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EFFECT OF TIMING OF ARTIFICIAL INSEMINATION RELATIVE TO SPONTANEOUS ESTRUS ON REPRODUCTIVE PERFORMANCE AND CALF GENDER RATIO IN REPEAT BREEDER HOLSTEIN COWS

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ABSTRACT

This study was conducted to investigate the effect of the interval between the onset of spontaneous estrus and artificial insemination (AI) on reproductive performance and calf sex ratio in repeat breeder Holstein cows. Two hundred eighty lactating Holstein cows were used in this study. The animals were artificially inseminated at different times (0–6, 7–12, 13–18, or 19–24 h) after the onset of spontaneous estrus. Reproductive performance did not differ between cows inseminated at 0–6 h (n = 70), 7–12 h (n = 70), 13–18 h (n = 70), or 19–24 h (n = 70) after the onset of estrus [pregnancy rate: 0–6 h, 57.1%;7–12 h, 65.7%;13–18 h, 54.3%; and19–24 h, 62.9%; embryonic mortality rate: 0–6 h, 8.5%;7–12 h, 5.7%; 13–18 h, 5.7%; and19–24 h, 7.1%; calving rate: 0–6 h, 48.6%;7–12 h, 60%;13–18 h, 48.6%; and19–24 h, 55.7%; twinning rate: 0–6 h, 2.9%;7–12 h, 0%;13–18 h, 5.7%; and 19–24 h 2.9%; and calf sex ratio (F/M): 0–6 h, 64/36%;7–12 h, 48/52%;13–18 h, 42/58%; and19–24 h, 46/54%; P>0.05]. In general, pregnancy rates of the groups inseminated at different postpartum times differed (P< 0.01) and were higher in the group inseminated at >151 days postpartum. Pregnancy rates were similar in groups inseminated in the second and third parities, but were lower in the group inseminated in the fourth parity. In conclusion, rates of pregnancy, embryonic mortality, calving, and twinning of repeat breeder Holstein cows did not differ between cows subjected to AI at different times after the onset of spontaneous estrus. The timing of AI in repeat breeder cows did not influence the calf sex ratio. Pregnancy rate of repeat breeder cows was influenced by postpartum time and parity number.

Key words: Repeat Breeder Cow, Calf Gender Ratio, Artificial Insemination Time, Pregnancy Rate.

INTRODUCTION

Repeat breeding has long been considered an important reproductive disorder in cattle (Canu *et al.,* 2010). The incidence of repeat breeding in lactating dairy cows varies among regions, environments, and management systems (Yusuf *et al.,* 2010).

Repeat breeder cows are usually defined as subfertile animals with no anatomic or infectious abnormality that require three or more services to become pregnant (Rodrigues *et al.,* 2010). Cows that fail to conceive after several attempts are a problem for the producer (Levine, 1999).Maurer and Echternkamp (1985) reported that repeat breeders show a significant level of embryonic deaths.Repeat breeding syndrome leads to large economic losses due to extended calving intervals; increased culling rates, insemination costs, and veterinary service costs; and for dairy cows, decreased milk production (García-Ispierto *et al.,* 2007; Yusuf *et al.,* 2010).

The causes of repeat breeding are multifactorial. Genetic factors, age, subclinical infections, nutritional imbalance, endocrine dysfunction, ovulation disturbances, early embryonic losses, low sperm quality, errors related to the technique and timing of artificial insemination (AI), and poor and incorrect estrus detection were listed among the reasons for repeat breeding in cows (Heuweiser *et al.,* 1997; Jordan *et al.,* 2002; Santos *et al.,* 2004; Yusuf *et al.,* 2010). Although conception rates in dairy cows have declined over the years, herd pregnancy rates could be improved by increasing the AI submission rate through increased estrus detection efficiency or the use of timed AI protocols that synchronize follicular growth, regression of the corpus luteum, and ovulation (Ambrose *et al.,* 2010). Probability and evolutionary equilibrium theories indicate that the secondary sex ratio, which is defined as the ratio of male-to-female offspring at birth, should be 50:50 (Roche *et al.,* 2006).Several studies that have been undertaken on calf sex ratio in cattle have variable and in some cases conflicting outcomes. For instances, Bayril and Yilmaz (2013), and Rorie *et al.* (1999) reported that timing of AI did not significantly affect calf sex ratio in dairy cattle. On the contrary, Emadi *et al.* (2014) and Martinez *et al.* (2004) observed that timing of AI did significantly affect calf sex ratio in dairy cattle.

The main objective of this study was to investigate the effect of the interval between the onset of spontaneous estrus and AI on reproductive performance and calf sex ratio in repeat breeder Holstein cows.

MATERIALS AND METHODS

The study was conducted on 280 lactating Holstein cows in a commercial dairy farm (Kazova Vasfi Diren Agriculture Farm) located in Turhal District of Tokat Province, Turkey. Cows were housed in free-stall barns and fed three times daily with a total mixed ration (TMR) that consisted of corn silage, alfalfa hay, concentrate, and mineral mix. They were given free access to water. Cows were milked three times a day at approximately 8-h intervals using an automatic milking system (Rotary Magnum 90 Milking Parlor; Westfalia Surge, Izmir, Turkey), and the average lactation milk yield of cows was 10,900 kg.

In this study, cows were selected on the basis of the following criteria:regular estrus, failure to conceive following three ormore inseminations during regular estrus, absence of purulent discharge during vaginal examination, and normal reproductive tract on rectal palpation and ultrasound examination.

Estrus was visually assessed daily (30 min each time) at approximately 8-h intervals and by monitoring increased signals from a pedometer system. The main criterion for estrus detection was acceptance of mounting by other cows, and the first detection of this behavior was considered the time of onset of standing estrus. Cows underwent AI in April and May. The animals were artificially once inseminated at different times (0–6, 7–12, 13–18, or 19–24 h) after the onset of spontaneous estrus. Insemination was carried out using frozen/thawed semen by an expert veterinarian at the farm.To remove theeffects of thebulls, a service bullwas identified by the herd manager as part of a herd management program (Dairy Plan C21; Westfalia Surge, Izmir, Turkey). Parity numbers (PNs), insemination numbers (INs), and postpartum times (PTs, days) of cows were retrieved from farm computer records. Cows were divided into three groups on the basis of PN: PN (1) (2 calves), PN(2) (3 calves), and PN (3) (4 calves); cows were allocated into four groups on the basis of IN: IN (1) (3times), IN (2) (4 times), IN (3) (5 times), and IN (4) (≥ 6 times); cows were classified into four groups according to PT: PT (1) (75–100 days), PT (2) (101–125 days), PT (3) (126–150 days), and PT (4) (>151 days). Pregnancy was determined by ultrasound with a 5–7.5 MHz linear probe (Agroscan L; MWM Medical, Istanbul, Turkey) at 28–35 days after AI as the observation of a fetal heart beat. Pregnancy was confirmed by both ultrasound and rectal palpation on the 90th day of gestation. Cows pregnant at 28–35 days, but not 90 days, were considered to have embryonic mortality their fetuses.

Data were analyzed by logistic regression, using the PROC LOGISTIC procedure of SAS. Interaction effects among the independent variables (time of AI, insemination number, parity number, postpartum time, and insemination month) were investigated for dependent variables (pregnancy, and embryonic mortality). Preliminary analyses showed that all first-order interaction effects among the independent variables were not statistically significant. Therefore, interaction effects among the independent variables were ignored for the model, and the model conducted main effect of independent variables for the dependent variables. Also, reproductive parameters between the groups inseminated at 0-6, 7-12, 13-18 or 19-24 h and pregnancy rates of cows inseminated at different parities and postpartum times were analyzed by chi-square using the PROC FREQ of SAS (SAS, 1995).

RESULTS

Results of logistic regression analyses for pregnancy and embryonic mortality are given in Tables 1 and 2. The last subcategory was taken as the reference for each independent variable in these tables. The odds ratio (OR) for the first subcategory of insemination number was statistically significant (P< 0.001). Furthermore, ORs for the first and second PN subcategories were significant (P<0.001), and the OR for the third PT subcategory was also significant (P<0.001). The OR for the first insemination month subcategory was significant (P<0.001).

Pregnancy rates, embryonic mortality rates, calving rates, twinning rates, and calf sex ratios of repeat breeder Holstein cows subjected to AI at different times after the onset of spontaneous estrus are shown in Table 3.Pregnancy rates, embryonic mortality rates, calving rates, twinning rates, and calf sex ratios of Holstein dairy cows inseminated at different times after estrus onset were statistically similar (P>0.05).

Pregnancy rates of cows inseminated at different parities and PTs are presented in Table 4. Pregnancy rates for cows inseminated at 75–100, 101–125, 126–150, and >151 days postpartum in the second parity were statistically different(P<0.001), but pregnancy rates for cows inseminated at 75–100, 101–125, 126–150, and >151 days postpartum in the third and fourth parities were statistically similar (P>0.05), In general, pregnancy rates of the groups inseminated at different PTs differed (P<0.01) and were lower in the group inseminated at 126-150 days postpartum. The pregnancy rate was similar between groups inseminated in the second and third parities, and was lower in the group inseminated in the fourth parity.

Table 1. Odds rates and confidence intervals for these odds rates are given based on independent variables for pregnancy

	[illegible]	SE	P-value	Odds	95 % CI for odds	
					Lower	Upper
Time of artificial insemination (TAI)			0.565			
TAI (1)	0.284	0.451	0.530	1.328	0.548	3.216
TAI (2)	0.440	0.466	0.345	1.553	0.623	3.870
TAI (3)	-0.153	0.456	0.736	0.858	0.351	2.096
Insemination number (IN)			0.000			
IN (1)	1.870	0.629	0.003	6.486	1.892	22.231
IN (2)	-0.674	0.614	0.272	0.510	0.153	1.696
IN (3)	-0.997	0.774	0.198	0.369	0.081	1.681
Parity number (PN)			0.000			
PN (1)	3.239	0.473	0.000	25.497	10.099	64.376
PN (2)	3.129	0.489	0.000	22.842	8.766	59.523
Postpartum time (PT)			0.000			
PT (1)	0.701	0.477	0.142	2.016	0.791	5.137
PT (2)	-0.240	0.486	0.621	0.786	0.303	2.040
PT (3)	2.092	0.546	0.000	8.102	2.779	23.627
Insemination month (IM) (1)	-0.266	0.319	0.404	0.766	0.410	1.431
Constant	-3.691	0.922	0.000	0.025		

TAI (1): 0-6 h, TAI (2): 7-12 h, TAI (3): 13-18 h
IN (1): three timesinseminatedgroup, IN (2):four timesinseminatedgroup, IN (3): five timesinseminatedgroup
PN (1):second timescalvinggroup, PN (2):three timescalvinggroup
PT (1): 75-100 d, PT (2): 101-125 d, PT (3): 126-150 d
IM (1): April

Table 2. Odds rates and respective confidence intervals for embryonic mortality in relation to time of artificial insemination, insemination number, parity number, postpartum time and insemination month. These odds rates are given based on independent variables for embryonic mortality

	[illegible]	SE	P-value	Odds	95 % CI for odds	
					Lower	Upper
Time of artificial insemination (TAI)			0.979			
TAI (1)	0.184	0.725	0.799	1.202	0.290	4.981
TAI (2)	0.274	0.770	0.722	1.315	0.290	5.950
TAI (3)	-0.013	0.767	0.987	0.987	0.220	4.435
Insemination number (IN)			0.863			
IN (1)	0.355	1.026	0.730	1.426	0.191	10.640
IN (2)	0.831	1.054	0.431	2.295	0.291	18.120
IN (3)	0.148	1.408	0.916	1.159	0.073	18,327
Parity number (PN)			0.464			
PN (1)	-18.332	8411.730	0.998	0.000	0.000	.
PN (2)	-18.999	8411.730	0.998	0.000	0.000	.
Postpartum time (PT)			0.759			
PT (1)	0.203	0.750	0.787	1.225	0.282	5.329
PT (2)	0.948	0.952	0.320	2.580	0.399	16.681
PT (3)	0.095	0.840	0.910	1.100	0.212	5.707
Insemination month (IM) (1)	-2.304	0.784	0.003	0.100	0.021	0.464
Constant	21.654	8411.730	0.998	2535434651.068		

Table 3. Reproductive parameters in Holstein cows inseminated at 0-6, 7-12, 13-18,or 19-24 h after of the onset of spontaneous estrus

	Inseminate time after the onset of spontaneous estrus									
	0-6 h (n=70)		7-12 h (n=70)		13-18 h (n=70)		19-24 h (n=70)		Total (n=280)	
	%	N	%	N	%	N	%	N	%	N
Pregnancy	57.1	40	65.7	46	54.3	38	62.9	44	60	168
Embryonic mortality	8.5	6	5.7	4	5.7	4	7.1	5	6.8	19
Calving	48.6	34	60.0	42	48.6	34	55.7	39	53.2	149
Twinning	2.9	2	0	0	5.7	4	2.9	2	2.9	8
Female/male calf	64/36	23/13	48/52	20/22	42/58	16/22	46/54	19/22	50/50	78/79

Indifferent from other groups within row (P>0.05)

Table 4. Pregnancy rates of Holstein cows inseminated at different parities and various postpartum times

	Postpartum times									
	75-100 days		101-125 days		126-150 days		>151 days		Total	
Parity	%	N	%	N	%	N	%	N	%	N
2	67.9 $^{ab, A}$	28	72.7 $^{ab, A}$	33	44.8 $^{b, A}$	29	88.4 $^{a, A}$	43	43.6 ^{A}	133
3	66.7 ^{A}	12	80.8 ^{A}	26	62.5 ^{A}	24	76.9 ^{A}	26	72.7 ^{A}	88
4	26.7 ^{B}	15	31.3 ^{B}	16	0 ^{B}	10	5.6 ^{B}	18	16.9 ^{B}	59
Total	56.4 ^{ab}	55	66.7 ^{a}	75	44.4 ^{b}	63	67.8 ^{a}	87		

Rows with different superscripts (a, b) differ (P<0.01)
Columns with different superscripts (A, B) differ (P<0.01)

DISCUSSION

Repeat breeder syndrome in cattle has gained importance in recent decades as a significant cause of subfertility and economic losses in the dairy industry (Zobel *et al.*,2011). Many repeat breeder cows are not kept long enough to lactate three or more times because they have long calving intervals and reduced daily production (Ghasemzadeh-Nava *et al.*, 2007).

In this study, repeat breeder Holstein cows were artificially inseminated at different times (0–6, 7–12, 13–18, or 19–24 h) after the onset of spontaneous estrus. We found that pregnancy and calving rates in repeat breeder Holstein dairy cows inseminated at different times after of the onset of spontaneous estrus were statistically similar. These results are similar to results reported by Bayril and Yilmaz (2013) and Pursley *et al.* (1998). Dransfield *et al.* (1998) reported that delaying AI lowered fertility, and recommended performing AI between 4 and 12 h from detection of the onset of estrus. Some researchers have recommended that animals should be inseminated between 12 and 18 h after the onset of estrus to achieve good fertility (Gwasdauskas *et al.*, 1986; Nebel *et al.*, 1994; Maatje *et al.*,1997).Ghasemzadeh-Nava *et al.* (2007) reported a pregnancy rate of 30% in repeat breeder cows, while Khoramian *et al.* (2011) reported a pregnancy rate of 42.7% in repeat breeder cows. The pregnancy rate in the present study (60%) was higher than the rates reported by the authors mentioned above. The disparity in results between studies could be due to differences in animal genotypes, age of cow, sire, semen, methodology, estrus detection methods, timing of AI, treatment,lactation number, season of the year, and herd management.

In our study, the pregnancy rate of cows inseminated at 126–150 days postpartum was lower than the pregnancy rates of cows inseminated at 75–100, 101–125, or>151 days postpartum. This result is not in agreement with findings reported by Pursley *et al.* (1998), Bayril and Yilmaz (2013). This difference in the pregnancy rates may be due to the showing pregnancy with Ovsynch protocol lower than showing estrus spontaneously. Also, Pursley*et al.* (1998)reported that is22% of embryonicdeathin their study. The highembryonicdeathmay have reduced thepregnancy rate.Yusuf *et al.* (2010) found pregnancy rates at 300 and 435 days postpartum in repeat breeder cows of 50 and 58.1%, respectively. Pregnancy rate in our study was slightly higher than values reported by Yusuf *et al.* (2010). Quintela *et al.* (2004) reported that calving season was a significant risk factor for low pregnacy rate.It is known that small ovarian follicles are susceptible to heat stress (Badinga *et al.*, 1993; Wolfenson *et al.*, 1995)

Embryonic mortality rate of 6.8% was found in the present study. Bayril and Yilmaz (2013) reported embryonic mortality rate of 6%, while Villarroel *et al.* (2004) described a rate of 14%, Hossein-Zadeh *et al.* (2008) reported 13.4%, and López-Gatius *et al.* (2004) published embryonic mortality rate of 9.2% after routine pregnancy diagnosis. These differences may be associated withmanagement, highmilk yield, season, lethal genotypes, infectious diseases, nutritional deficiencies and luteal insufficiency (Ayalon, 1978; Forar *et al.,* 1995: Rajala-Schultz *et al.,* 2003). Also, Boyd and Reed (1961) and Ball (1978) showed a higher frequency of embryo mortality in cows with more than 5 lactations than in cows between the second and the fourth lactations.

In dairy cattle production systems, the incidence of twin calving in herds ranges from 0.3 to 12% (Silva-del-Río*et al.,* 2007). Many factors are associated with the risk of twinning, including milk production, parity, genotype and season (Fricke *et al.,* 2001). Twin pregnancies in dairy cattle are undesirable as they pose serious management problems and reduce the profitability of the herd (Eddy *et al.,* 1991). Cows carrying twins have a risk of pregnancy loss 3.1–6.9 times greater than that of cows carrying singletons (Silva-del-Río *et al.,* 2007). Twinning rate of 2.9 % was found in our study. This value is similar compared to findings reported by Bayril and Yilmaz (2013) and Pursley *et al.* (1998), but it was lower than findings reported by some researchers (Andreu-Vázquez *et al.* 2012; Garcia-Ispierto *et al.* 2013). Differences among the results of these two studies with the present study might be due to different estrus synchronization protocols. Because the estrus synchronization protocol used before AI affected the twin pregnancy rate. Estrus synchronization protocols has been reported toprovide an importanttheincrease the incidence of twins compared to cows conceiving at natural estrus.

Controlling the sex ratio in farms gives many advantages, since it allows the yield of the operations to be improved based on the type of production (milk or meat)(Martinez *et al.,* 2004). In the present study, the calf sex ratio did not significantly differ between cows inseminated at different times after the onset of spontaneous estrus. This result agrees with those reported by Bayril and Yilmaz (2013), and Rorie *et al.* (1999). Emadi *et al.* (2014) reported that Holstein dairy cows inseminated at 12 h after observation of standing estrus had an altered secondary sex ratio toward male calves. Martinez *et al.* (2004) noted that the percentage of female offspring can be increased by performing AI within the first 18 h from the onset of estrus, whereas delaying AI significantly increased the percentage of males.Differences between the study results could be due to estrus detection method, artificial insemination protocols, sire, semen, season, and herd management.

Conclusions: In conclusion, rates of pregnancy, embryonic mortality, calving, and twinning of repeat breeder Holstein cows did not differ between cows subjected to AI at different times after the onset of spontaneous estrus. The timing of AI after the onset of spontaneous estrus in repeat breeder cows did not influence the calf sex ratio. Pregnancy rates of the groups inseminated at different PTs differed, and pregnancy rate was higher in the group inseminated at >151 days postpartum. The pregnancy rate was similar between groups inseminated in the second and third parities, and was lower in the group inseminated in the fourth parity.

Conflict interests: The authors declare that they have no competing interests.

Authors' contributions: Tahir Bayril conceived of the study, participated in its design and coordination, verification of data and helped draft the manuscript. Orhan Yilmaz and Bahattin Cak participated in collection of data and carried out descriptive statistics. All authors approved the final manuscript.

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