

## Relationships Age and Pregnancy Rates with Certain Oestrus Characteristics at the Insemination Time in Holstein Cows

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### ABSTRACT

The study was conducted to compare the age with respect to some oestrus signs on pregnancy success in spontaneously oestrus signed 96 Holstein cows under field conditions. Some oestrus findings were examined by visual observation and some clinical tests then all cows were artificially inseminated by frozen-thawing semen. The dominant follicle was located more often ( $P<0.01$ ) on the right (61.46%) than the left ovary (38.54%) in all animals, but there was no effect of location of the ovulatory follicle on the pregnancy rate. However, it was observed positive correlations between pregnancy rates and vaginal temperatures ( $r=0.661$ ,  $P<0.05$ ), and the ferning of cervical mucus ( $r=0.732$ ,  $P<0.01$ ), and also between the ferning of cervical mucus and OFS, and vaginal temperatures ( $P<0.05$ ) in all animals. In addition, the significantly ( $P<0.01$ ) high pregnancy rates were achieved in spontaneously oestrus occurred 4-7 years aged cows that ovulatory follicle sizes (OFS) were ranged from 14 mm to 18 mm. It was concluded that should be oestrus signs evaluated together with age, OFS and ferning of cervical mucus why pregnancy success were bigger in cows whose vaginal temperature and ferning of cervical mucus were maximum and OFS was moderate, pregnancy chance may be improved by means of optimizing insemination time in 4-7 years olded cows.

**Key Words:** Age, Cow, insemination, Oestrus, Pregnancy

## İneklerde Tohumlama Esnasındaki Bazı Östrus Belirtilerinin Yaş ve Gebe Kalma Oranı ile İlişkisi

### ÖZ

Sunulan araştırmada saha şartlarında östrus gösteren ineklerde gebelik üzerine tohumlama esnasındaki bazı östrus belirtilerinin yaşa göre etkisini karşılaştırmak amaçlanmıştır. Bu çalışmada 96 Holştayn inek kullanıldı, bazı östrus bulguları gözle muayene ve klinik testlerle değerlendirildikten sonra tüm ineklere dondurulmuş-çözdürülmüş sperma ile suni tohumlama yapıldı. Bütün hayvanlarda dominant follikülün soldan (%38.54) çok sağ (%61.46) ovaryumda yerleştiği ancak yerleşimin gebelik oranı üzerine etkisinin gözlenmediği belirlendi ( $p<0.01$ ). Bununla beraber, bütün hayvanlarda gebelik oranları ile vaginal sıcaklıklar ( $r=0.661$ ,  $P<0.05$ ) ve mukus kristalizasyonu ( $r=0.732$ ,  $P<0.01$ ) ayrıca mukus kristalizasyonu ile dominant follikül büyüklükleri ve vaginal sıcaklıklar arasındaki ( $P<0.05$ ) pozitif yöndeki ilişkiler önemli bulundu. Bununla birlikte önemli derecede ( $P<0.01$ ) yüksek gebelik oranları 4 ile 7 yaş arasındaki 14-18 mm arasında follikül çapına sahip kendiliğinden östrus gösteren ineklerin tohumlanmalarıyla elde edildi. Sonuç olarak, vaginal sıcaklık ile mukus kristalizasyonu yüksek ve orta büyüklükte folliküle sahip olan 4-7 yaşlı ineklerin tohumlanmalarıyla gebelik şansı yüksek olduğundan dominant follikül büyüklüğü ve mukus kristalizasyonun yaş ile birlikte değerlendirilerek tohumlama zamanının ayarlanmasıyla gebelik şansının artırılabilceği kanaatine varıldı.

**Anahtar Kelimeler:** Yaş, İnek, Tohumlama, Östrus, Gebelik

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## INTRODUCTION

Herd fertility is evaluated by the percentage of pregnant females and the litter size. In normal breeding conditions puberty occurs at about 12 months and age at first calving is about 30 months in cattle. The age of puberty is influenced by several factors such as physical environments. Especially nutritional levels modulate age at puberty. Apart from pathologic disturbances, the fertility parameters increase for a few years after puberty, reach a maximum, and then decrease slowly. The maximal pregnancy rate is reached around 5 to 7 years in cows. Also fertility could be increased by inseminating only cows in oestrus, proper semen thawing procedures, placement of semen in the uterus rather than cervix or vagina, uterine and clitoral massage following insemination and housing inseminated cows at temperatures below 23°C on the day after insemination (Barth 1993; Hafez and Hafez 2000; Roelofs et al., 2005; Schon et al., 2007; Sturman et al., 2000; Vaneerdenburg et al., 1996). Bovine follicles achieve ovulatory capacity at >10 mm in diameter (Acosta 2007; Bleach et al., 2004; Perry et al., 2007; Sartori et al., 2001; Townson et al., 2002). 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 (Perry et al., 2007; Cavalieri and Fitzpatrick 1995; Mosher et al., 1990; Vasconcelos et al., 2001).

The objectives of the current study were to determine the relations among some clinical signs of oestrus concerning the pregnancy success and to examine whether the oestrus signs especially the ferning of cervical mucus or/and ovulatory follicle sizes have positive effect on pregnancy success in spontaneously oestrus signed and different aged cows, and how it can be guide in decision about the optimal insemination time in field conditions alternatively.

## 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.

### Animals and management

Holstein cows were used in the research as material and all animals were divided into four subgroups consist of (1)  $\leq 3$  years (2) 4-5 years (3) 6-7 years and (4)  $\geq 8$  years considering to the age. First group was consisted of Nulliparous cows and they are not Heifers, and the other groups were consisted of Multiparous cows. Each animal was in oestrus according to the owner, and it was food conventionally. 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".

### Clinical and ultrasonographic observations

The clinical examinations were assessed (Kiddy et al., 1984; Thomas and Dobson 1989; Zartman and Dealba 1982) for vaginal temperature (was recorded directly via thermometer °C), vaginal pH (was measured by pH test paper.) and ferning of cervical mucus (score was calculated from 1 to 3 on mucus dried on a glass slide with light microscope equipment), and also it was observed the Duldungsreflex and the uterine tones were increased in all cows. Ovarian follicular examination was monitored using the method of transrectal ultrasonography (Pierson and Ginther 1988). Ultrasonography was carried out using a real-time B mode ultrasound scanner fitted with a 6 mHz Linear array probe. 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. In addition, cows with undetected oestrus and variety of other reproductive abnormalities during the research period must be excluded from consideration.

### Artificial insemination and pregnancy success

Clinically oestrus and follicle sized cows that have clear uterine body were artificially inseminated with recto-vaginal method by frozen-thawing semen. Semen which it was produced by official organ The Agriculture Ministry of The Republic of Turkey, were prepared within classical procedure, and also spermatozoa within straw had enough progressive motility. The pregnancy was determined by questioned the owner of cows about returning, and then it was confirmed within period of approximately 60 days after artificial insemination.

### Statistical analysis

All data were presented as mean  $\pm$  Standard Error Mean (SEM) for parametric variables. The comparisons of parameters were performed with the Student's t-test (Table 2) and ANOVA (Analysis of Variance, Table 1, Figure). The relationships between pregnancy and oestrus signs findings were studied by calculating Pearson coefficients. Data were analyzed using the SPSS® for Windows computing program (Version 13.0), and  $p < 0.05$  was considered statistically significant.

## RESULTS

Characteristics of the visual examination of oestrus signs at insemination times in cows are summarized in Table 1. The general mean values were found as  $5.46 \pm 0.25$  years for age,  $38.77 \pm 0.04$  °C for vaginal temperature,  $7.21 \pm 0.05$  for vaginal pH,  $2.54 \pm 0.06$  for ferning of cervical mucus,  $16.47 \pm 0.24$  mm for ovulatory follicle size and 70.83 % for pregnancy rate

in all cows. According to age value of vaginal pH and pregnancy rates were statistically significant difference in Table 1 ( $p < 0,05$ ). Therefore, the pregnancy rate was significantly higher compare to other age groups between 4-7 years. The vaginal pH value is less than 8 years and older cows. Also values of vaginal temperature, cervical mucus, follicle size do not differ in among the age group ( $p < 0,05$ ). The pregnancy rate and 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 than the left ovary in all cows. Also, all animals were divided into six subgroups consist of (1)  $< 12.0$  mm (2)  $12.0-13.99$  mm (3)  $14.0-15.99$  mm

(4)  $16.0-17.99$  mm (5)  $18.0-20.99$  mm (6)  $\geq 21.0$  mm considering to the ovulatory follicle sizes. But it was not observed the pregnancy in 1st and 6th groups. Possible associations among the visual examination findings of oestrus signs at insemination time in cows are studied. It was observed positive correlations between pregnancy rates and vaginal temperatures ( $r = 0.661$ ,  $P < 0.05$ , Table 3), and the ferning of cervical mucus ( $r = 0.732$ ,  $P < 0.01$ , Table 3), and also between the ferning of cervical mucus and ovulatory follicle sizes, and vaginal temperatures ( $P < 0.05$ ) in all animals. But vaginal pH was negatively correlated with pregnancy rate ( $r = -0.507$ ,  $P < 0.05$ , Table 3).

**Table 1:** Some examinations of oestrus signs at insemination time in different aged cows ( $X \pm S.E.M.$ ).

**Tablo 1:** Farklı yaşlı ineklerde tohumlama zamanında östrus belirtilerinin değerlendirilmesi ( $X \pm S.E.M.$ ).

<i>Agerange</i>	<i>n</i>	<i>Vaginal temperature (°C)</i>	<i>Vaginal pH</i>	<i>Ferning of cervical mucus</i>	<i>Ovulatory follicle size (mm)</i>	<i>Pregnancy rate (%)</i>
$\leq 3$	24	$38.88 \pm 0.10$	$7.21 \pm 0.07^a$	$2.50 \pm 0.12$	$16.25 \pm 0.52$	$62.50^b$
4-5	24	$38.74 \pm 0.07$	$7.23 \pm 0.10^a$	$2.58 \pm 0.12$	$16.42 \pm 0.49$	$83.34^a$
6-7	24	$38.73 \pm 0.08$	$7.27 \pm 0.07^a$	$2.58 \pm 0.12$	$16.63 \pm 0.39$	$79.17^a$
$\geq 8$	24	$38.73 \pm 0.05$	$7.13 \pm 0.11^b$	$2.50 \pm 0.12$	$16.58 \pm 0.51$	$58.33^b$
		NS		NS		NS

Different superscripts (a and d) within the same showed significant differences among the groups. ( $P < 0.05$ )

**Table 2:** Ovulatory follicle size and location, and pregnancy rates in cows ( $X \pm S.E.M.$ ).

**Tablo 2:** İneklere ovulatuvar folikül büyüklüğü ve konumu, ve gebelik oranları ( $X \pm S.E.M.$ ).

<i>Variable</i>	<i>Right</i>	<i>Left</i>	<i>P value</i>
Location of ovulatory follicle	61.46 %	38.54 %	$< 0.01$
Ovulatory follicle size (mm)	$16.65 \pm 0.29$	$16.19 \pm 0.40$	NS
Pregnancy rate	72.88 %	67.57 %	NS

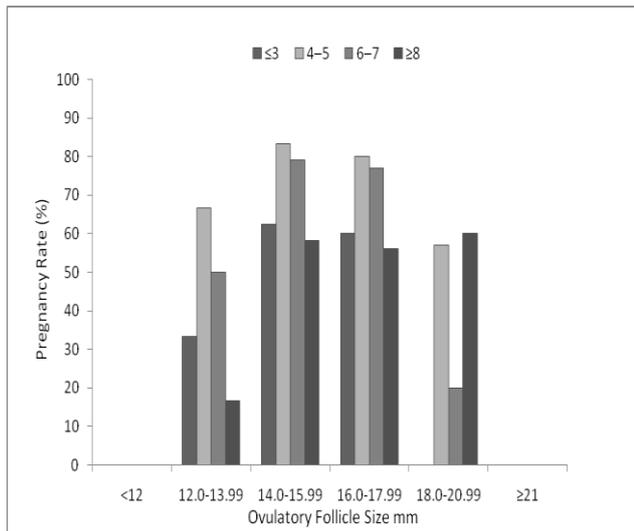
NS, not significant

**Table 3:** Pearson correlation coefficients and probabilities among the clinical parameters and pregnancy rate.

**Tablo 3:** Klinik parametreler ve gebelik oranı arasında Pearson korelasyon katsayıları ve olasılıkları

	Vaginal pH	Vaginal temperature	Ferning of cervical mucus	Ovulatory follicle size	Pregnancy rate
Vaginal pH	-	-	-	-	-
Vaginal temperature	-	-	-	-	-
Ferning of cervical mucus	-	$0.572^{**}$ $P < 0.05$	-	-	-
Ovulatory follicle size	-	-	$0.484^{**}$ $P < 0.05$	-	-
Pregnancy rate	$-0.507^*$ $P < 0.01$	$0.661^{**}$ $P < 0.05$	$0.732^*$ $P < 0.01$	-	-

Only correlation coefficient with  $P < 0.05$  are given



**Figure:** The pregnancy rates of different ovulatory follicle sized and aged cows.

## DISCUSSION

The insemination timing is still a problem particularly in herds under field condition. In practice, several procedures exist for timing of insemination. However, these procedures deliver very different results regarding fertility. Should be the fertility 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. The results of the present study indicate that pregnancy rate was noticeably high in cows that were inseminated at visual oestrus signs which degree of the vaginal temperature and ferning of cervical mucus were high and vaginal pH was low (Table 1). The same trends were noted previously for cows as increase in body temperatures and ferning of cervical mucus but also decrease in vaginal pH (Perry et al., 2007; Mosher et al., 1990; Zartman and Dealba 1982; Yoshida and Nakao 2005). However, age was no effect on pregnancy rate in this study and the finding was not in accordance with reference (Hafez and Hafez 2000). The difference may be resulted from limited material and arrangement of the artificial insemination time.

Follicular activity in cattle is known to be greater in the right than the left ovary. This is consistent with the present results as well as those of references (Hafez and Hafez 2000; Townson et al., 2002), in that ovulations occurred more frequently in the right ovary. Indeed, it was observed that approximately 61 % of dominant follicles developed in the right ovary. In contrast, other studies (Ginther et al., 1989; Sirois and Fortune 1988) found no such differences in location of the dominant follicles. These differing 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 pregnancy 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 pregnancy rate (Table 2).

Contributing factors for decreased inseminated pregnancy rates in cows that are subjected to premature oestrus and/or ovulation, ovulation from smaller sized follicles resulting in low lifespan and reduced function of CL or synchronization protocols were asynchrony of follicle wave emergence. The contributing factors from bulls might be differences in post-thaw sperm viability, progression of spermatozoa in the female internal genital tract and the resultant sperm reservoir, capacitation, acrosome reaction and fertilizing capacity (Vasconcelos et al., 2001; Phillips et al., 2004). Short lifespan of the male and female gametes in the female tract necessitate accurate timing of artificial insemination. In cows, delayed ovulation following oestrus minimizes the chances of successful fertilization due to the short fertile lifespan of bovine gametes (Roelofs et al., 2005; Acosta 2007; Dransfield et al., 1988; Walker et al., 1996).

The sperm reservoir serves to maintain the fertility of sperm until ovulation by regulating capacitation and preventing polyspermy. Because the capacitated sperm lifespan is very short, there is a need for continuous release of sperm from the sperm reservoir. This determines the presence of spermatozoa during different stages of capacitation and sperm viability before ovulation thus increasing the odds of fertilization (Kasimanickam et al., 2008). 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 for embryonic/fetal mortality when small follicles were induced to ovulate (Acosta 2007; Perry et al., 2007; Knopf et al., 1989). Little is known about variation that exists in oocyte quality among bovine preovulatory follicles. In the present study, spontaneously oestrus and ovulation occurred and the highest pregnancy rates were achieved ( $P < 0.01$ ) in 4-7 years aged cows which were inseminated the ovulatory follicle sizes were ranged from 14 mm to 18 mm (Figure). 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.

The present study evaluated the relationships between the pregnancy rate and the visual examination findings of oestrus signs at insemination times in cows. Especially, pregnancy rates were positively correlated with ferning of cervical mucus ( $P < 0.01$ ). Thus, when a female is about to ovulate, a distinct crystal or “ferning” pattern becomes present in its saliva due to an increase in the hormone estrogen (Hafez and Hafez, 2000; Thomas and Dobson, 1989). 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, ferning of cervical mucus was positively ( $P < 0.05$ ) correlated with vaginal temperature ( $r = 0.572$ ) and ovulatory follicle size ( $r = 0.484$ ) in all animals. 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 success were correlated with some oestrus signs (Barth 1993; Cavalieri and Fitzpatrick, 1995; Walker et al., 1996; Mihm et al., 1994; Saumande and Humblot, 2005).

In conclusion, pregnancy rate was greater in moderate ovulatory follicle sized cows undergoing spontaneous oestrus period inseminated that vaginal temperature and ferning of cervical mucus were high, and also age effect was no. Therefore, the exhibiting behavioral oestrus can be evaluated together with ovulatory follicle size and ferning of cervical mucus, then arrangement of insemination time may improve pregnancy success. If confirmed on large number and different aged animals from studies, this may have potential practical consequences, and further work is needed to develop new practicable methods for favouring ovulatory follicle size and ferning of cervical mucus estimation. Particularly, should be developed and/or created a practical, diminutive 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 pregnancy success may be boosted with using the apparatus.

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