



**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
2023

LUCAS GOMES DA SILVA

**FATORES QUE INFLUENCIAM A TAXA DE PREENHEZ E MORTALIDADE
EMBRIONÁRIA EM FÊMEAS NELORE SUBMETIDAS À INSEMINAÇÃO
ARTIFICIAL EM TEMPO FIXO NO BRASIL**

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

LUCAS GOMES DA SILVA
Orientador: Erikliis Nogueira

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

SUMÁRIO

RESUMO.....	04
Abstract.....	05
1. INTRODUÇÃO	06
2. REVISÃO DE LITERATURA.....	07
3. REFERENCIAS.....	12
4. ARTIGO ANIMAL REPRODUCTION SCIENCES.....	17
5. Considerações finais	41
6. Impacto econômico, social e inovação.....	41

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

39
40

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

259 **3 REFERÊNCIAS**

- 260 ABDALLA, H., ELGHAFGHUF, A., ELSOHABY, I., AND NASR, M. A. Maternal
261 and non-maternal factors associated with late embryonic and early fetal losses in
262 dairy cows. **Theriogenology** 100, 16-23, 2017.
- 263 AYALON N. A review of embryonic mortality in cattle. **Journal Reproduction**
264 **Fertility**. v.54 p. 483-93 1978.
- 265 BAMBER, R. L., SHOOK, G. E., WILTBANK, M. C., SANTOS, J. E. P., & FRICKE,
266 P. M. Genetic parameters for anovulation and pregnancy loss in dairy cattle.
267 **Journal of dairy science**, 92(11), 5739-5753, 2009.
- 268 BARBOSA, R. T., SILVA, J. D., BERGAMASCHI, M. A. C. M., BERTAN, C. M.,
269 SARTI, L. L., BINELLI, M. A Redução da Mortalidade Embrionária - Estratégia
270 Hormonal para Otimizar a Função Luteínica em Bovinos. 51. ed., p. 11, **São**
271 **Carlos: Embrapa**, 2006.
- 272 BARUSELLI, P. S., SANTOS, G. F. F. D., CREPALDI, G. A., CATUSSI, B. L. C.,
273 OLIVEIRA, A. C. D. S. IATF em números: evolução e projeção futura. **Revista**
274 **Brasileira de Reprodução Animal**, 76-83, 2022.
- 275 BAUERSACHS, S., AND WOLF, E. Immune aspects of embryo-maternal cross-
276 talk in the bovine uterus. **Journal Reproduction Immunology**. 97, 20-26, 2013.
- 277 BRIDGES G. A., MUSSARD M. L., BURKE C. R., DAY M. L. Influence of the
278 length of proestrus on fertility and endocrine function in female cattle. **Animal**
279 **Reproduction Science**. 117:208–215, 2010.
- 280 BRIDGES, G. A., DAY, M. L., GEARY, T. W., CRUPPE, L. H. Triennial
281 Reproduction Symposium: deficiencies in the uterine environment and failure to
282 support embryonic development. **Journal of Animal Science**, v. 91, n. 7, p.
283 3002-3013, 2013.
- 284 BRIDGES, G., KRUSE, S., FUNNELL, B., PERRY, G., GUNN, P., ARIAS, R.,
285 LAKE, S. Changes in body condition on oocyte quality and embryo survival. Proc.
286 Appl. **Reproduction Strategies in Beef Cattle** 23, 269-283, 2012.
- 287 BUTLER, M. L., BORMANN, J. M., WEABER, R. L., GRIEGER, D. M., ROLF, M.
288 M. Selection for bull fertility: a review. **Translational Animal Science**, 4(1), 423-
289 441, 2020.
- 290 CAPRA, E., TURRI, F., LAZZARI, B., CREMONESI, P., GLIOZZI, T. M.,
291 FOJADELLI, I., PIZZI, F. Small RNA sequencing of cryopreserved semen from
292 single bull revealed altered miRNAs and piRNAs expression between High-and
293 Low-motile sperm populations. **BMC genomics**, v. 18, n. 1, p. 1-12, 2017.
- 294 CARVALHO FILHO, I., MARQUES, D. B. D., DE CAMPOS, C. F., GUIMARÃES,
295 J. D., GUIMARÃES, S. E. F., LOPES, P. S., VERONEZE, R. Genetic parameters

- 296 for fertility traits in Nelore bulls. **Reproduction in Domestic Animals**, v. 55, n.
297 1, p. 38-43, 2020.
- 298 CHENG, Z., ABUDUREYIMU, A., OGUEJIOFOR, C. F., ELLIS, R., BARRY, A.
299 T., CHEN, X., ANSTAETT, O. L., BROWNLIE, J. WATHES, D. C. BVDV alters
300 uterine prostaglandin production during pregnancy recognition in cows.
301 **Reproduction** 151, 605-614, 2016.
- 302 CICCIOLI, N., WETTEMANN, R., SPICER, L., LENTS, C., WHITE, F., AND
303 KEISLER, D. Influence of body condition at calving and postpartum nutrition on
304 endocrine function and reproductive performance of primiparous beef cows.
305 **Journal Animal Science**. 81, 3107-3120, 2003.
- 306 DEROUEN, S., FRANKE, D., MORRISON, D., WYATT, W., COOMBS, D.,
307 WHITE, T., HUMES, P., AND GREENE, B. Prepartum body condition and weight
308 influences on reproductive performance of first-calf beef cows. **Journal Animal**
309 **Science**. 72, 1119-1125, 1994.
- 310 DISKIN MG, MORRIS DG. Embryonic and early foetal losses in cattle and other
311 ruminants. **Reproduction in domestic animals**. v. 2, p. 260-7, 2008.
- 312 DISKIN, M., WATERS, S., PARR, M., KENNY, D. Pregnancy losses in cattle:
313 potential for improvement. **Reproduction Fertility**. Dev. 28, 83-93, 2016.
- 314 FARIN, P. W., PIEDRAHITA, J. A., AND FARIN, C. E. Errors in development of
315 fetuses and placentas from in vitro-produced bovine embryos. **Theriogenology**
316 65, 178-191, 2006.
- 317 FERNANDEZ-NOVO, A., FARGAS, O., LOSTE, J. M., SEBASTIAN, F., PEREZ-
318 VILLALOBOS, N., PESANTEZ-PACHECO, J. L., ASTIZ, S. Pregnancy Loss (28–
319 110 Days of Pregnancy) in Holstein Cows: A Retrospective Study. **Animals**, v.
320 10, n. 6, p. 925, 2020.
- 321 FIELDS S. D., GEBHART K. L., PERRY B. L., GONDA M. G., WRIGHT C. L.,
322 BOTT R. C., PERRY G. A. Effects of Initiation of standing estrus prior to an
323 injection of GnRH on LH release, subsequent concentrations of progesterone,
324 and steroidogenic enzyme expression. **Domestic Animal Endocrinology**
325 42:11–19, 2012.
- 326 FRANCO, G. A., PERES, R. F. G., MARTINS, C. F. G., REESE, S. T.,
327 VASCONCELOS, J. L. M., POHLER, K. G. Sire contribution to pregnancy loss
328 and pregnancy-associated glycoprotein production in Nelore cows. **Journal of**
329 **Animal Science**, v. 96, n. 2, p. 632-640, 2018.
- 330 GREEN, J., OKAMURA, C., POOCK, S., AND LUCY, M. Measurement of
331 interferon-tau (IFN- τ) stimulated gene expression in blood leukocytes for
332 pregnancy diagnosis within 18–20d after insemination in dairy cattle. **Animal**
333 **Reproduction Science**. 121, 24-33, 2010.

- 334 HERMISDORFF, I. D. C., DIAZ, I. D. P. S., DE CAMARGO, G. M. F., DE
335 ALBUQUERQUE, L. G., COSTA, R. B. Effect of genomic X-chromosome regions
336 on Nelore bull fertility. **Journal of Applied Genetics**, v. 62, n. 4, p. 655-659,
337 2021.
- 338 ING N. H., TORNESI M. B. Estradiol up-regulates estrogen receptor and
339 progesterone receptor gene expression in specific ovine uterine cells. **Biology**
340 **Reproduction** 56:1205–1215, 1997.
- 341 JUNQUEIRA, JOSÉ R. C., ALFIERI, AMAURI A. Falhas da reprodução na
342 pecuária bovina de corte com ênfase para causas infecciosas. **Semina:**
343 **Ciências Agrárias**. 27, 2, 289-298, 2006.
- 344 KRUSE, S., BRIDGES, G., FUNNELL, B., BIRD, S., LAKE, S., ARIAS, R.,
345 AMUNDSON, O., LARIMORE, E., KEISLER, D., AND PERRY, G. Influence of
346 post-insemination nutrition on embryonic development in beef heifers.
347 **Theriogenology** 90, 185-190, 2017.
- 348 MANN G. E., LAMMING G. E. The influence of progesterone during early
349 pregnancy in cattle. **Reproduction Domestic Animals** 34:269–274, 1999.
- 350 MARTINI, A. P.; PESSOA, G. A.; RUBIN, M. I. B. Conception rate according to
351 sire, body condition score and estrus occurrence of suckled *Bos taurus* beef cows
352 submitted to timed artificial insemination. **Arquivo Brasileiro de Medicina**
353 **Veterinária e Zootecnia**, v. 74, p. 375-382, 2022.
- 354 MORAES, J. C. F.; DE SOUZA, C. J. H.; GONÇALVES, P. B. D. Controle do
355 estro e da ovulação em bovinos e ovinos. In: **Biotecnicas aplicadas à**
356 **reprodução animal**. São Paulo, Livraria, 2001.
- 357 NOGUEIRA, E., TIRPÁK, F., HAMILTON, L. E., ZIGO, M., KERNS, K.,
358 SUTOVSKY, M., SUTOVSKY, P. A Non-Synonymous Point Mutation in a WD-40
359 Domain Repeat of EML5 Leads to Decreased Bovine Sperm Quality and Fertility.
360 **Frontiers in cell and developmental biology**, v. 10, 2022.
- 361 ORTEGA, M. S., MORAES, J. G., PATTERSON, D. J., SMITH, M. F., BEHURA,
362 S. K., POOCK, S., SPENCER, T. E. Influences of sire conception rate on
363 pregnancy establishment in dairy cattle. **Biology of reproduction**, v. 99, n. 6, p.
364 1244-1254, 2018.
- 365 PERRY, G., PERRY, B., WALKER, J., WRIGHT, C., SALVERSON, R., AND
366 PATTERSON, H. Evaluation of prior grazing experience on reproductive
367 performance in beef heifers. **Prof. Animal Science**. 29, 595-600, 2013.
- 368 PERRY, G., SMITH, M., ROBERTS, A., MACNEIL, M., AND GEARY, T.
369 Relationship between size of the ovulatory follicle and pregnancy success in beef
370 heifers. **Journal of Animal Science**. 85, 684-689, 2007.

- 371 PESSOA, J. D. S., ROLIN FILHO, S. T., RIBEIRO, H. F. L., GARCIA, O. S.,
372 NUNES, K. D. B., AND AMORIM, B. S. D. Embryonic loss in cows submitted to
373 timed AI in the northeast of Para. **Ciencia Animal** 22, 239-241, 2012.
- 374 POHLER, K. G., GEARY, T. W., ATKINS, J. A., PERRY, G. A., JINKS, E. M.,
375 SMITH, M. F. Follicular determinants of pregnancy establishment and
376 maintenance. **Cell and tissue research**, 349(3), 649-664, 2012.
- 377 POHLER, K., PEREIRA, M., LOPES, F., LAWRENCE, J., KEISLER, D., SMITH,
378 M., VASCONCELOS, J., GREEN, J. Circulating concentrations of bovine
379 pregnancy-associated glycoproteins and late embryonic mortality in lactating
380 dairy herds. **Journal Dairy Science**. 99, 1584-1594, 2016.
- 381 POPE, W. Uterine asynchrony: a cause of embryonic loss. **Biology of**
382 **Reproduction**. 39, 999-1003, 1988.
- 383 PUGLIESI, G., MIAGAWA, B. T., PAIVA, Y. N., FRANÇA, M. R., SILVA, L. A.,
384 AND BINELLI, M. Conceptus-induced changes in the gene expression of blood
385 immune cells and the ultrasound-accessed luteal function in beef cattle: how early
386 can we detect pregnancy. **Biology Reproduction**. 91,95, 91-12, 2014.
- 387 REESE ST, FRANCO GA, POOLE RK, HOOD R, FERNADEZ MONTERO L,
388 OLIVEIRA FILHO RV, COOKE RF, POHLER KG. Pregnancy loss in beef cattle:
389 a meta-analysis. **Animal Reproduction Science**. 2019.
- 390 RICHARDSON, B. N., HILL, S. L., STEVENSON, J. S., DJIRA, G. D., AND
391 PERRY, G. A. Expression of estrus before fixed-time AI affects conception rates
392 and factors that impact expression of estrus and the repeatability of expression
393 of estrus in sequential breeding seasons. **Animal Reproduction Science**. 166,
394 133- 140, 2016.
- 395 ROBERTS, R. M., AND SCHALUE-FRANCIS, T. Maternal recognition of
396 pregnancy and embryonic loss. **Theriogenology** 33, 175-183, 1990.
- 397 SANTOS, S. A., ABREU, U. G. P. D., SOUZA, G. D. S., & CATTO, J. B. Condição
398 corporal, variação de peso e desempenho reprodutivo de vacas de cria em
399 pastagem nativa no Pantanal. **Revista Brasileira de Zootecnia**, 38(2), 354-360,
400 2009.
- 401 SANTOS, J.E.P., THATCHER, W.W., CHEBEL, R.C., CERRI, R.L.A., GALVÃO,
402 K.N., 2004. The effect of embryonic death rates in cattle on the efficacy of estrus
403 synchronization programs. **Anim. Reprod. Sci.** 82, 513–35.
404 <https://doi.org/10.1016/j.anireprosci.2004.04.015>.
- 405 SIGDEL, A.; BISINOTTO, R. S.; PEÑAGARICANO, F. Genes and pathways
406 associated with pregnancy loss in dairy cattle. **Scientific reports**, v. 11, n. 1, p.
407 1-11, 2021.

- 408 STONE G. M., MURPHY L., MILLER B. G. Hormone receptor levels and
409 metabolic activity in the uterus of the ewe: regulation by oestradiol and
410 progesterone. **Australian Journal Biology Science** 31:395–403, 1978.
- 411 TAYLOR, J. F.; SCHNABEL, R. D.; SUTOVSKY, P. Genomics of bull fertility.
412 **Animal**, v. 12, n. s1, p. s172-s183, 2018.
- 413 THUNDATHIL, J. C.; DANCE, A. L.; KASTELIC, J. P. Fertility management of
414 bulls to improve beef cattle productivity. **Theriogenology**, v. 86, n. 1, p. 397-405,
415 2016.
- 416 VASCONCELOS, J. L. M.; MENEGHETTI, M. Sincronização de ovulação como
417 estratégia para aumentar a eficiência reprodutiva de fêmeas bovinas, em larga
418 escala. **Simpósio de Produção de Gado de Corte**, v. 5, p. 529-541, 2006.
- 419 YANG, L., ZHANG, L., QIAO, H., LIU, N., WANG, Y., AND LI, S. Maternal immune
420 regulation by conceptus during early pregnancy in the bovine. **Asian Journal**
421 **Animal Veterinary**. Adv. 9, 610-620, 2014.
- 422 ZELINSKI M. B., NOEL P., WEBER D. W., STORMSHAK F. Characterization of
423 cytoplasmic progesterone receptors in the bovine endometrium during proestrus
424 and diestrus. **Journal Animal Science** 55:376–383, 1982.
- 425

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**

431 Lucas Gomes da Silva⁴, Luana Gomes da Silva², Luiz Carlos Louzada Ferreira³, Julia
432 Mascarello⁴, Joao Gabriel Nascimento Moraes⁵, Matthew C. Lucy⁶, Ériklis Nogueira^{1,4*}
433

434 1- Brazilian Agricultural Research Corporation- EMBRAPA, 21 de Setembro Street,
435 1880, 79320-900, Corumbá, MS, Brazil

436 2- Universidade Federal de Mato Grosso do Sul, Post-Graduation Program of Animal
437 Science, Campo Grande-MS, Brazil.

438 3- Cia Pecuária Assessoria, Campo Grande-MS, Brazil.

439 4- Universidade Federal de Mato Grosso do Sul, Post Graduation Program of Veterinary
440 Medicine- CIVET-UFMS, Campo Grande, Brazil.

441 5- Oklahoma State University, Stillwater, OK, USA

442 6- University of Missouri, Columbia, MO, USA

443 *eriklis.nogueira@embrapa.br
444

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; Ciccio 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.

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 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

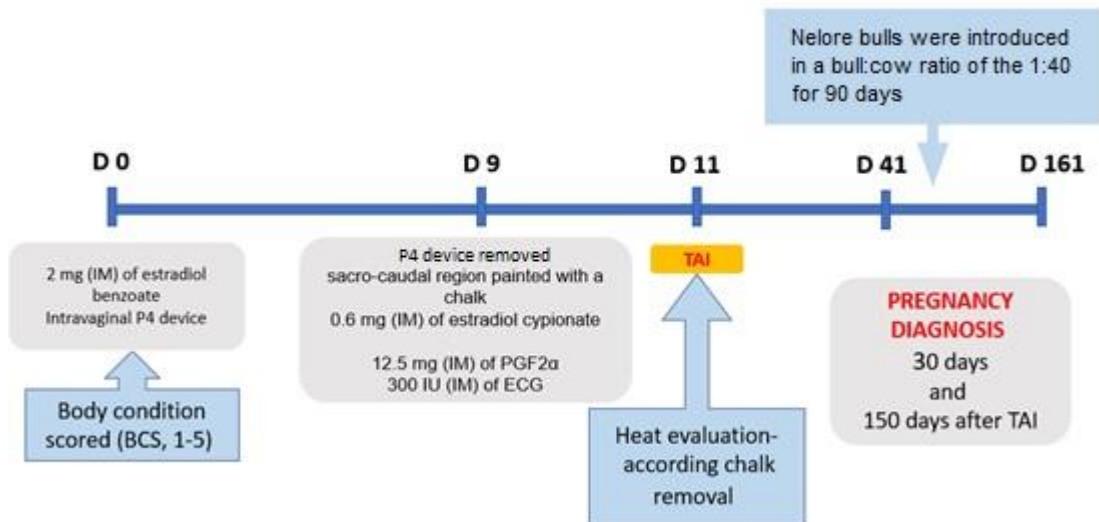
536

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.

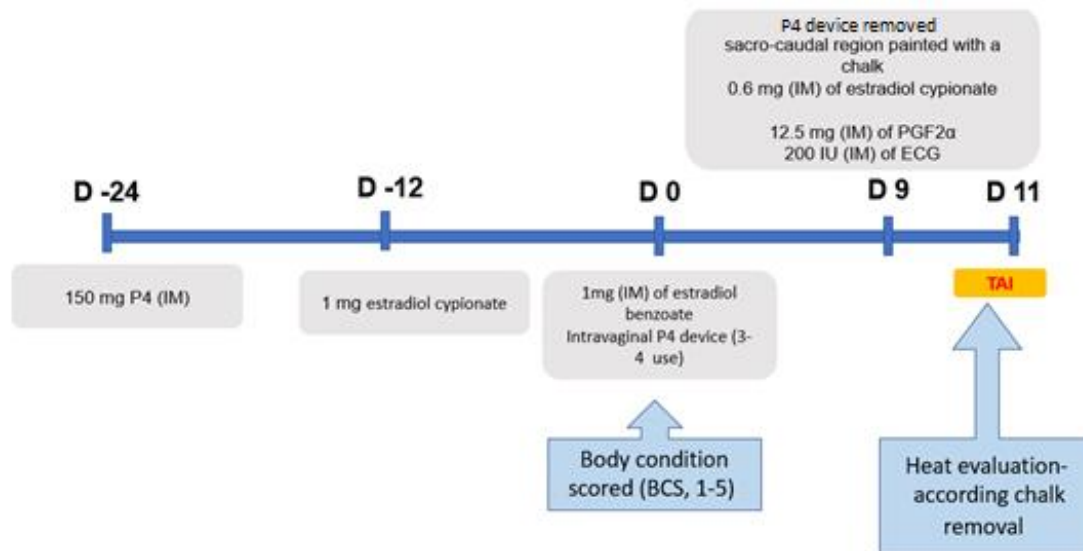


562 **Figure 1.** Artificial insemination protocol used for multiparous cows. On day (D0),
 563 multiparous cows received 2 mg (IM) of estradiol benzoate (Gonadiol®; Zoetis, São
 564 Paulo, SP, Brazil) and an intravaginal progesterone insert (CIDR®, 1.9 g progesterone;
 565 Zoetis). Body condition was 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.

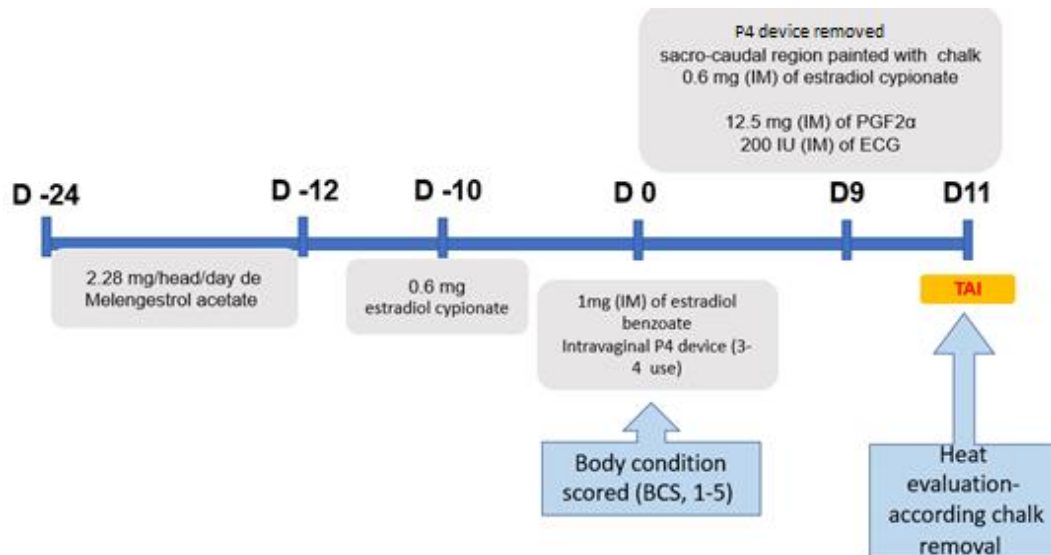


582

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 PGF2 α
 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
 612 with 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 PDIFF 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(%)
Category (n)		
Heifer (7,348)	47.90 ^d	5.03 ^c
Primiparous (8,181)	51.41 ^{bc}	7.11 ^b
Secondiparous (2,914)	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 (2,582)	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
<i>P</i>	<0.001	<0.001
Farm (n)		
A (923)	55.58% ^a	4.71% ^b
B (8,570)	51.06% ^b	5.77% ^b
C (5,313)	55.51% ^a	4.44% ^b
D (416)	49.28% ^b	3.91% ^b
E (3,936)	54.17% ^a	3.37% ^b
F (20,946)	53.11% ^a	6.89% ^a
<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

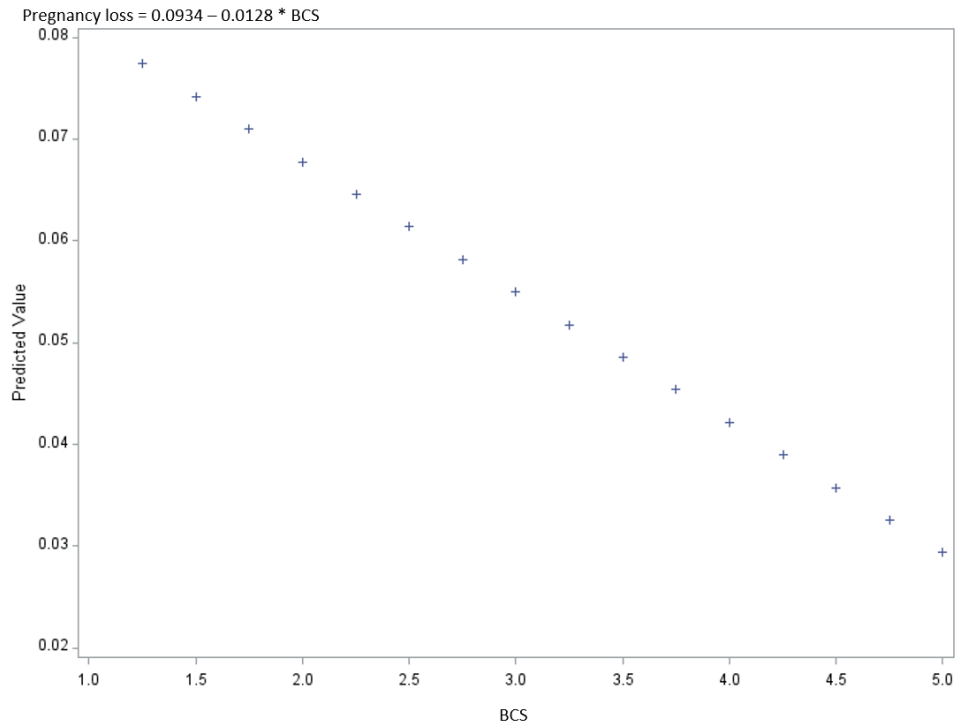
688 **Table 3.** Pregnancy per artificial insemination (P/AI) and pregnancy loss (%)
 689 according to month of parturition, sire breed, expression of estrus, and velocity
 690 score for exiting the chute.

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

691

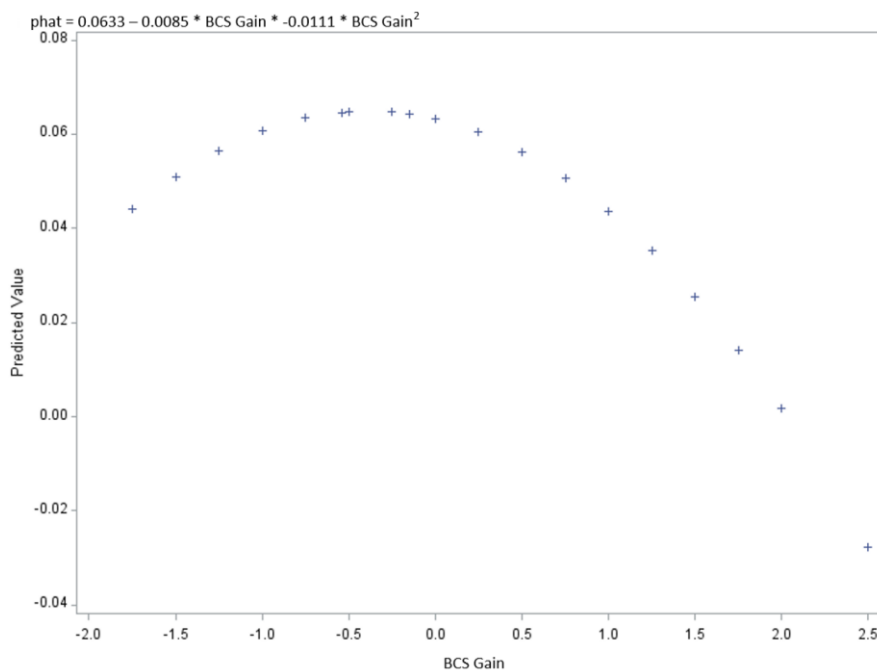
692 There was a significant effect of BCS at the onset of estrous
 693 synchronization on P/AI ($P < 0.001$) and pregnancy loss ($P < 0.001$). The
 694 probability of embryonic mortality decreased as BCS increased (Figure 4; $P <$

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 ($P < 0.05$).

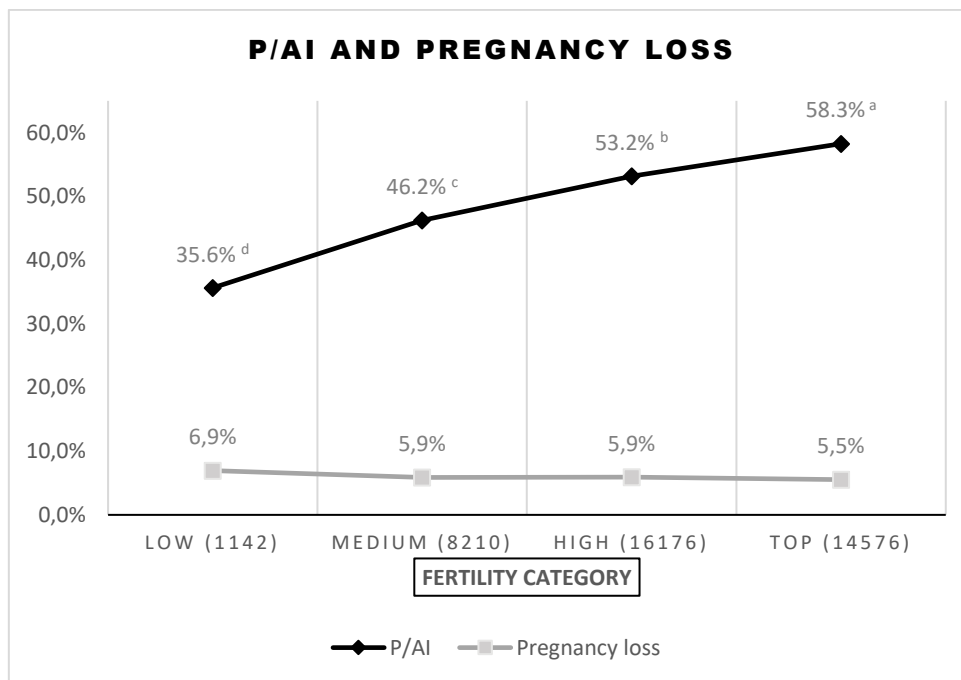


701

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

847 **4.6 References**

- 848 AHMAD, N., SCHRICK, F.N., BUTCHER, R.L., INSKEEP, E.K., 1995. Effect of
849 persistent follicles on early embryonic losses in beef cows. **Biol. Reprod.**
850 52,1129–35. <https://doi.org/10.1095/biolreprod52.5.1129>.
- 851 BAMBER, R. L., SHOOK, G. E., WILTBANK, M. C., SANTOS, J. E. P., & FRICKE,
852 P. M., 2009. Genetic parameters for anovulation and pregnancy loss in dairy
853 cattle. **J. Dairy sci.** 92, 5739-5753.
- 854 BARBOSA, R. T., SILVA, J. D., BERGAMASCHI, M. A. C. M., BERTAN, C. M.,
855 SARTI, L. L., BINELLI, M., 2006. A Redução da Mortalidade Embrionária -
856 Estratégia Hormonal para Otimizar a Função Luteínica em Bovinos.
857 **Documentos Embrapa.** 51,1-11.
- 858 BARTH, A. D., OKO, R.J.,1989. **Abnormal Morphology of Bovine**
859 **Spermatozoa.** Iowa State University Press.
- 860 BARUSELLI, P. S., SANTOS, G. F. F. D., CREPALDI, G. A., CATUSSI, B. L. C.,
861 OLIVEIRA, A. C. D. S., 2022. FTAI in numbers: evolution and future projection.
862 **Rev. Bras. Rep. Animal,** 46,76-83.
- 863 BOIVIN, X., LE NEINDRE, P., CHUPIN, J.M., 1992. Establishment of cattle-
864 human relationship. **App. Ani. Beh. Sci.**, 32, 325-335.
- 865 BRIDGES, G. A., MUSSARD, M. L., BURKE, C. R., DAY, M. L., 2010. Influence
866 of the length of proestrus on fertility and endocrine function in female cattle.
867 **Anim. Rep. Sci.** 117, 208–215.
- 868 BRIDGES, G., KRUSE, S., FUNNELL, B., PERRY, G., GUNN, P., ARIAS, R.,
869 LAKE, S., 2012. Changes in body condition on oocyte quality and embryo
870 survival. **Proc. Appl. Reprod. Strat. Beef Cattle.** 23, 269-283.
- 871 BUTLER, M. L., BORMANN, J. M., WEABER, R. L., GRIEGER, D. M., ROLF, M.
872 M., 2020. Selection for bull fertility: a review. **Trans. Ani. Sci.** 4, 423-441.
- 873 CAPRA, E., TURRI, F., LAZZARI, B., CREMONESI, P., GLIOZZI, T. M.,
874 FOJADELLI, I., PIZZI, F., 2017. Small RNA sequencing of cryopreserved semen
875 from single bull revealed altered miRNAs and piRNAs expression between High-
876 and Low-motile sperm populations. **BMC genomics.** 18, 1-12.
- 877 CARVALHO FILHO, I., MARQUES, D. B. D., DE CAMPOS, C. F., GUIMARÃES,
878 J. D., GUIMARÃES, S. E. F., LOPES, P. S., VERONEZE, R., 2020. Genetic
879 parameters for fertility traits in Nelore bulls. **Rep. Dom. Ani.** 55, 38-43.

880 CICCIOLO, N., WETTEMANN, R., SPICER, L., LENTS, C., WHITE, F., AND
881 KEISLER, D., 2003. Influence of body condition at calving and postpartum
882 nutrition on endocrine function and reproductive performance of primiparous beef
883 cows. **J. Ani. Sci.** 81, 3107-3120.

884 COOKE, R.F., ARTHINGTON, J.D., AUSTIN, B.R., YELICH, J.V., 2009. Effects
885 of acclimation to handling on performance, reproductive, and physiological
886 responses of Brahman-crossbred heifers. **J. Anim. Sci.** 87, 3403-3412

887 COOKE, R. F., BOHNERT, D. W., MENEGHETTI, M., LOSI, T. C.,
888 VASCONCELOS, J. L. M., 2011. Effects of temperament on pregnancy rates to
889 fixed-timed AI in *Bos indicus* beef cows. **Liv. Sci.** 142, 108-113.

890 CUSHMAN, R.A., KILL, L.K., FUNSTON, R.N., MOUSEL, E.M. AND PERRY,
891 G.A., 2013. Heifer calving date positively influences calf weaning weights through
892 six parturitions. **J. Ani. Sci.** 91(9), 4486-4491.

893 DEROUEN, S., FRANKE, D., MORRISON, D., WYATT, W., COOMBS, D.,
894 WHITE, T., HUMES, P., AND GREENE, B. 1994. Prepartum body condition and
895 weight influences on reproductive performance of first-calf beef cows. **J. Ani. Sci.**
896 72, 1119-1125.

897 DISKIN, M.G., MORRIS, D.G. 2008. Embryonic and early foetal losses in cattle
898 and other ruminants. **Reprod. Domest. Anim.** 43 (Suppl 2),260–7.
899 <https://doi.org/10.1111/j.1439-0531.2008.01171.x>.

900 DISKIN, M.G., SREENAN, J.M., 1980. Fertilization and embryonic mortality rates
901 in beef heifers after artificial insemination. **J. Reprod. Fertil.** 59, 463–8.
902 <https://doi.org/10.1530/jrf.0.0590463>.

903 DISKIN, M.G., KENNY, D.A., 2016. Managing the reproductive performance of
904 beef cows. **Theriogenology**, 86 (1), 379-387.

905 D'OCCHIO, M.J., BARUSELLI, P.S., CAMPANILE, G., 2019. Influence of
906 nutrition, body condition, and metabolic status on reproduction in female beef
907 cattle: A review. **Theriogenology**, 125, 277-284.

908 EALY, A.D., 2020. Pregnancy Losses in Livestock: An Overview of the
909 Physiology and Endocrinology Symposium for the 2020 ASAS-CSAS-WSASAS
910 Virtual Meeting. **J. Anim. Sci.** 98:skaa277.
911 [HTTPS://DOI.ORG/10.1093/JAS/SKAA277](https://doi.org/10.1093/JAS/SKAA277).

912 FERGUSON, J.D., BYERS, D., FERRY, J., 1994. Round table discussion: body
913 condition of lactating cows. **Agric. Practice.** 15, 7-21.

914 FIELDS S. D., GEBHART K. L., PERRY B. L., GONDA M. G., WRIGHT C. L.,
915 BOTT R. C., PERRY G. A., 2012. Effects of Initiation of standing estrus prior to
916 an injection of GnRH on LH release, subsequent concentrations of progesterone,
917 and steroidogenic enzyme expression. **Dom. Ani. End.** 42,11–19.

918 FORDYCE, G.; GODDARD, M.E., 1984. Maternal Influence on the temperament
919 of Bosindicus cross cows. **Proc. Aust. Soc. Ani. Prod.** 15, 345-348.

920 FRANCO, G.A.; PERES, R.F.G.; MARTINS, C.F.G.; REESE, S.T.;
921 VASCONCELOS, J.L.M; POHLER, K.G. 2018. Sire contribution to pregnancy
922 loss and pregnancy-associated glycoprotein production in Nelore cows **J. Anim.**
923 **Sci.** 96, 632–640 doi: 10.1093/jas/sky015

924 GOMES DA SILVA, L.;GOMES DA SILVA, L.; PEREIRA, JLG.; FERREIRA,
925 LCL.; MATEUS, RG.; NOGUEIRA, E., 2020. Influence of chute exit speed score
926 under the pregnancy rate in Nelore dams submitted to timed artificial
927 insemination. **Proceedings:** 34th Annual meeting of the brazilian embryo
928 technology society (sbte). Anim. Reprod. 17.

929 GREEN, J., OKAMURA, C., POOCK, S., AND LUCY, M., 2010. Measurement of
930 interferon-tau (IFN- τ) stimulated gene expression in blood leukocytes for
931 pregnancy diagnosis within 18–20d after insemination in dairy cattle. **Anim. Rep.**
932 **Sci.** 12, 24-33.

933 HEMSWORTH, P. H., COLEMAN, G. J., BARNETT, J. L., BORG, S., 2000.
934 Relationships between human-animal interactions and productivity of commercial
935 dairy cows. **J. Ani. Sci.** 78, 2821-2831.

936 ING N. H., TORNESI M. B., 1997. Estradiol up-regulates estrogen receptor and
937 progesterone receptor gene expression in specific ovine uterine cells. **Bio. Repr.**
938 56, 1205–1215.

939 KIM, I.H., JEONG, J.K., KIM, U.H., 2023. Impact of postpartum time period
940 associated body condition score on reproductive performance in dairy cows.
941 *Theriogenology* 209, 107-114.

942 KRUSE, S., BRIDGES, G., FUNNELL, B., BIRD, S., LAKE, S., ARIAS, R.,
943 AMUNDSON, O., LARIMORE, E., KEISLER, D., AND PERRY, G., 2017.
944 Influence of post-insemination nutrition on embryonic development in beef
945 heifers. **Theriogenology.** 90, 185-190.

946 LUCY, M.C., 2001. Reproductive loss in high-producing dairy cattle: where will it
947 end? **J. Dairy Sci.** 84, 1277–93. [https://doi.org/10.3168/jds.S0022-0302\(01\)70158-0](https://doi.org/10.3168/jds.S0022-0302(01)70158-0).

- 949 MAURER, R.R., CHENAULT, J.R., 1983. Fertilization failure and embryonic
950 mortality in parous and nonparous beef cattle. **J. Anim. Sci.** 56, 1186–9.
951 <https://doi.org/10.2527/jas1983.5651186x>.
- 952 MENEGHETTI, M., SÁ FILHO, O. G., PERES, R. F. G., LAMB, G. C., &
953 VASCONCELOS, J. L. M., 2009. Fixed-time artificial insemination with estradiol
954 and progesterone for *Bos indicus* cows I: basis for development of
955 protocols. **Theriogenology**. 72, 179-189.
- 956 MENEGHETTI, M.; VASCONCELOS, J. L. M., 2008. Calving date, body condition
957 score, and response to a timed artificial insemination protocol in first-calving beef
958 cows. **Arq. Bras. Med. Vet. Zoot.** 60, 786-793.
- 959 NOGUEIRA, E., SILVA, M. R., SILVA, J. C. B., ABREU, U. P. G., ANACHE, N.
960 A., SILVA, K. C., RODRIGUES, W. B., 2019. Timed artificial insemination plus
961 heat I: effect of estrus expression scores on pregnancy of cows subjected to
962 progesterone–estradiol-based protocols. **Animal**, 13, 2305-2312.
- 963 NOGUEIRA, E., TIRPÁK, F., HAMILTON, L. E., ZIGO, M., KERNS, K.,
964 SUTOVSKY, M., SUTOVSKY, P., 2022. A Non-Synonymous Point Mutation in a
965 WD-40 Domain Repeat of EML5 Leads to Decreased Bovine Sperm Quality and
966 Fertility. **Fro. cell dev. Bio.**10, article872740.
- 967 NORMAN, H.D., GUINAN, F.L., MEGONIGAL, J.H., DÜRR, J.W., 2020.
968 Reproductive Status of DHI Cows n.d.
969 <https://queries.uscdcb.com/publish/dhi/current/reproall.html>. (accessed August
970 8, 2023).
- 971 ORTEGA, M. S., MORAES, J. G., PATTERSON, D. J., SMITH, M. F., BEHURA,
972 S. K., POOCK, S., SPENCER, T. E., 2018. Influences of sire conception rate on
973 pregnancy establishment in dairy cattle. **Bio. Repr.** 99, 1244-1254.
- 974 PERRY, G., SMITH, M., ROBERTS, A., MACNEIL, M., AND GEARY, T., 2007.
975 Relationship between size of the ovulatory follicle and pregnancy success in beef
976 heifers. **J. Ani.Sci.** 85,684-689.
- 977 PERRY, G., DALTON, J., GEARY, T., 2011. Management factors influencing
978 fertility in beef cattle breeding programmes. Boise: **Proceedings**, Applied
979 Reproductive Strategies in Beef Cattle-Northwest.
- 980 PESSOA, J. D. S., ROLIN FILHO, S. T., RIBEIRO, H. F. L., GARCIA, O. S.,
981 NUNES, K. D. B., AND AMORIM, B. S. D., 2012. Embryonic loss in cows
982 submitted to timed AI in the northeast of Para. **Cie. Anim.** 22, 239-241.

- 983 PILAU, A., LOBATO, J. F. P., 2009. Reproductive performance of primiparous
984 cows at 22/24 months of age. **Rev. Bras. Zoo.** 38, 728-736.
- 985 POHLER, K. G., GEARY, T. W., ATKINS, J. A., PERRY, G. A., JINKS, E. M.,
986 SMITH, M. F., 2012. Follicular determinants of pregnancy establishment and
987 maintenance. **Cell and tissue research.** 349, 649-664.
- 988 REESE, S.T., FRANCO, G.A., POOLE, R.K., HOOD, R., MONTERO, L.F.,
989 OLIVEIRA FILHO, R.V., COOKE, R.F., POHLER, K.G., 2020. Pregnancy loss in
990 beef cattle: a meta-analysis. **Anim. Rep. Sci.** 2012, Article 106251
- 991 RIBEIRO, E.S., GOMES, G., GRECO, L.F., CERRI, R.L.A, VIEIRA-NETO, A.,
992 MONTEIRO, P.L.J., et al., 2016. Carryover effect of postpartum inflammatory
993 diseases on developmental biology and fertility in lactating dairy cows. **J. Dairy**
994 **Sci.** 99, 2201–20. <https://doi.org/10.3168/jds.2015-10337>.
- 995 RICHARDSON, B. N., HILL, S. L., STEVENSON, J. S., DJIRA, G. D., AND
996 PERRY, G. A., 2016. Expression of estrus before fixed-time AI affects conception
997 rates and factors that impact expression of estrus and the repeatability of
998 expression of estrus in sequential breeding seasons. **Anim. Rep. Sci.** 166,133-
999 140.
- 1000 RODRIGUES, A., COOKE, R., CIPRIANO, R., SILVA, L., CERRI, R., CRUPPE,
1001 L., MENEGHETTI, M., POHLER, K., VASCONCELOS, J., 2018. Impacts of
1002 estrus expression and intensity during a timed-AI protocol on variables
1003 associated with fertility and pregnancy success in Bos indicus-influenced beef
1004 cows. **J. Ani. Sci.** 96, 236-249.
- 1005 RUEDA, P. M., SANT'ANNA, A. C., VALENTE, T. S., DA COSTA, M. J. P., 2015.
1006 Impact of the temperament of Nelore cows on the quality of handling and
1007 pregnancy rates in fixed-time artificial insemination. **Liv. Sci.** 177,189-195.
- 1008 SÁ FILHO, O., DIAS, C., LAMB, G., AND VASCONCELOS, J., 2010.
1009 Progesterone-based estrous synchronization protocols in non-suckled and
1010 suckled primiparous Bos indicus beef cows. **Anim. Rep. Sci.** 119, 9-16.
- 1011 SANCHES, C., COSTA e SILVA, E.V., DODE, M.A.N., CUNHA, A.T.M., GARCIA,
1012 W.R., SAMPAIO, B.F.B., SILVA, J.C.B., VAZ, F.E.M., KERNS, K., SUTOVSKY,
1013 P., NOGUEIRA, E., 2023. Semen quality of Nelore and Angus bulls classified by
1014 fertility indices and relations with field fertility in fixed-time artificial insemination.
1015 **Theriogenology**, 212,148-156.
- 1016 SANTOS, J.E.P., THATCHER, W.W., CHEBEL, R.C., CERRI, R.L.A., GALVÃO,
1017 K.N., 2004. The effect of embryonic death rates in cattle on the efficacy of estrus

- 1018 synchronization programs. **Anim. Reprod. Sci.** 82, 513–35.
1019 <https://doi.org/10.1016/j.anireprosci.2004.04.015>.
- 1020 SARTORI, R., BASTOS, M.R., WILTBANK, M.C., 2010. Factors affecting
1021 fertilisation and early embryo quality in single- and superovulated dairy cattle.
1022 **Reprod. Fertil. Dev.** 22, 151–8. <https://doi.org/10.1071/RD09221>.
- 1023 SARTORI, R., SARTOR-BERGFELT, R., MERTENS, S.A., GUENTHER, J.N.,
1024 PARRISH, J.J., WILTBANK, M.C., 2002. Fertilization and early embryonic
1025 development in heifers and lactating cows in summer and lactating and dry cows
1026 in winter. **J. Dairy Sci.** 85, 2803–12. [https://doi.org/10.3168/jds.S0022-0302\(02\)74367-1](https://doi.org/10.3168/jds.S0022-0302(02)74367-1).
1027
- 1028 SATO, S., 1981. Factors associated with temperament of beef cattle, **Jap. J.**
1029 **Zoot. Sci.** 52, 595-605.
- 1030 SIGDEL, A., BISINOTTO, R. S., PEÑAGARICANO, F., 2021. Genes and
1031 pathways associated with pregnancy loss in dairy cattle. **Sci. Rep.** 11, 1-11.
- 1032 SILVA, C.S; COSTA E SILVA, E.V.; DODE, M.A.N; CUNHA, A.T.M.; GARCIA,
1033 W.R.; SAMPAIO, B.F.B; SILVA, J.C.B.; VAZ, F.E.M; KERNS, K.; SUTOVSKY,
1034 P.; NOGUEIRA. E. 2023. Semen quality of Nellore and Angus bulls classified by
1035 fertility indices and relations with field fertility in fixed-time artificial insemination.
1036 **Theriogenology**, 212, 148-156. <https://doi.org/10.1016/j.theriogenology.2023.09.001>
- 1037 SREENAN, J., DISKIN, M., 1986. The extent and timing of embryonic mortality in
1038 the cow. Embryonic mortality in farm animals, **Springer**. p. 1–11.
- 1039 STONE G. M., MURPHY L., MILLER B. G., 1978. Hormone receptor levels and
1040 metabolic activity in the uterus of the ewe: regulation by oestradiol and
1041 progesterone. **Aust. J. Bio. Sci.** 31, 395–403.
- 1042 TAYLOR, J. F., SCHNABEL, R. D., SUTOVSKY, P., 2018. Genomics of bull
1043 fertility. **Animal**. 12, 172-183.
- 1044 VASCONCELOS, J. L. M., SÁ FILHO, O. G., COOKE, R. F., 2014. Impacts of
1045 reproductive technologies on beef production in South America. **Cur. Fut. Rep.**
1046 **Tech. World Food Prod.** 161-180.
- 1047 WALSH, S.W., WILLIAMS, E.J., EVANS, A.C.O., 2011. A review of the causes
1048 of poor fertility in high milk producing dairy cows. **Anim. Reprod Sci.** 123, 127–
1049 38. <https://doi.org/10.1016/j.anireprosci.2010.12.001>.
- 1050 WILTBANK, M..C, BAEZ, G.M,, GARCIA-GUERRA, A., TOLEDO, M.Z.,
1051 MONTEIRO, P.L.J., MELO, L.F., et al., 2016. Pivotal periods for pregnancy loss

1052 during the first trimester of gestation in lactating dairy cows. **Theriogenology**. 86,
1053 239–53. <https://doi.org/10.1016/j.theriogenology.2016.04.037>.

1054 ZELINSKI M. B., NOEL P., WEBER D. W., STORMSHAK, F., 1982.
1055 Characterization of cytoplasmic progesterone receptors in the bovine
1056 endometrium during proestrus and diestrus. **J. Ani. Sci.** 55, 376–383.

1057

1058

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 genicas. 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 bezerras
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 subférteis 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 subférteis.