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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Fertility compairing with respect to oestrus signs at the time of artificial insemination in cows

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SUMMARY

The present study was conducted to compare the effect of oestrus signs on fertility and to investigate which of the oestrus signs have positive effect on pregnancy success in Brown Swiss (BS) and Simmental (S) cows under field conditions alternatively. The clinical findings of spontaneous oestrus signed cows were examined by visual observation and some clinical tests and then, all cows were artificially inseminated by frozen-thawing semen. The overall mean values of pregnancy rate were obtained as 69.09±6.29 % and 65.12±7.35 % in BS and S cows respectively, and breed effect was no. Average the highest rate of pregnancy (92.0% for BS and 82.68% for S) was achieved with inseminated range of 14.0-18.0 mm in ovulatory follicle sized cows, and breed effect was no, either. Also the extent that significantly (P<0.01) high fertility was observed to increase in rectal and vaginal temperature, vulva edema, vaginal hyperemia and cervical mucus ferning. The ovulatory follicle was located more often (P<0.01) on the right (67.27%, BS and 60.47%, S) than the left ovary (32.73%, BS and 39.53%, S), but there was no effect of location of the ovulatory follicle on the pregnancy rate. Nonetheless, it was observed positively common correlations among rectal and vaginal temperatures (P<0.01), vaginal hyperemia both ferning of cervical mucus and pregnancy rate (P<0.01), and also ferning of cervical mucus and pregnancy rate (P<0.05) in all animals. It was concluded that fertility was bigger spontaneously oestrus signed and inseminated cows of which vulva edema, vaginal hyperemia and ferning of cervical mucus were maximum and ovulatory follicle size was moderate. Therefore, should be behavioral oestrus signs evaluated with ovulatory follicle size and ferning of cervical mucus, fertility may be improved in case of optimizing insemination time in cattle.

KEY WORDS: Cow, fertility, follicle, insemination, mucus ferning, oestrus

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İneklerin tohumlama esnasındaki östrus belirtilerine göre dölverimlerinin karşılaştırılması

ÖZET

Bu çalışma saha şartlarındaki Simental (S) ve İsviçre Esmeri (BS) ineklerde gebelik üzerine tohumlama esnasındaki östrüs belirtilerinin etkisini ve bu belirtilerin hangisinin alternatif olarak daha olumlu olduğunu karşılaştırmak amacıyla yapılmıştır. Kendiliğinden östrus gösteren ineklerdeki klinik bulgular gözle muayene ve bazı klinik testlerle değerlendirildikten sonra tüm ineklere donmuş-çözünmüş sperma ile sun'i olarak tohumlandılar. Ortalama gebelik oranı sırasıyla BS için %69.09±6.29 ve S için %65.12±7.35 olarak bulundu ve en yüksek gebelik 14-18 mm arasında folliküle sahip ineklerin tohumlanmalarıyla elde edildi ve ırk etkisi gözlenmedi. Bunlara ek olarak rektal ve vaginal sıcaklık, vulva ödemi, vaginal hiperemi ve cervikal mucus kristalizasyonu artışıyla önemli düzeyde (P<0.01) fertilite gözlendi. Dominant follikül (P<0.01) sağdan (BS'de %67.27 ve S'de %60.47) ziyade sol (BS'de %32.73 ve S'de %39.53) ovaryumda yerleştiği belirlendi ancak yerleşimin gebelik oranı üzerine etkisi gözlenmedi. Bununa beraber, bütün hayvanlarda rektal ve vaginal sıcaklıklar arasında (P<0.01), vaginal hiperemi ile

hem mukus kristalizasyonu hem de gebelik oranları arasında (P<0.01) ve aynı zamanda mukus kristalizasyonu ile gebelik oranları arasındaki (P<0.05) pozitif yöndeki ilişkiler önemli bulundu. Sonuç olarak, vulva ödemi, vaginal hiperemisi, mukus kristalizasyonu yüksek ve orta büyüklükte folliküle sahip olan kendiliğinden östrus gösteren ineklerin tohumlanmalarıyla dölveriminde artış gözlenmiş olup bu amaçla östrus davranışları dominant follikül büyüklüğü ve mukus kristalizasyonu ile birlikte değerlendirilip tohumlamanın uygun zamanı belirlenerek fertilitenin arttırılabileceğinin hatırda tutulması yararlı olacaktır.

ANAHTAR KELİMELER: İnek, dölverimi, folikül, tohumlama, mukus ferning, östrus.

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INTRODUCTION

High fertility with artificial insemination (AI) depends upon accurate healthy females in sound breeding condition, heat detection, timing insemination, high quality semen and proper insemination technique. 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.[1-5] Viable sperm must be in the vicinity of the egg and capacitated shortly after ovulation.[6] 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.[7-11] Estrus and ovulation can also be synchronized in cyclic animals through a combination of progestagen and luteolytic agent. But individuals differ in their response to the hormones; therefore, it is important to understand the nature of these differences in order to induce ovulation successfully.[12-16] Should 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, synchronization protocols in re-breeding on the same animals have no information about the success then natural breeding (without hormones) can be preferred to exogenic applications for returning the normally hormonal levels. Bovine follicles achieve ovulatory capacity at >10 mm in diameter.[17-21] 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. [22-25] 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 comparison and relations among clinical signs of oestrus concerning the fertility and to examine whether the oestrus signs especially the ferning of cervical mucus or ovulatory follicle sizes have positive effect on pregnancy success of cows 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.

Multiparous Brown Swiss and Simmental cows were used in the research as material and the means of animal age were 5.38 ± 0.26 and 4.51 ± 2.12 years respectively. Each animal was in oestrus according to it owner were 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 Ultrasonic Observations

The clinical examinations were assessed for rectal and vaginal temperature (was recorded directly via thermometer °C), vaginal pH (was measured by pH test paper) and hyperemia (was estimated 1 to 3), ferning of cervical mucus (score was calculated from 1 to 3 on mucus

dried on a glass side with light microscope equipment) and vulva edema (was scored 1 to 3), and also it was observed the Duldungsreflex and the uterine tones were increased in all cows.[26-28] Ovarian follicular examination was monitored using the method of transrectal ultrasonography described by Pierson and Ginther[29] Ultrasonography (USG) 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 clearity. The uterine bodies dirty cows were excluded from evaluation. In addition, cows with undetected oestrus and variety of other reproductive abnormalities during the research period were excluded from consideration.

Artificial Insemination and Pregnancy Rate

Clinically oestrus and follicle sized cows that have clear uterine body were artificially inseminated with recto-vaginal method by frozen-thawed semen. Semen (produced by official organ The Agriculture Ministry of The Republic of Turkey) were prepared within classical procedure according to Jainudeen and Hafez[30], and also spermatozoa within straw had enough progressive motility. The pregnancy were determined by questioned the owner of cows about returning, and then it was confirmed with rectal palpation and/or USG on 16-60th days after AI.

Statistical Analysis

All statistical analyses were carried out using SYSTAT for windows (Inc. 1800 Sherman Ave. Evanston, IL USA, 1992). The characteristics oestrus signs and pregnancy in cows were compared by one-way analysis of variance (ANOVA). Pregnancy rates between cows and oestrus findings were compaired using least square means. The effects of breed were compared with the Wilcoxon signed-ranks test. The significant differences among means were detected using Duncan's multiple range test. The relationships between pregnancy and oestrus signs findings were studied by calculating Pearson coefficients. Differences were considered as statistically significant at the $P<0.05$ level. Results are presented as means ± standard error of the mean.

RESULTS

Characteristics of the visual examination of oestrus signs at insemination times in cows are summarized in Table 1. The parameters were ranged from 36.8°C to 39.4°C for rectal temperature, from 37.8°C to 39.8°C for vaginal temperature, from 1 to 3 for vulva edema, vaginal hyperemia and ferning of cervical mucus, form 6.0 to 8.0 for vaginal pH and from 7.0 mm to 25.0 mm for ovulatory follicle size in all cows, and breed effect was no. The pregnancy rate of vulva edema, vaginal hyperemia and ferning of cervical mucus was exhibited in Figure. Significantly high ($P<0.01$) rates of pregnancy were observed in maximum values of rectal and vaginal temperature, vulva edema, vaginal hyperemia and ferning of cervical mucus. 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. There was no effect of breed and location of the ovulatory follicle on the pregnancy rate. And also, all animals were divided into seven subgroups consist of (1) <12 mm (2) 12.0-13.99 mm (3) 14-15.99 mm (4) 16.0-17.99 mm (5) 18.0-19.99 mm (6) 20.0-21.99 mm and (7) ≥22.0 mm considering to the ovulatory follicle sizes (Figure). But it was not observed the pregnancy in 1st and 7th groups. The pregnancy rates were significantly ($P<0.01$) high in 3rd and 4th groups (Figure). Correlations among the visual examination findings of oestrus signs at insemination time in cows are given in Table 3. It was observed positively collective correlations among rectal and vaginal temperatures ($P<0.01$), vaginal hyperemia both ferning of cervical mucus and pregnancy rate ($P<0.01$), and also ferning of cervical mucus and pregnancy rate ($P<0.05$) in all animals.

DISCUSSION AND CONCLUSION

The best suitable 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 rectal and vaginal temperature, vulva edema, vaginal hyperemia and ferning of cervical mucus were high (Table 1 and 2 and Figure). The same trends were noted previously for cows as increase in body temperatures, vulva edema, vaginal hyperemia and ferning of cervical mucus but also decrease in vaginal pH [11,19,23,28,31.] However, vaginal pH was no effect on pregnancy rate in this study. The difference may be resulted from evaluation time and technique.

Follicular activity in cattle is known to be greater in the right than the left ovary.[30,32] This is consistent with the present results as well as those of Townson et al.[21], in that ovulations occurred more frequently in the right ovary. Indeed, it was observed that approximately 67% for BS and 60% for S of dominant follicles developed in the right ovary. In contrast, other studies [33,34] 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 s 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 estrus 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.[25,35]

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.[2,17,36,37] 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.[15] 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.[17,24,38] Little is known about variation that exists in oocyte quality among bovine preovulatory follicles. In the present study, spontaneous oestrus and ovulation occurred and the highest pregnancy rate was achieved in cows which were inseminated the ovulatory follicle size range were about 14-18 mm (Table 2 and 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 relationship between the pregnancy rate and the visual examination findings of oestrus signs at insemination times in cows Table 3. Especially, pregnancy rate was positively correlated with vaginal hyperemia and 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.[27,30] 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, rectal temperature was positively (P<0.01) correlated with vaginal temperature 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 rates were correlated with some oestrus signs.[1,6,9,13,22]

In conclusion, pregnancy rate 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 confirmed on large number and different breed of animals from studies in which females are not synchronized estrus or ovulation, this may have potential practical consequences when considering the timing of AI in relation to estrus detection and further work is needed to identify the role of feeding factor and seasonal effect without pharmacological manipulation and also 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, 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.

Table 1. Overall mean values of oestrus signs at insemination time in cows (Means± S.E.M.).

Cows	n	Rectal temperature (°C)	Vaginal temperature (°C)	Vulvar oedema (1-3)	Vaginal hyperemia (1-3)	Vaginal pH (5.5-9.0)	Mucus ferning (1-3)	Ovulatory follicle size (mm)	Pregnancy rate (%)
Brown Swiss	55	38.39 ± 0.04	38.66 ± 0.04	2.66 ± 0.07	2.64 ± 0.08	7.21 ± 0.07	2.56 ± 0.09	16.29 ± 0.44	69.09
Simmental	55	38.55 ± 0.59	38.91 ± 0.41	2.64 ± 0.07	2.56 ± 0.08	7.21 ± 0.05	2.51 ± 0.08	16.99 ± 0.51	65.12

Table 2. Overall mean values regarding the dominant follicle at insemination time in cows.

Cows	n	Location of follicle (%)		Ovulatory follicle size (Means± S.E.M., mm)		Pregnancy rate (%)	
		Right	Left	Right	Left	Right	Left
Brown Swiss	55	67.27[a]	32.73[b]	15.97 ± 0.59	16.96 ± 0.59	70.27	66.67
Simmental	55	60.47[a]	39.53[b]	16.80 ± 0.64	17.28 ± 0.87	65.39	64.71

a–b: The different superscript lowercase letters in each row are statistically different (P< 0.01).

Table 3. Pearson correlation coefficients and probabilities among the clinical parameters at insemination times in cows ([BS]: Brown Swiss, [S]: Simmental).

	Rectal temperature	*Vaginal temperature*	*Vulva oedema*	*Vaginal hyperemia*	*Vaginal pH*	*Ferning of cervical mucus*	*Ovulatory follicle size*
Vaginal temperature	0.849[(BS)] 0.729[(S)] P < 0.01						
Vulvar oedema	0.266[(BS)] P < 0.05	-					
Vaginal hyperemia	0.309[(S)] P < 0.05	-	0.575[(BS)] P < 0.01				
Vaginal pH	-	-	-	-			
Mucus ferning	-	0.320[(S)] P < 0.05	0.463[(BS)] P < 0.01	0.561[(BS)] 0.442[(S)] P < 0.01	-		
Ovulatory follicle size	-	-	0.452[(BS)] P < 0.01	-	-	-	
Pregnancy rate	-	-	0.369[(BS)] P < 0.01	0.460[(BS)] 0.430[(S)] P < 0.01	-	0.603[(BS)] 0.474 [(S)] P < 0.01	-

Only correlation coefficients with P < 0.05 are given.

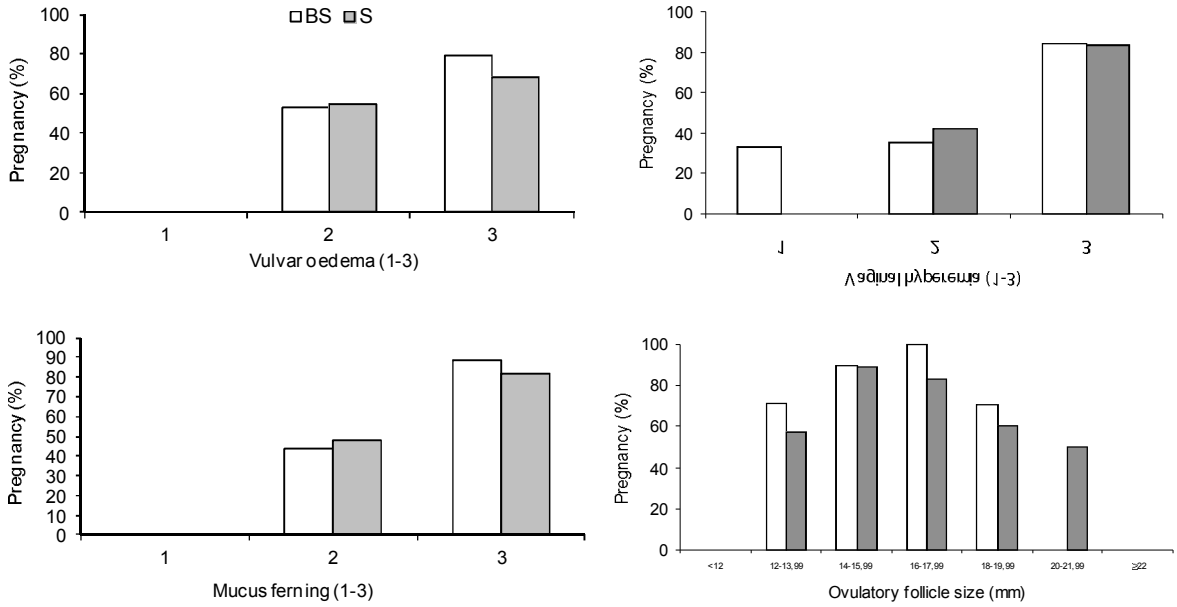

Figure. The pregnancy rates of vulva oedema, vaginal hyperemia, mucus ferning degree and different ovulatory follicle size at insemination time in Brown Swiss (BS) and Simmental (S) cows.

REFERENCES

1. **Barth AD** (1993) Factors affecting fertility with artificial insemination. Vet Clin N Am Food Anim Pract, 9, 275-289.
2. **Roelofs JB, Vaneerdenburg FJ, Soede NM, Kemp B** (2005) Various behavioral signs of estrous and their relationship with time of ovulation in dairy cattle. Theriogenology, 63, 1366-1377.
3. **Schon PC, Hamel B, Puppe B, Tuchscherer A, Kanitz W, Manteuffel G** (2007) Altered vocalization rate during the estrous cycle in dairy cattle. J Dairy Sci, 90, 202-206.
4. **Sturman H, Oltenacu EAB, Foote RH** (2000) Importance of inseminating only cows in estrus. Theriogenology, 53, 1657-1667.
5. **Vaneerdenburg FJ, Loeffler HS, Vanvliet JH** (1996) Detection of oestrus in dairy cows: a new approach to an old problem. Vet Q, 18, 52-54.
6. **Saumande J, Humblot P** (2005) The variability in the interval between estrus and ovulation in cattle and its determinants. Anim Reprod Sci, 85, 171-182.
7. **Ahmad N, Townsend EC, Dailey RA** (1997) Inskeep EK Relationships of hormonal patterns and fertility to occurrence of two or three waves of ovarian follicles, before and after breeding, in beef cows and heifers. Anim Reprod Sci, 49, 13-28.
8. **Cartmill JA, El-Zarkouny SZ, Hensley BA, Lamb GC, Stevenson JS** (2001) Stage of cycle, incidence, and timing of ovulation, and pregnancy rates in dairy cattle after three timed breeding protocols. J Dairy Sci, 84, 1051-1059.
9. **Kaim M, Rosenberg M, Folman Y** (1990) Management of reproduction in dairy heifers based on the synchronization of estrous cycles. Theriogenology, 34, 537-547.
10. **Mihm M, Baguisi A, Boland MP, Roche JF** (1994) Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. J Reprod and Fert, 102, 123-130.
11. **Yoshida C, Nakao T** (2005) Some characteristics of primary and secondary oestrous signs in high-producing dairy cows. Rep Dom Anim, 40, 150-155.
12. **Austin EJ, Mihm M, Ryan MP, Williams DH, Roche JF** (1999) Effect of duration of dominance of the ovulatory follicle on onset of estrus and fertility in heifers. J Anim Sci, 77, 2219-2226.
13. **Cavalieri J, Fitzpatrick LA** (1995) Artificial insemination of Bos indicus heifers: the effects of body weight, condition score, ovarian cyclic status and insemination regimen on pregnancy rate. Aust Vet J, 72, 441-447.
14. **Gumen A, Guenther JN, Wiltbank MC** (2003) Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. J Dairy Sci, 86, 3184-3194.

15. **Kasimanickam R, Hall JB, Currin JF, Whittier WD** (2008) Sire effect on pregnancy outcome in beef cows synchronized with progesterone based ovsynch and co-synch protocols. Anim Reprod Sci, 104, 1-8.

16. **Vasconcelos JL, Silcox RW, Rosa GJ, Pursley JR, Wiltbank MC** (1999) Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology, 52, 1067-1978.

17. **Acosta TJ** (2007) Studies of follicular vascularity associated with follicle selection and ovulation in cattle. J Rep Dev, 53, 39-44.

18. **Bleach ECL, Glencross BG, Knight PG** (2004) Association between ovarian follicle development and pregnancy rates in dairy cows undergoing spontaneous oestrous cycles. Reprod, 127, 621-629.

19. **Perry GA, Smith MF, Roberts AJ, Macneil MD, Geary TW** (2007) Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. J Anim Sci, 85, 684-689.

20. **Sartori R, Fricke PM, Ferreira JC, Ginther OJ, Wiltbank MC** (2001) Follicular deviation and acquisition of ovulatory capacity in bovine follicles. Biol Reprod, 65, 1403-1409.

21. **Townson DH, Tsang PC, Butler WR, Frajblat M, Griel LC, Johnson CJ, M ilvae RA, Niksic GM, Pate JL** (2002) Relationship of fertility to ovarian follicular waves before breeding in dairy cows. J Anim Sci, 80, 1053-1058.

22. **Busch DC, Atkins JA, Bader JF, Schafer DJ, Patterson DJ, Geary TW, Smith MF** (2008) Effect of ovulatory follicle size and expression of estrus on progesterone secretion in beef cows. J Anim Sci, 86, 553-563.

23. **Mosher MD, Ottobre JS, Haibel GK, Zartman DL** (1990) Estrual rise in body temperature in the bovine II. The temporal relationship with ovulation. Anim Reprod Sci, 23, 99-107.

24. **Perry GA, Smith MF, Lucy MC, Green JA, Parks TE, Macneil MD, Roberts AJ, Geary TW** (2005) Relationship between size of the ovulatory follicle and pregnancy success. Proc Natl Acad Sci, 102, 5268-5273.

25. **Vasconcelos JL, Sartori R, Oliveira HN, Guenther JG, Wiltbank MC** (2001) Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology, 56, 307-314.

26. **Kiddy CA, Mitchell DS, Hawk HW** (1984) Estrus-related odors in body fluids of dairy cows. J Dairy Sci, 67, 388-391.

27. **Thomas I, Dobson H** (1989) Oestrus during pregnancy in the cow. Vet Rec, 124, 387-390.

28. **Zartman DL, Hallford DM, Tierney LA, Hussain Y** (1983) Reproductive characteristics of Holstein heifers fitted with intravaginal temperature transmitters. Theriogenology, 19, 541-554.

29. **Pierson RA, Ginther OJ** (1988) Ultrasonic imaging of the ovaries and uterus in cattle. Theriogenology, 29, 21-37.

30. **Jainudeen MR, Hafez ESE (2000)** Cattle and Buffalo. 159-171. In: B. Hafez and E.S.E. Hafez (Ed), Reproduction in Farm Animals. Williams & Wilkins, Lippincott, Philadelphia, USA

31. **Zartman DL, Dealba E** (1982) Remote temperature sensing of oestrous cycles in cattle. Anim Reprod Sci, 4, 261-267.

32. **Savio JD, Keenan L, Boland MP, Roche JF** (1988) Pattern of growth of the dominant follicles during the oestrous cycle of heifers. J Reprod Fertil, 83, 663-671.

33. **Ginther OJ, Kastelic JP, Knopf L** (1989) Intraovarian relationships among dominant and subordinate follicles and the corpus luteum in heifers. Theriogenology, 32, 787-795.

34. **Sirois J, Fortune JE** (1988) Ovarian follicular dynamics during the estrous cycle monitored by real-time ultrasonograpy. Biol Reprod, 39, 308-317.

35. **Phillips NJ, Mcgowan MR, Johnston SD, Mayer DG** (2004) Relationship between thirty post-thaw spermatozoal characteristics and the field fertility of 11high-use Australian dairy AI sires. Anim Reprod Sci, 81, 47-61.

36. **Dransfield MBG, Nebel RL, Pearson RE, Warnick LD** (1998) Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. J Dairy Sci, 81, 1874-1882.

37. **Walker WL, Nebel RL, Mcgilliard ML** (1996) Time of ovulation relative to mounting activity in diary cattle. J Dairy Sci, 79, 1555-1561.

38. **Knopf L, Kastelic JP, Schallenberger E, Ginther OJ** (1989) Ovarian follicular dynamics in heifers: Test of two-wave hypothesis by ultrasonically monitoring individual follicles. Dom Anim Endoc, 6, 111-119.