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# Effect of Treatment With Gonadoreline at the Embryo Transfer on Pregnancy Outcomes in Bovine.

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# Abstract

The aim of this study was to assess whether administration of the gonadotropin-releasing hormone (GnRH) agonist gonadorelin would increase conception rates at 30 (P / ET 30) and 60 (P / ET 60) days of gestation and reduce pregnancy losses (PL) in embryo recipients and what would be the correlation between the animal category and the season of the year in which the embryo transfer would occur. The experiment was conducted on 11 commercial farms in the state of Minas Gerais, Brazil. Recipients were randomly divided into groups: treated (n = 624), cows that received 0.1 mg gonadorelin on the day of FTET, and control (n = 687), untreated cows. All embryos were produced in vitro from oocyte donors of different races. Recipients were classified as heifers, dry cows or lactating cows. Higher conception rate was observed at 30 days (p = 0.03) and 60 days (p = 0.01) in the treated group (45.8%; 43.0%, respectively). Dry and lactating cows had less pregnancy loss (p = 0.001) compared to heifers (2.70% and 2.47% vs 10.42%, respectively). During spring/summer, there was less P/FTET at 30 days (0.024). Embryo transfer carried out in the warmer seasons of the year reduced the pregnancy rate to 60 days and increased pregnancy loss. As an implication, the use of the GnRH analogue can be used as a tool to increase fertility in embryo recipients.


# Introduction

Embryo transfer (ET) is an important biotechnology for animal production, being an important tool for the multiplication of animals of superior genetic merit. (Batista et al. 2016). In the last decade, scientific and technological development innovated the embryo production processes leading to a considerable improvement of the ET technique. According to the International Embryo Technology Society (Viana, 2019), more than 1 million *in vitro* embryos were produced worldwide. This represents an increase of 37% in production since 2009. South America is the world leader in the number of *in vitro* embryos transferred, surpassing 300 thousand embryos. This shows great potential and importance of this technique in cattle reproduction (Viana, 2019).

Despite the growth in usage of ET in cattle, its results are still inconsistent. Recipients of *in vitro* embryo produced (IVEP) have lower conception rate and greater gestational loss compared to cows receiving *in vivo* produced embryos and artificial insemination (Sartori et al., 2016). Therefore, studies trying to create new strategies to increase the efficiency of *in vitro* embryo production and transfer programs should be developed.

Among several factors that can influence the success of ET, the quality of the corpus luteum (CL) plays an important role in the pregnancy maintenance (Diskin and Morris, 2008). The CL quality is correlated with serum progesterone (P4) concentration which is responsible for conception and intrauterine embryo development (Wiltbank et al., 2014).

The increase in serum progesterone (P4) concentration immediately after insemination has a function in maintaining pregnancy and promoting proper development of the conceptus (Inskeep, 2004; Stronge et al., 2005; Mcneil et al., 2006). Indirectly, the presence of P4 in the uterine environment modifies endometrial gene expression (Spencer et al., 2013; Lonergan and Forde, 2014), which in turn enables the development of the embryo and the production of interferon *tau*. The direct effect of P4 on embryo elongation has not been fully elucidated. However, the presence of P4 at correct levels, even before embryo deposition through inovulation, prepares the uterine environment, making it more receptive to the embryo and enhance its development (Geisert et al., 1991).

The postovulatory treatment with luteotropic hormones in the first week of the estrous cycle, such as human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH), can improve pregnancy rates and reduce pregnancy losses (Vasconcelos et al., 2011; Marques et al., 2012). These hormones promote the formation of an accessory CL and, consequently, an increase in serum P4 concentration.

Another explanation for the increase in the pregnancy rate has been described in cows treated with luteotropic hormones. The higher P4 concentration, caused by the formation of an accessory CL, leads to better modulation of follicular growth. Thus, the follicles growing under these conditions are usually smaller and, therefore, produce less estrogen. At this time, high concentrations of estrogen aren't desirable since it can increase the possibility of luteolysis, which would interrupt the pregnancy (Marques et al., 2012). Due to the inconsistent results of the FTET and the controversial effectiveness of luteotropic hormones in increasing the pregnancy rate (Galvão et al., 2014; García-Guerra et al., 2020)

The objectives of our study were to evaluate the effects of recipient category, season of the year, and administration of a GNRH analogue at the time of FTET on the conception rates at 30 (P/ET30) and 60 (P/ET60) days of gestation and on pregnancy losses.

## Material And Methods

The study was carried out in 11 commercial farms, in Uberlândia, Minas Gerais State, Southeast Brazil from July 2015 to July 2016. The climate, according to the Köppen classification, is the Aw type, megathermic, with hot and rainy summers (from October to March) and cold and dry winters (from April to September) (Rosa, 1991). In the spring / summer seasons, the average temperature was 22.29°C and the relative air humidity was 69.05% and, in the autumn / winter seasons, the average temperature was 24.97°C and the relative air humidity was 49.34% (IGUFU, 2017). This work was done according to the Ethical Principles in Animal Experimentation, approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Uberlândia (UFU) under the protocol number 041/14.

# Donors and embryos

The study used 144 oocytes donors and semen from 38 different sires. The donors were not submitted to any hormonal protocol and their oocytes were collected using the ovum pick-up method (OPU). The interval between OPU in a given donor was at least 2 months.

All the embryos were produced *in vitro*. Embryos were classified by laboratory technicians according to their stage of development (from 1 to 9) and their morphological quality (from 1 to 4) (IETS, 2014). For TETF, only embryos graded 1 for morphological quality and in developmental stages three (morula), four (Compact morula), five (early blastocyst), six (blastocyst), seven (expanded blastocyst) or eight (Hatched blastocyst) were used (Bó and Mapletoft, 2013). Nelore, Gir and Girolando embryos were used. The embryos were transferred fresh according to the order in which they were allocated in the embryo carrier.

# Recipient and treatment

To be eligible to the TETF program, recipients could not show any clinical disease. In addition, it was required a body condition score (BCS) ranging between 3 and 3.75, on a scale from 1 to 5 (Klopčič *et al.*, 2011).

On day 18 of the TETF protocol (Fig. 01), recipients were submitted to a transrectal ultrasound examination and only those that had at least one CL were considered eligible for the TETF program. The embryos were transferred to the ipsilateral uterine horn of the ovary that had the CL. Embryo were designated to recipients in a random way. The evaluations of health, BCS, and ovaries were performed by the same veterinarian, as well as all inovulations and pregnancy diagnoses.

The hormonal protocol used to synchronize the recipients' ovulation was: D0: insertion of the intravaginal P4 device with a dosage of 1.9 g and intramuscular application of 2.0 mg of estradiol benzoate; D7: administration of 0.526 mg of sodium cloprostenol, i.m.; D9: administration of 0.526 mg of sodium cloprostenol + 1 mg of estradiol cypionate i.m. + removal of the intravaginal device; D18: embryo transfer and administration i.m. or not of gonadorelin (Fig. 01).

The recipients were randomly divided into 2 groups: Treated group (n = 624, treated with 0.1 mg of gonadorelin on the day of TETF) and Control Group (n = 687, no treated).

# Pregnancy diagnoses and data collection

The pregnancy diagnosis was performed by transrectal ultrasound examination, 23 ± 5 days after TETF. Around 30 ± 5 days later, animals diagnosed as pregnant were reexamined. The conception rate at 30 days of gestation (P/ET 30) was calculated by dividing the number of pregnant animals in the first evaluation by the total number of TETF. The conception rate at 60 days of gestation (P/ET 60) was calculated by dividing the number of pregnant animals in the second evaluation by the total number of TETF. The pregnancy loss (PL) was calculated by dividing the number of pregnancy losses between the first and the second diagnoses by the number of pregnant animals in the first diagnosis. The result of these divisions was multiplied by 100 to get the percentage value. Data on season of the year when the TETF was performed (spring / summer and autumn / winter) and recipient category (heifers, lactating cows, and dry cows) were also collected.

# Statistical Analysis

The data with binomial distribution (P/ET 30, P/ET 60 and PL) were analyzed by multivariate logistic regression, using the GLIMIX procedure of SAS, version 9.2 (SAS / STAT, SAS Institute Inc., Cary, NC). In the first model, the effects of treatment, TETF season of the year, recipient category, as well as possible interactions effects on P/ET 30, P/ET 60 and PL were evaluated. Once no effect of the possible interactions was detected, they were removed from the final model. Statistical significance was considered when $P \leq 0.05$, and statistical tendency when $0.05 < P \leq 0.10$.

# Results

The treatment affected both P/ET 30 (P = 0.03) and P/ET 60 (P = 0.01). The treatment with gonadorelin on d 7 tended (P = 0.09) to reduce PL (7% and 4% for control and treated group, respectively, Table 1).

Table 1
Effect of treatment with gonadorelin at the time of embryo transfer on recipient’s pregnancy on 30 d (P/ET 30), 60 d (P/ET 60) and pregnancy loss (PL).

| Groups | P/ET30 % (n) | P/ET60 % (n) | PL % (n) |
|---|---|---|---|
| Control | 40.0 (247/624) | 37.0 (230/624) | 7.0 (17/247) |
| Treatment | 45.8 (312/687) | 43.0 (299/687) | 4.0 (13/312) |
| P value | 0.03 | 0.01 | 0.09 |

There was no effect of the recipient category on P/ET 30 and P/ET 60, however, heifers had a higher PL (P = 0.001), when compared with dry cows and lactating cows. The season of the TETF affected the conception rate at 60 days (P = 0,024) and tended to have an effect (P = 0.095) on pregnancy loss. During the spring / summer season the PL tended to be greater compared to the autumn / winter. Consequently, P/ET 60 was higher in the autumn / winter seasons (Table 2).

Table 2
Effect of recipient category and TETF season of the year on the conception rate at 30 (P/ET 30) and 60 days (P/ET 60) and on pregnancy loss (PL).

| Groups | P/ET 30 (%) | P/ET 60 (%) | PL (%) |
|---|---|---|---|
| Animal category (n) | | | |
| Heifer (607) | 45.14 | 41.52 | 10.42[a] |
| Dry cow (537) | 40.22 | 39.11 | 2.70[b] |
| Lactating cow (167) | 41.32 | 40.12 | 2.47[b] |
| P value | 0.160 | 0.544 | 0.001 |
| Season of TETF (n) | | | |
| Spring / Summer (775) | 40.77 | 37.10 | 7.71 |
| Autumn / Winter (536) | 45.34 | 44.03 | 4.43 |
| P value | 0.101 | 0.024 | 0.095 |
| Values with different letters are statistically different ($P \leq 0.05$) | | | |

## Discussion

One of the aims of this study was to evaluate whether the treatment with gonadorelin at the time of embryo transfer would improve the conception rate at 30 and 60 days or not. The results of this study showed that the groups treated with gonadorelin at the time of TETF had increased conception rates when compared to the control group (5.8% at 30 days and 6% at 60 days; Table 1). Similar results were found by (Marques et al., 2012), in which animals treated with GnRH had a higher conception rate compared to the control group. (Vasconcelos et al., 2011) also found a higher conception rate in recipients treated with hCG and GnRH, compared to the control group. Together, these studies show that the administration of an ovulation inducer can be effective for improving fertility of recipients in ET programs.

According to (García-Guerra et al., 2020), treatment with gonadorelin, causing the dominant follicle of the first follicular wave to ovulate and form an accessory CL, can improve recipient conception rate in ET programs. This treatment causes an increase in the P4 concentration, which is of fundamental importance for the development of the conceptus and the maintenance of pregnancy. Additionally, the high concentration of P4 provides a more favorable uterine environment for the lengthening of the conceptus, which positively influences the production of interferon *tau* and the maternal recognition of pregnancy (Maillo et al., 2012).

A second possible explanation for the results found is that ovulation of the dominant follicle in the first follicular wave leading to the formation of an accessory CL and the consequent increase in the P4 concentration inhibit the presence of a new large dominant follicle at the time of maternal pregnancy recognition, reducing the possibility of elevating circulating estradiol and the development of the luteolytic trigger this critical period of pregnancy (Marques et al., 2012).

In contrast, some studies have shown that the use of drugs that promote the formation of an accessory CL do not improve the fertility of recipients (Galvão et al., 2014; García-Guerra et al., 2020). (García-Guerra et al., 2020) did not find any improvement in the conception rate of animals treated with GnRH. According to the authors, this result may have occurred because both groups, treated and control, had high fertility and low pregnancy loss. Therefore, GnRH treatment 5 to 7 days after ovulation may not be efficient in herds with good fertility results.

(Galvão et al., 2014) reported a lower conception rate for the group treated with GnRH. However, the protocol that they used included an administration of equine chorionic gonadotrophin (eCG). According to (Marques et al., 2012), the protocol used for recipient synchronization can influence the P/ET. Protocols that use eCG usually lead to a greater development of the dominant follicle and, consequently, to the formation of a larger CL. A larger CL has an increased capacity of P4 production, leading to a greater conception rate. The use of eCG can also increase the double ovulation rate when administered during the pre-dominant phase of the follicular wave, increasing the P4 concentrations after ovulation. Since the use of eCG already increases the P4 concentrations, the treatment with GnRH after ovulation may not influence P/ET rates in this case. This may explain the results found by (Galvão et al., 2014) that diverges from what was found in the present study.

The treatment with gonadorelin tended to reduce the pregnancy loss rate (P = 0.095; Table 1). Some studies that administrated different luteotropic drugs found similar results as the present study ( Niles et al., 2019; García-Guerra et al., 2020). Niles *et al*. (2019), observed a reduction in pregnancy loss (P = 0.04) between the first and second pregnancy diagnoses (days 32 to 67) in heifers treated with hCG on the day of TETF (7 days after ovulation). Guerra et al. (2020), studying the administration of GnRH on day 5 of the estrous cycle of Holstein and crossbred heifers, reported an effect of embryo quality on the rate of pregnancy loss. Animals that received embryos in development stage 7 (incubation blastocyst), obtained a lower percentage of pregnancy losses than the other recipients. In addition, the effect of the presence of accessory CL in these animals potentiated the reduction of pregnancy loss. This reduction in pregnancy loss reached up to 50%. According to the authors, the embryo stage and the presence of an accessory CL (which is responsible for the higher P4 concentrations) are factors that possibly contributed with the decrease in pregnancy loss.

There was no effect of the recipient categories (heifers, dry cows and lactating cows) on the conception rate at 30 days and 60 days, however heifers showed greater PL when compared to the other groups (P = 0.001; Table 2). These results were contrary to what was expected since most data in the literature show that heifers tend to have better conception rates and less pregnancy loss (Hasler, 2014). However, the PL rate for heifers found in this study met the values found in other studies (Scanavez et al., 2013; García-Guerra et al., 2020). Thus, this difference may be because of the low PL rate found for the other two categories of recipients (dry and lactating cows). Normally, reduced P/ET and PL rates are observed for high producing lactating cows (Vasconcelos et al., 2006). Since the recipient cows used in the present study where either low producing or dry cows, the negative effect of milk production may not have impacted the PL rates, thus explaining the low values found.

An effect of season of the year (P = 0.024) on the conception rate at 60 days was found. Recipients that were submitted to TETF in the autumn / winter showed better reproductive performance compared to animals that TETF was performed in the spring / summer (Table 2). (Demetrio et al., 2007) reported that an increase in body temperature of animals on the 7th day after ovulation has a negative effect on the conception rate at 28 days in animals submitted to embryo transfer. Similarly, (Ferraz et al., 2016), evaluated the relationship between the temperature and humidity index (THI) with the conception rate, they observed that the conception rate for cows and heifers decreased with the increase in THI. They also found that cows are more sensitive to heat stress indicated by THI compared with heifers. According to (Sartori et al., 2002), when compared to heifers, it is more difficult for cows to regulate their body temperature in response to a warm weather. This bigger susceptibility to heat stress contributes to lower conception rates during the warm seasons.

In the present study, there was a tendency of influence of the season of the year in which the TETF was performed on the PL (P = 0.095). During the warmer seasons (spring / summer) the PL was greater than in the colder seasons (autumn / winter) (Table 2). (García-Ispierto et al., 2006) also evaluated the influence of heat stress on PL. They also observed a greater PL rate during the warmer months when compared the coldest periods. According to the authors, a high THI during the fourth week of gestation can negatively impact the pregnancy rate and contribute with the PL due to the embryo sensitivity to heat stress during the implantation period.

As a conclusion, the treatment with gonadorelin at the time of bovine embryo transfer increased the pregnancy per embryo transfer at day 30 and 60, and reduce pregnancy loss. Additionally, dry and lactating recipient cows showed a lower pregnancy loss rate compared to heifers. Furthermore, embryo transfer performed in the hottest seasons of the year (spring / summer) had result in lower pregnancy rate at day 60 and a greater pregnancy loss.

# Declarations

**Contributions:**

ML and FM: Experiment development, literature review and writing;

AFF, GC and MF: Analysis and interpretation of results and writing;

RS: Planning, analysis and interpretation of results and review.

All authors read and approved the final manuscript.

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**Conflict of interest:** The authors declare that they have no conflict of interest.

**Ethical standards:** This work was done according to the Ethical Principles in Animal Experimentation, approved by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Uberlândia (UFU) under the protocol number 041/14.

**Data availability:** All authors allow the availability of the data exposed in this document.

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# Figures

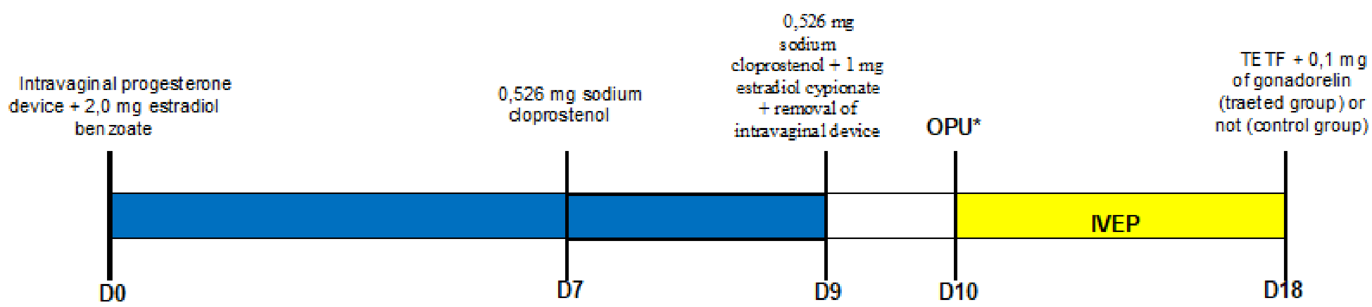


**Figure 1**

Schematic representation of the hormonal protocol used to synchronize the ovulation of embryo recipients and the chronology of the actions performed for TETF protocol. *OPU in oocyte donors.