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Induction of twin pregnancy: a tool for sustainability in beef cattle

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ABSTRACT

Beef cattle stand out in the national agribusiness sector; however the forms of production must seek to be economically and environmentally sustainable. In this area, the use of reproduction biotechnologies, such as the induction of twin pregnancies which can increase meat production per area, is necessary. Thus, the objective of this study was to evaluate this technique of inducing twin pregnancy in cattle through double embryo transfer. The study was conducted at Unicesumar Farm and 100 bovine recipients, heifers and cows, mixed breed, were used, divided into groups: G1 – implanted females with 1 embryo and G2 – implanted females with 2 embryos. Recipients were synchronized and, on D17, implanted with 1 or 2 Wagyu embryos produced in vitro, according to the experimental group. Pregnancy rate at 30 and 60 days, parturition rate, occurrence of retained placenta and dystocia, and calf weight both at birth and at 30 days of age were evaluated. The double transfer of embryos improved the pregnancy rate at 30 and 60 days of gestation ($p<0.05$). Heifers that received two embryos stood out in the pregnancy rate at 60 days and in the parturition rate ($p<0.05$). Calves from recipients that received two embryos were born lighter ($p<0.05$). The induction of twin pregnancy in cattle through double embryo transfer is promising and can promote meat production and contribute to the sustainability of beef cattle.

KEYWORDS: Beef cattle. Double birth. Embryo transfer.

1 INTRODUCTION

The global consumption of animal protein has increased significantly, making it necessary to increase meat production. This scenario is favorable for Brazil, especially in beef production, since the country has the largest commercial herd of beef cattle in the world and is the world's leading exporter of fresh beef (FARMNEWS, 2023).

However, improvements in the beef cattle production chain, based on the use of new techniques and management, are constantly needed. Among these techniques, the induction of twin or multiple pregnancies can be an important tool in the search for better production rates and sustainability of this chain (CAVALIERI *et al.*., 2018).

Induction can be based on the use of different reproductive biotechniques, such as stimulation of ovulation of ovarian follicles through the use of hormones; simultaneous use of Fixed-Time Artificial Insemination (FTAI) and Embryo Transfer (ET) (CAVALIERI *et al.*, 2018); and double embryo transfer. These techniques may or may not be associated with other strategies, such as changes in nutritional management.

Twin pregnancy in cattle is a rare condition, ranging from 3 to 5% (FACIOLI *et al.*, 2020). The occurrence of twin pregnancy in dairy cattle, natural or induced, can compromise cow performance and herd productivity indices (LOPEZ GATIUS *et al.*, 2017).

But unlike dairy cattle, the induction of twin pregnancy in beef cows can be a viable alternative as it shows better results from the use of reproduction biotechniques, being a viable option in promoting the biological and economic efficiency of beef herds and increasing the meat production potential by 20 to 25% (QUARESMA *et al.,* 2004).

This incremental increase may be related to the fact that double pregnancies improve maternal recognition of the pregnancy due to the greater production of interferon-tau (ITF-τ), a protein produced by the conceptus that, among its various functions, inhibits the expression of receptor of estrogen and oxytocin in the endometrium, preventing the release of prostaglandin F2α, the hormone responsible for luteolysis (ANTONIAZZI *et al.*, 2011).

Cavalieri et al. (2018) reported that the production of twins is a quick option to

ISSN 1980-0827 – Volume 19, Number 4, Year 2023

increase the biological and economic efficiency of bovine females from beef herds, without the need to increase the number of matrices in the herd and the area destined for production.

However, the practice of inducing twin pregnancies in beef cattle is controversial, as twinning can increase cases of retained placenta, the occurrence of freemartinism, dystocia deliveries (ANDOLFATO; DELFIOL, 2014), and delays the resumption of ovarian activity in postpartum cows (QUARESMA *et al.,* 2004). In addition, Hossein-Zadeh and Ardalan (2011) also stated that calves originating from twin births were lighter at calving and weaning, when compared to those originating from single births.

Cavalieri *et al.* (2018) conducted a study on induction of twin pregnancies in Nellore cows and did not observe problems at the time of calving, but reported that the double births increased the rejection rate by the cows of the calves and reduced the performance of the calves.

Thus, considering the current need to increase the production of proteins of animal origin such as beef, supported by the fact that the Wagyu breed has adapted in several regions of Brazil and has great economic importance due to the quality of its meat (GOTOH *et al.*, 2018; SILVA; SANTOS; MAFEI, 2020), and additionally the fact that Wagyu calves have low birth weights which favors twin pregnancy, studies on the induction of twin pregnancy of Wagyu calves are promising.

Thus, the objective of this study was to evaluate the use of the twin pregnancy induction technique through the double transfer of Wagyu bovine embryos in mixed breed recipients.

2 MATERIALS AND METHODS

The experiment was carried out at the Cesumar University School Farm/ UNICESUMAR, Maringá, Paraná, Brazil (23°25'S, 51°57'W and altitude of 550 meters), from 12/01/2021 to 11/30/2022. The biotechniques used in this research were approved by the Ethics Committee on the Use of Animals of the Cesumar University/ UNICESUMAR, under protocol No. 09/2021.

This study used 100 recipients, both heifers and cows, mixed breed, with heifers aged between 16 to 18 months and average live weight of 350 kg, and cows aged between 48 to 60 months and average live weight of 400 kg.

The animals were kept in brachiaria (*Brachiaria brizantha* cv MG-5) paddocks with mineral supplementation, water ad libitum, and submitted to the hygienic management adopted by the property.

The 100 recipients were distributed into 2 groups: G1 - recipients submitted to ET with only 1 embryo; and G2 - recipients submitted to ET with 2 embryos. For implantation, all recipients were synchronized using the routine protocol of Cesumar University School Farm (COLOMBO et al., 2017), and on day 17 (D17) the recipients were taken to the containment trunk to undergo transfer of embryos, either receiving one or two embryos according to the experimental group.

The embryos used for implantation were produced in vitro from oocytes from donor Wagyu cows, produced at the Biotechnology Laboratory - BIOTEC/ Unicesumar, following the

ISSN 1980-0827 – Volume 19, Number 4, Year 2023

laboratory's standard technical procedure (ZAMAI et al., 2021). Implantation was performed in the uterine horn ipsilateral to the ovary containing the corpus luteum (CL).

Pregnancy rates at 30 and 60 days were evaluated by ultrasonography (ALOKA SSD 500TM ultrasound device), as well as parturition rate, occurrence of abortions, stillbirths, retained placenta and dystocia, live weight of calves at birth and at 30 days of age, and the mortality rate.

In cases where twins were born, where the calf had very low birth weight or was rejected by the mother, the calf was removed from the paddock and transferred to calf-housing, being suckled with bovine milk. All calves were weighed within the first 24 hours of life and again at 30 days of age.

For the statistical analysis, the variables pregnancy rate and parturition rate were analyzed using the PROC GENMOD procedure of the SAS statistical program (2000), version 8.01, using binomial distribution and an identity linkage function. The variables weight at birth and weight at 30 days were analyzed using the PROC GLM procedure of the SAS statistical program (2000), version 8.01. Means were analyzed using the Least Squares Means (LSM) method.

2 RESULTS AND DISCUSSION

There was no interaction ($p>0.05$) between group and category (heifer and cow) for pregnancy rate at 30 days. The animals that received two embryos had a higher pregnancy rate at 30 and 60 days ($p<0.05$) (Table 1).

Regardless of the group or category, it was observed that the pregnancy rate values at 30 days for Wagyu breed embryo recipients were higher than those reported in the literature. With the aim of evaluating whether synchronization using estradiol benzoate in the presence of the corpus luteum can improve the efficiency of PIVE in Wagyu oocyte donors, Zamai *et al.* (2021) transferred Wagyu breed embryos into mixed-breed recipients and reported a pregnancy rate of 32.26% at gestation for the animals in the control group, a value lower than those found in this study.

Table 1 - Pregnancy rate at 30 (PR30) and 60 days (PR60) of mixed breed heifer and cow recipients, implanted with one (Group 1) or two embryos (Group 2).

Variable	Group 1		Group 2		p valor
PR30 (%)	46.00		70.00		0.0145
PR60 (%)	30.00		60.00		0.0024
	Heifer	Cow	Heifer	Cow	
PR30 (%)	53.33	42.82	69.70	70.59	0.1955
PR60 (%)	40.00	25.71	60.61	58.82	0.0760

Pregnancy rate at 60 days showed a significant interaction ($p<0.05$) between groups and categories (Table 2). In general, females that received two embryos had a higher pregnancy rate at 60 days, however the analysis revealed a more expressive increase in the rate among cows that received one and two embryos ($p<0.05$).

Part of these superior results of the pregnancy rates at 30 and 60 days of the animals in group 2 were attributed to the greater production of Interferon-Tau (IFN-τ), since there

ISSN 1980-0827 – Volume 19, Number 4, Year 2023

were two embryos. IFN-τ is a cytokine synthesized by the conceptus, responsible for maternal recognition of pregnancy (FORDE; LONERGAN, 2017).

Table 2 - Interaction of pregnancy rate at 60 days (PR60) of mixed breed heifers and cows recipients, implanted with one (Group 1) or two embryos (Group 2).

Category	Group		p valor
	1	2	
Heifer (%)	40.00	60.61	0.0166
Cow (%)	25.71	58.82	

The gestational period is determined by a high concentration of the hormone progesterone produced by the corpus luteum, which is essential for the maintenance of pregnancy. Among its main functions, endometrial development, maintenance of placental integrity, reduction of myometrial activity, sensitivity to oxytocin and inhibition of prostaglandin stand out (KLEIN, 2021). During pregnancy, the CL needs to remain active, producing high concentrations of P4. Any failure in the production of P4 by the CL leads to impaired embryonic growth and development (MANN; LAMMING, 2001; RIBEIRO *et al.,* 2013).

IFN-τ, a type I interferon secreted exclusively by trophectoderm cells of the ruminant conceptus, is considered the main agent for maternal recognition of pregnancy in ruminants, as it produces an antiluteolytic effect on the CL by inhibiting the expression of oxytocin receptors in uterine epithelial cells, preventing the pulsatile and luteolytic secretion of prostaglandin F2α by the uterine endometrium (ANTONIAZZI *et al.*, 2011; FORDE; LONERGAN, 2017). Thus, it is indicated that this effect was potentiated in the animals of group 2, due to having received two embryos, resulting in better pregnancy rates.

Regarding the pregnancy rate values observed at 30 and 60 days depending on the heifer or cow category, it was inferred that the rates observed in this study for the heifer category, mainly for G2 (60.61%), were similar, compared to that described by Scanavez, Campos and Santos (2013), who evaluated the results of 1,100 embryo transfers of ½ Holstein/Gir and ¾ Holstein/Gir in recipient ½ Nellore/Simmental heifers, and reported a pregnancy rate at 35 days of 57.8%.

However, other works reported much lower values than this study; Borges Filho (2018) evaluated the pregnancy rate in recipient heifers and cows implanted with in vitro produced embryos transferred fresh, vitrified or frozen in a Nellore beef herd. The authors did not find differences in pregnancy rates related to the categories of recipient cows or heifers, with pregnancy rates of 31.06% for recipient cows at 30 and 70 days, and 23.02% and 20.86 % at 30 and 70 days, respectively, for recipient heifers.

It is believed that this superior result in the pregnancy rate, especially for the category of heifers that were implanted with two embryos, is related, in part, to the fact that heifers have a smaller uterine structure when compared to cows. Therefore, the signaling promoted by the embryos via IFN-τ was more effective and translated into better maternal recognition of the pregnancy and consequently, better pregnancy rates.

In addition, it is known that embryonic mortality during the pre-implantation period is a factor that reduces herd fertility and occurs between days 7 to 8 of gestation (THATCHER *et al.*, 2011). This phase of embryonic loss coincides with the inhibitory phase of the effects of

ISSN 1980-0827 – Volume 19, Number 4, Year 2023

IFN-τ (FLINT, 1995; ROBINSON *et al.*, 2006). This suggests that perhaps, a few embryos are not enough to inhibit the release of PGF2α, maintain CL and progesterone production (THATCHER; MEYER; DANET-DESNOYERS, 2006), and as a consequence, the pregnancy is terminated (FLINT, 1995; ROBINSON et al., 2006). Therefore, the quality and especially the ability of the embryo to communicate with the uterine cells is an essential factor for the establishment and continuation of pregnancy (FOULADI-NASHTA *et al.,* 2006) and can be enhanced by the presence of more embryos, justifying the findings of this study.

Monteiro *et al.* (2001) verified the variations that occur in the uterus and uterine tubes of Nellore cows and heifers and pointed out that the uterine horns and uterine tubes of cows have, on average, a length of 26.0 cm and 17.6 cm, respectively, while in heifers the values are 14.7 cm and 15.3 cm respectively, that is, much lower.

The greater increase in pregnancy rates at 60 days among cows implanted with 2 embryos compared to cows implanted with only one embryo is intriguing data; however, it is considered that part of this result is due to the low pregnancy rate observed in the G1 cows, which was 25.71%, a value well below that expected for recipients.

Andrade et al. (2012) reported values of pregnancy rates of cows submitted to ET of 37.6% for Senepol cows and 39.1% for Nellore cows. Jaguszeski *et al.* (2019) showed even higher values, with a 45.15% pregnancy rate for Nellore recipient cows after ET.

The analysis showed that there was a significant interaction ($p<0.05$) between the groups and the category for the delivery rate variable (Table 3). Once again, the results showed that recipients implanted with two embryos had the best parturition rates, especially heifers; however, the increase was greater among cows implanted with 2 embryos compared to cows implanted with only one embryo.

Table 3 - Parturition rate of mixed breed heifer and cow recipients implanted with one (Group 1) or two embryos (Group 2).

Category	Group		p valor
	1 (n:50)	2 (n:50)	
Heifer (%)	40.00	59.35	0.0166
Cow (%)	25.71	55.56	

Regarding the number of calves born, in absolute values, the total number of calves born to the recipients implanted with only one embryo (n: 50) was 15: 6 from heifers and 9 from cows (average 30%). For recipients implanted with two embryos (n: 50), the total was 53 calves: 33 heifers and 20 cows (average 106%).

No interaction was observed between group and category for the variables birth weight and weight at 30 days of age, with or without correction for single or double delivery ($p>0.05$); however, calves from recipients that received two embryos were born lighter ($p<0.05$) (Table 4).

ISSN 1980-0827 – Volume 19, Number 4, Year 2023

Table 4 - Mean live weight at birth (LBW) and live weight at 30 days (LW30) of Wagyu calves, born from mixed breed heifer and cow recipients implanted with one (Group 1) or two embryos (Group 2).

Variável	Group 1		Group 2		p valor
LBW (kg)	35.77		33.10		0.0106
LW30 (kg)	63.02		63.31		0.7094
	Heifer	Cow	Heifer	Cow	
LBW (kg)	37.50	34.60	32.47	34.15	0.2388
LW30 (kg)	64.00	63.71	62.77	63.50	0.6593

In fact, an important aspect of twin pregnancy is the birth of lighter products. Hossein - Zadeh and Ardalan (2011) stated that calves originating from a double pregnancy are lighter at birth and weaning when compared to those born from a single pregnancy.

Cavalieri *et al*. (2018) conducted a study on induction of twin pregnancy in Nellore cows and did not observe problems at the time of parturition, but reported that the double birth reduced the weight and performance of the calves, a fact also observed in this research.

Regardless of the evaluated groups and categories, the calf weights at birth and at 30 days of age observed in this study were as expected for the breed. According to the Brazilian Association of Wagyu Bovine Breeders (ABCBRW, 2023), the average birth weight of Wagyu calves is 34.45 kg: for males the weight is 39.0 kg, with a standard minimum of 32.9 kg and maximum of 45.2 kg; for females, the standard weight at birth is 29.9 kg, with a minimum standard of 23.9 kg and a maximum of 35.9 kg. With regard to weight at 30 days of age, the Association considers an average live weight of 51.1 kg, in which the average weight of males is 56.6 kg, with a minimum of 47.6 kg and a maximum of 65.5 kg and the average weight of females is 45.6 kg, with a minimum of 36.4 kg and a maximum of 54.7 kg.

Despite the significant difference between the groups for the birth weight variable, we recalled that among the animals in group 2, there were single and double births, therefore, the average live weight at birth for calves in this group was not so low. Thus, we report that considering only the variable of weight of the calf at birth, born from single births or twin births, the birth of 20 calves from single births was observed to have an average live weight of 37.5 kg, while a total of 48 calves that were born from twin births had an average live weight of 32.83 kg. In any case, the difference existed, but in this case it was more evident.

Based on the same reasoning, the data showed that for the variable of live weight of calves at 30 days of age, a total of 13 calves born from single births had an average live weight of 64 kg, while 36 calves born from twin births had an average weight of 62.97 kg, showing that, in fact, the weights at 30 days were similar.

The mortality rate from birth to 30 days was similar between the evaluated groups ($p>0.05$), being 35% for calves born from single births and 33.33% for calves born from twin births. The main cause of mortality, in both groups, was the occurrence of diarrhea.

In fact, the neonatal period of calves, that is, up to 30 days of life, is a critical period and presents the challenge of diarrhea; several studies show that the main cause of calf deaths is diarrhea, ultimately causing dehydration, especially in the first months of life, with reports of the disease occurring in 20% to 50% of US herds (WINDEYER *et al.*, 2014).Typically, oral contamination via viral agents is the most common cause, while imported breeds are the most sensitive. Radostits *et al.* (2021) stated that in heifers up to 30 days old, mortality can vary from 3% to 30%, depending on the environment and husbandry management. Láu (2001)

ISSN 1980-0827 – Volume 19, Number 4, Year 2023

described in his study an overall mortality rate of 18% in herds of zebu type cattle (60% Nellore and 10% Gir-Nelore crossbreeds) and cattle from a cross between these two zebu breeds and the Dutch.

There were no cases of retained placenta and dystocia in the recipients who underwent double ET, and in cases of twin births the observed rejection rate was minimal.

Finally, it is inferred that the technique of inducing twin pregnancy can be useful for the beef production chain, however we emphasize that the criteria used in this study must be respected, such as performing the double implantation of embryos in cattle breeds that will result in small calves, as is the case with the Wagyu breed; implantation in recipients of greater weight/size and with a good body score; and, in case of rejection, rearing the rejected calves separately. However, the cost of this process and the value of the animal must be weighed. Due to the economic value of Wagyu calves, this management is justified.

3. CONCLUSION

Based on the results obtained, it is concluded that the use of the technique of inducing twin pregnancy in bovines, through the double transfer of Wagyu breed embryos in recipients of mixed breed, improved the pregnancy rate of the recipients at 30 and 60 days of gestation, and the heifers that received two embryos stood out both in the pregnancy rate at 60 days and in the parturition rate.

The calves from the recipients that received two embryos were born lighter, however at 30 days of age, no differences were observed in terms of live weight, evidencing a compensatory weight gain in the first month of life.

Considering the current need to increase the production of proteins of animal origin, the technique of inducing twin pregnancy in cattle through double TE is promising, and can promote meat production and contribute to the sustainability of beef cattle; however, more studies should be carried out in order to prove the efficiency of this technique.

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