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The effect of ovulatory follicle size at the time of insemination on pregnancy rate in lactating dairy cows

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Abstract: The aims of this study were to determine the factors that affect follicle size at the time of artificial insemination (AI) and to detect the relationship between ovulatory follicle size at the time of AI and pregnancy rate in dairy cows. A total of 1428 follicle size measurements were obtained from Holstein–Friesian (HF) and Swedish Red (SR) cows that were inseminated with the fixed-timed AI protocols used in this study. Follicle size was affected by breed ($P = 0.0001$), milk production ($P = 0.01$), parity ($P = 0.05$), and season ($P = 0.04$). Follicle size was greater ($P = 0.0001$) in the HF (15.55 mm) than the SR cows (14.88 mm). Multiparous cows had larger ($P = 0.04$) follicles (15.35 mm) than the primiparous cows (15.07 mm). Cows with follicle sizes between 13.5 and 17.5 mm were more likely to be pregnant than cows with other sizes follicles ($P < 0.01$). Embryonic loss was lower ($P < 0.01$) in cows with follicle sizes between 13.5 and 16.5 mm. Thus, the follicle size was affected by breed, milk production, parity, and season. Pregnancy and embryonic loss in lactating dairy cows were significantly related to follicle size.

Key words: Cow, follicle size, timed AI protocol, pregnancy rate

1. Introduction

A clear decline in the reproductive performance of lactating dairy cows has been observed over the past several decades. There are many factors that affect reproductive performance, such as high milk production, negative energy balance, low detection rates for estrus, and increased embryonic loss in dairy cows. The size of the ovulatory follicle could be an important factor that affects levels of estrogen, which induces estrous behavior and prepares the follicular cells for luteal formation and function (1–3). Subsequent formation of the corpus luteum (CL) and serum progesterone levels, which are related to the ovulatory follicle size, are known to affect embryonic survival (3,4). Thus, several studies have focused on the follicle size at the time of artificial insemination (AI) and the relations between follicle size and several aspects of the subsequent reproductive hormonal profile and fertility in lactating dairy cows. Previous studies have indicated that the ovulatory follicle size affects pregnancy rate, but the results are inconsistent (1–19).

Some studies have shown that larger ovulatory follicles at the time of AI increased pregnancy per AI (P/AI) (6,7,9–15,19,20). These studies indicated that larger follicles were associated with higher estradiol and progesterone levels and a larger CL. Higher estradiol levels improve sperm and/or oocyte transportation in the reproductive tract and uterine environment for early embryonic development (21,22). In addition, the larger CL, which forms following ovulation from larger follicles, is capable of producing more progesterone. High progesterone level is an important factor in early embryonic development in lactating dairy cows (2,4,9). However, larger follicles were also found to be associated with reduced fertility due to aging of the oocyte in some studies (23,24). Additionally, some researchers have shown that pregnancy rates are higher in dairy cows after ovulation from smaller and younger follicles (5,25). Smaller follicles were also found to be related to high embryo survival (25). However, other studies showed that follicle size does not affect pregnancy in cows (8,16–18). Thus, the objectives of this study were

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to detect to relationship between follicle size and P/AI and to determine the factors that affect follicle size at the time of AI in lactating dairy cows.

2. Materials and methods

2.1. Cows, housing, and reproductive management

Size measurements of 1428 follicles at the time of AI obtained from Holstein–Friesian (HF; n = 1114) and Swedish Red (SR; n = 314) cows were used in this study; animals were from a commercial dairy herd composed of HF and SR cows in Bursa, Turkey. Cows were housed together in free-stall barns. All cows were grouped according to their milk production and milked three times daily. The herd had an average annual milk production of 9880 kg (305 days) per cow. Cows were fed a total mixed ration formulated to meet the nutritional requirements, and the ration was in line with National Research Council recommendations (26). Daily milk yield, reproduction, health, and management records for each cow were collected with an ALPRO 2000 (DeLaval, Sweden). The average milk production for each cow was recorded for 7 days before and after AI. The protocols involving animals used in this research were approved by the Lalahan Livestock Central Research Institute Animal Care Committee. The voluntary waiting period of the farm was 50–55 days in milk (DIM). The main reproductive management of the herd was based on the detection of estrus, and timed AI protocols for cows are not able to detect estrus in cows. Only cows with at least one CL and a follicle on their ovaries that were assigned to one of the timed artificial insemination (TAI) protocols and inseminated with fixed-timed AI within the study protocol were included in the study. During the study, 6 different Ovsynch-based TAI protocols (PresynchOvsynch, DoubleOvsynch, Ovsynch, 6-day Ovsynch, Progesterone-based Ovsynch, GnRH before 7-day Ovsynch) were used on the farm. Cows were inseminated with commercial, same-breed, fertile bull semen by farm veterinarians.

Body condition score (BCS) was assessed using a scale from 1 to 5, with 1 = very thin and 5 = obese (27). To detect the seasonal effect on follicle size and P/AI, seasons were classified as hot and cold periods (hot season: June, July, and August; cold season: September to May).

2.2. Ultrasonographic examinations

Ultrasonographic examinations were performed at TAI to measure the maximum follicle (Honda HS 2000 equipped with a 7.5 MHz transducer, Honda, Japan). Measurements of ovulatory follicle sizes were made on a single frozen image of the apparent ovulatory area of the ovulatory follicle, using the average diameters in two directions at right angles. All cows were confirmed as having ovulated from the ovulatory follicle at 7 days after TAI. Cows that had two ovulatory follicles resulting in double ovulation and cows that did not ovulate were not included in the study. Pregnancy status was determined by using ultrasonography at 31 and 62 days after TAI. Visualizations of a fluid-filled uterine horn with embryonic vesicles (31 days) and the presence of a fetus (62 days) were used as positive indications of pregnancy. The value for P/AI was calculated by the number of cows diagnosed as pregnant at 31 days, divided by the number of cows receiving AI. Pregnancy loss was calculated as the number of cows diagnosed as nonpregnant at the second pregnancy check, expressed as a percentage of the cows diagnosed as pregnant at the check for pregnancy at 31 days.

2.3. Statistical analysis

All statistical procedures were performed using the computational software of SAS (Release 9.2, SAS Institute, Cary, NC, USA). The whole statistical model included the breed of the lactating dairy cows (HF or SR), parity (primiparous or multiparous), service number (first or more than one service), BCS, DIM, TAI protocols, season (cold or hot season), and milk production.

PROC LOGISTIC was used to analyze the following: the effects of milk production, DIM, BCS, parity, service number, TAI protocols, and hot/cold period on P/AI at the pregnancy diagnosis at 31 and 62 days. The PROC GLM procedure was used to analyze the effects of milk production, DIM, BCS, breed, season, parity, TAI protocols, and number of services on follicle size at TAI. A stepwise selection procedure was utilized to construct the final model.

Higher-order polynomial regression analysis was used to analyze the effect of follicle size on P/AI at the pregnancy diagnosis at 31 and 62 days and on embryonic loss. For the regression analyses, the values for P/AI and follicle size were considered as dependent and independent variables in the model.

3. Results

The effects of breed, parity, and season on the follicle size at TAI are given in the Table. The size of the ovulatory follicle was found to be influenced by breed (P = 0.0001), parity (P = 0.05), and season (P = 0.04). The average milk production was greater (P = 0.0001) in HF (36.2 ± 0.27 kg) than SR (33.2 ± 0.56 kg) cows during the study and milk production had an effect (P = 0.01) on follicle size. Follicle size increased by 0.02 ± 0.01 mm with a 1 kg increase in milk production. In addition, when follicle size was evaluated in breeds according to parity, follicle sizes were greater (P = 0.04) in multiparous (16.04 ± 0.09 mm in HF and 15.54 ± 0.14 mm in SR) than primiparous (15.66 ± 0.10 mm in HF and 14.78 ± 0.22 mm in SR) cows. No interactions among the main factors (breed, parity, season, and milk production) affected the ovulatory follicle size. In addition, follicle size was not affected by BCS, DIM, or the number of services in this study.

Table. The effects of breed, parity, and season on follicle size at TAI in lactating dairy cows.

		Follicle size (mm ± SE[1])	P-value
Breed	Holstein–Friesian	15.55 ± 0.19	0.0001
	Swedish Red	14.88 ± 0.23	
Parity	Primiparous	15.07 ± 0 .21	0.05
	Multiparous	15.35 ± 0.20	
Season	Hot period	15.05 ± 0.20	0.04
	Cold period	15.38 ± 0.21	

[1]SE: standard error.

The distribution of the size of the ovulatory follicles at TAI is shown in Figure 1. According to this distribution, when the follicle sizes were classified as a small (≥10 and <14 mm), medium (≥14 and <18 mm), and large (≥18 mm), the percentage of the cows with medium-sized ovulatory follicles was 63.9% (913/1428), whereas the percentage of cows with small and large follicles was 18.3% (261/1428) and 17.8% (254/1428), respectively. Likewise, regression analysis indicated that inseminated cows with a follicle size between 13.5 and 17.5 mm had achieved pregnancy more often ($P < 0.01$) than the others at 31 and 62 days, as shown in Figures 2 and 3, respectively. The relationship between follicle size and embryonic loss is shown in Figure 4. In the inseminated cows with a follicle size between 13.5 and 16.5 mm, embryonic loss was decreased ($P < 0.01$).

The pregnancy rates observed in dairy cows inseminated with the Ovsynch-based TAI protocols in this study were 45.9% (655/1428) and 42.1% (601/1428) at 31 and 62 days, respectively. Embryonic loss was 8.2% (54/655). The effects of other covariant factors, such as the type of TAI protocol, breed, parity, season, service number, milk production, DIM, and BCS, on P/AI were also evaluated. Only the season affected P/AI at 31 days ($P = 0.04$; 48.3%, 413/854 in the cold season and 42.1%, 242/574 in the hot season) and 62 days (45.1%, 386/854 in the cold season and 37.4%, 215/574 in the hot season), whereas other covariant factors did not affect P/AI.

4. Discussion

The results of the present study show that follicle size at TAI was affected by breed, season, milk production, and parity. These results showed that ovulatory follicle size at

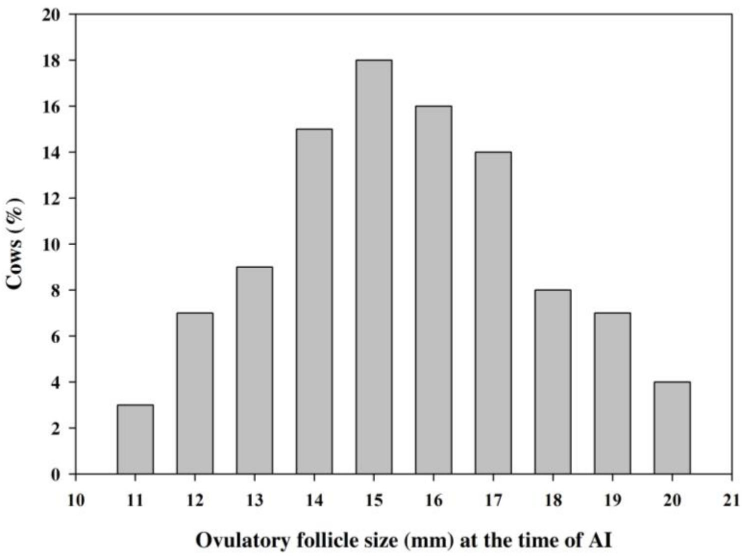

Figure 1. Distribution of follicle size at the time of AI in lactating dairy cows.

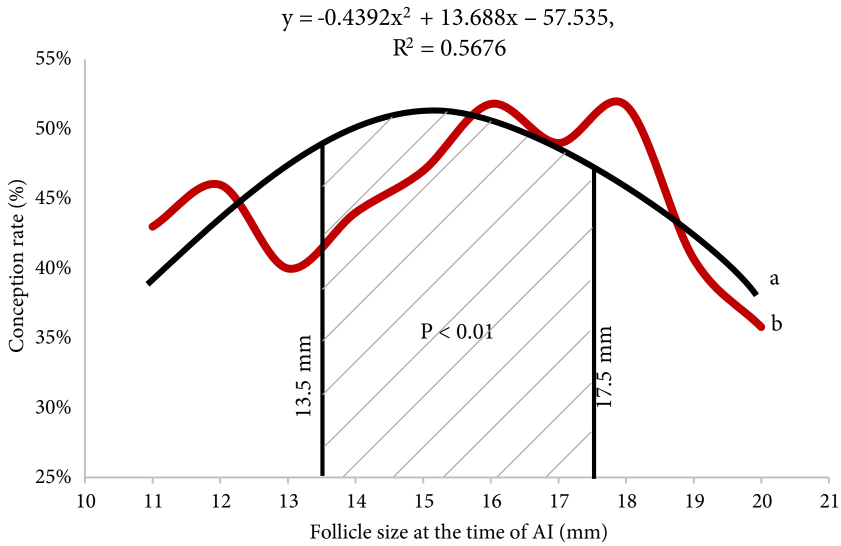

Figure 2. Relationship between the follicle size and P/AI at 31 days: a) estimated conception rate and b) real P/AI at 31 days.

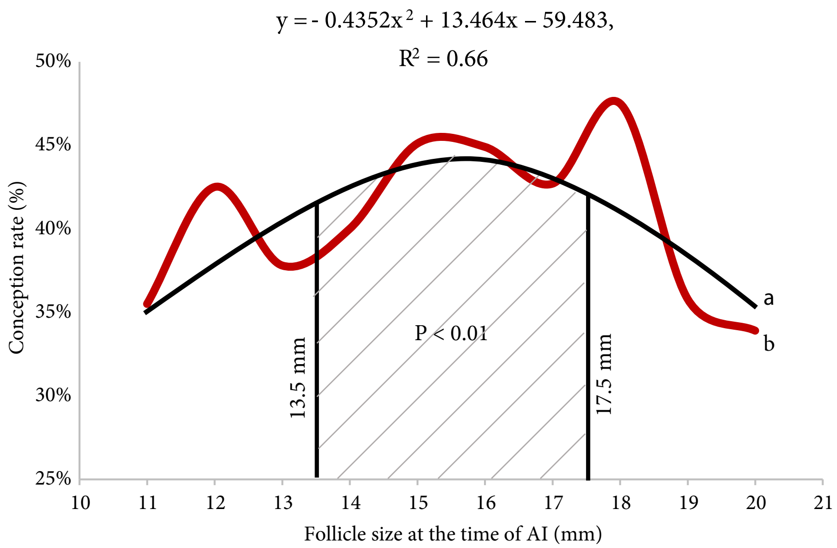

Figure 3. P/AI at 62 days according to follicle size: a) estimated P/AI at 62 days and b) real P/AI at 62 days.

TAI is affected not only by hormonal interactions during the estrous cycle (2,28) and hormonal treatments for synchronization (6,9–11,19,29), but also by other factors such as parity (8), milk production (5,30), season, and breed. For instance, milk production has been correlated with an increase in the size of the ovulatory follicle. For each 0.45 kg increase in milk production there was a 0.032 mm increase in follicle size (5). Similarly, in this study, when milk production increased by 1 kg, follicle size increased by 0.02 ± 0.01 mm ($P = 0.01$). Increased metabolism of steroid hormones has been found to be related to feed intake in high-producing cows compared to low-producing ones (30). The feed intake increases the metabolic clearance rate of progesterone and estradiol (30). Decreased circulating progesterone and estradiol affected the development, size, and number of follicles in lactating dairy cows (28–30). In addition, our results revealed larger follicles ($P = 0.05$) in multiparous cows (15.35 mm) than in primiparous (15.07 mm) cows. Similarly, Tenhagen et al. (8) indicated that follicle size is affected by parity, with primiparous cows producing smaller follicles than multiparous cows. Multiparous cows

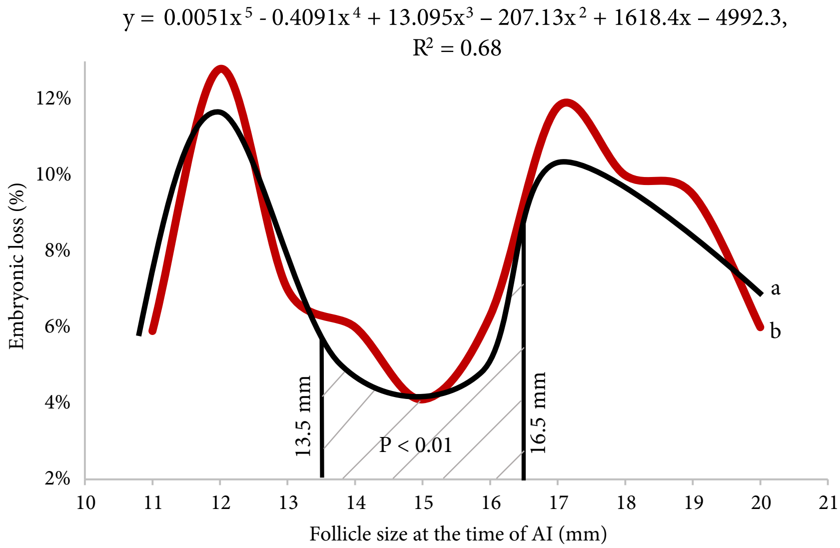

Figure 4. Relationship between embryonic loss and follicle size in lactating dairy cows: a) estimated embryonic loss at 62 days and b) real embryonic loss at 62 days.

are known to produce more milk than primiparous cows, and increasing milk production, feed intake, and steroid metabolism could have potential effects on the increasing follicle size in multiparous cows (8,30).

Heat stress during the warm season is accepted as a major cause of low fertility in dairy cows. Heat stress has been shown to compromise ovarian follicle development, steroidogenic capacity, oocyte quality, and luteal progesterone secretion (14). In our study, follicle size was smaller ($P = 0.04$) in the hot period (15.05 mm) compared to the cold period (15.38 mm). These results are not in line with the results of Lopes et al. (4); those authors reported that follicle size was not affected by season. Our data confirmed our expectations because feed intake and milk production are higher in the cold season than in the hot season. In this study, the HF cows produced larger ($P = 0.0001$) follicles (15.55 mm) than the SR (14.88 mm) cows. This difference between the breeds indicates that different breeds of lactating dairy cows may produce differences in sizes of ovulatory follicles at TAI, which may result in different subsequent fertility rates.

The reproductive performance of dairy cows depends on many factors, such as the embryo quality, adequate progesterone production by the CL, and the timing of ovulation. The ovulatory follicle size is associated with embryo quality and circulating steroid hormones (progesterone and estrogen) (4,9,25). This suggests that the follicle size at the time of AI is directly related to fertility in lactating dairy and beef cows. For instance, the concentration of estradiol, which is produced by the ovulatory follicles, plays an important role in the emergence of behavioral estrus, the preparation of follicular cells for luteal formation and function such as the enhanced acquisition of luteinizing hormone receptors, and increased cellular proliferation (9,23). In addition, higher estradiol levels improve sperm and/or oocyte transport in the reproductive tract and uterine environment for early embryonic development (21,22). The follicle must be mature to produce a high enough estradiol concentration for these actions (28). Perry et al. (9) reported that if cows exhibit standing estrus, regardless of follicle size, the follicles are capable of producing the estradiol necessary to adequately prepare the follicular cells for luteinization. The follicle size did not affect pregnancy in cows inseminated during spontaneous estrus (9). In contrast, Revah et al. (24) showed that smaller/medium-sized follicles induced more fertility in heifers undergoing spontaneous ovulation. In the present study, follicle sizes were obtained from TAI protocols based on Ovsynch. Previous studies using TAI protocols indicated that the ovulatory follicle size affected pregnancy rate, but the results were inconsistent (1,4–19). Our results show that ovulatory follicle size significantly affects the pregnancy rate and embryonic loss in lactating dairy cows. When cows with medium-sized follicles (between 14.5 and 17.5 mm) were inseminated according to the TAI protocol, the P/AI rate detected was greater ($P < 0.01$) than in cows with follicles of other sizes. Medium-sized follicles produced higher P/AI in dairy cows. Our results are consistent with previous studies that reported that medium/large follicles have higher fertility (4,6,7,9,11–13,19). As mentioned above, larger follicle size affects embryo quality and survival in lactating dairy cows. Estrogen concentration is related positively to follicle size at the time of AI (4,9,25) and P/AI (4). In addition, the size of the follicle at the time of AI has an effect on progesterone levels and CL volume after ovulation (4–6). Previous studies have shown that larger follicles produced larger CL volume and high progesterone levels (4,6,9). Our results are consistent with these studies because the medium-sized follicles had higher ($P < 0.01$) fertility than the smaller ones. However, some researchers indicated that smaller follicles more frequently produce pregnancy than larger follicles (5,25). Likewise, some studies have indicated that larger follicles produce lower fertility due to the aging of the oocyte (23,24).

In conclusion, follicle size at TAI in dairy cows is affected by breed, milk production, parity, and season. The follicle size at TAI tended to be medium-sized. Additionally, this size of follicle (between 13.5 and 17.5 mm) more often produced pregnancy and resulted in less embryonic loss than observed with other sizes of follicles in the lactating dairy cows in this study. When Ovsynch-based TAI protocols for herd reproductive management are used, inseminated cows with medium-sized ovulatory follicles may have higher fertility.

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