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

Mohammad Javad Mozaffari Makiabadi[1], Vahid Akbarinejad[1]*, Farid Heidari[2], Faramarz Gharagozlou[1], Mehdi Vojgani[1]

1. *Department of Theriogenology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
2. *Department of Animal Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.*

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.

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

METHODS: AGD of Holstein dairy cows of a commercial dairy herd (n = 290) was measured in millimeter at the first postpartum examination (days 28 to 32 postpartum). The cows were classified into three categories based on the AGD length, including short (20% of cows with lowest values), intermediate (60% of cows with moderate values) and long (20% of cows with highest values) AGDs. Additionally, data of postpartum reproductive variables were retrieved form the herd database. Data was analyzed using SAS software version 9.4.

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

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

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

Correspondence

Vahid Akbarinejad, Department of Theriogenology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. Tel: +98 (0912) 5624230, Fax: +98 (021) 66933222, Email: v_akbarinejad@ut.ac.ir

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Introduction

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

Postpartum uterine infections, including metritis and endometriosis, are considered as contributors to the suboptimal fertility in cows by delaying uterine involution, rendering the uterus susceptibility to chronic infections, and causing ovarian dysfunction (Sheldon *et al.*, 2006; Williams *et al.* 2007; Giuliodori *et al.*, 2013; Sheldon and Owens, 2017). Furthermore, various postpartum items have been indicated as risk factors for postpartum uterine infections, including twinning birth, dystocia, retained fetal membranes, excessive calf birth weight, and male offspring (Ghavi Hossein-Zadeh and Ardalan, 2011; Giuliodori *et al.*, 2013). To the best of our knowledge, there is no data available on whether AGD is related to postpartum uterine infections and their corresponding risk factors.

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

Materials and Methods

Ethical statement and study design

The Animal Ethics Committee approved this study at the University of Tehran concerning animal welfare and ethics (6/6/30854). The study was carried out at a commercial farm in Tehran province from August 2018 to March 2019. The voluntary waiting period was 50 days in the herd and cows were subjected to insemination 12 hours after detection of standing heat. Estrus detection was performed thrice a day by visual observation for at least 30 minutes each time. All artificial inseminations were done by the same technician and insemination of the sexed semen was merely performed in heifers. The pregnancy of cows was routinely diagnosed 40 to 45 days after insemination using the rectal examination. The research plan of this study was to measure the AGD period at the first postpartum examination of cows in order to associate AGD with reproductive parameters in dairy cows and the sample size of the study was 290 dairy cows.

Assessment of AGD

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

Nomenclature of reproductive parameters

Data associated with dystocia, twinning, retained fetal membranes (RFM), calf birth weight, offspring gender, puerperal metritis, clinical endometritis, days to first service (DFS) and first service conception rate (FSCR) were retrieved from herd database using individual ID number of cows. Sex ratio of offspring was defined as the proportion of male offspring (Gharagozlou et al., 2016). Cows were considered dystocia when calf delivery could not proceed spontaneously and required assistance (Giuliodori et al., 2013; Sheldon and Owens, 2017). The cow was considered with retained fetal membranes when the fetal membranes were not expelled by 24 hours after the commencement of parturition (Giuliodori et al., 2013; Sheldon and Owens, 2017). Puerperal metritis was defined as fetid watery red-brown uterine discharge during the first week postpartum (Sheldon et al., 2006). Clinical endometritis was defined as mucopurulent or purulent vaginal discharge at the time of first postpartum examination (Sheldon et al., 2006; Sheldon and Owens, 2017). DFS was the interval from calving to the first service postpartum (Akbarinejad et al. 2019, 2020). FSCR was the percentage of cows determined pregnant following the first service postpartum (Akbarinejad et al. 2019, 2020).

Statistical analysis

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

Results

AGD length in dairy cows

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

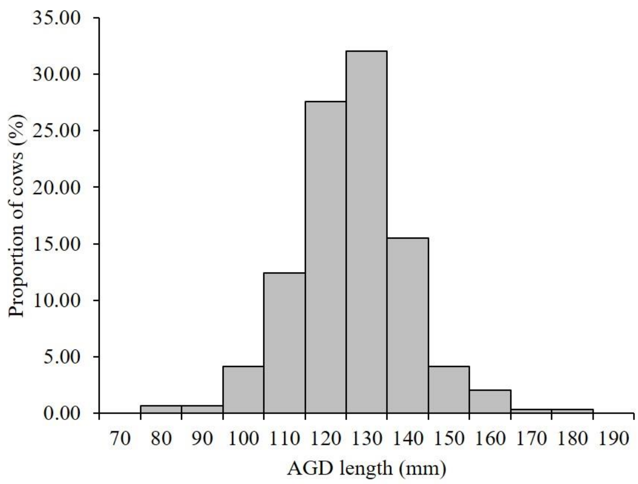

Figure 1. Histogram of frequency distribution regarding the length of AGD in Holstein dairy cows (n = 290).

Table 1. Reproductive parameters in dairy cows with short, intermediate and long anogenital distance (AGD). Data are presented as mean ± SEM or percentage. Values in parenthesis are actual numbers.

Parameter	Short AGD (n = 58)	Intermediate AGD (n = 174)	Long AGD (n = 58)
Mean ± SEM of AGD (mm)	107.66 ± 1.10	125.78 ± 0.40	145.11 ± 1.15
Median (range) of AGD (mm)	110.23 (75.64-116.85)	126.00 (117.07-136.42)	141.77 (136.66-178.20)
Dystocia rate (%)	18.97 (11/58)	18.97 (33/174)	13.79 (8/58)
Twinning rate (%)	3.45 (2/58)	4.02 (7/174)	1.72 (1/58)
Rate of retained fetal membranes (%)	5.17 (3/58)	5.75 (10/174)	8.62 (5/58)
Sex ratio of offspring (%)	30.36 (17/56)[a]	47.31 (79/167)[b]	56.14 (32/57)[b]
Calf birth weight (kg)	37.50 ± 0.55	38.67 ± 0.38	39.93 ± 0.59
Rate of puerperal metritis (%)	5.17 (3/58)	2.30 (4/174)	10.34 (6/58)
Rate of clinical endometritis (%)	17.24 (10/58)	14.94 (26/174)	17.24 (10/58)
Days to first service (day)	60.98 ± 1.35	62.39 ± 0.73	59.83 ± 1.36
First service conception rate (%)	24.14 (14/58)[a]	45.98 (80/174)[b]	27.59 (16/58)[a]

[a,b] Values with different superscripts within rows differ (P<0.05).

Effect of AGD on reproductive parameters

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

Table 2. Reproductive parameters in primiparous and multiparous dairy cows. Data are presented as mean ± SEM or percentage. Values in parenthesis are actual numbers.

Parameter	Primiparous (n = 90)	Multiparous (n = 200)
Dystocia rate (%)	22.22 (20/90)	16.00 (32/200)
Twinning rate (%)	2.22 (2/90)	4.00 (8/200)
Rate of retained fetal membranes (%)	3.33 (3/90)	7.50 (15/200)
Sex ratio of offspring (%)	30.68 (27/88)[a]	52.60 (101/192)[b]
Calf birth weight (kg)	37.42 ± 0.43[a]	39.26 ± 0.35[b]
Rate of puerperal metritis (%)	1.11 (1/90)	6.00 (12/200)
Rate of clinical endometritis (%)	13.33 (12/90)	17.00 (34/200)
Days to first service (day)	62.82 ± 1.19	61.05 ± 0.65
First service conception rate (%)	44.44 (40/90)	35.00 (70/200)

[a,b] Values with different superscripts within rows differ (P<0.05).

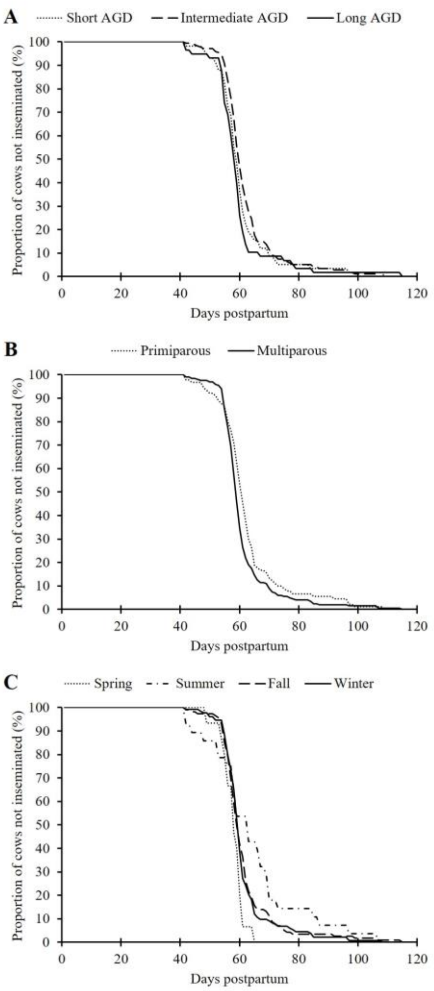

Figure 2. A) Time to first service postpartum in short (n = 58), intermediate (n = 174) and long (n = 58) AGD cows. B) Time to first postpartum insemination in primiparous (n = 90) and multiparous (n = 200) cows. C) Time to first postpartum insemination in cows during spring (n =15), summer (n = 28), fall (n = 115) and winter (n = 132).

Effect of parity on reproductive parameters

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

Effect of season on reproductive parameters

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

Table 3. Reproductive parameters in dairy cows during spring, summer, fall and winter. Data are presented as mean ± SEM or percentage. Values in parenthesis are actual numbers.

Parameter	Spring (n = 15)	Summer (n = 28)	Fall (n = 115)	Winter (n = 132)
Dystocia rate (%)	6.67 (1/15)	10.71 (3/28)	17.39 (20/115)	21.21 (28/132)
Twinning rate (%)	6.67 (1/15)	0.00 (0/28)	4.35 (5/115)	3.03 (4/132)
Rate of retained fetal membranes (%)	6.67 (1/15)	0.00 (0/28)	3.48 (4/115)	9.85 (13/132)
Sex ratio of offspring (%)	28.57 (4/14)	46.43 (13/28)	45.45 (50/110)	47.66 (61/128)
Calf birth weight (kg)	39.20 ± 1.05^{ab}	36.25 ± 1.07^{a}	37.48 ± 0.41^{a}	40.20 ± 0.40^{b}
Rate of puerperal metritis (%)	0.00 (0/15)	0.00 (0/28)	4.35 (5/115)	6.06 (8/132)
Rate of clinical endometritis (%)	0.00 (0/15)^{ab}	32.14 (9/28)^{a}	13.91 (16/115)^{b}	15.91 (21/132)^{b}
Days to first service (day)	58.00 ± 0.96^{a}	64.50 ± 2.88^{b}	61.66 ± 0.93^{ab}	61.33 ± 0.77^{ab}
First service conception rate (%)	40.00 (6/15)^{a}	7.14 (2/28)^{b}	39.13 (45/115)^{a}	43.18 (57/132)^{a}

^{a,b}Values with different superscripts within rows differ (P<0.05).

Discussion

The present study revealed that cows with intermediate length of AGD had a superior conception rate at the first postpartum insemination compared to the cows with short and long lengths of AGD; however, postpartum uterine infections and their contributing risk factors were not different among them AGD categories. These findings implicate that the effect of AGD on fertility was not mediated through alteration in the rate of postpartum complications. Previous studies merely reported suboptimal fertility of long AGD cows as compared to the short AGD cows since in those studies; dairy cows were simply classified in two quantiles (Gobikrushanth *et al.*, 2017; Akbarinejad *et al.*, 2019). In this context, the classification of cows into three AGD categories imparted this study the advantage to more accurately elucidate the association between AGD and reproductive competence in bovine. Given that prenatal exposure to the androgens is the main determinant of AGD length (Gore *et al.*, 2015; Kita *et al.*, 2016), it could be surmised that under-exposure, as well as over-exposure of fetus to the androgens, could lead to carry-over effects disrupting fertility of cows during adulthood, yet the corresponding underlying mechanisms remain to be unraveled by further studies.

Furthermore, the present study showed a positive association between maternal AGD and the sex ratio of calves. Likewise, the proportion of male offspring has been reported to be higher in dams with larger AGD in mice, rabbit and porcine (Drickamer *et al.*, 1997; Bánszegi *et al.*, 2010; Szenczi *et al.*, 2013), and the association between the maternal AGD and the sex ratio of offspring has been attributed to the androgens (Edwards *et al.*, 2016). In this regard, circulating and intra-follicular concentrations of testosterone have been positively associated with the sex ratio of offspring in bovine and non-bovine species (Grant and Irwin, 2005; Grant *et al.*, 2008; Helle *et al.*, 2008). Moreover, it has been suggested that this impact of testosterone is mediated through the interaction of androgens with their receptor (Gharagozlou *et al.*, 2016). Nevertheless, although a positive correlation of AGD with circulating testosterone has been observed in humans (Mira-Escolano *et al.*, 2014), the correlation between AGD and plasma testosterone was weak and insignificant in dairy cows (Gobikrushanth *et al.*, 2017). However, it is worth noting that the variety in androgen synthesis among cows with different lengths of AGD might be at paracrine level, which appears to play a determining role in terms of offspring sex allocation (Grant and Irwin, 2005; Grant *et al.*, 2008), and it is not manifested at an endocrine level.

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

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

Season of calving influenced the offspring's birth weight. The calves born during summer and fall were lighter than calves born during winter. Given that heat stress is higher during warm seasons than cold seasons, the negative effect of summer and fall on the offspring birth weight could be attributed to indirect exposure of the fetus to heat stress during the late stages of gestation, which are the most critical timeframes in terms of fetal growth, and in turn, neonatal birth weight (Akbarinejad *et al.*, 2017). In corroboration of this notion, a previous study has also indicated the adverse effects of maternal exposure to heat stress during the late pregnancy on the calf birth weight (Akbarinejad *et al.*, 2017). Indeed, heat stress could diminish total placental and umbilical blood flow (Reynolds *et al.*, 2006), compromise placental vascularization (Regnault *et al.*, 2003; Reynolds *et al.*, 2006), intensify placental resistance to oxygen, which would hinder transplacental oxygen diffusion and culminate in hypoxia (Regnault *et al.*, 2003), and disrupt the transport of nutrients to fetus (Regnault *et al.*, 2005). Besides, heat stress decreases maternal dry matter intake, aggravating fetal nutritional restriction (Wheelock *et al.*, 2010; Gorniak *et al.*, 2014; Conte *et al.*, 2018).

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

Eventually, it was observed that parturition in summer led to delayed first postpartum service and diminished first service conception rate, which is consistent with the results of previous studies (Emadi *et al.*, 2014; Akbarinejad *et al.*, 2017; Hansen, 2019). The negative impact of summer on fertility might have also originated from the heat stress given that heat stress postpones resumption of postpartum ovarian activity (Díaz *et al.*, 2020), deteriorates heat detection rate (Emadi *et al.*, 2014), impairs embryo development (Sakatani, 2017), decreases progesterone production, and abrogates endometrial function (Wolfenson *et al.*, 2000).

Conclusion

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

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Conflict of Interest

The authors have no conflict of interest to declare.

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

محمد جواد مظفری مکی آبادی[۱]، وحید اکبری نژاد[۱*]، فرید حیدری[۲]، فرامرز قراگوزلو[۱]، مهدی وجگانی[۱]

[۱] گروه مامایی و بیماری‌های تولیدمثل دام، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران

[۲] گروه فناوری تولیدمثل دام، پژوهشکده ملی مهندسی ژنتیک و زیست‌فناوری، تهران، ایران

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زمینه مطالعه: مطالعات پیشین مبین رابطه منفی فاصله آنوجنیتال با باروری در گاوها است، اما مکانیسم این رابطه معکوس به طور کامل مشخص نیست. در این رابطه، عفونت‌های رحمی پس از زایش و عوامل مخاطره مرتبط با این عفونت‌ها می‌توانند سبب کاهش باروری گاوها گردند، ولی هیچ پژوهشی تا به حال به بررسی ارتباط فاصله آنوجنیتال و مشکلات پس از زایش نپرداخته است.

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

روش کار: فاصله آنوجنیتال گاوها (تعداد = ۲۹۰) همزمان با اولین معاینه پس از زایش به میلی‌متر اندازه‌گیری شد. گاوها بر اساس طول فاصله آنوجنیتال به سه دسته شامل فاصله آنوجنیتال کوتاه (۲۰٪ جمعیت گاوها دارای کمترین مقادیر)، فاصله آنوجنیتال متوسط (۶۰٪ جمعیت گاوها دارای مقادیر متوسط) و فاصله آنوجنیتال بلند (۲۰٪ جمعیت گاوها دارای بیشترین مقادیر) تقسیم‌بندی شدند. به‌علاوه، داده‌های متغیرهای تولیدمثلی پس از زایش از پایگاه داده گله بازیابی شدند. داده‌ها با استفاده از نرم‌افزار SAS ویرایش شماره ۹/۴ آنالیز شدند.

نتایج: نرخ سخت‌زایی، دوقلوزایی و جفت‌ماندگی، متریت پس از زایش و آندومتریت بالینی، وزن تولد گوساله و فاصله زایش تا اولین تلقیح در میان دسته‌های مختلف فاصله آنوجنیتال متفاوت نبود ($P > ۰/۰۵$). اما، درصد موالید نر در گاوهای با فاصله آنوجنیتال کوتاه نسبت به گاوهای با فاصله آنوجنیتال متوسط و بلند کمتر بود ($P < ۰/۰۵$). علاوه بر این، نرخ باروری در تلقیح اول پس از زایش در گروه فاصله آنوجنیتال متوسط بالاتر از گروه‌های فاصله آنوجنیتال کوتاه و بلند بود ($P < ۰/۰۵$).

نتیجه‌گیری نهایی: در نتیجه، مطالعه حاضر بیانگر نرخ آبستنی در اولین تلقیح پس از زایش نامناسب در گاوهای دارای حداقل و حداکثر طول فاصله آنوجنیتال بود و نشان داد که این باروری کمتر از طریق تغییر در نرخ مشکلات تولیدمثلی پس از زایش حاصل نشده بود.

واژه‌های کلیدی: فاصله آنوجنیتال؛ گاو شیری؛ سخت زایی؛ باروری؛ عفونتهای رحمی

نویسندهٔ مسئول: وحید اکبری‌نژاد، گروه مامایی و بیماری‌های تولیدمثل دام، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران

ایمیل: v_akbarinejad@ut.ac.ir