs. 39.4% (39/99), respectively] than those receiving PGF treatment only on Day 8, demonstrating the possibility of a protocol with two managements for FTET programs.

V. FACTORS THAT AFFECT THE EFFICIENCY OF FTET PROGRAMS

Similar to previous described for FTAI programs, there are several factors that can influence the FTET results when applied in commercial farms. Recently we evaluate the possible factors that could influence the conception and embryo survival of the recipients receiving an *in vitro* produced embryo at fixed time. In this study, 10,195 embryo recipients *Bos taurus x Bos indicus* during the years 2004-2007 were evaluated. In all FTET only females with CL and absence of uterine abnormalities were selected to synchronization. All females were fixed-time embryo transferred after synchronization of ovulation using intravaginal P4 device and EB at the beginning of treatment. The time of P4 device withdraw, a PGF injection were administered along with eCG (400 IU) and ECP (0.5 mg). All recipients received an *in vitro* produced embryo and were evaluated by ultrasonography at the time of embryo transfer to verify the presence of CL, diameter (16, 18, 20 or 22 mm; Figure 6) and also to quantify the number of CL. Pregnancy diagnoses were done by ultrasound at 30 and 60 days after transfer, where it was also evaluated the occurrence of pregnancy loss.

The conception rate varied depending on the time of year. The conception rate was lower during the months of autumn and winter (41.1%; 448/1090) in relation to the months of spring and summer (48.1%; 1760/3658). These results may be related to the dry climate and/or lower availability/quality of pasture supply typically observed during these periods in tropical regions (Figure 7).

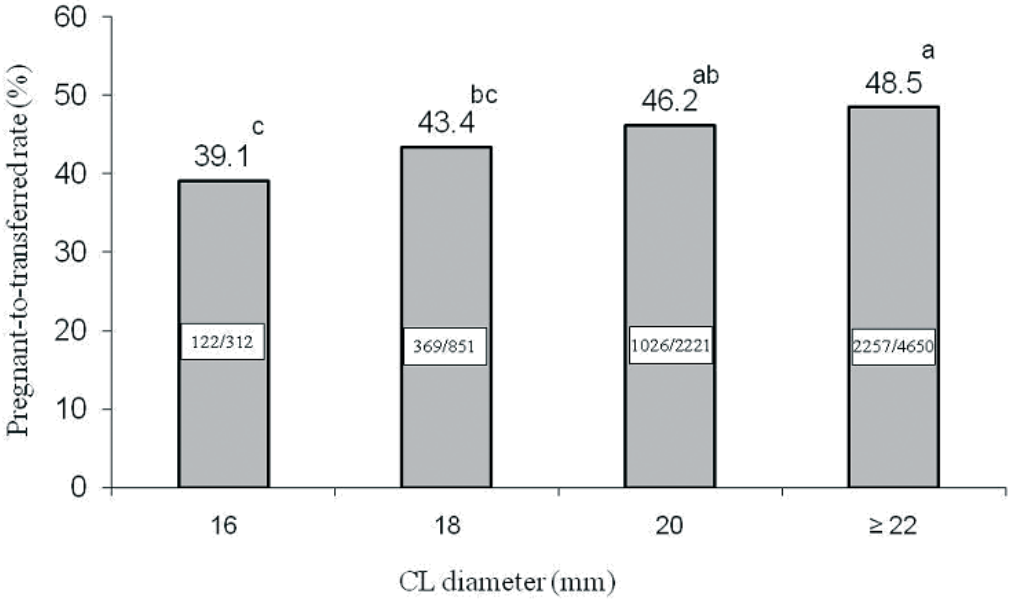

Figura. 6. Pregnant-to-transferred rate according to the diameter of the corpus luteum (CL) in *Bos indicus x Bos taurus* recipients (n=8,034) receiving *in vitro*-produced embryos ($P < 0.0001$).

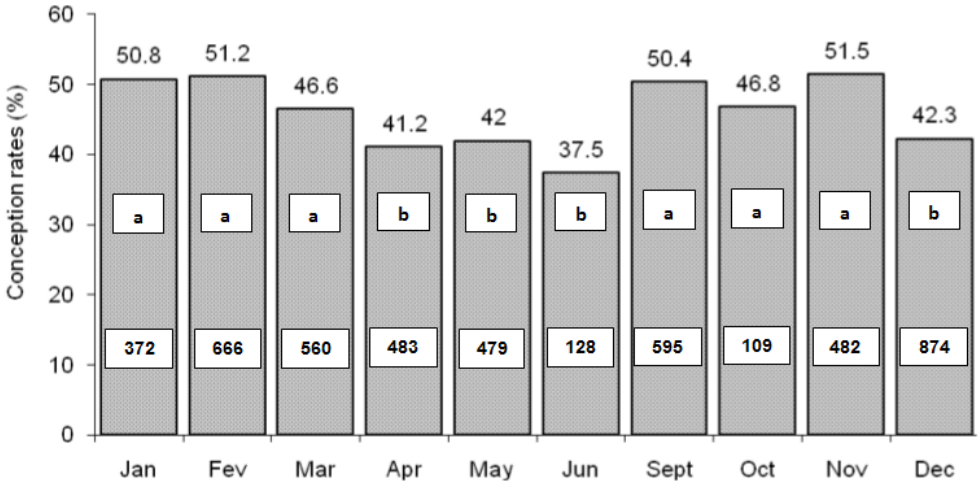

Figure 7. Conception rates in bovine embryo recipients receiving an *in vitro* produced embryo according the month of the year (n=4748 FTET).

Besides, pregnancy rate at 30 days was also affected by the diameter of the single CL [Figure 5; $P < 0.0001$]; by recipient superovulation response [superovulated=51.3 (328/639) or non-superovulated=45.8% (1880/4109); $P = 0.01$; Figure 8] and by the age of the embryo [6 days=55.0% (132/240)[a]; 7 days=46.5% (2046/4404)[b]; 8 days=28.9% (30/104)[b]; $P < 0.0001$].

The average gestational loss rate (between 30 to 60 days) was 11.7% (258/2208), and was not being influenced by the diameter of the single CL or by recipient superestimulation, by season or by stage of embryonic development. However, the gestational loss was influenced by the parity of the recipient. Heifers have higher rate of gestational loss (14.5%, 42/269) than cows with calves (9.2%; 27/294; $P = 0.05$; Figure 9).

VI. CONCLUSIONS

Currently, it is possible to manipulate the follicular dynamics and luteal phase, abolishing the need of the heat detection for FTAI or FTET. The

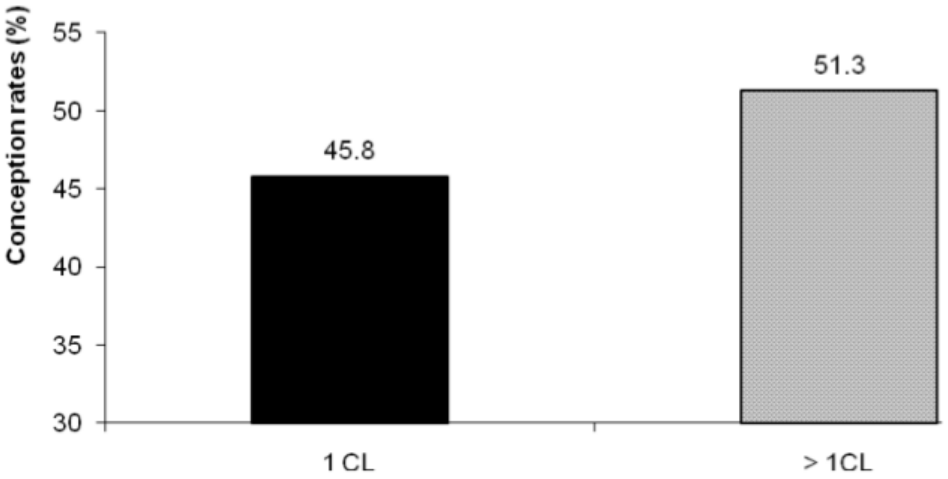

Figura 8. Conception rates after embryo transfer according to the number of corpus luteum (CL) at fixed time embryo transfer [>1CL (n= 639) vs 1 CL (n=4109); *P* = 0.01].

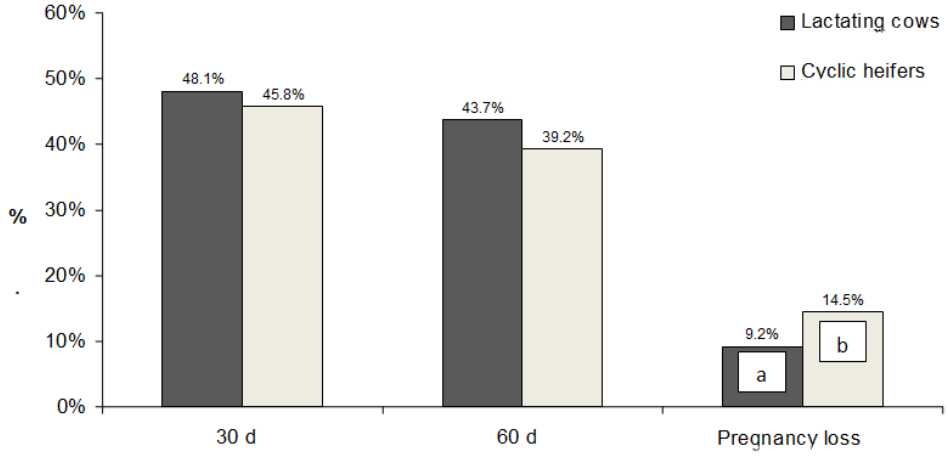

Figure 9. Conception rates at 30, at 60 and pregnancy loss occurred from 30 to 60 days in bovine in vitro embryo recipients after fixed time embryo transfers according the animal category (lactating cows or cyclic heifers). [Cyclic heifers (n=611) and Lactating cows (n=587)].

correct application of such biotechnologies enhances reproductive efficiency of livestock bringing sustainable and economic return, increasing the viability of the activity. It is important to mention that other factors such as nutrition and sanitary management as well as availability of personal qualification or training can influence significantly on the successful use of these biotechnologies.

Therefore, the standardization of the procedures necessary for the commercial application of FTAI and FTET in Brazil are fundamental for obtaining expressive results. This reality has placed the country in a prominent position as an exporter of biotechnologies for other countries that use similar production systems.

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