Fertility Parameters in Heifers

Mehmet Cengiz^{1,a,*}, VefaTohumcu^{1,b}, Armağan Hayırlı^{2,c}

- ¹ Atatürk University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Erzurum, Turkey
- ² Atatürk University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Disorders, Erzurum, Turkey

^aORCID: 0000-0001-9913-3468; ^bORCID: 0000-0003-4062-7513; ^cORCID: 0000-0002-4446-0848

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Abstract

This retrospective study was conducted to elucidate reproductive parameters of heifers. The age of insemination resulted with pregnancy (AIRP), the number of inseminations per pregnancy (NIPP), length of the gestation period (LGP), age at first calving (AFC), maternal body weight at the first calving (BWFC), and bodyweight of calves at parturition (BWCP) data were compiled from 179 heifers (Holstein, n = 32; Simmental, n = 147), which were born between June 22, 2013 and October 31, 2019 and raised under the same managerial program in a private farm. The data were subjected to 2-way ANOVA and regression analysis. The AIRP was longer in Simmental heifers than Holstein heifers (515 ± 5 d vs. 482 ± 7 d; P<0.01) while NIPP values were similar (1.99 ± 0.10 vs. 2.06 ± 0.10, P>0.05). Likewise, the AFC was 39 d shorter for Holstein heifers than that for Simmental heifers (753 ± 7 d vs. 796 ± 5 d, P<0.001). The LGP was shorter in Holstein heifers than Simmental heifers (271 ± 1 d vs. 280 ± 1 d; P<0.0001). However, the BWFC (576 ± 10 kg vs. 585 ± 5 kg) and BWCP (39.0 ± 0.8 kg vs. 40.7 ± 0.5) were not different. The sire, calf sex, and maternal weight accounted for 83.1% (P<0.01), 9.3% (P<0.05), and 7.6% (P>0.05) of variations in the BWCP. The linear models describing relationships were 1) LPG (d) = 0.46-BWCP (kg) + 260.4, R² = 0.12 (P<0.0001), 2) AFC (d) = 28.05 NIPP + 731.36, R² = 0.35, (P<0.0001), and 3) BWFC (kg) = 0.32-AFC (d) + 328.47, R² = 0.11 (P<0.0001). In conclusion, reproductive management covering accurate observation for estrus sign, inseminated by experienced inseminator, checked for pregnancy status as early as possible is crucial to improve life–time productivity. Sire selection should be considered for minimizing dystocia incidence.

 $\textbf{Keywords:} \ \textbf{Body weight, heifer, insemination, reproduction.}$

INTRODUCTION

Heifers are the genetic future of dairy farms, and they had economically determinative roles for sustainable dairy production (Zwald 2007; Wiegel et al., 2012; Çolakoğlu and Küplülü, 2016). Heifer breeding cost accounts for 15 - 20% of the total costs in dairy farming (Heinrichs, 1993; Gümüş and Akın, 2019). Puberty begins with the first ovulation in 6 - 18 months of age cattle (Atkins et al., 2013), and they are inseminated in 14 - 22 months of age (Heinrich, 1993). However, puberty is associated with live body weight rather than age. Thus, appropriate feeding strategy has a critical role in shortening the first insemination thereby enhancing age, productivity and reducing production costs (Gümüş and Akın, 2019).

The herds should be supported by the heifer population continuously for maintaining sustainable production (Hinman and Willett, 2021; Ateş and Arık, 2021). That is, culling cows/heifers must be compatible with presence of primiparous cows (involving heifer cows) in herd (Orpin and Eslemont, 2010). Increased age, decreased milk production, infertility, and diseases are the major causes for culling (Allaire, 1981; Kossaibati and Esslemont, 1995). If herd faces reduced breeder cows, it is recommended to apply strategies including sexed semen usage (Cottle et al., 2018), decreasing first insemination age (Davod and Elbaz, 2020), and the sexual cycle synchronization (Yizengaw, 2017).

Some parameters such as the age of insemination resulted in pregnancy (AIRP) (Wathes et al., 2014), the number of inseminations per pregnancy (NIPP) (Demiral

et al., 2007; Arslan et al., 2017), the length of the gestation period (LGP) (Tao and Dahl, 2013), the age at the first calving (AFC) (Gill and Allaire, 1976), the body weight at the first calving (BWFC) (Han et al., 2021), and the body weight of calves at parturition (BWCP) (Nogalski, 2003) have a definitive effect on sustainable production and productivity in dairy herds. This study aimed to compare the reproductive parameters of the Holstein and Simmental heifers and body weights of their calves.

MATERIALS AND METHODS *Heifers*

(Holstein, n = 32; Simmental, n = 147), which were born between June 22, 2013 and October 31, 2019 and raised under the same managerial programs (reproduction, nutrition, and health) in a private dairy farm (TR250001027418; Nail Cinisli Agriculture Livestock Food Industry and Trade Inc.) located in Aşkale Province of Erzurum (39°54′N, 40°51′E). Heifers were housed in a 2X2 free–stall nylon–covered tent barn. In this barn model,

the cows were protected from wind and rain and exposed

to outdoor temperature and humidity changes.

This retrospective study was carried on 179 heifers

Heifer calves were fed total mixed ration to meet nutrient requirements from calving till parturition, as well as thereafter (NRC, 2001). All heifers were taken the estrus induction and artificial insemination program if they reached 420 day of age, weighed at least 370 kg, and had at least one corpus luteum on ovaries. All retrospective data about estrus induction, insemination, pregnancy control, and postpartum measurements were achieved

from the herd management system software (DeLaval: Alpro Windows 6.60, Tumba, Sweden).

Estrus induction

According to farm records, a routine estrus induction protocol was used by veterinarians to synchronize the heifers in the farm between 2013 and 2019. The hormonal induction was initiated by injecting 2 mL of $PGF_{2\alpha}$ analog intramuscularly (d0) (PGveyx Forte®, 250 µg/mL cloprostenol, Veyx-Pharma, Schwarzenborn, Germany) as described by Lapp et al. (2020). After the estrus induction, heifers that exhibit pronounced sexual activity such as mounting other heifers, presence of cervical mucus around vulvar lips and tail were inseminated artificially by the same technician. The heifers that did not show estrus sign in 5 d (d5) after the first cloprostenol injection, were subjected to the second injection on the 11th day (d11). These heifers were then artificially inseminated 60 h following the second cloprostenol injection. Immediately after artificial insemination, the heifers were administered with 2.5 ml of GnRH analog (Receptal®, 4 μg/mL, buserelin acetate, Intervet International GmbH. Unterschleissheim, Germany).

Pregnancy confirmation

The heifers, which were non–return the estrus in 21 d after insemination, were taken to the pregnancy control between the $28-30\,d$ after the artificial insemination via a 7.5 MHz linear probe ultrasound (Wed $3000^{\$}$, Shenzen WELLD Medical Electronics Co. Ltd., Shenzen, China). According to farm data, veterinarians performed the consecutive pregnancy controls on d 45 (\pm 5d), 60 (\pm 10d), and 90 (\pm 10d) relative to the artificial insemination.

Statistical analysis

The AIRP, NIPP, LGP, AFC, BWFC, and BWCP data were obtained the herd management system software (DeLaval: Alpro Windows 6.60, Tumba, Sweden). Data analyses were performed using statistical software (Statistical Analysis System, SAS software, Version 9.0,

2012, Cary, NC). In a 2–way ANOVA, the linear model $(Y_{ijk} = \mu + B_i + S_j + (BxS)_{ij} + e_{ijk})$ included the main effect of breed of heifer (B_i) and sex of calf (S_j) as well as breed of heifer by sex of calf interaction $[(BxS)_{ij}]$ to evaluate reproductive parameters.

Pearson's correlation (r) and linear regression $(y = b_0 + b_1 \cdot x)$ analyses were computed to determined relationships among reproductive parameters. Finally, factor analysis based on type III sums of squares were processed to elucidate factors affecting calf weight. For this, BWFC were categorized as heavy, moderate, and light if it was >600 kg, 550-600 kg, and <550 kg, respectively and sires were grouped by their tag number. Statistical significance was declared at P<0.05.

RESULTS

Data from 1 Holstein heifers and 1 Simmental heifer were excluded due to twinning, data from 2 Holstein heifers and 2 Simmental heifers were excluded due to abortions. Thus, data were compiled from 32 Holstein heifers and 147 Simmental heifers.

Table 1 summarizes the effect of breed of heifer and sex of calf on the AIRP, NIPP, LGP, AFC, BWFC, and BWCP. Although the AIRP was significantly longer in Simmental heifers than Holstein heifers (515 ± 5 d vs. 482 \pm 7 d; P<0.01), their NIPP values were was similar (1.99 ± 0.10 for Simmental heifers vs. 2.06 \pm 0.10 for Holstein heifers; P>0.05). The AFC was 39 d shorter for Holstein heifers than that for Simmental heifers (753 ± 7 d vs. 796 \pm 5 d, P<0.001). Moreover, the LGP was shorter in Holstein heifers than Simmental heifers (271 ± 1 d vs. 280 \pm 1 d; P<0.0001). However, the BWFC (271 ± 1 d 271 ± 1 d 271 for Holstein heifers and 271 ± 1 d $271 \pm$

The AIRP, NIPP, LGP, AFC, BWFC, and BWCP values did not change due to the sex of calves (Table 1). There was no interaction effect of the breed of heifer by the sex of calf (Table 1).

Table 1. The effect of breed of heifer and sex of calf on reproductive parameters in Holstein and Simmental heifers entering first lactation.

	Response Variables*							
Factors	AIRP	NIPP	LGP	AFC	BWFC	BWCP		
Breed of Heifer (BH)								
Holstein (n=32)	482±7	2.06±0.18	271±1	753±7	576±10	39.0±0.8		
Simmental (n=147)	515±5	1.99±0.10	280±1	796±5	585±5	40.7±0.5		
Sex of Calf (SC)								
Female (n=84)	508±6	1.96±0.11	280±1	787±7	590±6	39.4±0.6		
Male (n=95)	510±6	2.03±0.14	279±1	789±6	577±5	41.3±0.6		
BH x SC								
Holstein-Female (n=16)	482±11	2.13±0.27	270±1	752±11	584±15	38.1±1.1		
Holstein-Male (n=16)	481±10	2.00±0.26	273±1	754±10	568±13	40.0±1.3		
Simmental–Female (n=68)	515±7	1.93±0.12	280±1	795±7	592±7	39.8±0.7		
Simmental–Male (n=79)	516±7	2.04±0.17	280±1	797±7	579±6	41.6±0.7		
ANOVA								
ВН	0.0031	0.7387	0.0001	0.0002	0.3833	0.1163		
SC	0.9774	0.9776	0.3181	0.8694	0.1753	0.0726		
BH x SC	0.9196	0.6232	0.3691	0.9816	0.8815	0.9573		

*AIRP = The age of insemination resulted in pregnancy; NIPP = The number of inseminations per pregnancy; LGP = The length of the gestation period; AFC = The age at the first calving; BWFC = The body weight at the first calving; BWCP = The body weight of calves at parturition. Data are least square means ± standard errors.

The AIRP was strongly correlated with the AFC (r = 0.99; P<0.0001) and moderately correlated with the NIPP (r = 0.59; P<0.0001) (Table 2). Despite statistical significance, correlations among other reproductive

parameters were either weak or lacking. The sire, calf sex, and maternal weight accounted for 83.1% (P<0.01), 9.3% (P<0.05), and 7.6% (P>0.05) of variations in the BWCP (Figure 1).

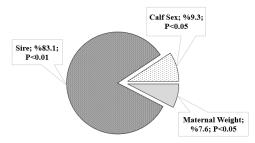
Table 2. The interrelationships among reproductive parameters in Holstein and Simmental heifers

entering first lactation.

	AIRP	NIPP	LGP	AFC	BWFC	BWCP
		0.59	0.11	0.99	0.34	0.20
AIRP	1.00	(P<0.0001)	(P>0.05)	(P<0.0001)	(P<0.0001)	(P<0.05)
			-0.07	0.57	0.39	0.10
NIPP		1.00	(P>0.05)	(P<0.0001)	(P<0.0001)	(P>0.05)
				0.25	0.12	0.34
LGP			1.00	(P<0.001)	(P>0.05)	(P<0.0001)
					0.35	0.24
AFC				1.00	(P<0.0001)	(P<0.01)
						0.19
BWFC					1.00	(P<0.05)
BWCP						1.00

*AIRP = The age of insemination resulted in pregnancy; NIPP = The number of inseminations per pregnancy; LGP = The length of the gestation period; AFC = The age at the first calving; BWFC = The body weight at the first calving; BWCP = The body weight of calves at parturition. Data are Pearson's correlation coefficients (r) and statistical significance.

Variance Sources in Calf Weights at the First Parturition



■ Maternal Weight ■ Sire □ Calf Sex

Figure 1. Contributions of sire, maternal weight, and calf sex on the body weights of calves at the first parturition.

The best fit to describe the relationship between the BWCP and the LGP was LPG (d) = $0.46 \cdot BWCP$ (kg) + 260.4, $R^2 = 0.12$ (P<0.0001) (Figure 2). As the NIPP increased, the AFC increased linearly [AFC (d) = $28.05 \cdot \text{NIPP} + 731.36$, $R^2 = 0.35$, P < 0.0001] (Figure 3), and consequently, it led to higher BWFC (BWFC (kg) = $0.32 \cdot AFC$ (d) + 328.47, $R^2 = 0.11$, P<0.0001] (Figure 4).

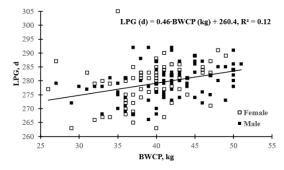


Figure 2. The relationship between the body weights of calves at parturition (BWCP) and the length of the gestation period (LGP) in heifers.

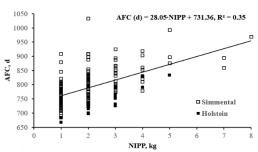


Figure 3. The relationship between the number of inseminations per pregnancy (NIPP) and the age at first calving (AFC) in heifers.

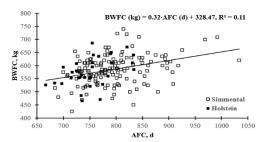


Figure 4. The relationship between the age at first calving (AFC) and the body weight at first calving (BWFC) in heifers.

DISCUSSION AND CONCLUSION

Due to breeding before 13 months of age causing reproductive problems in heifers, breeding approaches should be initiated at the 14 - 15 months of age (Cooke et al., 2013; Sivkin and Strekozov, 2017). In the presented study, the first insemination was started at 420 d of age as advised by Gabbler et al. (2000) and Ettema and Santos (2004). Results showed nearly 62 d and 95 d of prolongation in the AIRP for Holstein heifers and Simmental heifers, respectively, resulting in 33 d of difference between the breeds. This result was compatible with Brickell et al. (2009), who reported 473 ± 5 d for Holstein heifers. This prolongation in the AIRP could be associated with the effect of possible seasonal differentiation in the study. The heifers were housed in the tent barn, which allowed cold exposure that led to stress and disturbed physiological order such as ovulation and implantation by affecting homeostasis (Thatcher, 1974; Stott, 1981; Blackshaw and Blackshaw 1994).

In the dairy farm industry, the NIPP is a useful fertility parameter to evaluate productivity, estrus observation efficiency, and skills of inseminator. The efficiency of estrus detection and regular observation supports the efficiency of inseminations. The other significant factor is insemination at the appropriate time interval after the beginning of estrus (Boztepe and Aydın, 2017). The NIPP values were similar for Holstein heifers and Simmental heifers, but greater than those reported in the literature (Brickell et al., 2009). During the harsh winter season in the region where study was conducted, insemination efficiency was poor. Differences could also be related to natural mating vs. artificial insemination.

An interesting result was noted in the LGP, which was about 9 d shorter in Holstein heifers than Simmental heifers. The gender of calves, sire of the bull, and heat stress are major factors affecting the LGP (Healy et al. 2013). The pregnant cows with male fetuses had longer LGP than those with female fetuses (McClintock et al., 2003; Healy et al., 2013). There was no effect of sex of calf on the LGP. A shorter LGP in Holstein heifers could be linked to tolerance of environmental ambient temperatures (Andersen and Plum 1965; Mc Clintoc et al., 2003; McManus et al., 2009). Both species are large-frame cattle, which may explain lacking difference in the BWFC, despite difference in the LGP (Anderson and Plum, 1965).

Prolonged AFC (> 30 months of age) adversely affects future fertility parameters as well as reduces life—time productivity, leading to economical loss. Thus, Simmental and Holstein heifers should calve at 22-25 months of age (Day and Nogueira, 2013; Lim et al., 2015; Krpalkova et al., 2017). In the presented study, the AFC differed by the breed (\sim 43 d shorter in Holstein heifer than Simmental heifers), due to the AIRP because the NIPP was similar. However, the AFC values were within the acceptable intervals for both breeds.

Although there was no breed effect, female calves were about 1 kg lighter than male calves. The BWCP could be affected by various factors such as gender, season, twinning, and sire of the bull (Kertz et al., 1997; McClintock et al., 2003; Nelson et al., 2016). In the present study, among factors the sire (83.1%) was a predominant factor in determining the BWCP, followed by the sex of calf (9.3%) and the maternal weight (7.6%). The BWFC affects dystocia incidence. The controversial results about the cut-off point of the BWFC (560 kg) to decrease the rate of dystocia were reported in previous studies (Heinrichs, 1993; Tozer and Heinrichs, 2001; Zavalidova and Stipkova, 2013) There was a higher frequency of dystocia in Simmental heifers than Simmental heifers, independent from the BWFC. This could be related more to the body fat composition (Nelson et al. 2016).

In agreement with Nelson et al. (1986) who showed a prolonged AFC at a cut-off point of the BWCP>43 kg. Each kg increase in BWCP was associated with a 0.46 d prolongation in the AFC. Increase in the NIPP (each service) was associated with 28.05 d prolongation in the AFC, which may change depending on fertility surveillance and management practices. Therefore, accurate observation of the estrus by new technologies (*i.e.*, infrared thermography) and traditional methods (*i.e.*, visual observation of standing to be mounted) in herds maintain significant future productivity (Marquez et al., 2021). Prolonged AFC could increase the BWFC (about 0.32 kg/d), and consequently the BWCP. Thus, poor reproductive efficiency may trigger dystocia and other periparturient problems.

In conclusion, the AIRP, NIPP, LGP, AFC, BWFC, and BWCP are interrelated regardless of the breed of heifers. The heifers should be taken to accurate observation for estrus sign, inseminated by experienced inseminator, checked for pregnancy status as early as possible to maximize the future productivity and minimize the costs. Above all, the management should be careful in the selection of appropriate semen to prevent dystocia and periparturient problems.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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