Animal Reproduction Science 265 (2024) 107475

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Factors influencing pregnancy per artificial insemination (AI) and embryonic mortality in Nelore females subjected to timed-AI in Brazil

Lucas Gomes Da Silva [d], Luana Gomes Da Silva [b,c], Luiz Carlos Louzada Ferreira [c], Julia Mascarello [d], Joao Gabriel Nascimento Moraes [e], Matthew C. Lucy [f], Ériklis Nogueira [a,d,*]

[a] Brazilian Agricultural Research Corporation- EMBRAPA Beef Cattle, Av. Rádio Maia, 830, Campo Grande - MS, 79106-550, Brazil
[b] Universidade Federal de Mato Grosso do Sul, Post-Graduation Program of Animal Science, Campo Grande, MS, Brazil
[c] Cia Pecuaria Assessoria, Campo Grande, MS, Brazil
[d] Universidade Federal de Mato Grosso do Sul, Post Graduation Program of Veterinary Medicine- CIVET-UFMS, Campo Grandes, Brazil
[e] Oklahoma State University, Stillwater, OK, USA
[f] University of Missouri, Columbia, MO, USA

ARTICLE INFO

Keywords:
Embryonic loss
Bos indicus
Pregnancy rate

ABSTRACT

A greater understanding of factors influencing fertility is essential to improve pregnancy rates and reduce the occurrence of embryonic mortality in beef herds. The objective of the current study was to evaluate retrospective data of pregnancy per artificial insemination (P/AI) and pregnancy loss in Nelore females subjected to timed-AI (TAI) in Brazil. Data from 40,104 TAI collected from six breeding seasons (2016–2022) were analyzed, and the effects of animal category (e.g., classification based on age and parity), farm, month of parturition, sire, sire breed (Nelore vs Angus), estrus expression at TAI, animal temperament, and body condition scores (BCS) were evaluated. P/AI and pregnancy loss were affected ($P < 0.001$) by animal category. There was also an effect of farm ($P = 0.0013$) on P/AI and pregnancy loss ($P = 0.001$), as P/AI ranged from 49.28% and 55.58% and pregnancy loss from 3.37% to 6.89% across the herds evaluated. Month of parturition also affected ($P < 0.001$) P/AI and was higher for cows that became pregnant at the beginning of the previous breeding season. Calmer animals, presenting lower velocity scores while exiting the chute following TAI, achieved higher P/AI ($P < 0.001$). Lower BCS at TAI was associated ($P < 0.001$) with increased pregnancy loss, and BCS gain following AI was associated ($P < 0.001$) with reduced rates of embryonic mortality. There was a major effect ($P < 0.001$) of sire on P/AI and pregnancy loss, as P/AI ranged from 11% to 79%, and embryonic mortality from 0% to 40% for the bulls used in the study, highlighting the importance of the sire fertility on overall pregnancy success. Results from the current study reinforce the idea that animal age and parity at the beginning of the breeding season, BCS at the onset of estrous synchronization, BCS gain following AI, estrus expression at TAI, sire, and month of parturition are important factors influencing P/AI and rates of embryonic mortality in beef herds.

* Corresponding author at: Brazilian Agricultural Research Corporation- EMBRAPA Beef Cattle, Av. Rádio Maia, 830, Campo Grande - MS, 79106-550, Brazil.

E-mail address: eriklis.nogueira@embrapa.br (É. Nogueira).

https://doi.org/10.1016/j.anireprosci.2024.107475
Received 20 October 2023; Accepted 14 April 2024
Available online 15 April 2024

1. Introduction

The number of cows subjected to timed-artificial insemination (TAI) in Brazil is increasing and has reached approximately 27 million in 2021 (Baruselli et al., 2022). The expected pregnancy rates of herds undergoing TAI is close to 50% in Brazil and is significantly affected by sire, female fertility, herd management, estrus expression at TAI, rates of embryonic loss, and compliance during the implementation of TAI protocols (Nogueira et al., 2019; Reese et al., 2020). The paternal influence on pregnancy success (Ortega et al., 2018; Sanches et al., 2023) and pregnancy loss (Franco et al., 2018) are often overlooked, despite the substantial impact sires can exert on breeding outcomes.

Among factors inherent to the female that influence herd reproductive performance, resumption of postpartum fertility along with body condition scores (BCS) at the onset of estrous synchronization plays a major role in the success rates of TAI programs (Kim et al., 2023; D'occhio et al., 2019). Reduced BCS and inadequate nutrition have been associated with diminished pregnancy rates, increased pregnancy loss, and the development of low-quality embryos (2012; Ciccioli et al., 2003) underscoring the importance of maintaining appropriate BCS levels at calving and ensuring proper postpartum nutrition to optimize fertility outcomes. Animal temperament and behavior, independently of breed (*Bos t. taurus* and *Bos t. indicus*) have also been shown to affect reproductive outcomes in beef cattle (Cooke et al., 2009, Cooke et al., 2011).

While the success rate of fertilization in beef cattle can be as high as 90%, embryonic mortality following conception is especially high during the first month of gestation (Maurer and Chenault, 1983; Santos et al., 2004; Diskin and Kenny, 2016). In the context of embryonic and fetal losses in cattle, early embryonic mortality has been defined as those occurring between conception and day 24 of gestation, late embryonic losses between days 25 and 50, and fetal losses manifesting after day 50 of pregnancy (Santos et al., 2004). Regarding rates of pregnancy loss in beef herds, Reese et al. (2020) reported in a recent meta-analysis that 50% of pregnancy failure occurred prior to day 16, around 15.5% from days 16 and 32, and less than 6% after the first month of gestation. Of note, cow breed, parity, and breeding method were associated with early or late rates of embryonic/fetal losses.

The objective of the current study was to identify factors influencing pregnancy rate and embryonic mortality in Nelore heifers and cows subjected to TAI in central-west Brazil.

2. Material and methods

2.1. Animals

Data was collected from six herds located in the state of Mato Grosso do Sul, Brazil. All farms perform a complete cycle with breeding, rearing, and finishing. All animal procedures used were approved by the Committee of Ethics and Animal Use of Embrapa under the protocol number (Ceua Protocol N° 006/2022).

All animals enrolled in the study were maintained on pasture with *Urochloa humidicola* and *Urochloa brizantha* grass and received mineral supplementation and *ad libitum water.* Vaccination for foot-and-mouth disease, and against respiratory and reproductive diseases were administered before the initiation of TAI protocols.

The experiment included 40,104 Nelore females from six breeding seasons expanding from 2016–2022. Cows and heifers enrolled in the study were categorized based on age and parity at the start of the breeding season into 9 distinct categories (1) precocious heifer, (2) precocious primiparous, (3) precocious secondiparous, (4) precocious multiparous, (5) conventional heifer, (6) conventional primiparous, (7) conventional secondiparous, (8) conventional multiparous, and (9) non-lactating. The description of each category is presented in Table 1.

2.2. Description of the breeding seasons, TAI programs, and body condition scoring

All breeding seasons began in November and ended in February, lasting 120 days. For primiparous, secondiparous, and multiparous categories (precocious and conventional), cows were subjected to TAI protocols after at least 30 days postpartum following the calving season (July-December).

The TAI protocols utilized (Figs. 1, 2, and 3) were based on Meneghetti et al. (2009). For multiparous, primiparous, and secondiparous cows [precocious and conventional] (Fig. 1), on the first day of the protocol (D-0), cows received 2 mg (IM) of estradiol

Table 1
Classification of cows and heifers according to age and parity.

Category	Description
Precocious Heifer	Heifers with breeding starting up at 15 months of age
Precocious Primiparous	Cows from precocious heifers with first calf
Precocious Secondiparous	Cows from precocious heifers after their second calf
Precocious Multiparous	Cows from precocious heifers with more than two offspring
Conventional Heifer	Heifers with breeding starting at 15–26 months of age
Conventional Primiparous	Cows from conventional heifers with first calf
Conventional Secondiparous	Cows from conventional heifers after their second calf
Conventional Multiparous	Cows from conventional heifers with more than two offspring
Non-lactating	Cow without any offspring at the time of TAI

benzoate (Gonadiol®; Zoetis, São Paulo, SP, Brazil) and an intravaginal progesterone insert (CIDR ®, 1.9 g progesterone; Zoetis). On D9, progesterone inserts were removed and injections of 0.6 mg (IM) of estradiol cypionate (ECP®, Zoetis, Brazil), 12.5 mg (IM) of PGF2α (Lutalyse®; Zoetis), and 300 IU (IM) of ECG (Novormon®; Zoetis) were administered. Non-lactating cows received the same protocol, but with no application of ECG at P4 device removal. Furthermore, at the time of intravaginal devices removal on D9, all dams were painted in the sacrum-caudal region with marker sticks (Raidl-Maxi; RAIDEX GmbH, Dettingen/Erms, Germany) to aid in the visual detection of estrus. Cows were inseminated on D11, 48 h after implant removal. At the time of insemination, the occurrence of standing heat was evaluated based on the visual appearance of the estrus patches. Heat was scored as (1) all ink present (no heat); (2) partial removal of the ink (low heat); (3) all ink removed (high-intensity heat). For analysis, scores 2 and 3 were considered evidence of standing heat (Nogueira et al., 2019). Pregnancy diagnoses were conducted on days 30 and 150 following breeding.

For conventional heifers (Fig. 2) cyclicity was induced (prior to the initiation of the 11-day synch program) with an injection of 150 mg (I.M.) of P4 (Sincrogest ®, Ouro Fino, São Paulo, Brazil) on D-24, followed by 1 mg of estradiol cypionate on D-12. The induction protocol was followed by the 11-day synchronization program as described for the multiparous cows, with changes only in the dosage of estradiol benzoate on D0 (1 mg, IM) and eCG (200 IU, IM) on D9.

For precocious heifers (Fig. 3), induction of cyclicity was performed with oral administration of melengestrol acetate (MGA®, Zoetis, Brazil) from D-24 through D-12 at the dosage of 2.28 mg/head/day. On D-10, precocious heifers received 0.6 mg of estradiol cypionate. The induction protocol was followed by the 11-day synchronization program as described for the conventional heifers.

All animals enrolled in the study were scored on D0 by a trained technician for body condition (1 = emaciated and 5 = obese; 0.25-unit increments as described by Ferguson et al., 1994).

Angus (n=35) and Nelore (n=191) sires were used for breeding. All batches of semen selected underwent evaluation for motility and vigor, spermatic pathology, and thermoresistence test. All semen used was required to have a minimum of 30% motility and vigor 3 after thawing, with a minimum of 6 million viable spermatozoa presenting a maximum of 30% of sperm pathologies (Barth and Oko, 1989).

On the day of the AI, the speed which each animal exited the chute was evaluated by a trained technician performing only this role. The observed behavior of each animal was evaluated, and the speed which each animal exited the chute was recorded and classified as (1) walking, (2) trotting, or (3) running (Gomes da Silva et al., 2020).

The first pregnancy diagnosis was performed approximately 30 days after AI by ultrasonography (SD 2200, Mindray, China). Pregnant cows were sorted at this point to facilitate management. Approximately 7 days after insemination, Nelore bulls were turned in, maintaining a bull:cow ratio of 1:40. Bulls were kept with the cows until the end of the breeding season. Cows were re-evaluated by ultrasonography at 120–150 days after the first pregnancy diagnosis, to confirm pregnancy or detect pregnancy loss.

At the end of the breeding season, sires were classified based on average pregnancy rates as low- pregnancy rates equal or less than 39.9; average- pregnancy rates between 40% and 49.9%; high- pregnancy rates between 50% and 55.9%; and top- pregnancy rates equal or above 56% (Sanches et al., 2023).

2.3. *Statistical analysis*

All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc.). The effects of animal category, farm, month of parturition, breed of sire, standing heat at TAI, and chute speed score were evaluated using generalized linear mixed models using the

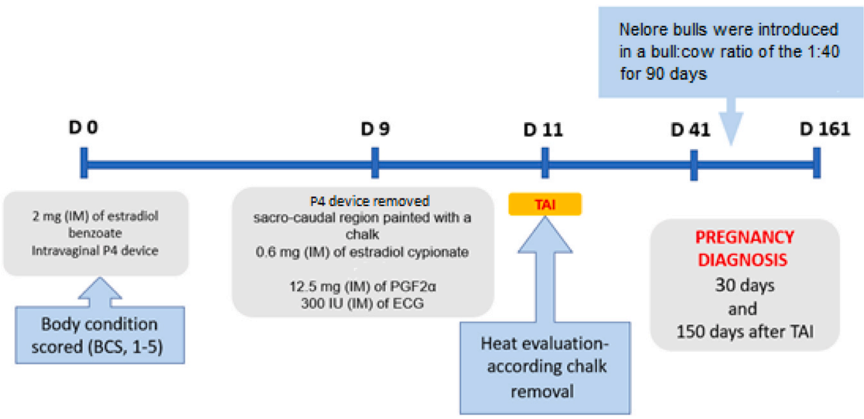

Fig. 1. Artificial insemination protocol used for multiparous cows. On day (D0), multiparous cows received 2 mg (IM) of estradiol benzoate (Gonadiol®; Zoetis, São Paulo, Brazil) and an intravaginal progesterone insert (CIDR ®, 1.9 g progesterone; Zoetis, Brazil). Body condition was scored on D0. On D9, progesterone inserts were removed and injections of 0.6 mg (IM) of estradiol cypionate (ECP®, Zoetis, Brazil), 12.5 mg (IM) of PGF2α (Lutalyse®; Zoetis, Brazil), and 300 IU (IM) of ECG (Novormon®; Zoetis, Brazil) were administered. Furthermore, on D9, cows were painted in the sacrum-caudal region with a marker stick (Raidl-Maxi; RAIDEX GmbH, Dettingen/Erms, Germany) to aid in the visual detection of estrus. On day 11 (D11) multiparous cows were examined for chalk removal (e.g., as an indication of standing estrus) and AI was performed. Pregnancy diagnoses were conducted on days 30 and 150 following breeding.

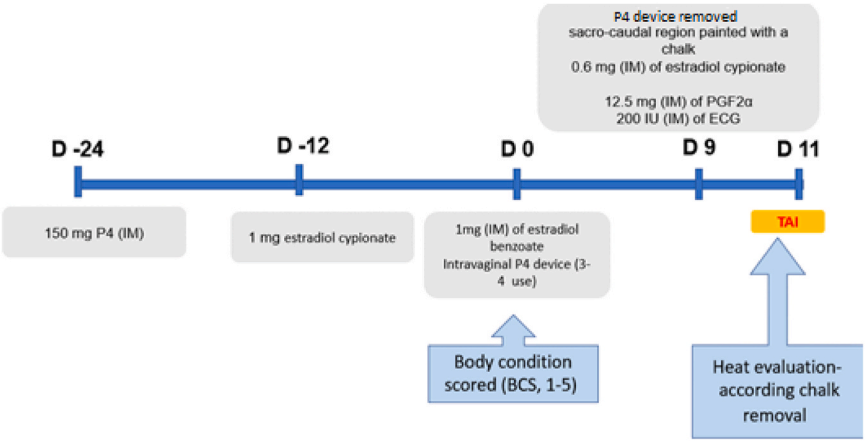

Fig. 2. Artificial insemination (AI) protocol for heifers. Cyclicity was induced with an injection of 150 mg (I.M.) of P4 (Sincrogest ®, Ouro Fino, São Paulo, Brazil.) on D-24, followed by 1 mg of estradiol cypionate on D-12. On day (D0), conventional heifers received 1 mg (IM) of estradiol benzoate (Gonadiol®; Zoetis, Brazil) and an intravaginal progesterone insert (CIDR ®, 1.9 g progesterone; Zoetis, Brazil). Body condition was scored on D0. On D9, progesterone inserts were removed and injections of 0.6 mg (IM) of estradiol cypionate (ECP®, Zoetis, Brazil), 12.5 mg (IM) of PGF2α (Lutalyse®; Zoetis, Brazil), and 200 IU (IM) of ECG (Novormon®; Zoetis, Brazil) were administered. Furthermore, on D9, heifers were painted in the sacrum-caudal region with a marker stick (Raidl-Maxi; RAIDEX GmbH, Dettingen/Erms, Germany) to aid in visual detection of estrus. On day 11 (D11) conventional heifers were examined for chalk removal (e.g., as an indication of standing estrus) and AI was performed. Pregnancy diagnoses were conducted on days 30 and 150 following breeding.

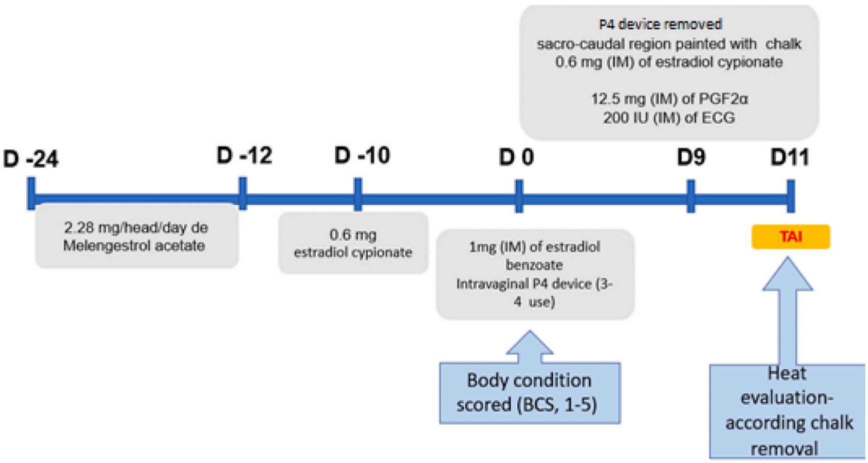

Fig. 3. Artificial insemination (AI) protocol used to synchronize ovulation in precocious heifers. Induction of cyclicity was performed with oral administration of melengestrol acetate (MGA®, Zoetis, Brazil) from D-24 through D-12 at the dosage of 2.28 mg/head/day. On D-10, precocious heifers received 0.6 mg of estradiol cypionate. On day (D0), heifers received 1 mg (IM) of estradiol benzoate (Gonadiol®; Zoetis, Brazil) and an intravaginal progesterone insert (CIDR ®, 1.9 g progesterone; Zoetis). Body condition was scored on D0. On D9, progesterone inserts were removed and injections of 0.6 mg (IM) of estradiol cypionate (ECP®, Zoetis, Brazil), 12.5 mg (IM) of PGF2α (Lutalyse®; Zoetis, Brazil), and 200 IU (IM) of ECG (Novormon®; Zoetis, Brazil) were administered. Furthermore, on D9, heifers were painted in the sacrum-caudal region with a marker stick (Raidl-Maxi; RAIDEX GmbH, Dettingen/Erms, Germany) to aid in the visual detection of estrus. On day 11 (D11) heifers were examined for chalk removal (e.g., as an indication of standing estrus) and AI was performed. Pregnancy diagnoses were conducted on days 30 and 150 following breeding.

GLIMMIX procedure. Means were separated using the LSMEANS PDIFF option with Tukey's adjustment.

The relationship between pregnancy loss, BCS, and BCS gain from the beginning of the TAI protocol to the day of the pregnancy diagnosis was determined using linear regression models, and when significant ($P < 0.05$), a probability graph was generated using the REG procedure of SAS. Statistical significance was defined as $P \leq 0.05$ and statistical tendencies as $0.05 < P < 0.10$.

3. Results

The overall results of P/AI and pregnancy loss are summarized in Tables 2 and 3. Considering all the data collected, the average P/

AI and pregnancy loss were 53.11% and 5.78%, respectively. P/AI was significantly ($P < 0.001$) affected by animal category. Precocious secondiparous (64.65%), precocious multiparous (60.00%), and multiparous (57.09%) had the highest P/AI, whereas precocious heifers (44.50%), precocious primiparous (46.43%) and conventional heifers (47.90%) had the lowest P/AI (Table 2).

Rates of pregnancy loss were also affected by animal category ($P < 0.001$), with higher rates of pregnancy loss detected in precocious primiparous (10.94%), precocious secondiparous (8.13%), and non-lactating cows (7.19%), and the lowest rates of pregnancy loss was detected in precocious multiparous (4.26%), multiparous (4.93%), heifers (5.03%), and secondiparous (6.44%) cows.

There was an effect of farm ($P = 0.0013$) on P/AI. The average pregnancy rates ranged from 49.28% and 55.58% among the herds enrolled in the study. Regarding pregnancy loss, there was also a significant effect ($P = 0.001$) of farm on embryonic mortality, as rates of pregnancy loss varied from 3.37% to 6.89% across the herds evaluated.

Animals with a low chute speed score (e.g., walked out of the chute as opposed to ran) had higher ($P < 0.001$) P/IA (54.42%) than those who trotted (52.19%) or ran (50.32%) out of the chute. However, the speed which animals exited the chute did not influence rates of pregnancy loss ($P = 0.2558$).

There was a significant effect ($P < 0.001$) of month of parturition on P/AI and pregnancy loss. Animals calving in July had the highest P/AI, whereas animals that calved in December (50.50%) or January (50.53%) had the lowest P/AI. Regarding the effect of month of parturition on embryonic mortality, pregnancy loss was highest in animals calving in December (14.29%), and lowest in animals calving in January (2.08%).

There was a significant effect of BCS at the onset of estrous synchronization on P/AI ($P < 0.001$) and pregnancy loss ($P < 0.001$). The probability of embryonic mortality decreased as BCS increased (Fig. 4; $P < 0.05$). Interestingly, BCS gains during the subsequent 30 days following the first measurement were associated with a reduced probability of pregnancy loss (Fig. 5; $P < 0.05$).

There was no association ($P = 0.408$) of sire classification based on fertility (e.g., P/AI at the end of the breeding season: low, average, high, and top) on rates of embryonic mortality (Fig. 6). As expected, P/AI was significantly different ($P > 0.001$) among sires allocated to the distinct fertility classification (Fig. 6).

Regarding the breed of the sire used for AI, Nelore bulls had higher ($P < 0.001$) P/AI than Angus bulls, with a difference of 1.13% between them. However, the breed of the sire did not influence ($P = 0.4089$) the rates of pregnancy loss. Furthermore, there was an effect ($P < 0.001$) of sire on P/AI, as P/AI ranged from 11% to 79% among the bulls used in the study. Similarly, there was an effect of sire on pregnancy loss ($P < 0.001$), as rates of embryonic mortality ranged from 0% to 40% for the bulls used in the study.

4. Discussion

Similar to the current investigation, Sá Filho et al. (2010) examined factors associated with P/AI in beef herds. Interestingly, they observed that heifers, as well as primiparous and non-lactating cows, displayed notably lower pregnancy rates, with only multiparous cows achieving pregnancy rates that exceeded 50%. Our results align with these findings, as heifers and primiparous cows had the lowest P/AI, while cows beyond their second calf exhibited the highest pregnancy rates. Primiparous cows are still growing at time of parturition and thus reproductive efficiency in the subsequent breeding season is often compromised (Vasconcelos et al., 2014). As noted by Pilau and Lobato (2009), primiparous cows typically experience a period of reduced body condition postpartum, which hinders their return to estrus and consequently impairs fertility.

The average pregnancy loss observed in the current study (5.78%) was slightly higher than the average pregnancy loss reported in a recent meta-analysis investigating rates of embryonic mortality in beef cattle (4.9%; Reese et al., 2020). The current study and the aforementioned meta-analysis concurred on the observation that the highest rates of embryonic mortality were identified in heifers

Table 2
Pregnancy per artificial insemination (P/AI) and pregnancy loss according to female category and farm.

	P/AI (%)	Pregnancy loss(%)
Category (n)		
Heifer (7348)	47.90[d]	5.03[c]
Primiparous (8181)	51.41[bc]	7.11[b]
Secondiparous (2914)	56.59[b]	6.44[c]
Multiparous (17,151)	57.09[ab]	4.93[c]
Non-lactating (722)	49.45[c]	7.19[ab]
Precocious heifer (2582)	44.50[e]	7.11[b]
Precocious Primiparous (812)	46.43[d]	10.94[a]
Precocious secondiparous (314)	64.65[a]	8.13[ab]
Precocious Multiparous (80)	60.00[ab]	4.26[c]
P	<0.001	<0.001
Farm (n)		
A (923)	55.58%[a]	4.71%[b]
B (8570)	51.06%[b]	5.77%[b]
C (5313)	55.51%[a]	4.44%[b]
D (416)	49.28%[b]	3.91%[b]
E (3936)	54.17%[a]	3.37%[b]
F (20,946)	53.11%[a]	6.89%[a]
P-value	0.0013	<0.001

Table 3
Pregnancy per artificial insemination (P/AI) and pregnancy loss (%) according to month of parturition, sire breed, expression of estrus, and velocity score for exiting the chute.

	P/AI (%)	Pregnancy loss(%)
Parturition Month (n)		
JULY (165)	68.48%[a]	7.21%[b]
AUGUST (3333)	57.67%[b]	5.90%[d]
SEPTEMBER (5127)	56.33%[b]	5.55%[d]
OCTOBER (5331)	55.00%[c]	6.01%[c]
NOVEMBER (4286)	54.01%[c]	6.80%[c]
DECEMBER (1705)	50.50%[d]	14.29%[a]
JANUARY (190)	50.53%[d]	2.08%[e]
P-value	<0.001	<0.001
Sire breed *(n)*		
ANGUS (9089)	52.24%	5.31%
NELORE (30,988)	53.37%	5.92%
P-value	<0.001	0.6886
HEAT		
0	40.9%[b]	6.7[a]
1	58.5%[a]	5.7[b]
P-value	<0.001	0.030
Chute velocity score *(n)*		
1 (20,367)	54.42%[a]	5.43%
2 (6611)	52.19%[b]	5.33%
3 (3283)	50.32%[b]	4.78%
P-value	<0.001	0.2558

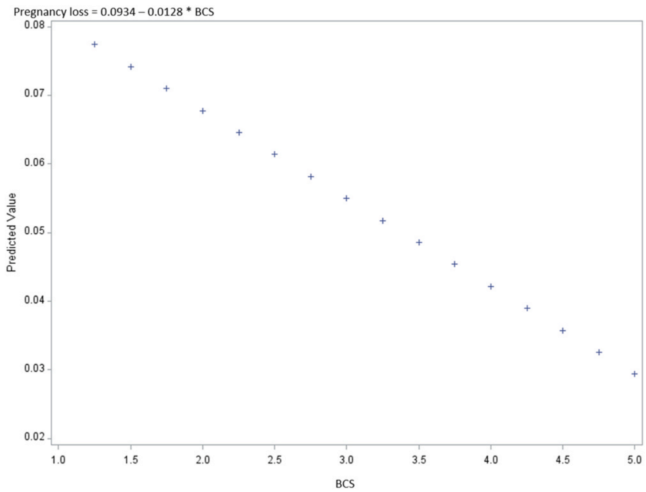

Fig. 4. Association of pregnancy loss and body condition score (BCS) in Nelore females subjected to timed- artificial insemination ($P<0.05$).

(8.1%), followed by primiparous (5.4%) and multiparous (5.1%) cows. Likewise, in a study conducted by Pessoa et al. (2012) with 658 Nelore females, heifers (5.99%) exhibited the highest rates of embryonic mortality. Primiparous cows, however, experienced lower rates (3.30%) of embryonic loss compared to their multiparous counterparts (5.30%).

Regarding the effects of cattle temperament and stress at time of insemination on fertility outcomes, the findings of our study align with those of Cooke et al. (2011) who reported that calm heifers, presenting lower velocity scores while exiting the chute following TAI, achieved higher conception rates. One strategy to reduce stress during handling is the adoption of cattle training, which involves positive reinforcement and gradual habituation of management practices (Rueda et al., 2015). Another effective strategy involves selecting animals with calmer temperaments and implementing farm-personnel training to improve human-animal interactions and reduce overall stress during handling (Hemsworth et al., 2000, Barbosa et al., 2006, Sato, 1981, Fordyce and Goddard, 1984, Boivin et al., 1992).

When considering the influence of the month of parturition on reproductive performance, cows calving in July displayed the highest P/AI, followed by those calving in August and September. Cows calving in July and August are the ones that conceived earliest in the breeding season, and consequently, calved early in the calving season, having a longer interval to rebreeding. This extended

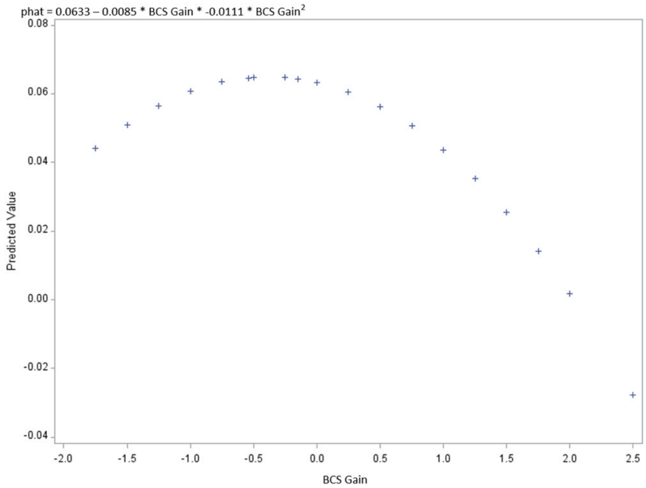

Fig. 5. Pregnancy loss as a function of body condition score (BCS) gain during the period from the initiation of the timed- artificial insemination (TAI) protocol to the day of pregnancy diagnosis conducted 30 days following breeding in Nelore females ($P < 0.05$).

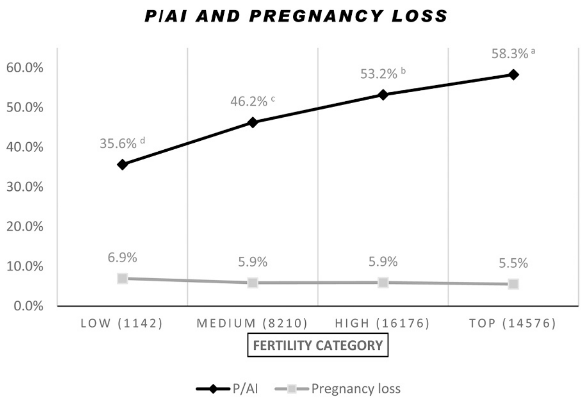

Fig. 6. Pregnancy per artificial insemination (P/AI) and pregnancy loss according to sire fertility category. (P/AI, $P < 0.001$; Pregnancy loss, $P = 0.408$).

postpartum interval is beneficial to reproductive success (Cushman et al., 2013).

The reduced incidence of pregnancy loss in cows calving in January can be attributed to the fact that confirmation of pregnancy following the first pregnancy diagnosis was conducted within a shorter interval compared to other months. The highest rates of pregnancy loss in cows calving in December might be attributed to the higher temperatures observed in the Brazilian mid-west region during the summer months.

In the current study, lower BCS was associated with increased pregnancy loss, and BCS gain after AI was associated with reduced rates of embryonic mortality. The negative effects of low BCS on reproduction are well-established (Meneghetti and Vasconcelos, 2008). Subjecting cows to nutrient restriction immediately following AI, during a period of 6 days, resulted in production of lower-quality embryos with delayed developmental competence (Kruse et al., 2017). Furthermore, superovulation of cows with low BCS resulted in reduced response to the superovulation protocol, reduced size of ovulatory follicles, reduced corpus luteum size and postovulatory progesterone concentrations, and impaired embryo recovery rates and quality (Bridges et al., 2012). Postovulatory progesterone concentrations play a major role regulating conceptus development and uterine receptivity (Stone et al., 1978, Zelinski et al., 1982, Ing and Tornesi, 1997, Bridges et al., 2010, Fields et al., 2012). The effect of BCS on reproduction is especially important for primiparous cows, due to the concomitant demand for growth and reproduction (Derouen et al., 1994, Ciccioli et al., 2003, Richardson et al., 2016).

Expression of estrus on the day of AI was associated with higher P/AI and lower rates of embryonic mortality as previously reported by Nogueira et al. (2019) and Rodrigues et al. (2018). Estrus expression occurs as a result of high circulating concentrations of estradiol produced by the dominant follicle. Preovulatory estradiol concentrations play a major role in pregnancy success, through its effects on the oocyte, follicular cells, oviduct, and uterus for the establishment of uterine receptivity (Pohler et al., 2012, Perry et al., 2007).

There was a major effect of sire on P/AI and pregnancy loss in the current study, as P/AI from individual bulls ranged from 11% to 79% and embryonic mortality from 0% to 40% despite only using semen that had passed a thorough evaluation. This underscores the significance of conducting additional research into the genetic and molecular mechanisms that govern the paternal influence on pregnancy establishment and pregnancy loss. Of note, Ortega et al. (2018) reported significant effects of sire conception rate (SCR) on preimplantation embryo development using semen that had passed all the standard quality control procedures before being tested in the field. Currently, the quality parameters commonly employed in semen evaluation do not offer complete reliability in predicting bull fertility (Capra et al., 2017). The identification of new genetic variants in the bovine paternal genome governing fertility and early embryonic mortality is highly desirable (Butler et al., 2020). Nogueira et al. (2022) identified a deleterious non-synonymous point mutation in the autosomal EML5 gene that when present in homozygosis resulted in prominent abnormal sperm phenotypes. Future studies should focus on identifying new genetic variants to accurately predict sire fertility to improve reproductive efficiency (Taylor et al., 2018). Selection for scrotal circumference has great potential to improve sire fertility, due to its high heritability and positive association with semen quality traits (Carvalho Filho et al., 2020).

In cattle, successful fertilization (or conception) generally occurs following breeding, but embryonic mortality is especially high during the first month of gestation. Fertilization rates have been estimated to be around 90% or higher in heifers, beef cows, and moderate-producing dairy cows (Diskin and Sreenan, 1980, Diskin and Morris, 2008, Maurer and Chenault, 1983, Sreenan and Diskin, 1986, Ahmad et al., 1995, Sartori et al., 2002), ~80% in high-yielding dairy cows under thermoneutral conditions, and ~60% in high-producing dairy cattle under heat stress (Wiltbank et al., 2016, Sartori et al., 2010, Ribeiro et al., 2016, Santos et al., 2004). Conception rates, however, range from 35% to 40% in dairy (Lucy, 2001, Walsh et al., 2011, Norman et al., 2020) and 50–70% in beef cattle (Perry et al.,2011). Thus, under thermoneutral conditions, there is a high rate of embryonic death following fertilization, which is especially high during the first two weeks of gestation in both beef (Ealy, 2020) and dairy cattle (Wiltbank et al., 2016).

Important biological events take place during the first couple weeks of preimplantation development, such as the formation of a blastocyst from a one-cell zygote, and the process of conceptus elongation, marked by trophectoderm cell proliferation and an exponential increase in conceptus length. Because embryonic mortality is a major factor limiting reproductive efficiency in cattle operations (Ealy, 2020), future efforts should focus on understanding the paternal and maternal influence on embryonic mortality particularly during the first two weeks of gestation.

5. Conclusion

Results from the current study reinforce the idea that animal age and parity at the beginning of the breeding season, BCS at the onset of estrous synchronization, BCS gain following AI, estrus expression at TAI, sire, and month of parturition are important factors influencing P/AI and rates of embryonic mortality in beef herds. Notably, there was a wide variation in P/AI and pregnancy loss among sires used in the study, reinforcing the importance of sire fertility in the success of breeding programs. The observed major effect of sire on fertility outcomes highlights the importance of further understanding the molecular basis orchestrating the paternal effects on pregnancy success and embryonic mortality. Collectively, this work provides foundational knowledge for future studies aiming to identify factors influencing fertility and pregnancy loss in beef herds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by EMBRAPA and National Council for Scientific and Technological Development - CNPq - Brazil (Grant #421687/2021-5), Post-Graduation Program of Veterinary Sciences at Federal University of Mato Grosso do Sul (UFMS), Brazil. The authors express their gratitude to Rafael Batista Trannin, and farms - Seriema, Agropecuaria 3B, and Santa Anna for their generous collaboration and invaluable assistance during the data collection process.

References

Ahmad, N., Schrick, F.N., Butcher, R.L., Inskeep, E.K., 1995. Effect of persistent follicles on early embryonic losses in beef cows. Biol. Reprod. 52, 1129–1135. https://doi.org/10.1095/biolreprod52.5.1129.

Barbosa, R.T., Silva, J.D., Bergamaschi, M.A.C.M., Bertan, C.M., Sarti, L.L., Binelli, M., 2006. A Redução da Mortalidade Embrionária - Estratégia Hormonal para Otimizar a Função Luteínica em Bovinos. Doc. Embrapa 51, 1–11.

Barth, A.D., Oko, R.J., 1989. Abnormal Morphology of Bovine Spermatozoa. Iowa State University Press.

Baruselli, P.S., Santos, G.F.F.D., Crepaldi, G.A., Catussi, B.L.C., Oliveira, A.C.D.S., 2022. FTAI in numbers: evolution and future projection. Rev. Bras. Rep. Anim. 46, 76–83.

Boivin, X., Le Neindre, P., Chupin, J.M., 1992. Establishment of cattle-human relationship. Appl. Ani. Beh. Sci. 32, 325–335.

Bridges, G.A., Mussard, M.L., Burke, C.R., Day, M.L., 2010. Influence of the length of proestrus on fertility and endocrine function in female cattle. Anim. Rep. Sci. 117, 208–215.

Bridges, G., Kruse, S., Funnell, B., Perry, G., Gunn, P., Arias, R., Lake, S., 2012. Changes in body condition on oocyte quality and embryo survival. Proc. Appl. Reprod. Strat. Beef Cattle 23, 269–283.

Butler, M.L., Bormann, J.M., Weaber, R.L., Grieger, D.M., Rolf, M.M., 2020. Selection for bull fertility: a review. Trans. Ani. Sci. 4, 423–441.

Capra, E., Turri, F., Lazzari, B., Cremonesi, P., Gliozzi, T.M., Fojadelli, I., Pizzi, F., 2017. Small RNA sequencing of cryopreserved semen from single bull revealed altered miRNAs and piRNAs expression between High-and Low-motile sperm populations. BMC Genom. 18, 1–12.

Carvalho Filho, I., Marques, D.B.D., De Campos, C.F., Guimarães, J.D., Guimarães, S.E.F., Lopes, P.S., Veroneze, R., 2020. Genetic parameters for fertility traits in Nelore bulls. Rep. Dom. Ani. 55, 38–43.

Ciccioli, N., Wettemann, R., Spicer, L., Lents, C., White, F., Keisler, D., 2003. Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. J. Ani. Sci. 81, 3107–3120.

Cooke, R.F., Arthington, J.D., Austin, B.R., Yelich, J.V., 2009. Effects of acclimation to handling on performance, reproductive, and physiological responses of Brahman-crossbred heifers. J. Anim. Sci. 87, 3403–3412.

Cooke, R.F., Bohnert, D.W., Meneghetti, M., Losi, T.C., Vasconcelos, J.L.M., 2011. Effects of temperament on pregnancy rates to fixed-timed AI in Bos indicus beef cows. Liv. Sci. 142, 108–113.

Cushman, R.A., Kill, L.K., Funston, R.N., Mousel, E.M., Perry, G.A., 2013. Heifer calving date positively influences calf weaning weights through six parturitions. J. Ani. Sci. 91 (9), 4486–4491.

Derouen, S.M., Franke, D., Morrison, D.G., Wyatt, W.E., Coombs, D.F., White, T.W., Humes, P.E, Greene, B, B., 1994. Prepartum body condition and weight influences on reproductive performance of first-calf beef cows. J. Ani. Sci. 72, 1119–1125.

Diskin, M.G., Morris, D.G., 2008. Embryonic and early foetal losses in cattle and other ruminants. Reprod. Domest. Anim. 43 (Suppl 2), 260–267. https://doi.org/10.1111/j.1439-0531.2008.01171.x.

Diskin, M.G., Sreenan, J.M., 1980. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. J. Reprod. Fertil. 59, 463–468. https://doi.org/10.1530/jrf.0.0590463.

Diskin, M.G., Kenny, D.A., 2016. Managing the reproductive performance of beef cows. Theriogenology 86 (1), 379–387.

D'occhio, M.J., Baruselli, P.S., Campanile, G., 2019. Influence of nutrition, body condition, and metabolic status on reproduction in female beef cattle: A review. Theriogenology 125, 277–284.

Ealy, A.D., 2020. Pregnancy Losses in Livestock: An Overview of the Physiology and Endocrinology Symposium for the 2020 ASAS-CSAS-WSASAS Virtual Meeting. J. Anim. Sci. 98, skaa277. HTTPS://DOI.ORG/10.1093/JAS/SKAA277.

Ferguson, J.D., Byers, D., Ferry, J., 1994. Round table discussion: body condition of lactating cows. Agric. Pract. 15, 7–21.

Fields, S.D., Gebhart, K.L., Perry, B.L., Gonda, M.G., Wright, C.L., Bott, R.C., Perry, G.A., 2012. Effects of Initiation of standing estrus prior to an injection of GnRH on LH release, subsequent concentrations of progesterone, and steroidogenic enzyme expression. Dom. Ani. End. 42, 11–19.

Fordyce, G., Goddard, M.E., 1984. Maternal Influence on the temperament of Bosindicus cross cows. Proc. Aust. Soc. Ani. Prod. 15, 345–348.

Franco, G.A., Peres, R.F.G., Martins, C.F.G., Reese, S.T., Vasconcelos, J.L.M., Pohler, K.G., 2018. Sire contribution to pregnancy loss and pregnancy-associated glycoprotein production in Nelore cows. J. Anim. Sci. 96, 632–640. https://doi.org/10.1093/jas/sky015.

Gomes Da Silva, L., Gomes Da Silva, L., Pereira, J.L.G., Ferreira, L.C.L., Mateus, R.G., Nogueira, E., 2020. Influence of chute exit speed score under the pregnancy rate in Nelore dams submitted to timed artificial insemination. Proc.: 34th Annu. Meet. Braz. embryo Technol. Soc. (sbte). Anim. Reprod. 17.

Hemsworth, P.H., Coleman, G.J., Barnett, J.L., Borg, S., 2000. Relationships between human-animal interactions and productivity of commercial dairy cows. J. Ani. Sci. 78, 2821–2831.

Ing, N.H., Tornesi, M.B., 1997. Estradiol up-regulates estrogen receptor and progesterone receptor gene expression in specific ovine uterine cells. Bio. Repr. 56, 1205–1215.

Kim, I.H., Jeong, J.K., Kim, U.H., 2023. Impact of postpartum time period associated body condition score on reproductive performance in dairy cows. Theriogenology 209, 107–114.

Kruse, S., Bridges, G., Funnell, B., Bird, S., Lake, S., Arias, R., Amundson, O., Larimore, E., Keisler, D., Perry, G., 2017. Influence of post-insemination nutrition on embryonic development in beef heifers. Theriogenology 90, 185–190.

Lucy, M.C., 2001. Reproductive loss in high-producing dairy cattle: where will it end? J. Dairy Sci. 84, 1277–1293. https://doi.org/10.3168/jds.S0022-0302(01)70158-0.

Maurer, R.R., Chenault, J.R., 1983. Fertilization failure and embryonic mortality in parous and nonparous beef cattle. J. Anim. Sci. 56, 1186–1189. https://doi.org/10.2527/jas1983.5651186x.

Meneghetti, M., Sá Filho, O.G., Peres, R.F.G., Lamb, G.C., Vasconcelos, J.L.M., 2009. Fixed-time artificial insemination with estradiol and progesterone for *Bos indicus* cows I: basis for development of protocols. Theriogenology 72, 179–189.

Meneghetti, M., Vasconcelos, J.L.M., 2008. Calving date, body condition score, and response to a timed artificial insemination protocol in first-calving beef cows. Arq. Bras. Med. Vet. Zoot. 60, 786–793.

Nogueira, E., Silva, M.R., Silva, J.C.B., Abreu, U.P.G., Anache, N.A., Silva, K.C., Rodrigues, W.B., 2019. Timed artificial insemination plus heat I: effect of estrus expression scores on pregnancy of cows subjected to progesterone–estradiol-based protocols. Animal 13, 2305–2312.

Nogueira, E., Tirpák, F., Hamilton, L.E., Zigo, M., Kerns, K., Sutovsky, M., Sutovsky, P., 2022. A Non-Synonymous Point Mutation in a WD-40 Domain Repeat of EML5 Leads to Decreased Bovine Sperm Quality and Fertility. Fro. Cell Dev. Bio. 10 article872740.

Norman, H.D., Guinan, F.L., Megonigal, J.H., Dürr, J.W., 2020. Reproductive Status of DHI Cows n.d. ⟨https://queries.uscdcb.com/publish/dhi/current/reproall.html⟩. (accessed August 8, 2023).

Ortega, M.S., Moraes, J.G., Patterson, D.J., Smith, M.F., Behura, S.K., Poock, S., Spencer, T.E., 2018. Influences of sire conception rate on pregnancy establishment in dairy cattle. Bio. Repr. 99, 1244–1254.

Perry, G., Smith, M., Roberts, A., Macneil, M., Geary, T., 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. J. Ani. Sci. 85, 684–689.

Perry, G., Dalton, J., Geary, T., 2011. Management factors influencing fertility in beef cattle breeding programmes. Boise: Proceedings, Applied Reproductive Strategies in Beef Cattle-Northwest.

Pessoa, J.D.S., Rolin Filho, S.T., Ribeiro, H.F.L., Garcia, O.S., Nunes, K.D.B., Amorim, B.S.D., 2012. Embryonic loss in cows submitted to timed AI in the northeast of Para. Cie. Anim. 22, 239–241.

Pilau, A., Lobato, J.F.P., 2009. Reproductive performance of primiparous cows at 22/24 months of age. Rev. Bras. Zoo. 38, 728–736.

Pohler, K.G., Geary, T.W., Atkins, J.A., Perry, G.A., Jinks, E.M., Smith, M.F., 2012. Follicular determinants of pregnancy establishment and maintenance. Cell Tissue Res. 349, 649–664.

Reese, S.T., Franco, G.A., Poole, R.K., Hood, R., Montero, L.F., Oliveira Filho, R.V., Cooke, R.F., Pohler, K.G., 2020. Pregnancy loss in beef cattle: a meta-analysis. Anim. Rep. Sci. 2012, Article 106251.

Ribeiro, E.S., Gomes, G., Greco, L.F., Cerri, R.L.A., Vieira-Neto, A., Monteiro, P.L.J., et al., 2016. Carryover effect of postpartum inflammatory diseases on developmental biology and fertility in lactating dairy cows. J. Dairy Sci. 99, 2201–2220. https://doi.org/10.3168/jds.2015-10337.

Richardson, B.N., Hill, S.L., Stevenson, J.S., Djira, G.D., Perry, G.A., 2016. Expression of estrus before fixed-time AI affects conception rates and factors that impact expression of estrus and the repeatability of expression of estrus in sequential breeding seasons. Anim. Rep. Sci. 166, 133–140.

Rodrigues, A., Cooke, R., Cipriano, R., Silva, L., Cerri, R., Cruppe, L., Meneghetti, M., Pohler, K., Vasconcelos, J., 2018. Impacts of estrus expression and intensity during a timed-AI protocol on variables associated with fertility and pregnancy success in Bos indicus-influenced beef cows. J. Ani. Sci. 96, 236–249.

Rueda, P.M., Sant'anna, A.C., Valente, T.S., Da Costa, M.J.P., 2015. Impact of the temperament of Nelore cows on the quality of handling and pregnancy rates in fixed-time artificial insemination. Liv. Sci. 177, 189–195.

Sá Filho, O., Dias, C., Lamb, G., Vasconcelos, J., 2010. Progesterone-based estrous synchronization protocols in non-suckled and suckled primiparous Bos indicus beef cows. Anim. Rep. Sci. 119, 9–16.

Sanches, C., Costa, E., Silva, E.V., Dode, M.A.N., Cunha, A.T.M., Garcia, W.R., Sampaio, B.F.B., Silva, J.C.B., Vaz, F.E.M., Kerns, K., Sutovsky, P., Nogueira, E., 2023. Semen quality of Nellore and Angus bulls classified by fertility indices and relations with field fertility in fixed-time artificial insemination. Theriogenology 212, 148–156.

Santos, J.E.P., Thatcher, W.W., Chebel, R.C., Cerri, R.L.A., Galvão, K.N., 2004. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Anim. Reprod. Sci. 82, 513–535. https://doi.org/10.1016/j.anireprosci.2004.04.015.

Sartori, R., Bastos, M.R., Wiltbank, M.C., 2010. Factors affecting fertilisation and early embryo quality in single- and superovulated dairy cattle. Reprod. Fertil. Dev. 22, 151–158. https://doi.org/10.1071/RD09221.

Sartori, R., Sartor-Bergfelt, R., Mertens, S.A., Guenther, J.N., Parrish, J.J., Wiltbank, M.C., 2002. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. J. Dairy Sci. 85, 2803–2812. https://doi.org/10.3168/jds.S0022-0302(02)74367-1.

Sato, S., 1981. Factors associated with temperament of beef cattle. Jpn. J. Zoot. Sci. 52, 595–605.

Sreenan, J., Diskin, M., 1986. The extent and timing of embryonic mortality in the cow. Embryonic mortality in farm animals. Springer, pp. 1–11.

Stone, G.M., Murphy, L., Miller, B.G., 1978. Hormone receptor levels and metabolic activity in the uterus of the ewe: regulation by oestradiol and progesterone. Aust. J. Bio. Sci. 31, 395–403.

Taylor, J.F., Schnabel, R.D., Sutovsky, P., 2018. Genomics of bull fertility. Animal 12, 172–183.

Vasconcelos, J.L.M., Sá Filho, O.G., Cooke, R.F., 2014. Impacts of reproductive technologies on beef production in South America. Cur. Fut. Rep. Tech. World Food Prod. 161–180.

Walsh, S.W., Williams, E.J., Evans, A.C.O., 2011. A review of the causes of poor fertility in high milk producing dairy cows. Anim. Reprod. Sci. 123, 127–138. https://doi.org/10.1016/j.anireprosci.2010.12.001.

Wiltbank, M.C., Baez, G.M., Garcia-Guerra, A., Toledo, M.Z., Monteiro, P.L.J., Melo, L.F., et al., 2016. Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows. Theriogenology 86, 239–253. https://doi.org/10.1016/j.theriogenology.2016.04.037.

Zelinski, M.B., Noel, P., Weber, D.W., Stormshak, F., 1982. Characterization of cytoplasmic progesterone receptors in the bovine endometrium during proestrus and diestrus. J. Ani. Sci. 55, 376–383.