



**FUNDAÇÃO UNIVERSIDADE FEDERAL DE MATO GROSSO DO SUL  
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS VETERINÁRIAS  
CURSO DE MESTRADO**



**FATORES QUE INFLUENCIAM AS TAXAS DE PRENHEZ E PERDA  
GESTACIONAL EM FÊMEAS NELORE SUBMETIDAS À INSEMINAÇÃO  
ARTIFICIAL EM TEMPO FIXO NO BRASIL**

**LUCAS GOMES DA SILVA**

Campo Grande – MS  
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***FACTORS INFLUENCING PREGNANCY PER ARTIFICIAL INSEMINATION  
(AI) AND PREGNANY LOSS IN NELORE FEMALES SUBJECTED TO  
TIMED-AI IN BRAZIL***

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Veterinárias da Universidade Federal de Mato Grosso do Sul, como requisito à obtenção do título de Mestre em Ciências Veterinárias.

Campo Grande – MS  
2023

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## RESUMO

SILVA, L. G. Fatores que influenciam a taxa de prenhez e mortalidade embrionária em fêmeas Nelore submetidas à inseminação artificial em tempo fixo no Brasil. 2023. MESTRADO – Programa de Pós-Graduação em Ciências Veterinárias. Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, 2023

A inseminação artificial no Brasil vem evoluindo com aumento o de vacas inseminadas com taxa de prenhez média esperada de 50%, e para que a cadeia seja produtiva, é desejável que a matriz entregue um bezerro ao ano, tornando aquela matriz que perdeu o bezerro um animal com custo alto na propriedade. Porém, a fertilidade bovina é um processo multifatorial, dependente da qualidade do sêmen, fertilidade da fêmea, manejo, tempo preciso, anestro pós-parto e escores de condição corporal (ECC). Outro ponto importante, alguns estudos com avaliação de perdas gestacionais, observaram grande variação nos índices e que foram também influenciados pela raça, ECC, categoria, fazenda, porém são poucas fazendas que avaliam e conseguimos identificar as perdas somente após o primeiro diagnóstico de gestação, com aproximadamente 30 dias. Uma maior compreensão dos fatores que influenciam a fertilidade é essencial para melhorar as taxas de prenhez e reduzir a ocorrência de mortalidade embrionária em rebanhos de corte. O objetivo do presente estudo foi avaliar dados retrospectivos de gestação por inseminação artificial (P/IA) e perda de gestação em fêmeas Nelore submetidas à IATF em tempo fixo (IATF) no Brasil. Foram analisados dados de 40.104 IATF coletados em seis estações reprodutivas (2016 a 2022) e os efeitos da categoria do animal (por exemplo, classificação baseada em idade e paridade), fazenda, mês de parto, pai, raça do pai (Nelore vs Angus). Foram avaliados a expressão de estro na IATF, o temperamento dos animais e o ECC. P/IA e perda de gestação foram afetados ( $P < 0,001$ ) pela categoria da matriz. Houve também efeito da fazenda ( $P = 0,0013$ ) sobre P/IA e perda de prenhez ( $P = 0,001$ ), pois P/IA variou de 49,28% e 55,58% e perda de prenhez de 3,37% a 6,89% nos rebanhos avaliados. O mês do parto também afetou ( $P < 0,001$ ) o P/IA e foi maior para vacas que emprenharam no início da estação reprodutiva anterior. Animais mais calmos, apresentando menores escores de velocidade na saída do corredor após a IATF, obtiveram maior P/IA ( $P < 0,001$ ). O menor ECC na IATF foi associado ( $P < 0,001$ ) ao aumento da perda gestacional, e o ganho do ECC após a IA foi associado ( $P < 0,001$ ) à redução das taxas de mortalidade embrionária. Houve um efeito importante ( $P < 0,001$ ) do touro no P/IA e na perda de prenhez, pois o P/IA variou de 11 a 79%, e a mortalidade embrionária de 0 a 40% para os touros utilizados no estudo, destacando a importância da fertilidade do touro no sucesso geral da gestação. Os resultados do presente estudo reforçam categoria dos animais, o ECC no início do protocolo de IATF, o ganho do ECC após a IA, a expressão do estro na IATF, o touro e o mês do parto são fatores importantes que influenciam a P/IA e taxas de mortalidade embrionária em rebanhos de corte.

*Palavras-chave:* bovino, fertilidade, IATF, perdas gestacionais

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## ABSTRACT

41 Artificial insemination in Brazil has been evolving with an increase in inseminated cows with an  
42 expected average pregnancy rate of 50% and for the chain to be productive, it is desirable that  
43 the mother delivers one calf per year, with the mother who lost the calf a high cost on the property.  
44 However, bovine fertility is a multifactorial process, counting on semen quality, female fertility,  
45 management, precise timing, postpartum anestrus and BCS. On the other hand, semen fertility,  
46 often neglected, must be considered, due to the great impact it can have on pregnancy rates. On  
47 another important point, some studies evaluating pregnancy losses observed great variation in  
48 rates and were also influenced by breed, BCS, category, farm, but there are few farms that  
49 evaluate and we were able to identify losses only after the first diagnosis of pregnancy, with  
50 approximately 30 days. A greater understanding of the factors that influence fertility is essential  
51 to improve pregnancy rates and reduce the occurrence of embryonic mortality in beef herds. The  
52 objective of the current study was to evaluate retrospective data of pregnancy per artificial  
53 insemination (P/AI) and pregnancy loss in Nelore females subjected to timed-AI (TAI) in Brazil.  
54 Data from 40,104 TAI collected from six breeding seasons (2016 to 2022) were analyzed, and  
55 the effects of animal category (e.g., classification based on age and parity), farm, month of  
56 parturition, sire, sire breed (Nelore vs Angus), estrus expression at TAI, animal temperament, and  
57 body condition scores (BCS) were evaluated. P/AI and pregnancy loss were affected ( $P < 0.001$ )  
58 by animal category. There was also an effect of farm ( $P = 0.0013$ ) on P/AI and pregnancy loss ( $P$   
59  $= 0.001$ ), as P/AI ranged from 49.28% and 55.58% and pregnancy loss from 3.37% to 6.89%  
60 across the herds evaluated. Month of parturition also affected ( $P < 0.001$ ) P/AI and was higher  
61 for cows that became pregnant at the beginning of the previous breeding season. Calmer animals,  
62 presenting lower velocity scores while exiting the chute following TAI, achieved higher P/AI ( $P <$   
63  $0.001$ ). Lower BCS at TAI was associated ( $P < 0.001$ ) with increased pregnancy loss, and BCS  
64 gain following AI was associated ( $P < 0.001$ ) with reduced rates of embryonic mortality. There  
65 was a major effect ( $P < 0.001$ ) of sire on P/AI and pregnancy loss, as P/AI ranged from 11 to 79%,  
66 and embryonic mortality from 0 to 40% for the bulls used in the study, highlighting the importance  
67 of the sire fertility on overall pregnancy success. Results from the current study reinforce the idea  
68 that animal category, BCS at the onset of estrous synchronization, BCS gain following AI, estrus  
69 expression at TAI, sire, and month of parturition are important factors influencing P/AI and rates  
70 of embryonic mortality in beef herds.

71 *Keywords:* cattle, fertility, FTAI, pregnancy losses.

## 72 **1 INTRODUÇÃO**

73 A inseminação artificial no Brasil vem evoluindo com aumento de vacas  
74 inseminadas, alcançando em 2021, aproximadamente 27 milhões de protocolos  
75 comercializados (Baruselli, 2022). Em relação à técnica de IATF, a taxa de  
76 prenhez média esperada é próxima a 50%. A fertilidade bovina é um processo  
77 multifatorial, contando com qualidade do sêmen, fertilidade da fêmea, manejo  
78 adequado do rebanho e tempo preciso ao usar a inseminação artificial (IA).

79 Os fatores inerentes à fêmea, salienta-se o anestro pós-parto e a baixa  
80 condição corporal no início dos protocolos. Por outro lado, a fertilidade do sêmen,  
81 muitas vezes negligenciada, deve ser considerada, pelo grande impacto que  
82 pode promover nas taxas de prenhez.

83 Em outro ponto importante, alguns estudos com avaliação de perdas  
84 gestacionais, observaram grande variação nos índices (3,3%-32,3%), que foram  
85 também influenciados pela raça, ECC, categoria, fazenda (REESE et al., 2019).  
86 Vale ressaltar que este é um índice difícil de ser avaliado rotineiramente, devido  
87 ao aumento de manejo nas propriedades, porém muito impactante na eficiência  
88 reprodutiva do rebanho.

89 Bamber et al. (2009), estimaram a herdabilidade e variabilidade genética  
90 de perdas gestacionais e a variação do valor genético dos efeitos embrionários  
91 foi três vezes superior a variação do valor genético dos efeitos maternos,  
92 indicando que, do ponto de vista genético, a capacidade de sobrevivência do  
93 embrião tem um efeito maior na perda da gestação do que a capacidade da vaca  
94 de manter a prenhez.

95 O uso da ultrassonografia pode ser uma ferramenta de auxílio na  
96 avaliação de perdas gestacionais, porém suas conclusões são certamente mais  
97 demoradas devido ao diagnóstico inicial ser realizado com 30 dias (DISKIN et  
98 al., 2016).

99 Portanto, é fundamental desenvolver ferramentas para conhecer a relação  
100 da prenhez de perdas gestacionais com a paternidade, sobretudo em bovinos  
101 Nelore jovens, com genética superior, que não apresentam informações  
102 suficientes para prever taxas de prenhez e morte embrionária. Dessa forma,  
103 otimizando a utilização desses touros nos programas de seleção e acasalamento  
104 que utilizam a inseminação artificial ou IATF.

105

## 106 **2 REVISÃO DE LITERATURA**

107 Para que a cadeia produtiva da bovinocultura de corte proporcione bons  
108 retornos econômicos para o produtor, o desempenho reprodutivo é um dos  
109 principais fatores (SANTOS et al., 2009) associado ao elevado índice de  
110 produção (VASCONCELOS E MENEGETTI, 2006).

111 Para o aumento produtivo, é desejável que cada vaca produza um bezerro  
112 por ano, diminuindo o intervalo entre partos e aumentando o número de vacas  
113 prenhes no início da estação de monta (MORAES et al, 2001). Uma vaca que  
114 não produz um bezerro ao ano devido a perda gestacional ou mortalidade  
115 neonatal, o custo médio de produção se torna uma despesa sem retorno  
116 (JUNQUEIRA E ALFIERI, 2006; REESE, et al 2019)

117

### 118 **2.1 Taxa de prenhez de touros**

119 As taxas de prenhez na IATF são dependentes de diversos fatores  
120 relacionados a matriz, ao ambiente e ao touro (THUNDATHIL et al., 2016). Entre  
121 os principais fatores podemos destacar o escore de condição corporal da matriz  
122 (valor 3 é ideal numa escala de 1-5), a expressão do cio e a taxa de concepção  
123 do touro, podendo este, ainda ter efeitos da partida utilizada no momento da  
124 IATF (MARTINI et al., 2022)

125 Exames andrológicos e avaliação do sêmen congelado identificam touros  
126 grosseiramente anormais e servem de controle, mas existem touros que se  
127 mostram bons nos exames e com classificações satisfatórias e seus índices são  
128 ruins, levando a questões genômicas envolvidas. Isso mostra a importância de  
129 identificar marcadores moleculares para fertilidade, onde poderíamos eliminar os  
130 touros ou seu sêmen ou até mesmo pensar em soluções para preservar sua  
131 fertilidade (THUNDATHIL et al., 2016).

132

### 133 **2.2 Efeitos genéticos na fertilidade**

134 Utilizando touros com características para altas taxas de mortalidade  
135 embrionária, Ortega et al. (2018) identificaram que estes bovinos apresentam  
136 problemas durante a fertilização, implantação e até mesmo durante a  
137 embriogênese, pensando em fatores envolvendo o genoma dos animais.

138 Capra et al. (2017), identificou a presença de alguns miRNAs expressos  
139 de forma diferentes de acordo com a motilidade espermática. Esses miRNAs

140 estão associados a à apoptose celular, potencial de membrana mitocondrial e  
141 alteração da espermatogênese e isso afeta diretamente sua fertilidade.

142 Hermisdorff et al. (2021), destacaram a importância de estudos no  
143 cromossomo x, pois identificou genes que indicam baixa fertilidade em touros  
144 que também são presentes em vacas na mesma região com variâncias genéticas  
145 maiores que 1%.

146 Nogueira et al. (2022) encontraram uma mutação em touro da raça Angus  
147 que apresenta defeitos na formação da manchete, levando a formação de  
148 espermatozoides com defeitos e conseqüentemente baixa fertilidade. Além  
149 disso, essa mutação pode ser passada pelo gameta e quando acasalado com  
150 fêmeas portadoras do mesmo alelo, podem comprometer o desenvolvimento  
151 embrionário.

152 Ao avaliar características fenotípicas relacionadas com fertilidade de  
153 touros, Carvalho Filho et al., (2020), verificaram uma alta correlação entre  
154 perímetro escrotal e qualidade seminal, o que acarreta em animais de alta  
155 fertilidade. E perímetro escrotal foi uma característica de alta herdabilidade e sua  
156 correlação com as características seminais foi maior do a morfologia e  
157 características visuais dos touros. Motilidade e morfologia espermática também  
158 são características com altas correlações genéticas para fertilidade (Butler et al.,  
159 2020)

160 De acordo com Taylor et al. (2018) poucos polimorfismos específicos  
161 estão associados a fertilidade e menos ainda a perdas gestacionais, porém  
162 sabemos que existem e podemos utilizar como ferramenta para seleção. Ainda  
163 salienta sobre o uso de biomarcadores como citometria ou imagens para facilitar  
164 e identificar as causas genômicas de formas visuais.

165

### 166 **2.3 Perdas Gestacionais**

167 A morte embrionária pode acarretar prejuízos econômicos significativos  
168 para os produtores em relação à produção de ruminantes, pois altas taxas de  
169 concepção vêm acompanhadas de perdas gestacionais, diminuindo assim o  
170 número de bezerros nascidos na propriedade (DISKIN et al., 2016). Ayalon  
171 (1978) publicou uma das primeiras revisões sobre perdas gestacionais, a partir  
172 de então poucos trabalhos foram executados a fim de revisar esse conteúdo de  
173 extrema importância para a produção pecuária.



174 Denomina-se como morte embrionária precoce aquelas ocorridas entre a  
175 concepção até 24 dias de gestação, depois até 50 dias como morte embrionária  
176 tardia e aquelas ocorridas após 50 dias como aborto e considera-se taxas  
177 aceitáveis de até 5% no geral da produção (Santos et al., 2004).

178 A morte embrionária precoce é mais variável devido ao menor número de  
179 avaliações, que normalmente ocorrem no diagnóstico de gestação com 30 dias  
180 (DISKIN et al., 2016). Já a morte fetal, por ser de mais fácil avaliação, mais  
181 conhecida, e pode ocorrer devido a mutações genéticas, irreconhecimento fetal,  
182 insuficiência placentária ou algum tipo de doença (POPE, 1988; FARIN et al.,  
183 2006; DISKIN E MORRIS, 2008; CHENG et al., 2016; POHLER et al., 2016;  
184 ABDALLA et al., 2017).

185 Os valores das perdas gestacionais, destacados por Barbosa et al. (2006),  
186 afirmam que a mortalidade embrionária precoce pode chegar até 40% e é o  
187 principal momento da perda gestacional. Reese et al (2019) destacaram a  
188 ausência de estudos que comprovem quais os principais fatores que estão  
189 ocasionando essas perdas gestacionais de forma fidedigna.

190 Pensando nessas taxas aceitáveis para perdas gestacionais sem  
191 atrapalhar o desempenho produtivo do rebanho, Reese et al. (2019)  
192 identificaram em sua meta-análise uma perda gestacional total de 4.9%, com  
193 valor maior para as novilhas (8.1%), seguindo das primíparas (5.4%) e  
194 múltíparas (5.1%).

195 Pessoa et al. (2012) selecionou 658 matrizes sendo 283 Múltíparas, 91  
196 primíparas e 284 nulíparas, de bom ECC e com atividade ovariana, realizando  
197 um diagnóstico de gestação aos 30 dias e outro como confirmação aos 90 dias  
198 após a IATF. Obteve os resultados de 5,30% para múltíparas, 3,30% para  
199 primíparas e 5,99% para nulíparas.

200 Fernandez-Novo et al. (2020), fizeram um estudo retrospectivo para a  
201 prevalência de perdas gestacionais com 19.437 vacas Holandesas em 3 anos.  
202 Os fatores estação, protocolo de IA de tempo fixo, tempo de lactação, touro,  
203 inseminador ou tipo de sêmen não foram significativamente associadas com as  
204 taxas de perdas gestacionais. Havendo diferença apenas para categoria com  
205 primíparas (10,8%) e múltíparas (15,3%) maior do que novilhas (6,9%), IATF  
206 obtendo um valor menor que IA (IA 12,7 vs. IATF 11,9%) e o primeiro serviço  
207 perdendo mais que o segundo (IATF 13,8% vs. RESSINC 11,2).

## 208 **2.4 A matriz nas perdas gestacionais**

209 O escore de condição corporal (ECC) da matriz, que quando baixo  
210 apresenta maiores perdas gestacionais, embriões de baixa qualidade e muitos  
211 deles imaturos (BRIDGES et al., 2012; PERRY et al., 2013; KRUSE et al., 2017),  
212 sendo um fator crítico na redução da fertilidade de vacas primíparas (DeROUEN  
213 et al., 1994; CICCIONI et al., 2003) e é uma variável correlacionada com taxas  
214 de prenhez (RICHARDSON et al., 2016).

215 A maturidade fisiológica de um folículo dominante na indução ovulação  
216 pode afetar manutenção da gestação (POHLER et al., 2012), sendo assim  
217 hormônios como o estradiol contribui para manutenção da gestação com a  
218 preparação do ambiente uterino (PERRY et al., 2007) e a progesterona como  
219 principal hormônio regulador dessa gestação (BRIDGES et al., 2010).

220 A progesterona é responsável por manter a prenhez, com isso, baixas  
221 concentrações séricas resultam num ambiente uterino incapaz de manter o  
222 embrião (STONE et al., 1978, ZELINSKI et al., 1982; ING E TORNESI 1997;  
223 FIELDS et al., 2012) e a suplementação após a inseminação pode ser uma  
224 alternativa para acréscimo de até 5% nas taxas de prenhez de vacas que  
225 apresentam esse déficit (MANN E LAMMING, 1999).

226

## 227 **2.5 Efeitos genômicos nas perdas gestacionais**

228 No momento que o reconhecimento materno e a proteção do embrião  
229 contra o sistema imunológico materno ocorrem, são os fatores mais críticos na  
230 manutenção gestacional (ROBERTS E SCHALUE-FRANCIS, 1990;  
231 BAUERSACHS E WOLF, 2013; YANG et al., 2014). Porém esse período ainda  
232 é pouco avaliado devido ao tempo gestacional, o diagnóstico de gestação ocorre  
233 com 30 dias e os métodos de diagnósticos mais precoces envolvem genes  
234 estimulados por interferon e tornam inviável a avaliação (GREEN et al., 2010;  
235 PUGLIESI et al., 2014, DISKIN et al., 2016).

236 Fatores genéticos podem estar associados durante esse período inicial da  
237 gestação. Segundo Bamber et al. (2009), a herdabilidade e variabilidade  
238 genética de perdas gestacionais tem prevalência geral de 14,4%, com o modelo  
239 pai-avô materno produzindo uma herdabilidade para efeito direto de 0,489 (PSD  
240 = 0,221) e para efeitos maternos de 0,166 (PSD = 0,113). Neste estudo, a  
241 variação do valor genético dos efeitos embrionários foi três vezes a variação do

242 valor genético dos efeitos maternos, indicando que, no nível dos valores  
243 genéticos, a capacidade de sobrevivência do embrião tem um efeito maior na  
244 perda gestacional do que a capacidade da vaca de manter a prenhez.

245 Franco et al. (2018) utilizou 9 diferentes touros, sendo 3 da raça Nelore e  
246 6 Angus, dividindo-os em grupos de alta perda embrionária e baixa perda  
247 embrionária. Após isso inseminou em dois lotes, e ao avaliar a taxa de prenhez  
248 e perda gestacional verificaram que os touros da Raça Nelore apresentavam  
249 maior taxa de prenhez e maior taxa de perda gestacional e aqueles touros que  
250 apresentavam altas taxas de perdas gestacionais continuaram com essa  
251 característica nessa estação de monta.

252 Numa análise genômica integrativa para entender a base genética e  
253 biológica da perda de prenhez em gado leiteiro e favorecer a taxa de prenhez  
254 por meio de marcadores moleculares, SIGDE et al. (2021), identificaram sete  
255 regiões genômicas ligadas a crescimento feto-placentário, modulação imune,  
256 sinalização de cálcio, vascularização e organogênese que explicaram mais de  
257 0,5% das variações genéticas aditivas para perda fetal.

258

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426 *Artigo Submetido e aceito após revisões na Revista Animal Reproduction*  
427 *Science*

428 **4. FACTORS INFLUENCING PREGNANCY PER ARTIFICIAL INSEMINATION**  
429 **(AI) AND EMBRYONIC MORTALITY IN NELORE FEMALES SUBJECTED TO**  
430 **TIMED-AI IN BRAZIL**

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445

446 **4.1 Abstract**

447 A greater understanding of factors influencing fertility is essential to improve  
448 pregnancy rates and reduce the occurrence of embryonic mortality in beef herds.

449 The objective of the current study was to evaluate retrospective data of  
450 pregnancy per artificial insemination (P/AI) and pregnancy loss in Nelore females

451 subjected to timed-AI (TAI) in Brazil. Data from 40,104 TAI collected from six  
452 breeding seasons (2016 to 2022) were analyzed, and the effects of animal

453 category (e.g., classification based on age and parity), farm, month of parturition,  
454 sire, sire breed (Nelore vs Angus), estrus expression at TAI, animal temperament,

455 and body condition scores (BCS) were evaluated. P/AI and pregnancy loss were  
456 affected ( $P < 0.001$ ) by animal category. There was also an effect of farm ( $P =$

457  $0.0013$ ) on P/AI and pregnancy loss ( $P = 0.001$ ), as P/AI ranged from 49.28%  
458 and 55.58% and pregnancy loss from 3.37% to 6.89% across the herds

459 evaluated. Month of parturition also affected ( $P < 0.001$ ) P/AI and was higher for  
460 cows that became pregnant at the beginning of the previous breeding season.

461 Calmer animals, presenting lower velocity scores while exiting the chute following  
462 TAI, achieved higher P/AI ( $P < 0.001$ ). Lower BCS at TAI was associated ( $P <$

463  $0.001$ ) with increased pregnancy loss, and BCS gain following AI was associated

464 (P < 0.001) with reduced rates of embryonic mortality. There was a major effect  
465 (P < 0.001) of sire on P/AI and pregnancy loss, as P/AI ranged from 11 to 79%,  
466 and embryonic mortality from 0 to 40% for the bulls used in the study, highlighting  
467 the importance of the sire fertility on overall pregnancy success. Results from the  
468 current study reinforce the idea that animal age and parity at the beginning of the  
469 breeding season, BCS at the onset of estrous synchronization, BCS gain  
470 following AI, estrus expression at TAI, sire, and month of parturition are important  
471 factors influencing P/AI and rates of embryonic mortality in beef herds.

472

473 **Keywords:** Embryonic loss; Bos indicus; pregnancy rate.

474

## 475 **4.2 Introduction**

476 The number of cows subjected to timed-artificial insemination (TAI) in  
477 Brazil is increasing and has reached approximately 27 million in 2021 (Baruselli  
478 et al., 2022). The expected pregnancy rates of herds undergoing TAI is close to  
479 50% in Brazil and is significantly affected by sire, female fertility, herd  
480 management, estrus expression at TAI, rates of embryonic loss, and compliance  
481 during the implementation of TAI protocols (Nogueira et al., 2019; Reese et al.,  
482 2020). The paternal influence on pregnancy success (Ortega et al., 2018;  
483 Sanches et al., 2023) and pregnancy loss (Franco et al., 2018) are often  
484 overlooked, despite the substantial impact sires can exert on breeding outcomes.

485 Among factors inherent to the female that influence herd reproductive  
486 performance, resumption of postpartum fertility along with body condition scores  
487 (BCS) at the onset of estrous synchronization plays a major role in the success  
488 rates of TAI programs (Kim et al., 2023; D'Occhio et al., 2019). Reduced BCS  
489 and inadequate nutrition have been associated with diminished pregnancy rates,  
490 increased pregnancy loss, and the development of low-quality embryos  
491 (Richardson et al., 2016, Kruse et al., 2017, Bridges et al., 2012; Cicciolelli et al.,  
492 2003) underscoring the importance of maintaining appropriate BCS levels at  
493 calving and ensuring proper postpartum nutrition to optimize fertility outcomes.  
494 Animal temperament and behavior, independently of breed (*Bos t. taurus* and  
495 *Bos t. indicus*) have also been shown to affect reproductive outcomes in beef  
496 cattle (Cooke et al., 2009, Cooke et al., 2011).

497 While the success rate of fertilization in beef cattle can be as high as 90%,  
498 embryonic mortality following conception is especially high during the first month  
499 of gestation (Maurer and Chenault, 1983; Santos et al., 2004; Diskin and Kenny,  
500 2016). In the context of embryonic and fetal losses in cattle, early embryonic  
501 mortality has been defined as those occurring between conception and day 24 of  
502 gestation, late embryonic losses between days 25 and 50, and fetal losses  
503 manifesting after day 50 of pregnancy (Santos et al., 2004). Regarding rates of  
504 pregnancy loss in beef herds, Reese et al. (2020) reported in a recent meta-  
505 analysis that 50% of pregnancy failure occurred prior to day 16, around 15.5%  
506 from days 16 and 32, and less than 6% after the first month of gestation. Of note,  
507 cow breed, parity, and breeding method were associated with early or late rates  
508 of embryonic/fetal losses.

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

512

### 513 **4.3 Material and Methods**

#### 514 **4.3.1 Animals**

515           Data was collected from six herds located in the state of Mato Grosso do  
516 Sul, Brazil. All farms perform a complete cycle with breeding, rearing, and  
517 finishing. All animal procedures used were approved by the Committee of Ethics  
518 and Animal Use of Embrapa under the protocol number (Ceua Protocol N°  
519 006/2022).

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

525           The experiment included 40,104 Nelore females from six breeding  
526 seasons expanding from 2016 to 2022. Cows and heifers enrolled in the study  
527 were categorized based on age and parity at the start of the breeding season into  
528 9 distinct categories (1) precocious heifer, (2) precocious primiparous, (3)  
529 precocious secondiparous, (4) precocious multiparous, (5) conventional heifer,  
530 (6) conventional primiparous, (7) conventional secondiparous, (8) conventional  
531 multiparous, and (9) non-lactating. The description of each category is presented  
532 in Table 1.

533

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

<b>Category</b>	<b>Description</b>
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 to 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

535

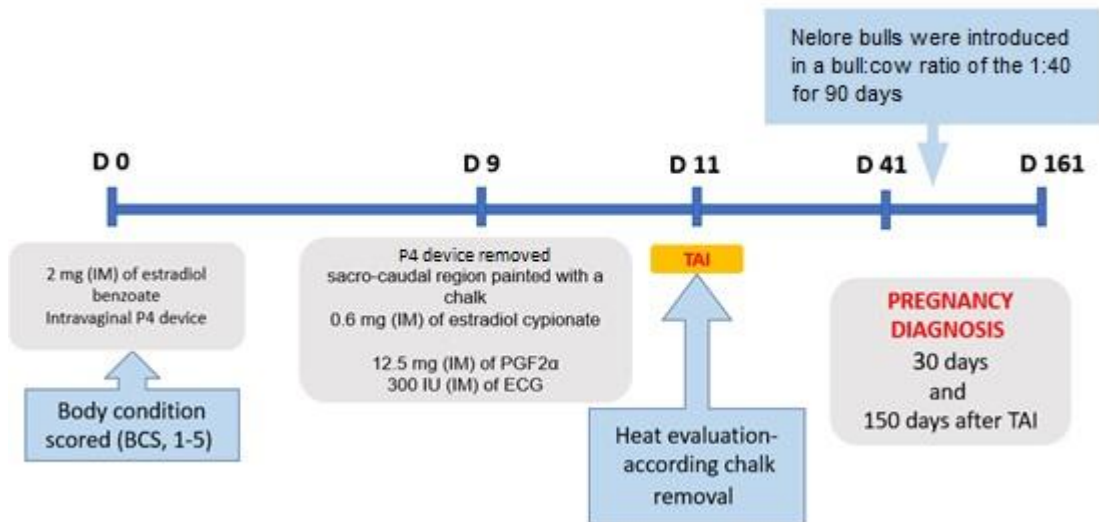
536

537 **4.3.2 Description of the breeding seasons, TAI programs, and Body**  
 538 **Condition Scoring**

539 All breeding seasons began in November and ended in February, lasting  
 540 120 days. For primiparous, secondiparous, and multiparous categories  
 541 (precocious and conventional), cows were subjected to TAI protocols after at  
 542 least 30 days postpartum following the calving season (July-December).

543 The TAI protocols utilized (Figures 1, 2, and 3) were based on Meneghetti  
 544 et al. (2009). For multiparous, primiparous, and secondiparous cows [precocious  
 545 and conventional] (Figure 1), on the first day of the protocol (D-0), cows received  
 546 2 mg (IM) of estradiol benzoate (Gonadiol®; Zoetis, São Paulo, SP, Brazil) and  
 547 an intravaginal progesterone insert (CIDR®, 1.9 g progesterone; Zoetis). On D9,  
 548 progesterone inserts were removed and injections of 0.6 mg (IM) of estradiol  
 549 cypionate (ECP®, Zoetis, Brazil), 12.5 mg (IM) of PGF2α (Lutalyse®; Zoetis), and  
 550 300 IU (IM) of eCG (Novormon®; Zoetis) were administered. Non-lactating cows  
 551 received the same protocol, but with no application of ECG at P4 device removal.  
 552 Furthermore, at the time of intravaginal devices removal on D9, all dams were  
 553 painted in the sacrum-caudal region with marker sticks (Raidl-Maxi; RAIDEX  
 554 GmbH, Dettingen/Erms, Germany) to aid in the visual detection of estrus. Cows  
 555 were inseminated on D11, 48h after implant removal. At the time of insemination,

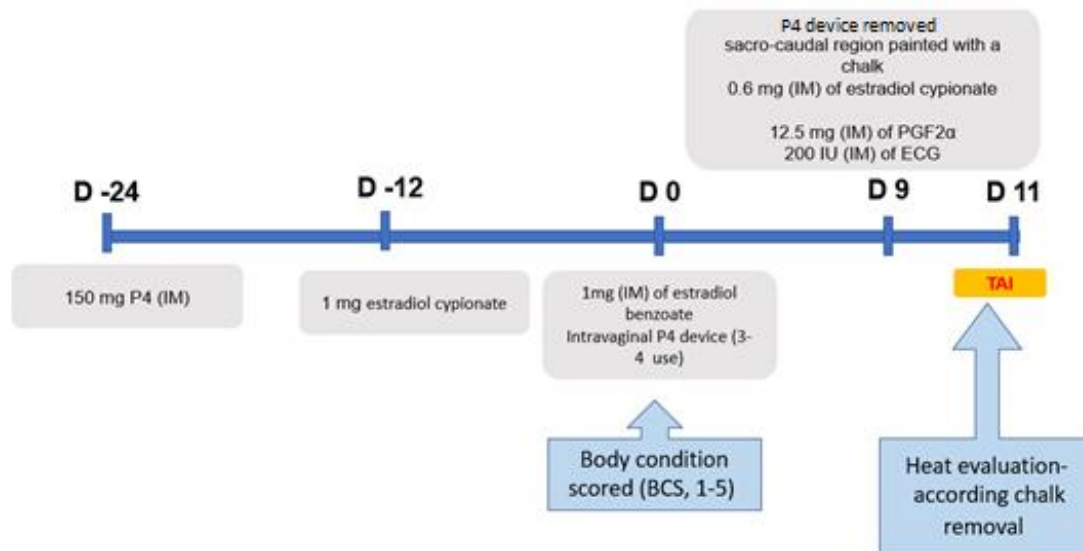
556 the occurrence of standing heat was evaluated based on the visual appearance  
 557 of the estrus patches. Heat was scored as (1) all ink present (no heat); (2) partial  
 558 removal of the ink (low heat); (3) all ink removed (high-intensity heat). For  
 559 analysis, scores 2 and 3 were considered evidence of standing heat (Nogueira et  
 560 al. 2019). Pregnancy diagnoses were conducted on days 30 and 150 following  
 561 breeding.



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

573

574  
 575 For conventional heifers (Figure 2) cyclicity was induced (prior to the  
 576 initiation of the 11-day synch program) with an injection of 150 mg (I.M.) of P4  
 577 (Sincrogest®, Ouro Fino, São Paulo, Brazil) on D-24, followed by 0,6 mg of  
 578 estradiol cypionate on D-12. The induction protocol was followed by the 11-day  
 579 synchronization program as described for the multiparous cows, with changes  
 580 only in the dosage of estradiol benzoate on D0 (1 mg, IM) and eCG (200 IU, IM)  
 581 on D9.

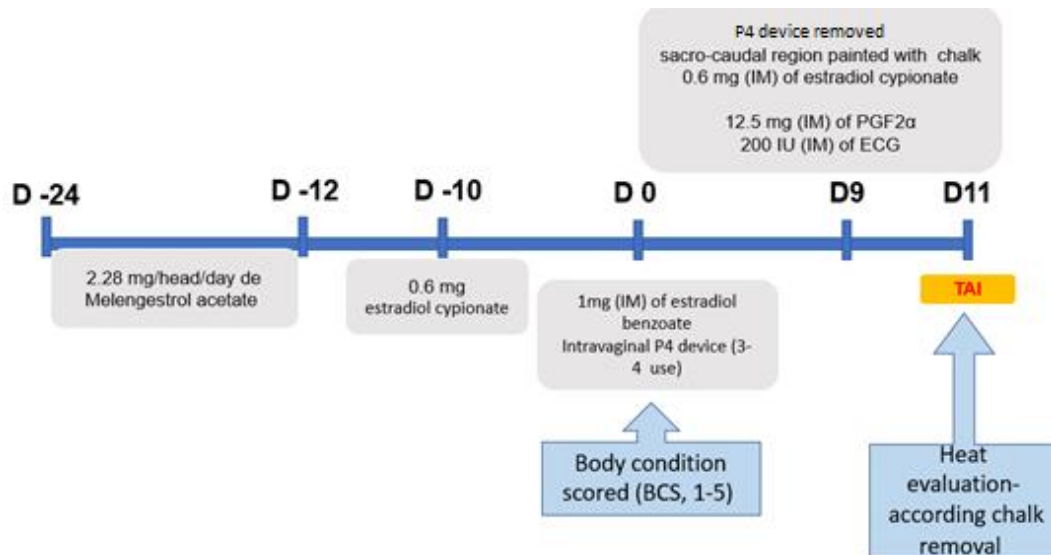


582

583 **Figure 2.** Artificial insemination (AI) protocol for heifers. Cyclicity was induced with an  
 584 injection of 150 mg (I.M., Sincrogest® - Ouro Fino, Brazil) of P4 on D-24, followed by  
 585 0,6 mg of estradiol cypionate on D-12. On day (D0), conventional heifers received 1 mg  
 586 (IM) of estradiol benzoate (Gonadiol®; Zoetis, Brazil) and an intravaginal progesterone  
 587 insert (CIDR®, 1.9 g progesterone; Zoetis). Body condition was scored on D0. On D9,  
 588 progesterone inserts were removed and injections of 0.6 mg (IM) of estradiol cypionate  
 589 (ECP®, Zoetis, Brazil), 12.5 mg (IM) of PGF2α (Lutalyse®; Zoetis, Brazil), and 200 IU  
 590 (IM) of ECG (Novormon®; Zoetis, Brazil) were administered. Furthermore, on D9, heifers  
 591 were painted in the sacrum-caudal region with a marker stick (Raidl-Maxi; RAIDEX  
 592 GmbH, Dettingen/Erms, Germany) to aid on visual detection of estrus. On day 11 (D11)  
 593 conventional heifers were examined for chalk removal (e.g., as an indication of standing  
 594 estrus) and AI performed.

595

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



601

602 **Figure 3.** Artificial insemination (AI) protocol used to synchronize ovulation in precocious  
 603 heifers. Induction of cyclicity was performed with oral administration of melengestrol  
 604 acetate (MGA®, Zoetis, Brazil) from D-24 through D-12 at the dosage of  
 605 2.28mg/head/day. On D-10, precocious heifers received 0.6 mg of estradiol cypionate.  
 606 On day (D0), heifers received 1 mg (IM) of estradiol benzoate (Gonadiol®; Zoetis, Brazil)  
 607 and an intravaginal progesterone insert (CIDR®, 1.9 g progesterone; Zoetis). Body  
 608 condition was scored on D0. On D9, progesterone inserts were removed and injections  
 609 of 0.6 mg (IM) of estradiol cypionate (ECP®, Zoetis, Brazil), 12.5 mg (IM) of PGF2α  
 610 (Lutalyse®, Zoetis, Brazil), and 200 IU (IM) of ECG (Novormon®; Zoetis, Brazil) were  
 611 administered. Furthermore, on D9, heifers were painted in the sacrum-caudal region with  
 612 a marker stick (Raidl-Maxi; RAIDEX GmbH, Dettingen/Erms, Germany) to aid on visual  
 613 detection of estrus. On day 11 (D11) heifers were examined for chalk removal (e.g., as  
 614 an indication of standing estrus) and AI performed.

615

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

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

625 On the day of the AI, the speed which each animal exited the chute was  
 626 evaluated by a trained technician performing only this role. The observed



627 behavior of each animal was evaluated, and the speed which each animal exited  
628 the chute was recorded and classified as (1) walking, (2) trotting, or (3) running  
629 (Gomes da Silva et al., 2020).

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

637 At the end of the breeding season, sires were classified based on average  
638 pregnancy rates as low- pregnancy rates equal or less than 39.9; average-  
639 pregnancy rates between 40% and 49.9%; high- pregnancy rates between 50%  
640 and 55.9%; and top- pregnancy rates equal or above 56%.

641

### 642 **4.3.3 Statistical Analysis**

643 All statistical analyses were conducted using SAS version 9.4 (SAS  
644 Institute Inc.). The effects of animal category, farm, month of parturition, breed of  
645 sire, standing heat at TAI, and chute speed score were evaluated using  
646 generalized linear mixed models using the GLIMMIX procedure. Means were  
647 separated using the LSMEANS PDIF option with Tukey's adjustment.

648 The relationship between pregnancy loss, BCS, and BCS gain from the  
649 beginning of the TAI protocol to the day of the pregnancy diagnosis was  
650 determined using linear regression models, and when significant ( $P < 0.05$ ), a  
651 probability graph was generated using the REG procedure of SAS. Statistical  
652 significance was defined as  $P \leq 0.05$  and statistical tendencies as  $0.05 < P <$   
653  $0.10$ .

654

## 655 **4.4 Results**

656 The overall results of P/AI and pregnancy loss are summarized in Tables  
657 2 and 3. Considering all the data collected, the average P/AI and pregnancy loss  
658 were 53.11% and 5.78%, respectively. P/AI was significantly ( $P < 0.001$ ) affected  
659 by animal category. Precocious secondiparous (64.65%), precocious multiparous  
660 (60.00%), and multiparous (57.09%) had the highest P/AI, whereas precocious

661 heifers (44.50%), precocious primiparous (46.43%) and conventional heifers  
 662 (47.90%) had the lowest P/AI (Table 2).

663

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

	P/AI (%)	Pregnancy loss(%)
<b>Category (n)</b>		
Heifer (7,348)	47.90 <sup>d</sup>	5.03 <sup>c</sup>
Primiparous (8,181)	51.41 <sup>bc</sup>	7.11 <sup>b</sup>
Secondiparous (2,914)	56.59 <sup>b</sup>	6.44 <sup>c</sup>
Multiparous (17,151)	57.09 <sup>ab</sup>	4.93 <sup>c</sup>
Non-lactating (722)	49.45 <sup>c</sup>	7.19 <sup>ab</sup>
Precocious heifer (2,582)	44.50 <sup>e</sup>	7.11 <sup>b</sup>
Precocious Primiparous (812)	46.43 <sup>d</sup>	10.94 <sup>a</sup>
Precocious secondiparous (314)	64.65 <sup>a</sup>	8.13 <sup>ab</sup>
Precocious Multiparous (80)	60.00 <sup>ab</sup>	4.26 <sup>c</sup>
<i>P</i>	<0.001	<0.001
<b>Farm (n)</b>		
A (923)	55.58% <sup>a</sup>	4.71% <sup>b</sup>
B (8,570)	51.06% <sup>b</sup>	5.77% <sup>b</sup>
C (5,313)	55.51% <sup>a</sup>	4.44% <sup>b</sup>
D (416)	49.28% <sup>b</sup>	3.91% <sup>b</sup>
E (3,936)	54.17% <sup>a</sup>	3.37% <sup>b</sup>
F (20,946)	53.11% <sup>a</sup>	6.89% <sup>a</sup>
<i>P</i>	0.0013	<0.001

666

667 Rates of pregnancy loss were also affected by animal category ( $P <$   
 668 0.001), with higher rates of pregnancy loss detected in precocious primiparous  
 669 (10.94%), precocious secondiparous (8.13%), and non-lactating cows (7.19%),  
 670 and the lowest rates of pregnancy loss was detected in precocious multiparous  
 671 (4.26%), multiparous (4.93%), heifers (5.03%), and secondiparous (6.44%) cows.

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

677 Animals with a low chute speed score (e.g., walked out of the chute as  
 678 opposed to ran) had higher ( $P < 0.001$ ) P/IA (54.42%) than those who trotted

679 (52.19%) or ran (50.32%) out of the chute. However, the speed which animals  
 680 exited the chute did not influence rates of pregnancy loss ( $P = 0.2558$ ).

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

687

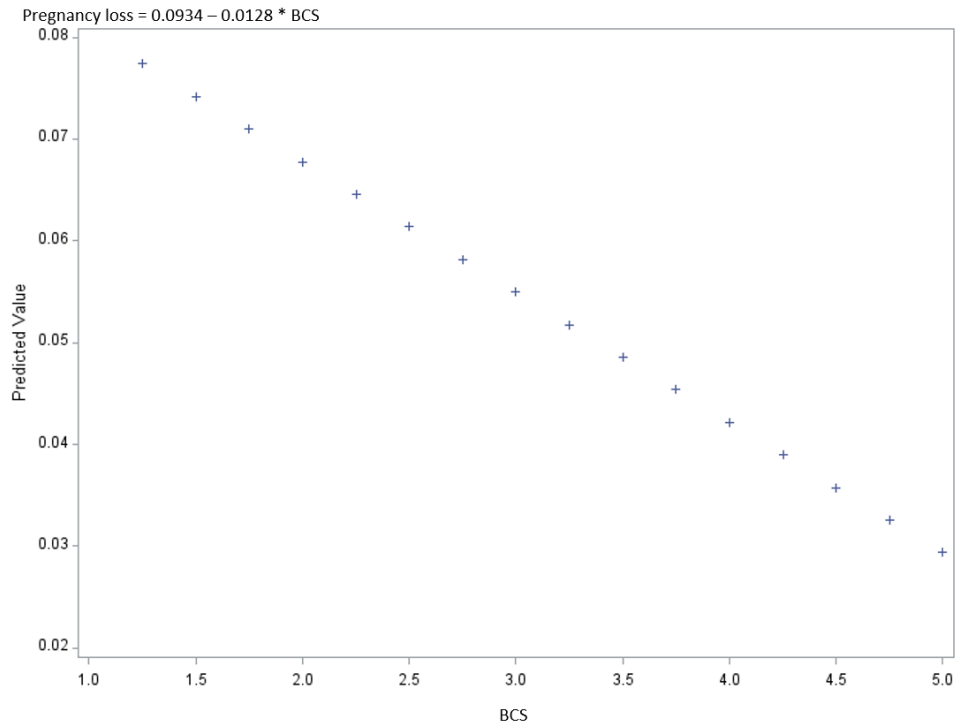
688 **Table 3.** Pregnancy per artificial insemination (P/AI) and pregnancy loss (%)  
 689 according to month of parturition, sire breed, expression of estrus, and velocity  
 690 score for exiting the chute.

	<b>P/AI (%)</b>	<b>Pregnancy loss(%)</b>
<b>Parturition Month (n)</b>		
JULY (165)	68.48% <sup>a</sup>	7.21% <sup>b</sup>
AUGUST (3,333)	57.67% <sup>b</sup>	5.90% <sup>d</sup>
SEPTEMBER (5,127)	56.33% <sup>b</sup>	5.55% <sup>d</sup>
OCTOBER (5,331)	55.00% <sup>c</sup>	6.01% <sup>c</sup>
NOVEMBER (4,286)	54.01% <sup>c</sup>	6.80% <sup>c</sup>
DECEMBER (1,705)	50.50% <sup>d</sup>	14.29% <sup>a</sup>
JANUARY (190)	50.53% <sup>d</sup>	2.08% <sup>e</sup>
<i>P</i>	<0.001	<0.001
<b>Sire breed (n)</b>		
ANGUS (9,089)	52.24%	5.31%
NELORE (30,988)	53.37%	5.92%
<i>P</i>	<0.001	0.6886
<b>HEAT</b>		
0	40.9% <sup>b</sup>	6.7 <sup>a</sup>
1	58.5% <sup>a</sup>	5.7 <sup>b</sup>
<i>P</i>	<0.001	0.030
<b>Chute velocity score (n)</b>		
1 (20,367)	54.42% <sup>a</sup>	5.43%
2 (6,611)	52.19% <sup>b</sup>	5.33%
3 (3,283)	50.32% <sup>b</sup>	4.78%
<i>P</i>	<0.001	0.2558

691

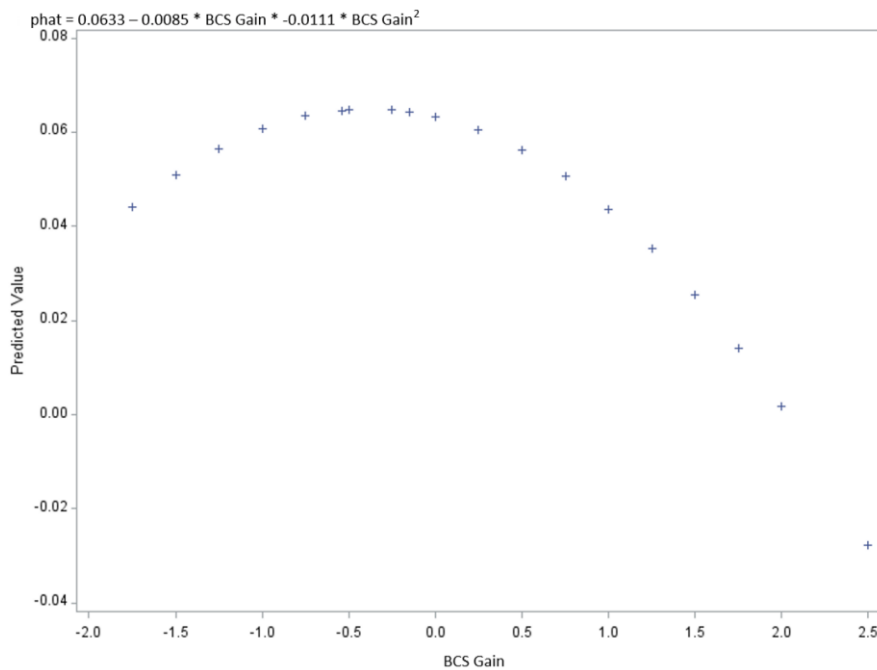
692 There was a significant effect of BCS at the onset of estrous  
 693 synchronization on P/AI ( $P < 0.001$ ) and pregnancy loss ( $P < 0.001$ ). The  
 694 probability of embryonic mortality decreased as BCS increased (Figure 4;  $P <$

695 0.05). Interestingly, BCS gains during the subsequent 30 days following the first  
696 measurement were associated with a reduced probability of pregnancy loss  
697 (Figure 5;  $P < 0.05$ ).



698

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

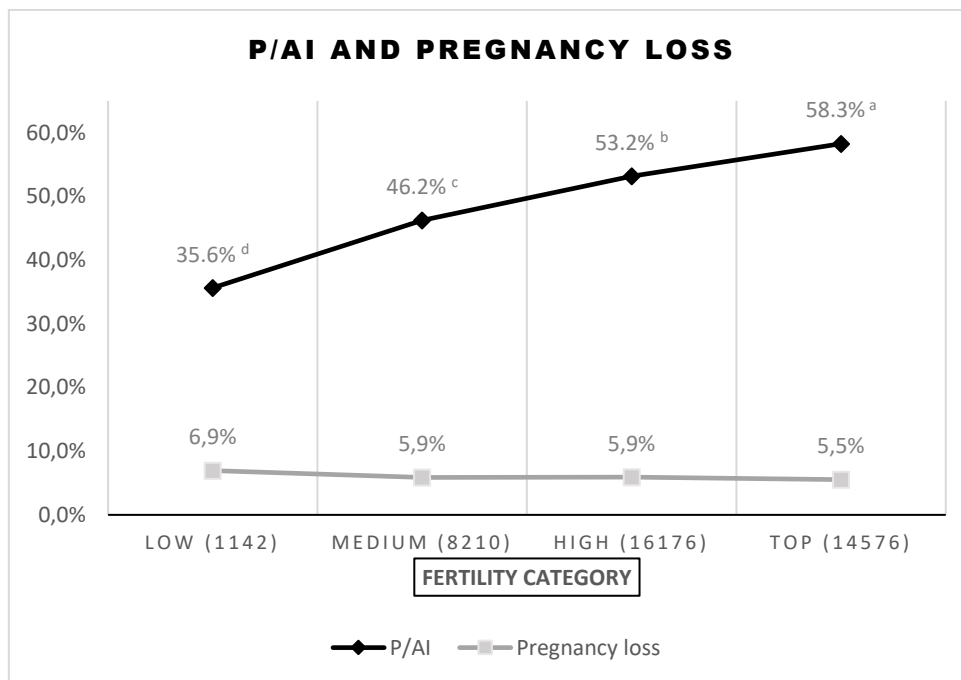


701

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

705

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



711

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

714

715 Regarding the breed of the sire used for AI, Nelore bulls had higher ( $P <$   
716  $0.001$ ) P/AI than Angus bulls, with a difference of 1.13% between them. However,  
717 the breed of the sire did not influence ( $P = 0.4089$ ) the rates of pregnancy loss.  
718 Furthermore, there was an effect ( $P < 0.001$ ) of sire on P/AI, as P/AI ranged from  
719 11 to 79% among the bulls used in the study. Similarly, there was an effect of sire  
720 on pregnancy loss ( $P < 0.001$ ), as rates of embryonic mortality ranged from 0 to  
721 40% for the bulls used in the study.

722

723

## 724 **4.5 Discussion**

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

736           The average pregnancy loss observed in the current study (5.78%) was  
737 slightly higher than the average pregnancy loss reported in a recent meta-  
738 analysis investigating rates of embryonic mortality in beef cattle (4.9%; Reese et  
739 al., 2020). The current study and the aforementioned meta-analysis concurred on  
740 the observation that the highest rates of embryonic mortality were identified in  
741 heifers (8.1%), followed by primiparous (5.4%) and multiparous (5.1%) cows.  
742 Likewise, in a study conducted by Pessoa et al. (2012) with 658 Nelore females,  
743 heifers (5.99%) exhibited the highest rates of embryonic mortality. Primiparous  
744 cows, however, experienced lower rates (3.30%) of embryonic loss compared to  
745 their multiparous counterparts (5.30%).

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

757           When considering the influence of the month of parturition on reproductive  
758 performance, cows calving in July displayed the highest P/AI, followed by those  
759 calving in August and September. Cows calving in July and August are the ones  
760 that conceived earliest in the breeding season, and consequently, calved early in  
761 the calving season, having a longer interval to rebreeding. This extended  
762 postpartum interval is beneficial to reproductive success (Cushman et al., 2012).

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

769           In the current study, lower BCS was associated with increased pregnancy  
770 loss, and BCS gain after AI was associated with reduced rates of embryonic  
771 mortality. The negative effects of low BCS on reproduction are well-established  
772 (Meneghetti et al., 2008). Subjecting cows to nutrient restriction immediately  
773 following AI, during a period of 6 days, resulted in production of lower-quality  
774 embryos with delayed developmental competence (Kruse et al., 2017).  
775 Furthermore, superovulation of cows with low BCS resulted in reduced response  
776 to the superovulation protocol, reduced size of ovulatory follicles, reduced corpus  
777 luteum size and postovulatory progesterone concentrations, and impaired  
778 embryo recovery rates and quality (Bridges et al., 2012). Postovulatory  
779 progesterone concentrations play a major role regulating conceptus development  
780 and uterine receptivity (Stone et al., 1978, Zelinski et al., 1982, Ing and Tornesi,  
781 1997, Bridges et al., 2010, Fields et al., 2012). The effect of BCS on reproduction  
782 is especially important for primiparous cows, due to the concomitant demand for  
783 growth and reproduction (DeRouen et al., 1994, Ciccioli et al., 2003, Richardson  
784 et al., 2016).

785           Expression of estrus on the day of AI was associated with higher P/AI and  
786 lower rates of embryonic mortality as previously reported by Nogueira et al (2019)  
787 and Rodrigues et al. (2018). Estrus expression occurs as a result of high  
788 circulating concentrations of estradiol produced by the dominant follicle.  
789 Preovulatory estradiol concentrations play a major role in pregnancy success,

790 through its effects on the oocyte, follicular cells, oviduct, and uterus for the  
791 establishment of uterine receptivity (Pohler et al., 2012, Perry et al., 2007)

792         There was a major effect of sire on P/AI and pregnancy loss in the current  
793 study, as P/AI from individual bulls ranged from 11 to 79% and embryonic  
794 mortality from 0 to 40% despite only using semen that had passed a thorough  
795 evaluation. This underscores the significance of conducting additional research  
796 into the genetic and molecular mechanisms that govern the paternal influence on  
797 pregnancy establishment and pregnancy loss. Of note, Ortega et al. (2018)  
798 reported significant effects of sire conception rate (SCR) on preimplantation  
799 embryo development using semen that had passed all the standard quality  
800 control procedures before being tested in the field. Currently, the quality  
801 parameters commonly employed in semen evaluation do not offer complete  
802 reliability in predicting bull fertility (Capra et al., 2017). The identification of new  
803 genetic variants in the bovine paternal genome governing fertility and early  
804 embryonic mortality is highly desirable (Butler et al., 2020). Nogueira et al. (2022)  
805 identified a deleterious non-synonymous point mutation in the autosomal EML5  
806 gene that when present in homozygosis resulted in prominent abnormal sperm  
807 phenotypes. Future studies should focus on identifying new genetic variants to  
808 accurately predict sire fertility to improve reproductive efficiency (Taylor et al.,  
809 2018). Selection for scrotal circumference has great potential to improve sire  
810 fertility, due to its high heritability and positive association with semen quality  
811 traits (Carvalho Filho et al., 2020).

812         In cattle, successful fertilization (or conception) generally occurs following  
813 breeding, but embryonic mortality is especially high during the first month of  
814 gestation. Fertilization rates have been estimated to be around 90% or higher in  
815 heifers, beef cows, and moderate-producing dairy cows (Diskin and Sreenan,  
816 1980, Diskin and Morris, 2008, Maurer and Chenault, 1983, Sreenan and Diskin,  
817 1986, Ahmad et al., 1995, Sartori et al., 2002), ~80% in high-yielding dairy cows  
818 under thermoneutral conditions, and ~60% in high-producing dairy cattle under  
819 heat stress (Wiltbank et al., 2016, Sartori et al., 2010, Ribeiro et al., 2016, Santos  
820 et al., 2004). Conception rates, however, range from 35-40% in dairy (Lucy, 2001,  
821 Walsh et al., 2011, Norman et al., 2020) and 50-70% in beef cattle (Perry et  
822 al., 2011). Thus, under thermoneutral conditions, there is a high rate of embryonic



823 death following fertilization, which is especially high during the first two weeks of  
824 gestation in both beef (Ealy, 2020) and dairy cattle (Wiltbank et al., 2016).

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

833

#### 834 **4.5 Conclusion**

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

846

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1059 **5 Considerações finais**

1060 Os resultados nos indicam que existem fatores fenotípicos relacionados a  
1061 matriz, assim como fatores relacionados ao meio, que influenciam da P/IA e  
1062 taxas de mortalidade embrionária no rebanho e que podem ser corrigidos na  
1063 propriedade. Consideravelmente, o efeito do touro, foi muito importante nessa  
1064 variação em relação a P/IA e perda gestacional, ressaltando a importância dos  
1065 estudos relacionados a fertilidade e identificação dos fatores genéticos que  
1066 modulam essas variações ou as expressões genéticas. Coletivamente, este  
1067 trabalho fornece conhecimento fundamental para estudos futuros com o objetivo  
1068 de identificar fatores que influenciam a fertilidade e a perda de prenhez em  
1069 rebanhos de corte.

1070

1071 **6 Impacto econômico, social e inovação**

1072 Com a identificação dos fatores ambientais e fenotípicos que causam  
1073 efeito sobre a prenhez e a manutenção dessa gestação, conseguimos minimizar  
1074 esses fatores que diminuem as taxas de ganho por ano de uma propriedade.  
1075 Com isso, conseguimos impactar positivamente na produção de mais bezerros  
1076 e principalmente da diminuição de matrizes que perdem a gestação e  
1077 permanecem na fazenda por um longo prazo sem produzir.

1078 Além dos ganhos econômicos com a identificação dos fatores que somam  
1079 esses prejuízos, podemos identificar os indivíduos subfértéis dentro do rebanho.  
1080 Com maiores estudos, a identificação de fatores genéticos correlacionado a  
1081 fertilidade aumentará a nossa capacidade de identificar indivíduos que, além de  
1082 diminuir as taxas de prenhez ou aumentar as perdas gestacionais, também  
1083 transferem essa genética para seus produtos.

1084 Outro fator importante é na identificação de indivíduos jovens e com alto  
1085 potencial genético, que com essas identificações de fertilidade, conseguimos  
1086 utilizá-los de forma mais eficiente, além de identificar de forma precoce os  
1087 indivíduos subfértéis.