



## Effect of gonadorelin treatment in embryo transfer on pregnancy outcomes in cattle

### Efeito do tratamento com gonadotrofina na transferência de embriões nos resultados de prenhez em bovinos

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**Abstract:** The objectives were to evaluate the effects of recipient category, season and administration of a gonadorelin analogue at the time of fixed-time embryo transfer (FTET) on pregnancy per FTET at 30 and 60 days after oestrus and on pregnancy losses (PL). Recipients were randomly assigned to: treated group (n = 624), in which recipients received an intramuscular injection of 0.2 mg of gonadorelin (Fertagyl®) at FTET; or a control group (n = 687) that remained untreated. Recipients were previously treated with a synchronisation protocol based on progesterone and oestradiol. All embryos were produced *in vitro*. The data with binomial distribution were analysed by multivariate logistic regression, using the GLIMIX procedure of SAS. Higher P/FTET was observed at 30 days (45.8 vs. 40.0%; P = 0.03) and 60 days (43.0 vs. 37.0%; P = 0.01) in the treated group. There was a tendency toward reduced PL in the treated group (4.0 vs. 7.0%; P = 0.09). Dry cows (2.70%) and lactating cows (2.47%) had less PL (P = 0.001) compared with heifers (10.42%). In the spring/summer season the P/FTET at 60 days was smaller (P = 0.024). Greater PL tended to occur in the warmer season. Treatment with gonadorelin at the time of bovine ET increased the pregnancy per ET at days 30 and 60 and reduced PL. Additionally, dry and lactating recipient cows showed a lower PL rate compared to heifers. Furthermore, ET performed in the warmer seasons of the year resulted in a lower pregnancy rate at day 60 and greater PL.

**Keywords:** reproductive efficiency; dairy cattle; reproduction.

**Resumo:** Objetivou-se avaliar os efeitos da categoria receptora, estação do ano e administração de análogo da gonadorelina no momento da transferência de embrião em tempo fixo (TETF) na prenhez por TETF aos 30 e 60 dias após o estro e nas perdas gestacionais (PG). As receptoras foram distribuídos aleatoriamente: grupo tratado (n = 624), onde as receptoras

receberam injeção intramuscular de 0,2 mg de gonadorelina (Fertagyl®) na TETF e um grupo controle (n =687) permaneceu sem tratamento. As receptoras foram previamente tratadas com protocolo de sincronização à base de progesterona e estradiol. Todos os embriões foram produzidos *in vitro*. Os dados foram analisados por regressão logística multivariada, utilizando o procedimento GLIMIX do SAS. Maior P/TETF foi observado em 30 dias (45,8 vs. 40,0%;  $p=0,03$ ) e 60 dias (43,0 vs. 37,0%;  $p=0,01$ ) no grupo tratado. Houve tendência de redução da PG no grupo tratado (4,0 vs. 7,0%;  $p=0,09$ ). Vacas secas (2,70%) e vacas em lactação (2,47%) apresentaram menor PG ( $p=0,001$ ), em comparação com novilhas (10,42%). Na estação primavera/verão a P/TETF aos 60 dias foi menor ( $p=0,024$ ). Maior PG tendeu a ocorrer na estação mais quente. O tratamento com gonadorelina no momento do TE aumentou a prenhez por TE aos dias 30 e 60 e reduziu o PG. Vacas receptoras secas e lactantes apresentaram menor taxa de PG em comparação com novilhas. Além disso, a TE realizada nas estações mais quentes do ano resultou em menor taxa de prenhez aos 60 dias e maiores PG.

**Palavras-chave:** eficiência reprodutiva; gado de leite; reprodução.

## 1. Introduction

Embryo transfer (ET) is an important biotechnology for animal production, constituting an important tool for the multiplication of animals of superior genetic merit<sup>(1)</sup>. According to Viana<sup>(2)</sup>, in 2020 more than 1.1 million *in vitro* embryos were produced worldwide. This shows great potential and the importance of this technique in cattle breeding<sup>(2)</sup>. Despite the growth in the usage of ET in cattle, its results remain inconsistent. Recipients of *in vitro* embryo production (IVEP) have lower conception rates and greater gestational loss compared to cows receiving *in vivo*-produced embryos and artificial insemination<sup>(3)</sup>. Therefore, studies should be developed to discover new strategies to increase the efficiency of *in vitro* embryo production and transfer programmes.

Among several factors that can influence the success of ET, the quality of the corpus luteum (CL) plays an important role in pregnancy maintenance<sup>(4)</sup>. Corpus luteum quality is correlated with serum progesterone (P4) concentration, which is responsible for conception and intrauterine embryo development<sup>(5)</sup>. The increase in serum P4 concentration immediately after insemination has a function in maintaining pregnancy and promoting proper development of the conceptus<sup>(6-8)</sup>. Indirectly, the presence of P4 in the uterine environment modifies endometrial gene expression<sup>(9)</sup>, 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 ovulation, prepares the uterine environment, making it more receptive to the embryo and enhancing its development<sup>(10)</sup>.

Postovulatory treatment with luteotropic hormones in the first week of the oestrous cycle, such as human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH), can improve pregnancy rates and reduce pregnancy losses<sup>(11-12)</sup>. These hormones promote the formation of an accessory CL and, consequently, an increase in serum P4 concentration.

Another explanation for the increase in 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 oestrogen. At this time, high concentrations of oestrogen are not desirable since it can increase the possibility of luteolysis, which would interrupt the pregnancy<sup>(11)</sup>. Due to the inconsistent results of fixed-time embryo transfer (FTET) and the controversial effectiveness of luteotropic hormones in increasing the pregnancy rate<sup>(13)</sup>, more research in this area is required.

The objectives of our study were to evaluate the effects of recipient category, season of the year and administration of a gonadorelin analogue (Fertagyl®) at the time of FTET on pregnancy per FTET at 30 (P/FTET 30) and 60 (P/FTET 60) days after oestrus and on pregnancy losses (PL).

## 2. Material and methods

The study was carried out on 11 commercial farms in Uberlândia, Minas Gerais state, Southeast Brazil, from July 2015 to July 2016. According to the Köppen classification, the climate is the Aw type, megathermal, with hot and rainy summers (from October to March) and cold and dry winters (from April to September)<sup>(14)</sup>. Average temperature and relative humidity were 24.97 °C and 69.05% in the spring/summer seasons and 22.29 °C and 49.34% in the autumn/winter seasons, respectively<sup>(15)</sup>. This study was conducted 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 approval number 041/14.

### 2.1 Donors and embryos

The study used 144 oocyte donors and semen from 38 different sires. The donors did not undergo 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 two 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)<sup>(2)</sup>. 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<sup>(16)</sup>. Nellore (80), Gyr (530) and Girolando (701) embryos were used. The embryos were transferred fresh according to the order in which they were allocated in the embryo carrier.

### 2.2 Recipients and treatment

To be eligible for the FTET programme, crossbred recipients could not show any clinical disease. In addition, a body condition score (BCS) ranging between 3 and 3.75, on a scale from 1 to 5<sup>(17)</sup>, was required. The selected recipients were randomly divided into two groups: the treated group (n = 624, treated with 0.2 mg of gonadorelin (Fertagyl) on the day of FTET; and the control group (n = 687, not treated).

The hormonal protocol used to synchronise the recipients' ovulation was: D0: insertion of the intravaginal P4 (FertilCare®) device at a dose of 1.2 g and intramuscular application of 2.0 mg of oestradiol benzoate (FertilCare Sincronização®); D7: administration of 0.526 mg of sodium cloprostenol (Sincrosin®) i.m. (intramuscularly); D9: administration of 0.526 mg of sodium cloprostenol (Sincrosin®) i.m. + 1 mg of oestradiol cypionate (FertilCare Ovulação®) i.m. + removal of the intravaginal device; D18: recipients underwent a transrectal ultrasound examination and only those that had at least one CL were considered eligible for the FTET programme. The embryos were transferred to the ipsilateral uterine horn of the ovary that had the CL, and administration or omission of 0.2 mg of gonadorelin i.m. (Fertagyl®) was carried out. Embryos were designated to recipients randomly. Evaluations of health, BCS and ovaries, as well as all inoconceptions and pregnancy diagnoses, were performed by the same veterinarian.

### 2.3 Pregnancy diagnoses and data collection

For pregnancy diagnosis, a transrectal ultrasound examination was performed  $23 \pm 5$  days after FTET. Around  $30 \pm 5$  days later, animals diagnosed as pregnant were re-examined. Pregnancy per FTET at 30 days of gestation (P/FTET 30) was determined as the ratio between the number of pregnant animals in the first evaluation and the total number of FTET. Pregnancy per FTET at 60 days of gestation (P/FTET 60) was calculated as the ratio between the number of pregnant animals in the second evaluation and the total number of FTET. Finally, pregnancy loss (PL) was calculated by the ratio between the number of pregnancy losses between the first and second diagnoses and the number of pregnant animals in the first diagnosis. The result of these divisions was multiplied by 100 to obtain a percentage value. Data on the season of the year when the FTET was performed (spring/summer and autumn/winter) and recipient category (heifers, lactating cows and dry cows) were also collected.

### 2.4 Statistical analysis

The data with binomial distribution (P/FTET 30, P/FTET 60 and PL) were analysed 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, FTET season of the year, recipient category, as well as possible interaction effects on P/FTET 30, P/FTET 60 and PL were evaluated. When 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 a statistical tendency when  $0.05 < P \leq 0.10$ .

## 3. Results

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

Table 1 Effect of treatment with gonadorelin on pregnancy at 30 and 60 days and pregnancy loss

Group	P/FTET30 % (n)	P/FTET60 % (n)	PL % (n)
Control	40.0 (247/624) <sup>a</sup>	37.0 (230/624) <sup>a</sup>	7.0 (17/247) <sup>a</sup>
Treatment	45.8 (312/687) <sup>b</sup>	43.0 (299/687) <sup>b</sup>	4.0 (13/312) <sup>b</sup>
P value	0.03	0.01	0.09

There was no effect of the recipient category on P/FTET 30 and P/FTET 60; however, heifers showed higher PL ( $P = 0.001$ ) than dry cows and lactating cows (Table 2). The season of FTET affected pregnancy per FTET at 60 days ( $P = 0.024$ ) and tended to affect ( $P = 0.095$ ) PL. During the spring/summer season, PL tended to be greater compared to the autumn/winter. Consequently, pregnancy per ET at 60 (P/ET 60) was higher in the autumn/winter seasons (Table 3).

Table 2 Effect of recipient category on pregnancy at 30 and 60 days and pregnancy loss.

Animal category (n)	P/FTET 30 (%)	P/FTET 60 (%)	PL (%)
Heifer (607)	45.14	41.52	10.42 <sup>a</sup>
Dry cow (537)	40.22	39.11	2.70 <sup>b</sup>
Lactating cow (167)	41.32	40.12	2.47 <sup>b</sup>
P value	0.160	0.544	0.001

<sup>a,b</sup> Values followed by different letters are statistically different ( $P \leq 0.05$ )

Table 3 Effect of season at FTET on pregnancy at 30 and 60 days and pregnancy loss.

Season of FTET (n)	P/FTET 30 (%)	P/FTET 60 (%)	PL (%)
Spring/Summer (775)	40.77	37.10 <sup>a</sup>	7.71 <sup>a</sup>
Autumn/Winter (536)	45.34	44.03 <sup>b</sup>	4.43 <sup>b</sup>
P value	0.101	0.024	0.095

4. Discussion

One of the aims of this study was to evaluate whether treatment with gonadorelin at the time of ET would improve pregnancy per FTET at 30 and 60 days or not. The results showed

that the groups treated with gonadorelin at the time of FTET had increased pregnancy outcomes 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.<sup>(11)</sup>, in which animals treated with GnRH had a higher P/ET compared to the control group. Vasconcelos et al.<sup>(12)</sup> also found a higher P/ET 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 in improving pregnancy outcomes in ET programmes.

According to Guerra et al.<sup>(18)</sup>, treatment with gonadorelin, causing the dominant follicle of the first follicular wave to ovulate and form an accessory CL, can improve recipient P/ET. This treatment causes an increase in P4 concentration<sup>(19)</sup>, which is of fundamental importance for the development of the conceptus and maintenance of pregnancy. Additionally, the high concentration of P4 provides a more favourable uterine environment for the elongation of the conceptus, which positively influences the production of interferon *tau* and the maternal recognition of pregnancy<sup>(20)</sup>.

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 oestradiol and the development of the luteolytic trigger at this critical period of pregnancy<sup>(11)</sup>. A study that characterized follicle and luteal dynamics and luteolysis in non-inseminated lactating Holstein cows that form accessory CL due to treatment with hCG after ovulation indicated that forming accessory CL with the use of hCG disrupted natural luteolysis and ovulation subsequent to the treatment. These are critical physiological events that regulate oestrous cycle length, but a possible delay in luteolysis could be beneficial to the pregnancy outcomes after ET<sup>(21)</sup>.

In contrast, some studies have shown that the use of drugs that promote the formation of an accessory CL does not improve pregnancy in recipients<sup>(13)</sup>. Guerra et al.<sup>(18)</sup> did not find any improvement in the conception rate of animals treated with GnRH. According to the authors, this may have occurred because both groups, treated and control, had high fertility and low PL. Therefore, GnRH treatment 5-7 days after ovulation may not be efficient in herds with good fertility results.

Loiola et al.<sup>(13)</sup> reported a lower pregnancy rate for a group treated with GnRH. However, the protocol they used included administration of equine chorionic gonadotrophin (eCG). According to Baruselli et al.<sup>(22)</sup>, the protocol used for recipient synchronisation 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 for P4 production, leading to higher pregnancy rates. The use of eCG can also increase the double ovulation rate when administered during the predominant phase of the follicular wave, increasing the P4 concentrations after ovulation. Since the use of eCG already increases the P4 concentrations, treatment with GnRH after ovulation may not influence P/ET rates in this case. This may explain the results found by Loiola et al.<sup>(13)</sup>, which diverge from those of the present study.



The treatment with gonadorelin tended to reduce the PL rate ( $P = 0.095$ ; Table 1). Some studies that administrated different luteotropic drugs found similar results to the present study<sup>(23)</sup>. Niles *et al.*<sup>(23)</sup> observed a reduction in PL between the first and second pregnancy diagnoses (days 32 to 67) in heifers treated with hCG on the day of TFET (seven days after ovulation). Guerra *et al.*<sup>(18)</sup>, studying the administration of GnRH on day 5 of the oestrous cycle of Holstein and crossbred heifers, reported an effect of embryo quality on the rate of PL. Animals that received embryos in development stage 7 (expanded blastocyst) had a lower percentage of pregnancy losses than the other recipients. In addition, the effect of the presence of an accessory CL in these animals potentiated the reduction of PL. This reduction in PL reached up to 50%. According to the authors, the embryo stage and the presence of an accessory CL (which is responsible for higher P4 concentrations) are factors that possibly contributed to the decrease in PL.

An effect of the recipient categories (heifers, dry cows and lactating cows) on pregnancy per ET at 30 days and 60 days was not detected; 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 pregnancy outcomes and fewer pregnancy losses<sup>(24)</sup>. However, the PL rate for heifers in this study met the values found in other studies<sup>(25)</sup>. Thus, this difference may be due to 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<sup>(26)</sup>. Since the recipient cows used in the present study were 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.

A study that used hCG as an ovulation inducer revealed a higher concentration of progesterone on day 12 after AI, and, regardless of parity, pregnancy was improved only in the primiparous cows, compared to the multiparous cow<sup>(27)</sup>. In contrast with the present results, Silva *et al.* (2023)<sup>(28)</sup> observed that a single dose of GnRH administration was able to induce ovulation in *Bos indicus* heifers, but not in cows. According to the authors, a possible reason for this finding is that ovulation in heifers may require a lower LH peak compared to cows, leading to a possible parity effect at the follicular level impacting the ovulatory response.

Season of the year was found to affect ( $P = 0.024$ ) pregnancy per ET at 60 days. Recipients that underwent FTET in the autumn/winter showed better reproductive performance compared to animals in which FTET was performed in the spring/summer (Table 3). Demétrio *et al.*<sup>(29)</sup> reported that an increase in the body temperature of animals on the seventh day after ovulation has a negative effect on pregnancy at 28 days in animals subjected to ET. Similarly, Ferraz *et al.*<sup>(30)</sup> evaluated the relationship between the temperature-humidity index (THI) and the conception rate and 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 the THI compared with heifers. According to Sartori *et al.*<sup>(31)</sup>, compared to heifers, cows have greater difficulty regulating their body temperature in response to warm weather. This greater susceptibility to heat stress contributes to lower conception rates during the warm seasons.

In the present study, the season of the year in which FTET was performed tended to affect PL ( $P = 0.095$ ). During the warmer seasons (spring/summer), PL was greater than in the colder seasons (autumn/winter) (Table 3). Garcia-Ispuerto *et al.*<sup>(32)</sup> also evaluated the influence of heat stress on PL. They also observed a greater PL rate during the warmer months when compared to the colder periods. According to the authors, a high THI during the fourth week of gestation can negatively impact pregnancy rate and contribute to PL due to the embryo's sensitivity to heat stress during the implantation period. Nanas *et al.* (2021)<sup>(33)</sup> analysed artificially inseminated cows and found a higher pregnancy rate in winter compared to summer. They also found greater PL in the summer. According to them, these results may be mainly due to luteal insufficiency. Based on this, we can infer that the treatment with GnRH after ET was not sufficient to prevent PL during the summer.

## 5. Conclusion

The treatment with gonadorelin at the time of bovine embryo transfer increased pregnancy per embryo transfer at days 30 and 60 and reduced pregnancy losses. Additionally, dry and lactating recipient cows showed a lower PL rate compared to heifers. Furthermore, embryo transfer performed in the warmer seasons of the year (spring/summer) resulted in a lower pregnancy rate at day 60 and a higher pregnancy loss rate.

## Conflict of interest

There is no conflict of interest between the authors.

## Author contributions

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*Software:* Not applicable.

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