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Ovulatory Follicle Size and Mucus Ferning Level in Relation To Non-Return Rate During Artificial Insemination Time in Spontaneously Oestrus Signed Cows

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The present study was conducted to compare the effect of follicle size and clinical examination on non-return rate (NRR) and to investigate whether the oestrus signs or ovulatory follicle sizes (OFS) have positive effect on pregnancy success of cows in field conditions alternatively. The clinical findings of oestrus in animals were examined by visual observation and some clinical tests undergoing spontaneous oestrous cycles and then, all cows were artificially inseminated (AI) by frozen-thawing semen. The overall mean value of NRR was obtained as 67.08 % in all cows, and also the NRR was affected by size of the ovulatory follicle ($P<0.05$), vulva edema, vaginal hyperemia and ferning of the cervical mucus ($P<0.01$). The highest rate of non-return (92.0 %) was achieved with inseminated range of 15.0-17.99 mm ovulatory follicle sized cows. However significantly ($P<0.01$) high NRR (84.78 %) was observed to increase the ferning of cervical mucus. The ovulatory follicle was located more often ($P<0.01$) on the right (64.10 %) than the left (35.90 %) ovary, but there was no effect of location of the ovulatory follicle on the NRR. In conclusion, NRR was greater spontaneously oestrous signed and inseminated cows of which vulva edema, vaginal hyperemia and ferning of cervical mucus were high and OFS was moderate. Therefore, the exhibiting behavioral oestrus can be evaluated with ovulatory follicle size and ferning of cervical mucus, the fertility may be improved.

Key words: Cow, ferning, follicle, insemination, oestrus.

Spontan Olarak Östrus Gösteren İneklerde Sun'i Tohumlama Esnasındaki Dominant Follikül Büyüklüğü ve Mukus Kristalizasyon Düzeyinin Geri Dönmeyenlerin Oranı İle İlişkisi

Bu çalışma geri dönmeme oranı üzerine follikül çapının ve klinik muayenenin etkisini karşılaştırmak ve saha şartlarında alternatif olarak gebeliğin elde edilmesi üzerine östrus belirtilerinin mi dominant follikül (DF)'ün mü daha pozitif etkili olduğunu araştırmak amacıyla yapılmıştır. Östrusun klinik bulguları görsel ve bazı klinik testlerle değerlendirildikten sonra bütün hayvanlar donmuş-çözünmüş sperma ile sun'i olarak tohumlandılar. Geri dönmeyenlerin oranı (GDO) ortalama % 67.08 olarak bulundu ve GDO üzerine DF'ün ($P<0.05$), vulva ödemi, vaginal hiperemi ve mukus kristalizasyonunun ($P<0.01$) etkileri önemli bulundu. Ortalama en yüksek GDO (% 92.0) DF çapı 15-17.99 mm arasında olan ineklerin tohumlanmalarıyla elde edildi. Bununla beraber mukus kristalizasyonundaki artışla birlikte GDO (% 84.78)'daki artış önemli ($P<0.01$) bulundu. Bu çalışmadan elde edilen diğer bir bulgu da DF'ün sol ovaryumdan (% 35.90) ziyade ($P<0.01$) sağ (% 64.10) ovaryumda şekillendiği fakat GDO üzerine etkisinin gözlenmediğidir. Sonuç olarak, kendiliğinden östrus gösteren ve müteakibinde tohumlanan vulva ödemi, vaginal hiperemisi ve mukus kristalizasyonu artmış ve DF çapı orta değerlerde olan ineklerde geri dönmeyenlerin oranı yüksek tespit edildiğinden sergilenen östrus davranışları DF ve mukus kristalizasyonu ile birlikte değerlendirilmesinin dölvemini arttırabileceği kanaatine varılmıştır.

Anahtar Kelimeler: İnek, kristalizasyon, follikül, tohumlama, östrus.

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Introduction

Fertility is important factor in sustainable animal breeding and it plays a major role in animal industry. High fertility with artificial insemination depends upon accurate and healthy females, heat detection, timing the insemination, using the high quality semen and proper insemination technique. Pregnancy can be achieved by insemination of only estrus signed cows. Viable sperm must be in the vicinity of the egg and capacitated shortly after ovulation (1-5). Because ovulation is difficult to detect, insemination should be timed relative to estrus. Reproductive rates of domestic animals are maximized when females are bred for first time at the earliest opportunity and then are rebred promptly after parturition (6-8). Synchronization of estrus and ovulation in a group of females allow predict the time of estrus with reasonable accuracy. This reduces the time required for detection of estrus. Several different hormones or combinations are used for induction of ovulation and synchronization of estrus in cattle (7, 9, 10). These hormones consist of progestagens and luteolytic agents. And ovulation can be induced in some anestrous postpartum cows that are in good body condition by administration of GnRH (11, 12). But individuals differ in their response to the hormones (13-15). If the estrus or ovulation synchronization is applied to cow, the insemination time must be

selected according to the estrus result from the synchronization method, proposed time or fixed-time AI. However, there is no information about the success of synchronization protocol in rebreeding. Bovine follicles achieve ovulatory capacity at >10 mm in diameter (16-18). The timing can be arranged according to dominant follicle size in terms of fertilization. Only a few studies have been carried out on cows to investigate to correlations between follicle size and pregnancy success (19, 20). None of the studies considered clinical examination associated with the follicle size of cows under field conditions. The objectives of the current study were to determine the relationship between ovulatory follicle sizes and clinical signs of oestrus concerning the non-return rates and to examine whether the ferning of cervical mucus or ovulatory follicle sizes have positive effect on pregnancy success of cows.

Materials and Methods

This work was carried out in Afyonkarahisar province, Turkey. Oestrus detection was carried out at the clinical unit of Veterinarian Faculty of Afyon Kocatepe University. A total of 161 multiparous Holstein bred cows were used in the research and overall mean of all animals age was 5.12 ± 0.24 years. Body conditions of all animals were subjectively scored from 1 to 5 and animals were neither "emaciated" nor "obese" and all animals were 3-4 "moderate and good".

The clinical observations were examined (21-23) for rectal and vaginal temperature (was recorded directly via thermometer °C), vaginal pH (was measured by pH test paper.), hyperemia (was estimated 1 to 3), ferning of cervical mucus (score was calculated from 1 to 3 on mucus dried on a glass slide with light microscope equipment) and vulva edema (was scored 1 to 3), and also the uterine tones were increased in all cows. Ovarian follicular examination was monitored using the method of transrectal ultrasonography described by Pierson and Ginther (24). Ultrasonography was carried out using a real-time B mode ultrasound scanner fitted with a 6 MHz Linear array probe (Concept 2000, Falco, Esaote pie Medical). The dominant follicle sizes were measured and further to that the uterine bodies were examined appertaining to clarity. It was evaluated excepting the research the uterine body was dirty. Clinically oestrus and follicle sized cows that have clear uterine body were artificially inseminated with recto-vaginal method by frozen-thawing semen. Semen was prepared within classical procedure according to Jainudeen and Hafez (2), and also spermatozoa within straw had enough progressive motility. The NRR of cows were determined by questioned the owner of cows, and then it was confirmed with USG on 60th days after following AI. All statistical analyses were carried out using SYSTAT for windows (Inc. 1800 Sherman Ave. Evanston, IL USA, 1992). The characteristics of follicle size, oestrus signs and return or non-return cows were compared by student *t*-test. The mean differences in the

NRR for cows in oestrus signs were analyzed using chi-square test. The relationships between the ovulatory follicle size and the visual examination findings of oestrus signs were studied by calculating Pearson coefficients. Differences were considered as statistically significant at the $P < 0.05$ level. Results are presented as means \pm standard error of the mean.

Results

The characteristics of oestrus signs at insemination times in cows are summarized, and also the non-return rates are exhibited in Table 1. The parameters were ranged from 37.2°C to 39.5°C for rectal temperature, from 37.9°C to 40.5°C for vaginal temperature, from 1 to 3 for vulva edema, vaginal hyperemia and ferning of cervical mucus, from 6.0 to 8.5 for vaginal pH and from 7.0 mm to 24.4 mm for OFS. The general NRR mean value was obtained as 67.08 % in all cows. The NRR were influenced by the visual examination characteristics of oestrus signs except for rectal and vaginal temperature and vaginal pH of which the effect were not significant on the NRR. The self interactions in size of the ovulatory follicle ($P < 0.05$), vulva edema, vaginal hyperemia and ferning of the cervical mucus ($P < 0.01$) were statistically significant. Significantly high ($P < 0.01$) rates of non-return (84.78 %) was observed in increased ferning cows. The non-return rates of the ultrasonic findings of ovulatory follicle sizes and locations at insemination times in cows are presented in Table 2. The ovulatory follicle was located more often ($P < 0.01$) on the right (64.10 %) than the left (35.90 %) ovary. There was no effect of location of the ovulatory follicle on the NRR. All animals were divided into six subgroups consist of (1) ≤ 7 mm (2) 12.0-14.99 mm (3) 15-17.99 mm (4) 18.0-20.99 mm (5) 21.0-23.99 mm and (6) > 24.0 mm considering to the OFS (Table 3). But it was not observed the pregnancy in 1st and 6th groups. Overall mean values of NRR were obtained as 74.19%, 92.0%, 78.26% and 25.0% respectively other groups and NRR significantly high ($P < 0.01$) in 3rd group. Correlations among the visual examination findings of oestrus signs at insemination time in cows are studied. Rectal temperature was positively correlated with vaginal temperature and ovulatory follicle size ($P < 0.01$), but negatively correlated with vaginal pH ($P < 0.05$). Vaginal hyperemia and edema were positively both interrelated ($P < 0.01$) and correlated with ovulatory follicle size ($P < 0.05$) and ferning of cervical mucus ($P < 0.01$), and also OFS was correlated with ferning of cervical mucus ($P < 0.01$). Further to that the NRR was positively correlated with ovulatory follicle size ($P < 0.05$), vulva edema, vaginal hyperemia and ferning of cervical mucus ($P < 0.01$). NRR was exhibited the high rates in the same time the values of rectal and vaginal temperature, vulva edema, vaginal hyperemia and ferning of cervical mucus were recorded to increase, but also to decrease in vaginal pH while ovulatory follicle size was moderate during insemination time.

Table 1. Overall mean values of the visual examination of oestrus signs at insemination time in cows studied (X ± s.e.m.).

NRR (%)	n	Rectal temperature (°C)	Vaginal temperature (°C)	Vulva edema (1-3)	Vaginal hyperemia (1-3)	Vaginal pH (5.5-9.0)	Mucus ferning (1-3)	Ovulatory follicle size (mm)
		-	-	**	**	-	**	*
0	53	38.36 ± 0.10	38.76 ± 0.08	2.11 ± 0.11 ^b	2.07 ± 0.11 ^b	7.33 ± 0.08	1.85 ± 0.16 ^b	15.13 ± 0.88 ^b
100	108	38.49 ± 0.07	38.81 ± 0.06	2.73 ± 0.06 ^a	2.65 ± 0.07 ^a	7.19 ± 0.06	2.73 ± 0.08 ^a	17.01 ± 0.27 ^a
Mean	161	38.45 ± 0.06	38.79 ± 0.05	2.51 ± 0.07 ^b	2.45 ± 0.07 ^a	7.24 ± 0.05	2.42 ± 0.09 ^a	16.36 ± 0.36 ^{ab}

a-b: The different superscript lowercase letters in each column are statistically different (P < 0.05).

Table 2. Overall mean values of the size and location of ovulatory follicles at insemination time and NRR in cows studied.

Variable	Right	Left	P value
Location of ovulatory follicle	64.10 %	35.90 %	< 0.01
Ovulatory follicle size (mm)	16.49 ± 0.32	16.12 ± 0.53	NS
Non-return rate	68.75 %	63.27 %	NS

NS, not significant

Table 3. The values of the size and location of ovulatory follicles at insemination time and NRR in different follicle sized cows studied.

Groups	Location (%)		OFS (X ± s.e.m.)		NRR (%)	
	Right	Left	Right	Left	Right	Left
< 12.0	66.67 ^A	33.33 ^B	10.26 ± 0.59	9.54 ± 0.88	-	-
12.0-14.99	70.97 ^A	29.03 ^B	13.47 ± 0.22	13.23 ± 0.31	77.27 ^b	66.67 ^b
15.0-17.99	72.0 ^A	28.0 ^B	16.48 ± 0.14	17.01 ± 0.19	94.44 ^a	85.71 ^a
18.0-20.99	69.57 ^A	30.43 ^B	19.03 ± 0.15	18.58 ± 0.19	78.13 ^b	78.57 ^{ab}
21.0-23.99	50.0 ^A	50.0 ^A	21.30 ± 0.21	21.78 ± 0.40	33.33 ^c	16.67 ^c
≥ 24.0	100 ^A	0 ^B	24.24 ± 0.08	-	-	-

A-B: The different superscript uppercase letters in row of location column are statistically different (P < 0.01).

a-c: The different superscript lowercase letters in NRR column are statistically different (P < 0.05).

Discussion

The NRR high or low was depended upon that it can be attributed in part to visual oestrus signs examination and follicle sizes evaluation, which led to optimal timing of insemination. In addition, cows with undetected oestrus and variety of other reproductive abnormalities during the research period must be excluded from consideration. The results of the present study indicate that NRR was noticeably high in cows that were inseminated at visual oestrus signs which overall mean values of the rectal and vaginal temperature, vulva edema, vaginal hyperemia and pH, ferning of cervical mucus and ovulatory follicle size were founded about 38.36-38.49°C and 38.76-38.81°C, 2.73, 2.45-2.65, 7.19-7.33, 2.42-2.73 and 16.36-17.01mm respectively. This same trend was noted previously for cows as increase in body temperatures, vulva edema, vaginal hyperemia and ferning of cervical mucus but also decrease in vaginal pH (8, 17, 23, 25, 26).

Follicular activity in cattle is known to be greater in the right than the left ovary (2, 27). This is consistent with

the present results as well as those of Townson et al. (18), in that ovulations occurred more frequently in the right ovary. Indeed, it was observed that approximately 64 % of dominant follicles developed in the right ovary. In contrast, other studies (28,29) found no such differences in location of the dominant follicles. The results cannot be attributed solely to reproductive status, as the study used cows, whereas the others used heifers. Of further interest is that in cows of the present study, there was a tendency for NRR to increase when ovulation occurred from the right ovary. The reasons for this observation are unclear at this time although it is not due to a higher incidence of fertile oocytes from the right ovary. In fact, the analysis showed that there was no interaction between location of the ovulatory follicle and NRR (Table 2).

Contributing factors for decreased inseminated pregnancy rates in cows that are subjected to premature estrus and/or ovulation, ovulation from smaller sized follicles resulting in low lifespan and reduced function of

CL. Short lifespan of the male and female gametes in the female tract necessitate accurate timing of artificial insemination. In cows, delayed ovulation following estrus minimizes the chances of successful fertilization due to the short fertile lifespan of bovine gametes (30-33). The lifespan of the oocyte is determining factor for successful fertilization, which means the oocyte is waiting for the arrival of eligible sperm. If the oocyte is aged before the arrival of the capable sperm cells than failure of fertilization and/or embryonic development may results. Inadequate oocyte development is another possible explanation when small follicles are induced to ovulate (19,34). Little is known about variation that exists in oocyte quality among bovine preovulatory follicles. Similar trends were observed in the present study and spontaneously oestrus and ovulation occurred, differences in minimum (0 %) and maximum (100 %) NRR rates were detected among different ($P<0.05$) follicle sizes (Table 1). The mean values of OFS was founded as 17.01 ± 0.27 mm when return was no. However, the highest NRR was achieved in cows which were inseminated the OFS range were about 15-18 mm. Consequently, when a follicle has matured and is capable of initiating the cascade of events leading to ovulation, a viable embryo can develop according to follicular size.

In this study evaluated the relationship between the non-return rates and the visual examination findings of oestrus signs at insemination times in cows. Especially, NRR was positively correlated with ovulatory follicle size

($P<0.05$), vulva edema, vaginal hyperemia and ferning of cervical mucus ($P<0.01$). It was showed that these findings support the hypothesis that visual estrous signs are indication of the ovulatory follicle size in cows undergoing spontaneous oestrous cycles and the findings were in accordance with the references findings that pregnancy rates were correlated with some oestrus signs (1, 5, 11, 22, 33). According to the result, mucus crystalization test may be offered to breeders and veterinarians a natural and affordable way to increase its chances of pregnancy. Another finding from the present study was that visual estrous signs of cows were correlated with ovulatory follicle sizes of its and were interrelated.

NRR was greater in cows undergoing spontaneous oestrus period inseminated that vulva edema, vaginal hyperemia and ferning of cervical mucus were very clear and high with moderate ovulatory follicle sized. Therefore, the exhibiting behavioral oestrus can be evaluated with ovulatory follicle size and ferning of cervical mucus, and management practices that optimize ovulatory follicle size may improve fertility. If it is developed new practicable methods for favouring ovulatory follicle size and ferning of cervical mucus estimation particularly, it is developed and/or created a practical, minor and mobile device insist of microscope equipment to use in veterinary practice for determination of the cervical mucus ferning, it may be guide to arrange of the optimal insemination time and thus NRR may be boosted with using the tool.

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