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Greater Reproductive Performance in Holstein Dairy Cows with Moderate Length of Anogenital Distance at First Service Postpartum

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Abstract

BACKGROUND: Previous studies have indicated negative association of anogenital distance (AGD) with fertility in dairy cows; however, the mechanism of inverse relationship is not completely understood. In this regard, postpartum uterine infections and their corresponding risk factors could diminish fertility of cows, yet there has been no research exploring the relationship between AGD and postpartum disorders.

22 **OBJECTIVES:** The aim of this study was to investigate the relationship between AGD and
23 postpartum reproductive performance in dairy cows.

24 **METHODS:** AGD of Holstein dairy cows of a commercial dairy herd ($n = 290$) was measured at
25 the first postpartum examination (days 28 to 32 postpartum) in millimeter. Cows were classified
26 into three categories based on AGD length, including short anogenital distance (20% of cows with
27 lowest values), intermediate anogenital distance (60% of cows with moderate values) and long
28 AGD (20% of cows with highest values). Additionally, data of postpartum reproductive variables
29 were retrieved from herd database. Data were analysed using SAS software version 9.4.

30 **RESULTS:** Rate of dystocia, twinning, retention of fetal membranes, puerperal metritis and
31 clinical endometritis, calf birth weight and days to first service did not differ among the various
32 AGD categories ($P > 0.05$). However, proportion of male offspring was lower in short AGD cows
33 than intermediate and long AGD cows ($P < 0.05$). Furthermore, first service conception rate was
34 greater in intermediate anogenital distance group than short and long anogenital distance groups
35 ($P < 0.05$).

36 **CONCLUSIONS:** In conclusion, the present study showed suboptimal first postpartum
37 conception rate in cows with minimal and maximal length of anogenital distance and indicated
38 that this inferior fertility was not mediated through alteration in rate of postpartum reproductive
39 disorders.

40 **KEYWORDS:** Anogenital distance; Dairy cows; Dystocia; Fertility; Uterine infections

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42 Introduction

43 Anogenital distance (AGD), which is defined as the distance between anus and clitoris in
44 female individuals, is an anthropometric index reflecting fetal exposure to androgens (Gore et al.,
45 2015; Thankamony et al., 2016; 2016; Gobikrushanth et al., 2017; Akbarinejad et al., 2019).
46 Hereby, AGD is suggested as an indicator for assessment of the impact of endocrine disruptor
47 chemicals on ontogeny of reproductive organs in male and female offspring (Gore et al., 2015;
48 Thankamony et al., 2016; Gobikrushanth et al., 2017; Akbarinejad et al., 2019). More recently,
49 Gobikrushanth et al. 2017 characterized AGD in dairy cows and found that length of AGD was
50 adversely associated with first service conception rate and likelihood of pregnancy in dairy cows
51 (Gobikrushanth et al., 2017). Furthermore, another study substantiated the inverse association of
52 AGD length with fertility in dairy cows and reported delayed first postpartum insemination,
53 diminished first service conception rate, escalated proportion of repeat breeders and prolonged
54 calving to conception interval in dairy cows with long AGD as compared to those with short AGD
55 (Akbarinejad et al., 2019). Yet the mechanisms underlying this negative association between
56 length of AGD and reproductive performance in bovine is not completely known (Gobikrushanth
57 et al., 2017; Akbarinejad et al., 2019).

58 Postpartum uterine infections, including metritis and endometrosis, are considered as
59 contributors to suboptimal fertility in cows by delaying uterine involution, rendering the uterus
60 susceptible to chronic infections and causing ovarian dysfunction (Sheldon et al., 2006; Williams
61 et al. 2007; Giuliadori et al., 2013; Sheldon and Owens, 2017). Furthermore, various postpartum
62 items have been indicated as risk factors for postpartum uterine infections, including twinning

63 birth, dystocia, retained fetal membranes, excessive calf birth weight and male offspring (Ghavi
64 Hossein-Zadeh and Ardalan, 2011; Giuliadori et al., 2013). Nevertheless, to the best of our
65 knowledge, there is no data available whether AGD is related to postpartum uterine infections and
66 their corresponding risk factors.

67 Accordingly, this study was primarily designed to understand whether the reverse
68 association of AGD with fertility in cows is attributable to different rates of postpartum
69 reproductive complications among cows with various length of AGD. In addition, given that this
70 study was carried out on Holstein dairy cows of different parities across various seasons, the effect
71 of parity as well as season was also investigated in the present research.

73 **Materials and Methods**

74 **Ethical statement and study design**

75 This study was approved by Animal Ethics Committee at University of Tehran with respect
76 to animal welfare and ethics (6/6/30854). The study was carried out at a commercial farm located
77 in Tehran province from August, 2018 to March, 2019. Voluntary waiting period was 50 days in
78 the herd and cows were subjected to insemination 12 hours after detection of standing heat. Estrus
79 detection was performed thrice a day by visual observation for at least 30 minutes each time. All
80 artificial inseminations were done by the same technician and insemination of sexed semen was
81 merely performed in heifers. Pregnancy of cows was routinely diagnosed 40 to 45 days after
82 insemination using rectal examination. The research plan of this study was to measure AGD period

83 at the first postpartum examination of cows in order to associate AGD with reproductive
84 parameters in dairy cows and the sample size of the study was 290 dairy cows.

85 **Assessment of AGD**

86 AGD was measured at the first examination postpartum (days 28 to 32 postpartum) by
87 determining the distance from anus center to clitoris base by a digital caliper in millimeter
88 (Hangzhou Instar Precision Machinery Co., Zhejiang, China). In total, AGD of dairy cows (n =
89 290) with different parities [primiparous (n = 90) and multiparous (n = 200) cows] over various
90 seasons [spring (n = 15), summer (n = 28), fall (n = 115) and winter (n = 128)] were collected.
91 Considering data of AGD length, cows were partitioned in three categories including short AGD
92 (20% of cows with lowest values; n = 58), intermediate AGD (60% of cows with moderate values;
93 n = 174) and long AGD (20% of cows with highest values; n = 58).

94 **Nomenclature of reproductive parameters**

95 Data associated with dystocia, twinning, retained fetal membranes (RFM), calf birth
96 weight, offspring gender, puerperal metritis, clinical endometritis, days to first service (DFS) and
97 first service conception rate (FSCR) were retrieved from herd database using individual ID number
98 of cows. Sex ratio of offspring was defined as the proportion of male offspring (Gharagozlou et
99 al., 2016). Cows were considered with dystocia when calf delivery could not proceed
100 spontaneously and required assistance (Giuliodori et al., 2013; Sheldon and Owens, 2017). The
101 cow was considered with retained fetal membranes when the fetal membranes were not expelled
102 by 24 hours after commencement of parturition (Giuliodori et al., 2013; Sheldon and Owens,
103 2017). Puerperal metritis was defined as the presence of fetid watery red-brown uterine discharge

104 during the first week postpartum (Sheldon et al., 2006). Clinical endometritis was defined as the
105 presence of mucopurulent or purulent vaginal discharge at the time of first postpartum examination
106 (Sheldon et al., 2006; Sheldon and Owens, 2017). DFS was the interval from calving to the first
107 service postpartum (Akbarinejad et al. 2019, 2020). FSCR was the percentage of cows determined
108 pregnant following the first service postpartum (Akbarinejad et al. 2019, 2020).

109 **Statistical analysis**

110 Continuous data (i.e., calf birth weight) were analyzed using generalized linear model
111 (GLM) procedure. Binary data (i.e., rate of dystocia, twinning, RFM, puerperal metritis and
112 clinical endometritis, sex ratio of offspring and FSCR) were analyzed using logistic regression by
113 GENMOD procedure considering function link logit in the statistical model. Logistic regression
114 analysis produced adjusted odds ratio (AOR) as the level of difference among various groups. DFS
115 as a time-to-event variable was analyzed using LIFETEST procedure and the hazard of interval
116 from calving to first service postpartum was analyzed using Cox regression by PHREG procedure.
117 Cox regression analysis generated adjusted hazard ratio (AHR) as the conditional daily likelihood
118 of the first service postpartum. AGD (short, intermediate and long AGD groups), parity
119 (primiparous and multiparous cows) and season (spring, summer, fall and winter) were included
120 as fixed effects in all statistical models. The LSMEANS statement was used to perform multiple
121 comparisons. All analyses were conducted in SAS version 9.4 (SAS Institute Inc., Carry, NC,
122 USA). Differences at $P < 0.05$ were considered statistically significant.

123

124 **Results**

125 **AGD length in dairy cows**

126 Mean \pm standard error of mean (SEM), median, minimum and maximum of AGD length
127 were 126.02 ± 0.80 mm, 126.00 mm, 75.64 mm and 178.20 mm, respectively, in investigated cows
128 ($n = 290$; Figure 1). Statistics of AGD length in short, intermediate and long AGD categories are
129 presented in Table 1.

130 **Effect of AGD on reproductive parameters**

131 Sex ratio of offspring was lower in short AGD group as compared with intermediate AGD
132 group (AOR = 0.509; 95% CI = 0.261-0.990; $P = 0.047$) and long AGD (AOR = 0.418; 95% CI =
133 0.187-0.939; $P = 0.035$; Table 1). Furthermore, FSCR was higher in intermediate AGD group than
134 short AGD (AOR = 2.526; 95% CI = 1.257-5.076; $P = 0.009$) and long AGD (AOR = 2.260; 95%
135 CI = 1.159-4.407; $P = 0.017$) groups (Table 1). However, rate of dystocia, twinning, retained fetal
136 membranes, calf birth weight, rate of puerperal metritis and clinical endometritis, hazard of first
137 postpartum insemination and DFS did not differ among various AGD categories ($P > 0.05$; Table
138 1; Figure 2, A).

139 **Effect of parity on reproductive parameters**

140 Calf birth weight was greater in multiparous than primiparous cows ($P = 0.032$; Table 2).
141 In addition, sex ratio of offspring was higher in multiparous cows as compared with primiparous
142 cows (AOR = 2.300; 95% CI = 1.323-3.997; $P = 0.003$; Table 2). But rate of dystocia, twinning,
143 retained fetal membranes, puerperal metritis and clinical endometritis, hazard of first postpartum
144 insemination, DFS and FSCR was not different between primiparous and multiparous cows ($P >$
145 0.05; Table 2; Figure 2, B).

146 Effect of season on reproductive parameters

147 Calf birth weight was greater in winter than summer and fall ($P < 0.01$; Table 3). Moreover,
148 rate of clinical endometritis was higher during summer as compared with fall (AOR = 3.006; 95%
149 CI = 1.133-7.972; $P = 0.027$) and winter (AOR = 2.637; 95% CI = 1.025-6.784; $P = 0.044$; Table
150 3). Further, the hazard of first postpartum insemination was higher during spring than summer
151 (AHR = 2.850; 95% CI = 1.327-6.119; $P = 0.007$; Figure 2, C), which culminated in shorter DFS
152 during spring than summer ($P = 0.010$; Table 3). Additionally, FSCR was lower during summer
153 as compared with spring (AOR = 0.120; 95% CI = 0.020-0.718; $P = 0.020$), fall (AOR = 0.123;
154 95% CI = 0.027-0.551; $P = 0.006$) and winter (AOR = 0.097; 95% CI = 0.022-0.433; $P = 0.002$;
155 Table 3). Yet rate of dystocia, twinning, retained fetal membranes, sex ratio of offspring and rate
156 of puerperal metritis did not differ among various seasons ($P > 0.05$; Table 3).

158 Discussion

159 The present study revealed that cows with intermediate length of AGD had superior
160 conception rate at first postpartum insemination as compared to cows with short and long length
161 of AGD; however, postpartum uterine infections and their contributing risk factors were not
162 different among AGD categories. These findings implicate that the effect of AGD on fertility was
163 not mediated through alteration in rate of postpartum complications. Previous studies merely
164 reported suboptimal fertility of long AGD cows as compared to short AGD cows since in those
165 studies, dairy cows were simply classified in two quantiles (Gobikrushanth et al., 2017;
166 Akbarinejad et al., 2019). In this context, classification of cows into three AGD categories

167 imparted this study the advantage to more accurately elucidate the relationship between AGD and
168 reproductive competence in bovine. Given that prenatal exposure to androgens is the main
169 determinant of AGD length (Gore et al., 2015; Kita et al., 2016), it could be surmised that under-
170 exposure as well as over-exposure of fetus to androgens could lead to carry-over effects disrupting
171 fertility of cows during adulthood, yet the corresponding underlying mechanisms remain to be
172 unraveled by further studies.

173 Furthermore, the present study showed positive association between maternal AGD and
174 sex ratio of calves. Likewise, proportion of male offspring has been reported to be higher in dams
175 with larger AGD in mice, rabbit and porcine (Drickamer et al., 1997; Bánszegi et al., 2010; Szenczi
176 et al., 2013), and the relationship between maternal AGD and sex ratio of offspring has been
177 attributed to androgens (Edwards et al., 2016). In this regard, circulating and intrafollicular
178 concentration of testosterone have been positively associated with sex ratio of offspring in bovine
179 and non-bovine species (Grant and Irwin, 2005; Grant et al., 2008; Helle et al., 2008). Moreover,
180 it has been suggested that this impact of testosterone is mediated through interaction of androgens
181 with their receptor (Gharagozlou et al., 2016). Nevertheless, although positive correlation of AGD
182 with circulating testosterone has been observed in human (Mira-Escolano et al., 2014), the
183 correlation between AGD and plasma testosterone was weak and insignificant in dairy cows
184 (Gobikrushanth et al., 2017). Yet, it is worth noting that the variety in androgen synthesis among
185 cows with different length of AGD might be at paracrine level, which appears to play a determining
186 role in terms of offspring sex allocation (Grant and Irwin, 2005; Grant et al., 2008), and it is not
187 manifested at endocrine level.

188 Maternal parity also affected sex ratio of calves and proportion of female offspring was
189 higher in primiparous than multiparous cows. Albeit the effect of parity on sex ratio of calves has
190 been previously reported (Hosseini-Zadeh, 2012), the substantial greater proportion of female
191 calves in primiparous cows in the current study might have resulted from application of sexed
192 semen in heifers in the herd rather than the effect of dam parity *per se*.

193 Moreover, it was observed that calves born to multiparous dams were heavier as compared
194 with calves born to primiparous dams and this finding was in accord with results of previous
195 studies (Akbarinejad et al., 2018). This observation could implicate dissimilar level of intrauterine
196 nutrition between primiparous and multiparous dams since intrauterine nutrition is one of the main
197 factors controlling offspring birth weight (Negrato and Gomes, 2013). To begin with, the parity-
198 related variation in intrauterine nutrition could be attributed to differential nutritional partitioning
199 between primiparous and multiparous cows owing to the fact that primiparous cows are still
200 growing over the course of gestation and allocate part of their nutritional intake to their own
201 development (Wathes et al., 2014). Alternatively, this phenomenon could have stemmed from less
202 developed uterine vasculature and placenta, supplying the fetus with oxygen and nutrients (Browne
203 et al., 2015), in primiparous than multiparous animals (Klewitz et al., 2015; Van Eetvelde et al.,
204 2016; Robles et al., 2018).

205 Season of calving influenced offspring birth weight as well and calves born during summer
206 and fall were lighter than calves born during winter. Given that during that the risk of heat stress
207 is higher during warm seasons than cold seasons, the negative effect of summer and fall on
208 offspring birth weight could be attributed to indirect exposure of fetus to heat stress during late

209 stages of gestation, which are the most critical timeframes in terms of fetal growth, and in turn,
210 neonatal birth weight (Akbarinejad et al., 2017). In corroboration of this notion, a previous study
211 has also indicated adverse effects of maternal exposure to heat stress during late pregnancy on calf
212 birth weight (Akbarinejad et al., 2017). Indeed, heat stress could diminish total placental and
213 umbilical blood flow (Reynolds et al., 2006), compromise placental vascularization (Regnault et
214 al., 2003; Reynolds et al., 2006), intensify placental resistance to oxygen, which would hinder
215 transplacental oxygen diffusion and culminate in hypoxia (Regnault et al., 2003), and disrupt
216 transport of nutrients to fetus (Regnault et al., 2005). Besides, heat stress decreases maternal dry
217 matter intake, which could aggravate fetal nutritional restriction (Wheelock et al., 2010; Gorniak et
218 al., 2014; Conte et al., 2018).

219 In addition, the present study showed higher rate of clinical endometritis during summer
220 than fall and winter. By contrast, other studies investigating the prevalence of clinical endometritis
221 across various seasons failed to detect any association between season of calving and occurrence
222 of clinical endometritis (Lee et al., 2018). Regardless, heat stress might have contributed to the
223 effect of summer on rate of clinical endometritis because heat stress could increase secretion of
224 glucocorticoids, which suppress immune system and predispose the animal to various diseases
225 including uterine infections (Bagath et al., 2019).

226 Eventually, it was observed that parturition in summer led to not only delayed first
227 postpartum service but also diminished first service conception rate, which is consistent with the
228 results of previous studies (Emadi et al., 2014; Akbarinejad et al., 2017; Hansen, 2019). The
229 negative impact of summer on fertility might have also originated from heat stress given that heat

230 stress postpones resumption of postpartum ovarian activity (Díaz et al., 2020), deteriorates heat
231 detection rate (Emadi et al., 2014), impairs embryo development (Sakatani, 2017), decrease
232 progesterone production and abrogate endometrial function (Wolfenson et al., 2000).

233

234 **Conclusion**

235 In conclusion, the present study showed that cows with intermediate length of AGD had
236 greater reproductive performance at the first postpartum insemination as compared with their
237 counterparts with shortest and longest lengths of AGD. Also, the probability of calf being male
238 augmented as the length of AGD increased. Moreover, it was observed that sex ratio of offspring
239 and calf birth weight were lower in primiparous than multiparous cows. Further, it was revealed
240 that calves born during summer and fall had lighter birth weight and cows calved during summer
241 were afflicted with higher rate of clinical endometritis, delayed first postpartum insemination and
242 suboptimal first service conception rate.

243

244 **Conflict of interest**

245 The authors have no conflict of interest to declare.

246

247 **References**

248 Akbarinejad V., Gharagozlou F., Vojgani M., Ranji A. (2020). Evidence for quadratic association
249 between serum anti-Müllerian hormone (AMH) concentration and fertility in dairy cows.
250 Anim Reprod Sci, 218, 106457. <https://doi.org/10.1016/j.anireprosci.2020.106457>.

- 251 Akbarinejad V., Gharagozlou F., Vojgani M. (2017). Temporal effect of maternal heat stress
252 during gestation on the fertility and anti-Müllerian hormone concentration of offspring in
253 bovine. *Theriogenology*, 99, 69-78.
254 <http://dx.doi.org/10.1016/j.theriogenology.2017.05.018>.
- 255 Akbarinejad V., Gharagozlou F., Vojgani M., Bagheri Amirabadi M. M. (2018). Nulliparous and
256 primiparous cows produce less fertile female offspring with lesser concentration of anti-
257 Müllerian hormone (AMH) as compared with multiparous cows. *Anim Reprod Sci*, 197,
258 222-230. <http://dx.doi.org/10.1016/j.anireprosci.2018.08.032>.
- 259 Akbarinejad V., Gharagozlou F., Vojgani M., Shourabi E., Makiabadi M. J. (2019). M. Inferior
260 fertility and higher concentrations of anti-Müllerian hormone in dairy cows with longer
261 anogenital distance. *Domest Anim Endocrinol*, 68, 47-53.
262 <http://dx.doi.org/10.1016/j.domaniend.2019.01.011>.
- 263 Bagath M., Krishnan G., Devaraj C., Rashamol V. P., Pragna P., Lees A. M., Sejian V. (2019).
264 The impact of heat stress on the immune system in dairy cattle: A review. *Res Vet Sci*, 126,
265 94-102. <https://doi.org/10.1016/j.rvsc.2019.08.011>.
- 266 Bánszegi O., Altbäcker V., Dúcs A., Bilkó A. (2010). Testosterone treatment of pregnant rabbits
267 affects sexual development of their daughters. *Physiol Behav*, 101, 422-427.
268 <https://doi.org/10.1016/j.physbeh.2010.07.020>.
- 269 Browne V. A., Julian C. G., Toledo-Jaldin L., Cioffi-Ragan D., Vargas E., Moore L. G. (2015).
270 Uterine artery blood flow, fetal hypoxia and fetal growth. *Philos Trans R Soc Lond B Biol*
271 *Sci*, 370, 20140068. <https://doi.org/10.1098/rstb.2014.0068>.

- 272 Conte G., Ciampolini R., Cassandro M., Lasagna E., Calamari L., Bernabucci U., Abeni F. (2018).
273 Feeding and nutrition management of heat-stressed dairy ruminants. *Ital J Anim Sci*, 2018,
274 1–17. <https://doi.org/10.1080/1828051X.2017.1404944>.
- 275 Díaz R. F., Galina C. S., Aranda E. M., Aceves L. A., Sánchez J. G., Pablos J. L. (2020). Effect of
276 temperature – humidity index on the onset of post- partum ovarian activity and
277 reproductive behavior in *Bos indicus* cows. *Anim Reprod*, 17, e20190074.
278 <http://doi.org/10.21451/1984-3143-AR2019-0074>.
- 279 Drickamer L. C., Arthur R. D., Rosenthal T. L. (1997). Conception failure in swine: importance
280 of the sex ratio of a female's birth litter and tests of other factors. *J Anim Sci*, 75, 2192-
281 2196. <https://doi.org/10.2527/1997.7582192x>.
- 282 Edwards A., Cameron E., Wapstra E. (2016). Are there physiological constraints on maternal
283 ability to adjust sex ratios in mammals? *J Zool*, 299, 1-9. <https://doi.org/10.1111/jzo.12327>.
- 284 Emadi S. R., Rezaei A., Bolourchi M., Hovareshti P., Akbarinejad V. (2014). Administration of
285 estradiol benzoate before insemination could skew secondary sex ratio toward males in
286 Holstein dairy cows. *Domest Anim Endocrinol*, 48, 110-118.
287 <https://doi.org/10.1016/j.domaniend.2014.03.001>.
- 288 Gharagozlou F., Youssefi R., Vojgani M., Akbarinejad V., Rafiee G. (2016). Androgen receptor
289 blockade using flutamide skewed sex ratio of litters in mice. *Vet Res Forum*, 7, 169-172.
- 290 Ghavi Hossein-Zadeh N., Ardalan M. (2011). Cow-specific risk factors for retained placenta,
291 metritis and clinical mastitis in Holstein cows. *Vet Res Commun*, 35, 345-354.
292 <https://doi.org/10.1007/s11259-011-9479-5>.

- 293 Giuliadori M. J., Magnasco R. P., Becu-Villalobos D., Lacau-Mengido I. M., Risco C. A., de la
294 Sota R. L. (2013). Metritis in dairy cows: risk factors and reproductive performance. *J*
295 *Dairy Sci*, 96, 3621-3631. <https://doi.org/10.3168/jds.2012-5922>.
- 296 Gobikrushanth M., Bruinje T. C., Colazo M. G., Butler S. T., Ambrose D. J. (2017).
297 Characterization of anogenital distance and its relationship to fertility in lactating Holstein
298 cows. *J Dairy Sci*, 100, 9815-9823. <https://doi.org/10.3168/jds.2017-13033>.
- 299 Gore A. C., Chappell V. A., Fenton S. E., Flaws J. A., Nadal A., Prins G. S., Toppari J., Zoeller
300 R. T. (2015). EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-
301 Disrupting Chemicals. *Endocr Rev*, 36, E1-E150. <https://doi.org/10.1210/er.2015-1010>.
- 302 Gorniak T., Meyer U., Sudekum K. H., Danicke S. (2014) Impact of mild heat stress on dry matter
303 intake, milk yield and milk composition in mid-lactation Holstein dairy cows in a temperate
304 climate. *Arch Anim Nutr*, 68, 358–369. <https://doi.org/10.1080/1745039X.2014.950451>.
- 305 Grant V. J., Irwin R. J. (2005). Follicular fluid steroid levels and subsequent sex of bovine
306 embryos. *J Exp Zool A Comp Exp Biol*, 303, 1120-1125. <https://doi.org/10.1002/jez.a.233>.
- 307 Grant V. J., Irwin R. J., Standley N. T., Shelling A. N., Chamley L. W. (2008). Sex of bovine
308 embryos may be related to mothers' preovulatory follicular testosterone. *Biol Reprod*, 78,
309 812-815. <https://doi.org/10.1095/biolreprod.107.066050>.
- 310 Hansen P. J. (2019). Reproductive physiology of the heat-stressed dairy cow: implications for
311 fertility and assisted reproduction. *Anim Reprod*, 16, 497-507.
312 <http://doi.org/10.21451/1984-3143-AR2019-0053>.

- 313 Helle S., Laaksonen T., Adamsson A., Paranko J., Huitu O. (2008). Female field voles with high
314 testosterone and glucose levels produce male-biased litters. *Anim Behav*, 75, 1031-1039.
315 <https://doi.org/10.1016/j.anbehav.2007.08.015>.
- 316 Hossein-Zadeh N. G. (2012). Factors affecting secondary sex ratio in Iranian Holsteins.
317 *Theriogenology*, 77, 214-219. <https://doi.org/10.1016/j.theriogenology.2011.07.040>.
- 318 Kita D. H., Meyer K. B., Venturelli A. C., Adams R., Machado D. L., Morais R. N., Swan S.
319 H., Gennings C., Martino-Andrade A. J. (2016). Manipulation of pre and postnatal
320 androgen environments and anogenital distance in rats. *Toxicology*, 368-369, 152-161.
321 <https://doi.org/10.1016/j.tox.2016.08.021>.
- 322 Klewitz J., Struebing C., Rohn K., Goergens A., Martinsson G., Orgies F., Probst J., Hollinshead
323 F., Bollwein H., Sieme H. (2015). Effects of age, parity, and pregnancy abnormalities on
324 foal birth weight and uterine blood flow in the mare. *Theriogenology*, 83, 721-729.
325 <https://doi.org/10.1016/j.theriogenology.2014.11.007>.
- 326 Lee S. C., Jeong J. K., Choi I. S., Kang H. G., Jung Y. H., Park S. B., Kim I. H. (2018). Cytological
327 endometritis in dairy cows: diagnostic threshold, risk factors, and impact on reproductive
328 performance. *J Vet Sci*, 19, 301-308. <https://doi.org/10.4142/jvs.2018.19.2.301>.
- 329 Mira-Escolano M. P., Mendiola J., Mínguez-Alarcón L., Roca M., Cutillas-Tolín A., López-Espín
330 J. J., Torres-Cantero A. M. (2014). Anogenital distance of women in relation to their
331 mother's gynaecological characteristics before or during pregnancy. *Reprod Biomed*
332 *Online*, 28, 209-215. <https://doi.org/10.1016/j.rbmo.2013.09.026>.

- 333 Negrato C. A., Gomes M. B. (2013). Low birth weight: causes and consequences. *Diabetol Metab*
334 *Syndr*, 5, 49. <https://doi.org/10.1186/1758-5996-5-49>.
- 335 Regnault T. R. H., de Vrijer B., Galan H. L., Davidsen M. L., Trembler K. A., Battaglia F. C.,
336 Wilkening R. B., Anthony R. V. (2003). The relationship between transplacental O₂
337 diffusion and placental expression of PlGF, VEGF and their receptors in a placental
338 insufficiency model of fetal growth restriction. *J Physiol*, 550, 641-656.
339 <https://doi.org/10.1113/jphysiol.2003.039511>.
- 340 Regnault T. R. H., Friedman J. E., Wilkening R. B., Anthony R. V., Hay W. W. Jr. (2005).
341 Fetoplacental transport and utilization of amino acids in IUGR—A review. *Placenta*, 26,
342 S52-S62. <https://doi.org/10.1016/j.placenta.2005.01.003>.
- 343 Reynolds L. P., Caton J. S., Redmer D. A., Grazul-Bilska A. T., Vonnahme K. A., Borowicz P. P.,
344 Luther J. S., Wallace J. M., Wu G., Spencer T. E. (2006). Evidence for altered placental
345 blood flow and vascularity in compromised pregnancies. *J Physiol*, 572, 51-58.
346 <https://doi.org/10.1113/jphysiol.2005.104430>.
- 347 Robles M., Dubois C., Gautier C., Dahirel M., Guenon I., Bouraima-Lelong H., Viguié C., Wimel
348 L., Couturier-Tarrade A., Chavatte-Palmer P. (2018). Maternal parity affects placental
349 development, growth and metabolism of foals until 1 year and a half. *Theriogenology*, 108,
350 321-330. <https://doi.org/10.1016/j.theriogenology.2017.12.019>.
- 351 Sakatani M. (2017). Effects of heat stress on bovine preimplantation embryos produced in vitro. *J*
352 *Reprod Dev*, 63, 347-352. <https://doi.org/10.1262/jrd.2017-045>.

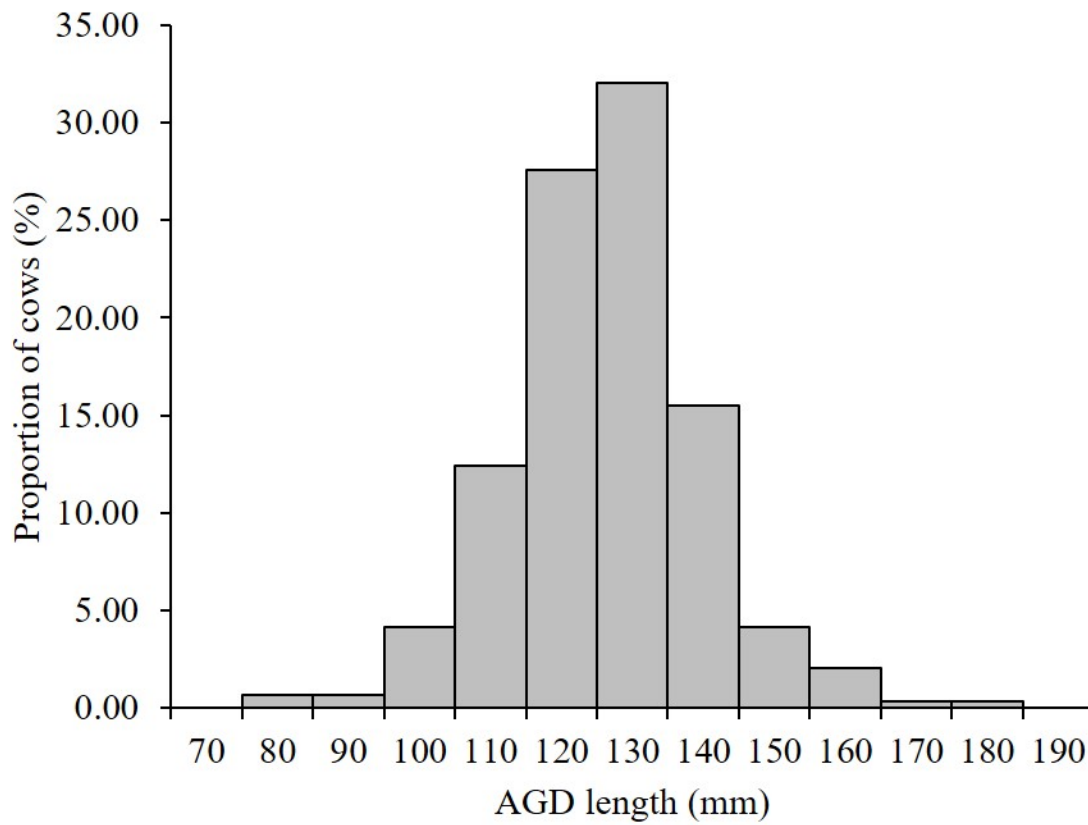
- 353 Sheldon I. M., Lewis G. S., LeBlanc S., Gilbert R. O. (2006). Defining postpartum uterine disease
354 in cattle. *Theriogenology*, 65, 1516-1530.
355 <https://doi.org/10.1016/j.theriogenology.2005.08.021>.
- 356 Sheldon I., Owens S. E. (2017). Postpartum uterine infection and endometritis in dairy cattle. *Anim*
357 *Reprod*, 14, 622-629. <https://doi.org/10.21451/1984-3143-AR1006>.
- 358 Szenczi P., Bánszegi O., Groó Z., Altbäcker V. (2013). Anogenital distance and condition as
359 predictors of litter sex ratio in two mouse species: a study of the house mouse (*Mus*
360 *musculus*) and mound-building mouse (*Mus spicilegus*). *PLoS One*, 8, e74066.
361 <https://doi.org/10.1371/journal.pone.0074066>.
- 362 Thankamony A., Pasterski V., Ong K. K., Acerini C. L., Hughes I. A. (2016). Anogenital distance
363 as a marker of androgen exposure in humans. *Andrology*, 4, 616-625.
364 <https://doi.org/10.1111/andr.12156>.
- 365 Van Eetvelde M., Kamal M. M., Hostens M., Vandaele L., Fiems L. O., Opsomer G. (2016).
366 Evidence for placental compensation in cattle. *Animal*, 10, 1342-1350.
367 <https://doi.org/10.1017/S1751731116000318>.
- 368 Wathes D. C., Pollott G. E., Johnson K. F., Richardson H., Cooke J. S. (2014). Heifer fertility and
369 carry over consequences for life time production in dairy and beef cattle. *Animal*, 8, 91-
370 104. <https://doi.org/10.1017/S1751731114000755>.
- 371 Wheelock J. B., Rhoads R. P., Vanbaale M. J., Sanders S. R., Baumgard L. H. (2010). Effects of
372 heat stress on energetic metabolism in lactating Holstein cows. *J Dairy Sci*, 93, 644-655.
373 <https://doi.org/10.3168/jds.2009-2295>.

374 Williams E. J., Fischer D. P., Noakes D. E., England G. C., Rycroft A., Dobson H., Sheldon I. M.
375 (2007). The relationship between uterine pathogen growth density and ovarian function in
376 the postpartum dairy cow. *Theriogenology*, 68, 549-559.
377 <https://doi.org/10.1016/j.theriogenology.2007.04.056>.

378 Wolfenson D., Roth Z., Meidan R. (2000). Impaired reproduction in heat-stressed cattle: basic and
379 applied aspects. *Anim Reprod Sci*, 60-61, 535-537. [https://doi.org/10.1016/S0378-](https://doi.org/10.1016/S0378-4320(00)00102-0)
380 [4320\(00\)00102-0](https://doi.org/10.1016/S0378-4320(00)00102-0).

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Uncorrected Proof



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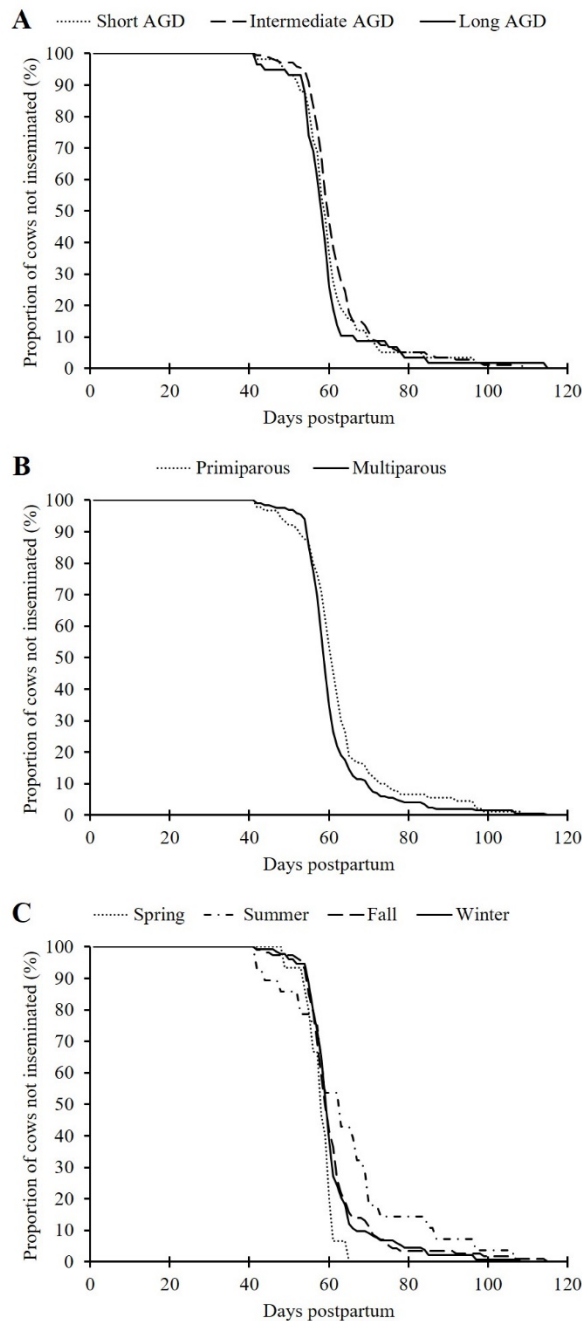
384 **Figure 1.** Histogram of frequency distribution regarding length of AGD in Holstein dairy cows (n
385 = 290).

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Accepted Proof

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392 **Figure 2.** A) Time to first service postpartum in short (n = 58), intermediate (n = 174) and long (n

393 = 58) AGD cows. B) Time to first postpartum insemination in primiparous (n = 90) and

394 multiparous (n = 200) cows. C) Time to first postpartum insemination in cows during spring (n
395 =15), summer (n = 28), fall (n = 115) and winter (n = 132).

Uncorrected Proof

396 عملکرد تولیدمثلی بهتر گاوهای شیری هلشتاین با طول فاصله آنوجنییتال متوسط در تلقیح اول پس از زایش

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404 چکیده

405 زمینه مطالعه: مطالعات پیشین مبین رابطه منفی فاصله آنوجنییتال با باروری در گاوها است، اما مکانیسم این رابطه معکوس به طور

406 کامل مشخص نیست. در این رابطه، عفونت‌های رحمی پس از زایش و عوامل مخاطره مرتبط با این عفونت‌ها می‌توانند سبب کاهش

407 باروری گاوها گردند، ولی هیچ پژوهشی تا به حال به بررسی ارتباط فاصله آنوجنییتال و مشکلات پس از زایش نپرداخته است.

408 هدف: مطالعه حاضر به منظور بررسی رابطه فاصله آنوجنییتال با عملکرد تولیدمثلی پس از زایش در گاوهای شیری به انجام رسید.

409 روش کار: فاصله آنوجنییتال گاوها (تعداد = 290) همزمان با اولین معاینه پس از زایش به میلی‌متر اندازه‌گیری شد. گاوها بر اساس

410 طول فاصله آنوجنییتال به سه دسته شامل فاصله آنوجنییتال کوتاه (20٪ جمعیت گاوها دارای کمترین مقادیر)، فاصله آنوجنییتال

411 متوسط (60٪ جمعیت گاوها دارای مقادیر متوسط) و فاصله آنوجنییتال بلند (20٪ جمعیت گاوها دارای بیشترین مقادیر) تقسیم‌بندی

412 شدند. به‌علاوه، داده‌های متغیرهای تولیدمثلی پس از زایش از پایگاه داده گله بازیابی شدند. داده‌ها با استفاده از نرم‌افزار SAS

413 ویرایش شماره 9/4 آنالیز شدند.

- 414 نتایج: نرخ سخت‌زایی، دوقلو‌زایی و جفت‌ماندگی، متریت پس از زایش و آندومتریت بالینی، وزن تولد گوساله و فاصله زایش تا اولین
- 415 تلقیح در میان دسته‌های مختلف فاصله آنوجنی‌تال متفاوت نبود ($P > 0/05$). اما، درصد موالید نر در گاوهای با فاصله آنوجنی‌تال
- 416 کوتاه نسبت به گاوهای با فاصله آنوجنی‌تال متوسط و بلند کمتر بود ($P < 0/05$). علاوه بر این، نرخ باروری در تلقیح اول پس از
- 417 زایش در گروه فاصله آنوجنی‌تال متوسط بالاتر از گروه‌های فاصله آنوجنی‌تال کوتاه و بلند بود ($P < 0/05$).
- 418 نتیجه‌گیری: در نتیجه، مطالعه حاضر بیانگر نرخ آبستنی در اولین تلقیح پس از زایش نامناسب در گاوهای دارای حداقل و حداکثر
- 419 طول فاصله آنوجنی‌تال بود و نشان داد که این باروری کمتر از طریق تغییر در نرخ مشکلات تولیدمثلی پس از زایش حاصل نشده
- 420 بود.
- 421 واژه‌های کلیدی: فاصله آنوجنی‌تال؛ گاو شیری؛ سخت‌زایی؛ باروری؛ عفونتهای رحمی