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How FTAI and FTET Impact Reproductive Efficiency of Brazilian Dairy Herds

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ABSTRACT

***Background*:** Throughout dairy cows evolution, milk production was always the key point to select the superior animal. Currently, several evidences has shown that high milk production have intensively contributed to the decline of dairy cattle fertility. Beyond milk production, dairy cows have their reproductive performance impaired by another factors, heat stress and repeat-breeding. Methods like fixed time artificial insemination and embryo transfer were developed to minimize the effects of these factors, and improve dairy herds profitability. This review aims to show some key-point experiments conducted to improve the efficiency of the self-appointed protocols for artificial insemination and embryo transfer in Brazil, overcoming several reproductive problems. Our goal is to develop cheap and easy self-appointed programs that facilitate animal handling and maximize their reproductive outcomes all over the year.

***Review*:** Failure in estrus detection is the mainly limiting factor for the use of artificial insemination in high-production dairy herd. An excellent alternative to overcome the need of estrus detection is fixed time artificial insemination. Many protocols with and without the use of estradiol have been developed to that end. Among the protocols for fixed time artificial insemination without estradiol, DoubleOvsynch has been extensively used recently in American dairy herds. In Brazil, similar pregnancy rate was obtained compared to progesterone-estradiol based protocols for fixed time artificial insemination. Particularities of progesterone-estradiol based protocols as (1) new progesterone device or devices previously used for eight days; (2) different doses of eCG; and (3) the use of estradiol cypionate for fixed time artificial insemination have been studied in Brazil. The use of self-appointed artificial insemination also enabled the reduction of the interval calving-conception compared to cows inseminated following the standing estrus. Regarding the low fertility of repeat breeders and the effect of heat stress at early pregnancy, other methods like embryo transfer became important tools to enhance reproductive efficiency of Brazilian dairy herds. Protocols were also developed to allow fixed time embryo transfer, eliminating the need of estrus detection and improving the reproductive efficiency of lactating recipients. As well as described for fixed time artificial insemination treatments, there is a large variety of hormone combination for fixed time embryo transfer (with and without estradiol). An experiment conducted in Brazil demonstrated that protocols for fixed time embryo transfer without estradiol can be as good as with estradiol to synchronize high-producing Holstein recipients, essentially during summer. Particularities related to embryos cryopreservation, synchronization of the estrus cycle of donors and recipients and the site of embryo release into the uterine horn were also investigated. Greater conception rates were achieved when fresh embryos were transferred compared to frozen-thawed ones. Also, the tight synchronization between donor and recipient (same day of estrus) resulted more pregnancies than when recipients were one day later or in advantage in relation to donors. Moreover, the site of embryo release into the uterine horn (ipsilateral to the corpus luteum) had no effect on pregnancy rates after *in vivo* produced embryo transfer.

***Conclusion*:** Both fixed time artificial insemination and fixed time embryo transfer are important tools to improve reproductive efficiency of high-producing dairy cows. These biotechnologies help bypassing some of the greatest challenges of dairy cattle reproduction: the difficulties of estrus detection, and the low fertility associated to heat stress and repeat breeding.

Keywords: FTAI, FTET, heat stress, repeat breeders, reproductive efficiency.

Descritores: IATF, TETF, estresse térmico, repetidoras de serviço, eficiência reprodutiva.

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I. INTRODUCTION

During the last decades, the selection of genetically superior cows for milk production has been correlated to the lower fertility of dairy herds. However, the decline in reproductive efficiency has become alarming [10,11,21]. Several evidences lead to a role of high milk production and heat stress (HS) in contributing with changes in reproductive physiology that may underlie the decline of cows profitability [10,27].

Aiming to avoid the need for estrus detection and enhance pregnancy rates, different protocols for synchronization of follicular wave emergence and ovulation with self-appointed managements have been developed. Mainly, the hormonal treatments are based on a combination of GnRH/PGF$_{2\alpha}$/GnRH – Ovsynch [14] or association of progesterone (P4) releasing devices and estradiol (E2) [3]. Currently, there are numerous protocols for fixed time artificial insemination (FTAI) and embryo transfer (FTET) with different combination.

Based on the knowledge that oocytes and embryos at early stages of development are extremely sensitive to HS [5,15,7], embryo transfer (ET) was employed as a potential tool to improve summer fertility by bypassing the effects of HS on early embryonic development [1,9]. Moreover, FTET can be successfully employed to bypass the conception failure observed in repeat breeding (RB) cows, especially during HS [reviewed by 6, 17].

This review aims to show some key-point experiments conducted to improve the efficiency of FTAI and FTET protocols in Brazil, overcoming several reproductive problems. Our goal is to develop cheap and easy self-appointed programs that facilitate animal handling and maximize their reproductive outcomes all over the year.

II. THE USE OF FTAI IN HIGH PRODUCTION HOLSTEIN CATTLE

The main limiting factors for the massive use of artificial insemination (AI) are the failures and difficulties to perform efficient estrus detection. Even in well-organized farms the rate of estrus detection barely achieves 50% [26], which in turn result in few number of AI. When FTAI is used the service rate achieves 100%, ending up with a larger number of cows been inseminated, increasing the percentage of P/AI. Protocols currently used combine GnRH and PGF$_{2\alpha}$ or P4 and E2. The choice for one or another mainly relies on the availability of the drugs, the permission to use them (federal laws) and their cost.

2.1 Protocols for FTAI without estradiol

A protocol developed to synchronize both cyclic and anovular cows was the Double-Ovsynch (DO) [23]. It consists of a pre-synchronization method using an Ovsynch 7d prior to the Ovsynch-timed AI protocol. In addition to treatment of anovular cows, it seems likely that DO more tightly synchronized the stage of the estrous cycle at initiation of Ovsynch compared to Presynch, increasing fertility to the FTAI [23]. Recently, DO was compared to a P4-E2 based protocol [P4 device and estradiol benzoate (EB) - 8d - device removal, eCG, PGF$_{2\alpha}$ and EC - 56h – GnRH and AI] commercially used in Brazilian Holstein cows (Ranieri & Baruselli, unpublished data). Both protocols resulted similar 30 ($P = 0.15$) and 60d pregnancy rate ($P = 0.20$) and pregnancy loss ($P = 0.83$; Figure 1). Also, when cows had a corpus luteum (CL) at the beginning of the treatment greater 30 and 60d pregnancy rates were observed, but pregnancy loss was similar (Figure 2).

In conclusion, the P4-E2 based protocols require lesser managements (3 vs 7) and have shorter duration (10 vs 25d) than DO with similar 30 and 60d P/AI (although it reached 12.5 and 9.6 points over DO, respectively).

2.2. Protocols for FTAI with estradiol

Currently, numerous P4-E2 based protocols for FTAI [2,12] with different combinations of hormones, doses and duration are available in Brazil. The possibility of using P4 devices previously used for eight days and the benefits of enlarging the proestrus period [by giving estradiol cypionate (EC) at device withdrawal] during protocols for FTAI was evaluated [22]. Neither the type of device (new or previously used) nor the administration of EC affected P/AI of

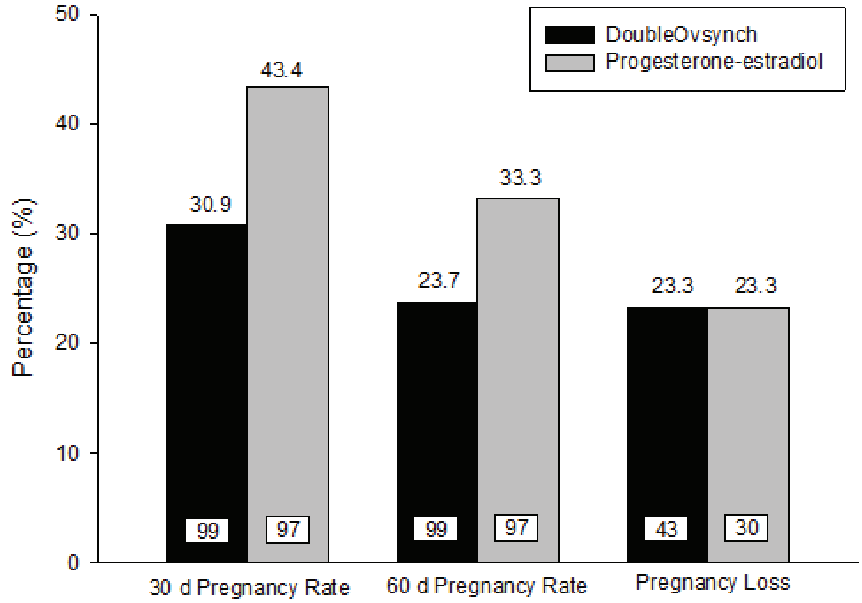

Figure 1. Percentage of 30 and 60 d pregnancy and pregnancy loss of high-producing Holstein cows treated with different protocols for FTAI. P = 0.15, 0.20 and 0.83, respectively (Adapted from Ranieri & Baruselli, unpublished data).

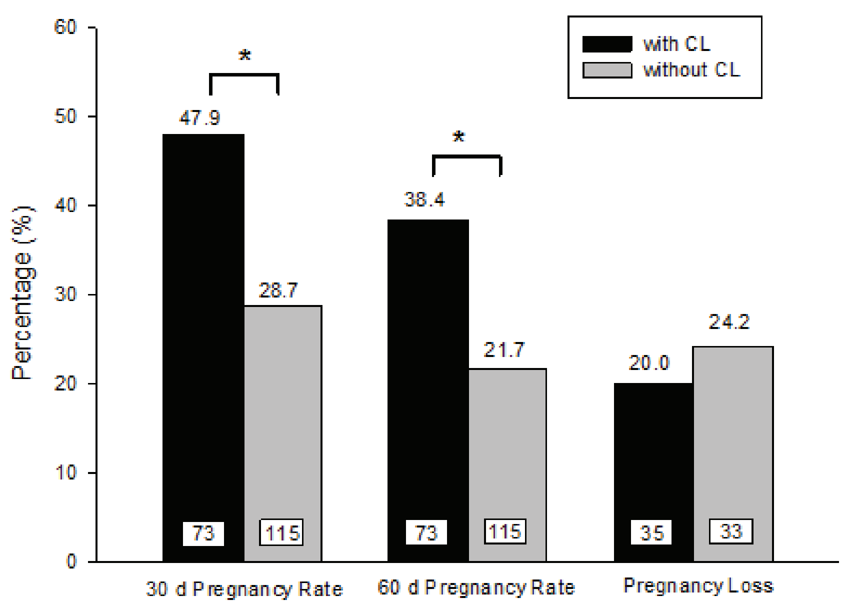

Figure 2. Percentage of 30 and 60 d pregnancy and pregnancy loss of high-producing Holstein cows with or without a CL at the begging of the protocol for FTAI.* P = 0.01. Adapted from Souza *et al*.[23].

cows receiving GnRH as ovulatory stimulus. Thus, the device previously used can be efficiently associated with EB in protocols for FTAI. Also, there was no additional effect of using EC to enlarge the proestrus duration when cows are treated with GnRH prior to AI (Figure 3). However, cows with BCS < 2.75 EC improved P/AI compared to GnRH (21.7% vs. 6.1%; P < 0.05)[22].

Another goal was to verify if additional eCG (400IU) at device removal could improve P/AI. Also, the type of ovulatory stimulus (GnRH or EC) was evaluated [22,24]. The average diameter of the ovulatory follicle (13.9 mm) and time to the LH peak (average 43.6 h after device removal) was unaffected by the type of ovulatory stimulus or the use of eCG. However, greater variability in time to the LH peak was found when cows where treated with EC than GnRH (S.E.M. 2.6 vs 1.8). Overall, time to ovulation averaged 73.2 h after device removal. The size of the CL, P4 concentrations in the subsequent diestrus, and P/AI (Figure 4) were unaffected by the use of eCG or type of ovulatory stimulus. However, thinner cows (body condition score below 2.75) receiving eCG had greater P/AI than those not treated with eCG (Figure 5).

Another study was done to reevaluate the need of using eCG (at device removal) and the benefit of increasing its dose (0, 400 or 600 IU) in protocols for FTAI. Although follicular growth rate tended to increase in cows treated with eCG (no eCG = 2.5 ± 0.5[a] vs 400IU eCG = 3.8 ± 0.6[b] and 600IU eCG = 3.5 ± 0.5[b]; P = 0.06), follicular diameter, ovulation rate, time to ovulation, size of the CL and P4 concentration were

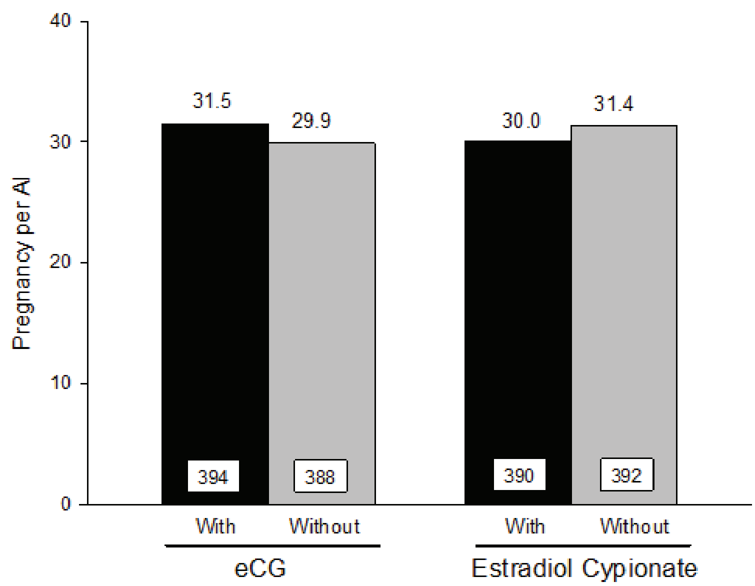

Figure 3. P/AI for Holstein cows treated for FTAI with new progesterone device or device previously used for 8 days, and receiving or not estradiol cypionate at device removal. P > 0.05. Adapted from Souza *et al.* [23].

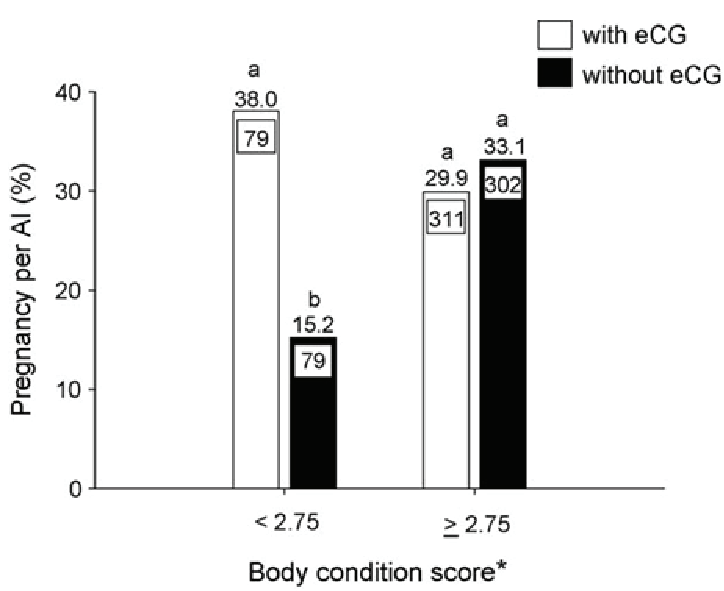

Figure 4. P/AI for Holstein cows treated or not with eCG, and receiving or not estradiol cypionate at device removal for FTAI. P > 0.05. Adapted from Souza *et al*. [23].

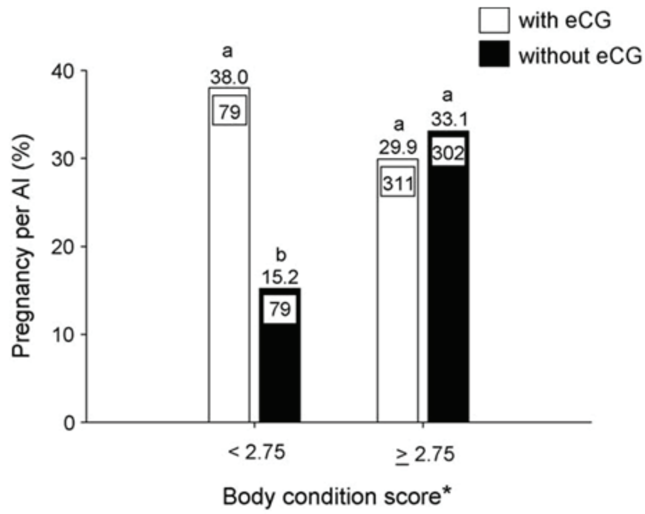

Figure 5. P/AI for Holstein cows receiving or not eCG for FTAI and with BCS < 2.75 or = 2.75. $P < 0.05$. Adapted from Souza *et al*. [23].

not affected by eCG or its dose (unpublished data). P/AI was also similar among groups (Figure 6), suggesting that eCG might not be essential to FTAI protocols in high-producing confined Holstein cows in Brazil.

Recently, the interval calving-AI and calving-conception and P/AI at first, second, third services, and 150 DIM were compared among FTAI and AI 12h after estrus detection [25]. Similar P/AI after first, second and third AI and at 150 DIM were achieved. However, pregnancy loss was greater with FTAI compared to AI following estrus (Figure 7). The interval calving-AI and calving-conception were reduced in FTAI cows, because they started the protocol around 57 DIM regardless the observation of estrus, anticipating the first and second AI postpartum with similar P/AI (Figure 8). Thus, although FTAI protocols initiated around 60 DIM decreased the interval calving-conception, the percentage of pregnant cows at 150 DIM was similar among cows subjected to FTAI and AI after estrus detection.

III. THE USE OF ET IN HIGH-PRODUCING HOLSTEIN CATTLE

The main advantages of implementing the ET is (1) to accelerate the herd genetic gain by producing more calves per selected donors, (2) to increase reproductive efficiency of RB cows, and (3) to reduce the deleterious effects of HS.

3.1 Benefits of applying embryo technology in Holstein cattle raised in tropical condition

It is already well established that HS strongly impairs Holstein cows fertility, decreasing the herd reproductive efficiency [9]. Oocytes and embryos at

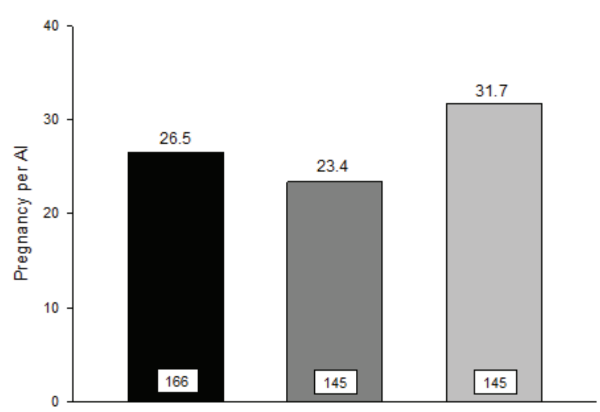

Figure 6. P/AI for high-producing Holstein cows receiving 0, 400 or 600 IU of eCG for FTAI. $P > 0.05$. Adapted from Ferreira & Baruselli, unpublished data.

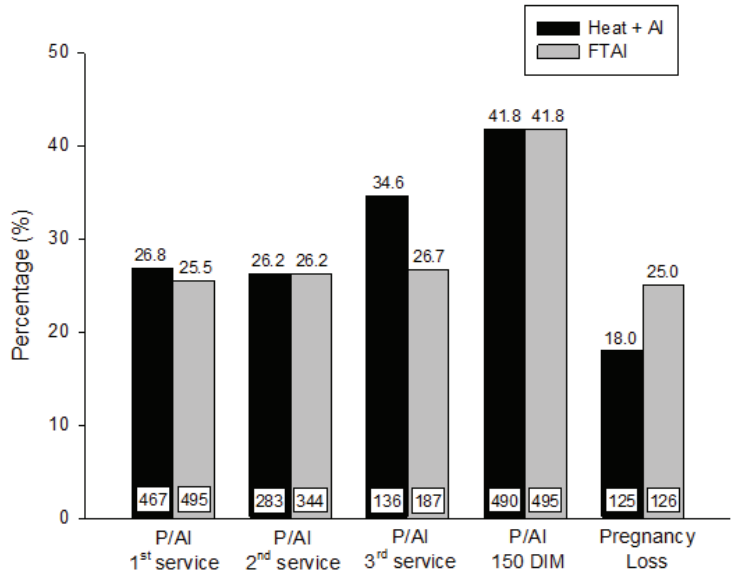

Figure 7. P/AI at first, second and third services and at 150 days in milk (DIM), and pregnancy loss (first to third services) of Holstein cows inseminated 12 h following estrus detection or treated for FTAI. *P* > 0.05. Adapted from Teixeira [25].

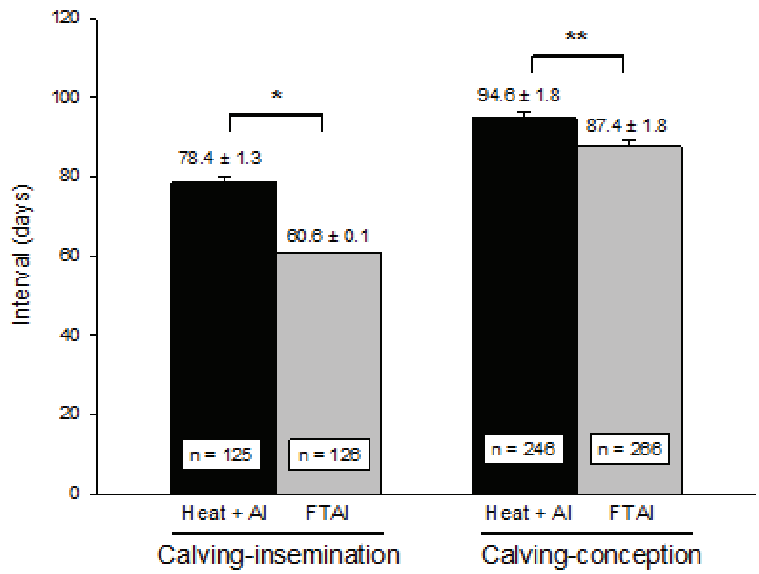

Figure 8. Interval calving-insemination and calving-conception of Holstein cows inseminated 12 h following estrus detection or treated for FTAI. **P* = 0.0001; ***P* = 0.02. Adapted from Teixeira [25].

early stages of development are extremely sensitive to heat stress [7,9]. A strategy that has been used to improve fertility during HS is ET.

In attempt to elucidate the reproductive performance of Brazilian high-producing Holstein cows after AI or ET, a retrospective study was conducted [16]. Cows receiving an embryo had higher P/AI (41.9%) than those inseminated (28.5%) throughout the year (Figure 9). Similar results were observed when RB were evaluated [17]; Figure 9. Therefore, ET came out as a valuable alternative to obtain satisfactory P/AI throughout the year, especially during HS and in RB.

Although ET enhances P/AI compared to AI, Freitas *et al.* [2010] demonstrated that pregnancy loss between 30 and 60 d pregnancy was higher when cows received embryos (20.5%) than AI (17.3%; *P* = 0.001).

RB had similar ($P = 0.39$) embryonic losses compared to non RB after AI (17.1 vs 17.9%) or ET (19.4 vs 20.7%). Overall, HS increased ($P = 0.001$) pregnancy loss regardless the use of AI (16.1 vs 19.1%) or ET (18.4 vs 23.1%).

3.2 Advantages of using FTET

As for FTAI, an alternative to avoid the need of estrus detection to transfer embryos is the use of FTET [2,4]. Rodrigues *et al.* [2009] compared the reproductive efficiency of high-producing RB recipients receiving embryo at fixed time or 7 d after estrus detection. The protocol (P4 implant and E2 - 8d - implant removal, eCG and PGF2α - 9d – ET and GnRH) increased transferred-treated and pregnancy rates. When cows had a CL at the beginning of the treatment transferred-treated rate was enhance, however similar pregnancy was obtained compared to cows that started the protocol without a CL (Figure 10). Thus, FTET increased the number of recipients suitable for ET compared to estrus detection, and allow the use of cows regardless the presence of a CL at the begging of the protocol, with same efficiency.

3.3 FTET protocols with or without E2

High-producing Holstein cows submitted to FTET using or not E2, and with or without eCG at device removal had similar 25 and 42 d pregnancy rates during winter. However, during summer the recipients treated with GnRH (without E2) had greater 25 d pregnancy rates. But protocols with E2 seem to be more efficient during winter (Figure 11; unpublished data). Similarly to FTAI, the use of eCG had no effect in RB recipients subjected to FTET (Figure 12). In conclusion, FTET without E2 can be an important strategy (need more studies) during HS in high-producing Holstein recipients

3.4 Particularities of ET process

A retrospective study with Holstein cows raised in tropical regions showed that greater conception rates were achieved when fresh embryos were transferred (43.9%; n = 2,634) compared to frozen-thawed ones (39.5%; n = 2,237) [18]. Further, the tight synchronization between donor and recipient (same day of estrus) showed higher efficacy (43.6%; n = 2,648) than when recipients were one day later (41,5%; n = 1,259) or advantage (37.6%; n = 964) in relation to donors, especially when cryopreserved embryos were used [19]. These results suggest that, in Holstein cows raised in tropical climates, the use of fresh embryos produce greater conception rates than frozen embryos. In general, the difference in conception rate between fresh and frozen embryos seems to be greater in later stages of embryo development.

Another study investigated the effect of the site of embryo release (proximal, median or distal) into the uterine horn ipsilateral to the ovary with the CL on pregnancy rates after fresh and frozen-thawed

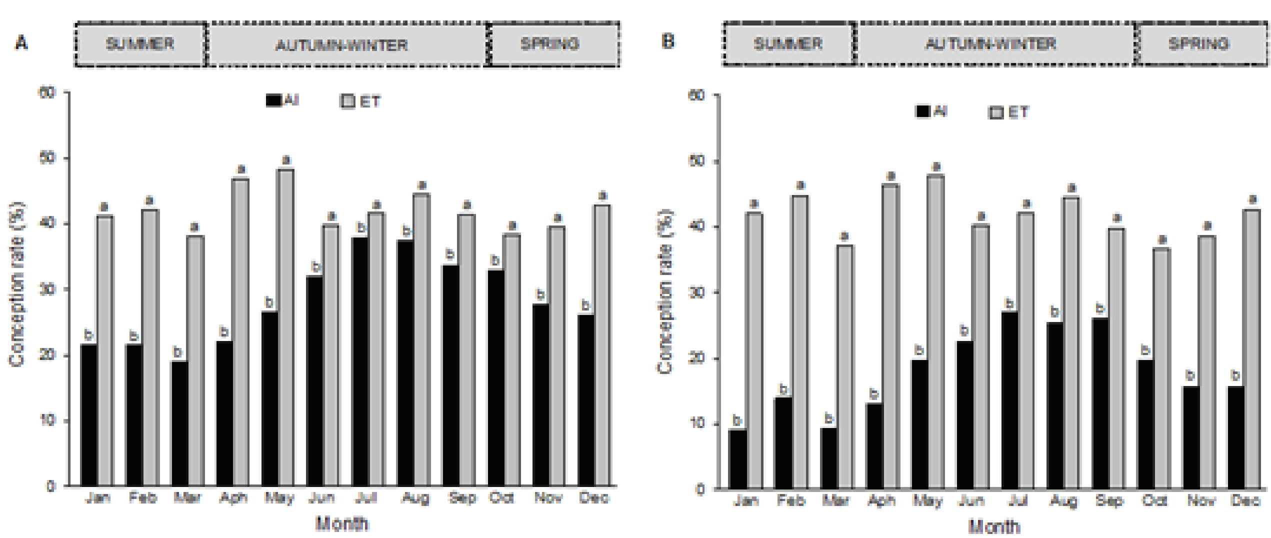

Figure 9. Monthly conception rate of high-producing Holstein cows after AI or ET over six years: (A) overall (all services), AI = 18,568 and ET = 4,871 and (B) repeat-breeder cows (= 3 services), AI = 5,693 and ET = 3,858. Adapted from Rodrigues *et al.* [16,17].

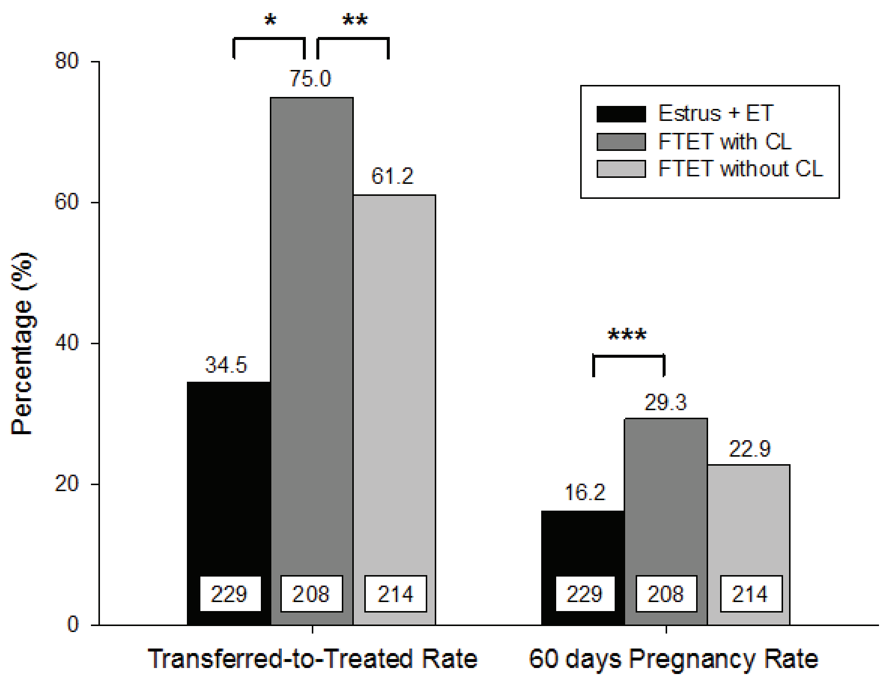

Figure 10. Transferred-to-treated and 60 d pregnancy rates of recipient Holstein cows receiving an embryo at fixed time (FTET) or following estrus detection. *$P < 0.0001$; ** $P = 0.003$; ***$P = 0.001$. Adapted from Rodrigues *et al*. [20].

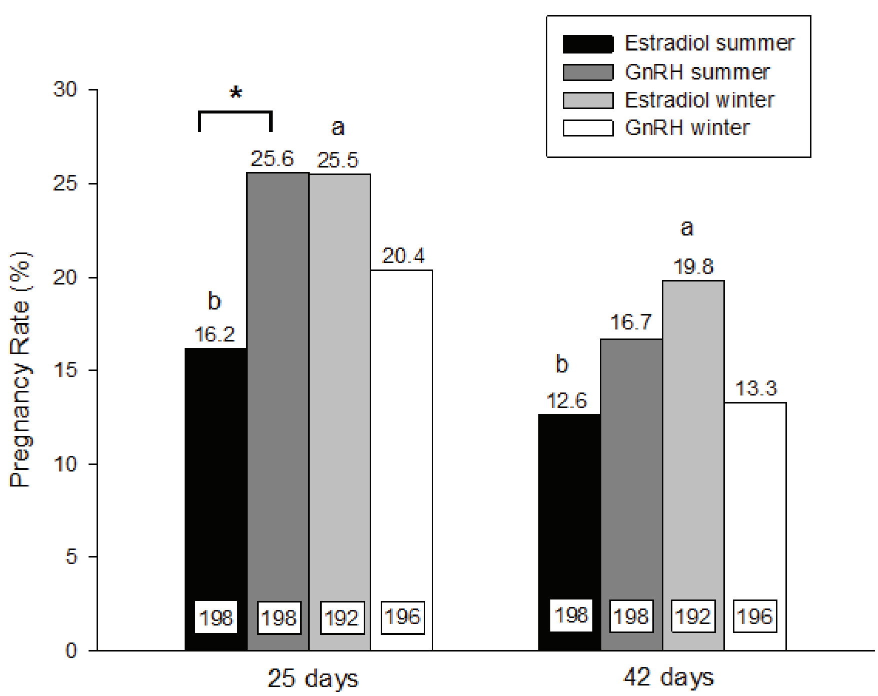

Figure 11. In sequence, 25 and 42 d pregnancy rate of recipient Holstein cows receiving an embryo following a FTET protocol using GnRH or estradiol. Values within treatment (ab) and within seasons (*) differ ($P < 0.05$). Adapted from Rodrigues *et al*. [20].

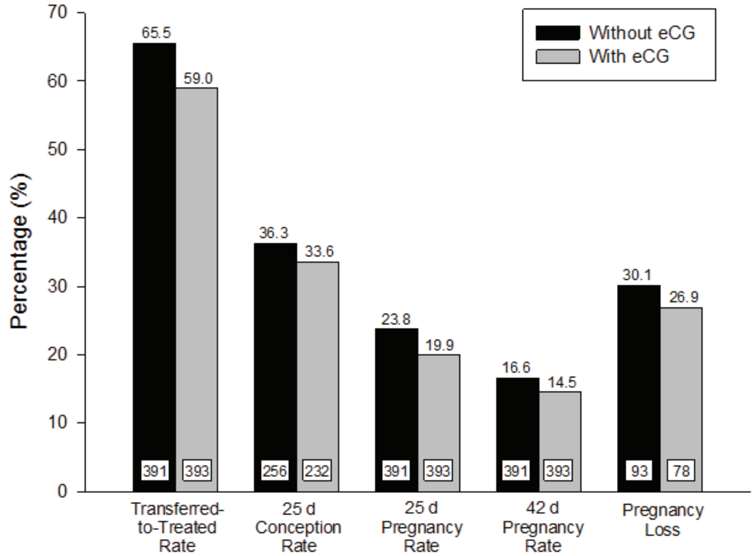

Figure 12. Reproductive parameters of recipient Holstein cows receiving an embryo following a FTET protocol using or not eCG. $P > 0.05$. Adapted from Rodrigues *et al*. 2010.

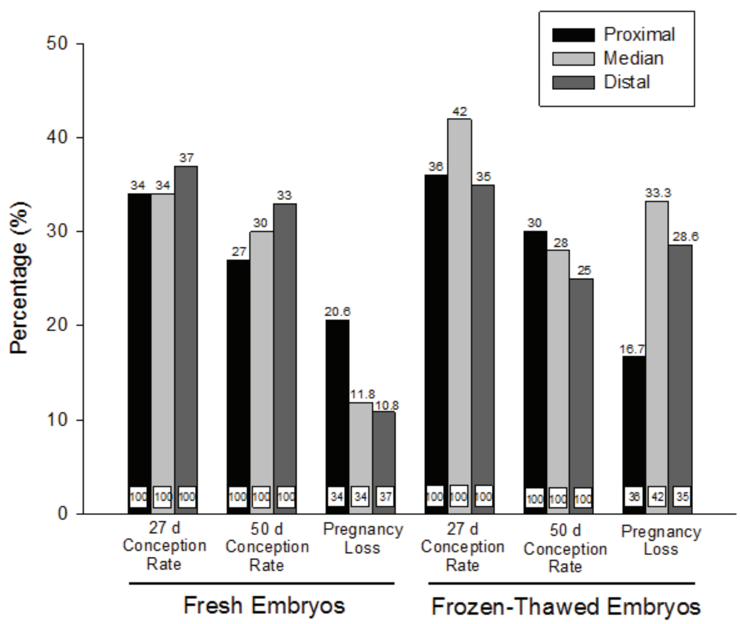

Figure 13. Conception rate and pregnancy loss of recipient Holstein cows receiving fresh or frozen-thawed embryo following a FTET. Embryos were released in different sites (proximal, median or distal) of the uterine horn adjacent to the corpus luteum. $P > 0.05$. Adapted from Pieroni *et al*. [13].

embryo transfer [13]. It was verified that the site of embryo release did not influence (P = 0.84) 27 and 50d pregnancy rates in recipients receiving fresh or frozen--thawed embryos. Also, pregnancy loss was similar among sites, regardless the type of embryo (Figure 13).

In conclusion, results are suggestive that fresh or frozen-thawed embryos can be transferred to any site of the uterine horn adjacent to the ovary containing the CL, without interfering in pregnancy rates.

IV. CONCLUSION

Both FTAI and FTET are important tools to improve reproductive efficiency of high-producing dairy cows. These biotechnologies help bypassing some of the greatest challenges of dairy cattle reproduction: the difficulties of estrus detection, and the low fertility associated to HS and RB.

Unpublished data. Ferreira & Baruselli (robertinhavet@yahoo.com.br); Ranieri & Baruselli (barusell@usp.br) - abstract sent to this meeting.

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