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Monitoring and Comparing Follicular and Luteal Function Between Genetically High- and Low-Producing Dairy Cows by Ultrasonography

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Abstract: The objectives of this study were to investigate the effectiveness of ultrasonography in monitoring ovarian activity by examining follicular growth and regression and studying the correlation between corpus luteum (CL) measurements and plasma progesterone (P4) concentrations, and to compare high-and low-production genetic lines of dairy cows regarding endogenous bovine somatotropin (bST) and milk production effects on follicular wave patterns, size and number of follicles, CL growth and plasma P4 concentrations during the estrous cycle monitored by ultrasound. Seventeen multiparous lactating Holstein cows were divided into two groups, a low-production group (control group, n=9) and a high-production group (select group, n=8). Ovaries were examined daily from the day after estrus until the subsequent estrus by ultrasonography to monitor follicular and luteal dynamics. Nine of the 17 estrous cycles investigated in this trial had two follicular waves with an estrous cycle length of 22.2±0.4 days with a ovulatory (Graafian) follicle (18.1±0.4 mm), while 8 cycles had three follicular waves with an estrous cycle length of with a ovulatory (Graafian) follicle (18.1±0.4 mm), while 8 cycles had three follicular waves with an estrous cycle length of 24.5±0.7 days with a Graafian follicle (16.0±0.9 mm). The average length of the 2-wave estrous cycle was significantly shorter (P<0.02) than that of the 3-wave estrous cycle. There was a positive correlation between products of lengths and widths, diameters and averages of lengths and widths of CL, and mean plasma P4 concentrations, r=0.92, r=0.89 and r=0.91, respectively. In conclusion, ultrasonography was determined to be an effective tool for monitoring follicles and CL in dairy cows. Moreover, no differences were found other than a larger (P<0.05) second largest follicle in genetically select cows.

Key Words: Cow, Follicle, Corpus luteum, Progesterone, Bovine somatotropin, Ultrasonography, Reproduction, Milk Production.

Yüksek ve Düşük Verimli Süt İneklerinde Ultrasonografi ile Foliküler ve Luteal Fonksiyonların Gözlenmesi ve Karşılaştırılması

Özet: Bu çalışmada ultrasonla yapılan corpus luteum (CL) ölçümleri ile plazma progesteron (P4) konsantrasyonları karşılaştırılarak ve folikül gelişim ve gerilemeleri ultrasonla incelenerek ultrasonografinin ovaryum aktivitelerini gözetlemedeki etkinliği araştırıldı. Ayrıca, genetik olarak yüksek ve düşük verimli portpartum (50-70 gün) süt ineklerinde endojen büyüme hormonunun (bovine somatotropin=bST) ve süt veriminin foliküler dalgalar, folikül büyüklüğü, sayısı, CL büyümesi ve plazma P4 konsantrasyonu üzerine olan etkileri karşılaştırıldı. 17 baş birden fazla doğum yapmış Holştayn inekler süt verimleri yönünden genetik özellikleri dikkate alınarak iki gruba ayrıldı; yüksek verimli grup: 10.216 kg/yıl 8 inek, düşük verimli grup (kontrol) 6.797 kg/yıl 9 inek. Ovaryumlar östrus görülen günün ertesi gününden bir sonraki östrusa kadar her gün ultrasonla muayene edilip, foliküler ve luteal bulgular ölçüldü. Bu çalışmadaki 9 ineğe ait östrus siklusları 2 foliküler dalga içerip, 22.2±0.4 gün sürdü ve Graafian follikülünün ortalama çapı 18.1±0.4 mm olarak saptandı. Diğer 8 ineğe ait östrus siklusları 3 foliküler dalga içerip, 24.5±0.7 gün sürdü ve Graafian folikülünün ortalama çapı 16.0±0.9 mm olarak ölçüldü. 2 foliküler dalga içeren östrus siklusunun uzunluğu, 3 dalga içeren östrus siklusundan istatistiksel bakımdan önemli olarak kısa bulundu (P<0.02). Bu çalışmada bir östrus siklusu boyunca ultrasonla ölçülen bütün ineklere ait CL uzunlukları ile enlerinin ortalaması, yalnız uzun çaplarının ortalaması ve CL uzunlukları ile enlerinin çarpımıyla elde edilen sonuçların ortalaması ile, ortalama serum P4 konsantrasyonu arasında pozitif ilişki bulundu, bu ilişkiler sırasıyla r=0.91, r=0.89 ve r=0.92 olarak hesaplandı. Özetle süt verimi yönünden farklı genetik özelliğe sahip Holştayn ineklerinde yapılan karşılaştırmalarda ovulasyona giden son foliküler dalgadaki ikinci büyük folikül yüksek süt verimli ineklerde, düşük verimli ineklere kıyasla istatistiksel olarak daha büyük bulundu (P<0.05). Süt verimi yönünden genetik olarak farklı bu iki grupta başka bir farklılık bulunamadı. Ayrıca ultrasonun süt ineklerinde foliküllerí ve CL ölçmede, gelişim ve gerilemelerini takip etmede etkili bir yöntem olduğu tekrar belirlendi.

Anahtar Sözcükler: İnek, Folikül, Corpus luteum, Progesteron, Büyüme hormonu, Ultrasonografi, Üreme, Süt Verimi.

Introduction

Transrectal real-time linear array ultrasound instruments were used to examine genital organs in bovine reproduction, contributing to the evaluation of reproductive organs and the understanding of reproductive physiology as a recent diagnostic tool. The ultrasound apparatus works by emitting and receiving high-frequency sound waves to produce images based on the amount reflected and absorbed by tissues with various densities. For instance, dense tissues such as the mature CL reflect more of the sound waves, and are seen as a brighter image (echogenic), whereas fluid-filled structures such as follicles appear as dark (non-echogenic) areas on the screen. Thereby, the three main components of ovaries (follicles, corpora lutea and stroma) can be differentiated. Follicles as small as 2 to 3 mm, and CL with a well-defined border from about three days after ovulation can be monitored until the next estrus. Ultrasonography can be used clinically to detect follicular and luteal development, ovulation, cystic ovarian conditions, and ovarian abscesses and tumors. In addition, ultrasonography can be used to monitor individual follicles at physiologically significant periods of the estrous cycle by injecting the contrast agents into the follicles via flank laparatomy. In light of previous studies, the first purpose of this paper was to investigate the effectiveness of ultrasonography in monitoring ovarian activity in dairy cows. This was accomplished by monitoring 17 estrous cycles for follicular waves, size and number of follicles, appearance of CL and correlation between CL and plasma P4 concentrations (1-7).

Higher-producing dairy cows have lower reproductive efficiency than lower-producing dairy cows (8). This suboptimal reproductive performance is thought to be caused by a negative energy balance due to superior genetics or bST treatment. In this regard, a slight retardation of cyclic ovarian activity and decreased first service conception rates contribute to increased days open in the higher-producing dairy cows. However, reduced reproductive efficiency in higher-producing dairy cows is attributed to loss of body weight, body condition and negative energy balance rather than being considered a direct effect of high milk production (8-10). Milk production in lactating dairy cows is increased (10 to 20%) by administration of recombinant bST. On the other hand, the administration of bST decreases energy balance as a galactopoietic agent, and affects both the ovarian follicles and the CL in dairy cattle. Similar to genetically higher-producing cows, bST-supplemented cows have been reported to have increased days open, calving interval and services-per-conception, causing lower conception rates than non-treated cows, when bST supplementation is started prior to breeding; other studies, however, indicate no bST effect on reproduction. Therefore, the physiological changes caused by bST administration are parallel to the characteristics of genetically higher-producing cows. The effects of bST supplementation on ovarian activity include increased growth of the second-largest follicles during the first and second follicular wave, increased number of class 2 follicles, and, according to some studies, enhanced P4 concentration (11). Earlier research at the University of Minnesota indicated that genetically higher-producing dairy cows had higher endogenous bST than genetically lower-producing cows due to selection for milk yield (12). Therefore, the aim of this study was to compare high versus low production genetic lines of dairy cows in terms of milk production and the endogenous bST effect on follicular wave patterns, size and number of follicles, CL growth and plasma P4 concentrations during the monitored estrous cycles.

Materials and Methods

Seventeen multiparous lactating Holstein cows were divided into two groups, low milk producers (control group; 6.797 kg milk/year, n=9) and high milk producers (select group; 10.216 kg milk/year, n=8), at the St. Paul Dairy Cattle Research Center, University of Minnesota, USA, from May, 1996, to October, 1996. This genetic selection project for milk production began in 1964, and the control line has been continuously bred with 1964 semen, whereas the select line has been bred with semen from the four highest indexing milk production sires of US bull studs each year (13).

Cows were observed twice daily for standing estrus after day 50 postpartum, after morning milking (6:00-6:30 AM) and before evening milking (3:30-4:00 PM). Cows which did not show estrus by day 70 postpartum were palpated to determine estrous cycle status and assess the beginning of the estrous cycle by examining the ovaries with ultrasound to monitor regressed CL, Graafian follicle and ovulation. Once cows were determined to be in standing estrus, either by observation or by palpation and ultrasound, ovaries were monitored daily by ultrasonography under optimized conditions to determine follicular and luteal dynamics with a Medison 500 linear-array real-time B-mode ultrasound scanner equipped with a 5-MHz rectal transducer (Universal Medical Systems, Inc., NY, USA) throughout the study until subsequent standing heat or ovulation if there was no standing heat. After fecal material was removed from the rectum, the ultrasound probe was inserted into the rectum with lubricant to cover the transducer for good contact to obtain better images, and the probe was moved along the dorsal surface of the uterine horns; then, the probe was moved laterally and positioned adjacent to each ovary for examination, and each ovary was scanned in several positions by moving the probe along its surface to monitor the follicles and CL. In the case of the presence of more than one follicle of similar size in the same ovary, it was attempted to view all such follicles in the same image in order to avoid inaccurate counts. Ovarian follicles ≥ 2mm were measured by using the built-in electronic calipers after freezing the image on the screen; then these follicles were assigned to three classes, Class I (2 to 5 mm), Class II (6 to 9 mm) and Class III (≥10 mm). They were then recorded onto follicular maps. Non-spherical follicles were measured by averaging the largest and widest diameters. Moreover, the size (length and width) and number of corpora lutea were recorded.

Blood samples (10 ml) were collected from each cow by coccygeal venipucture every Monday, Wednesday and Friday starting approximately 14 days before the expected calving date to the end of the monitored estrous cycle. Then, blood samples were placed into evacuated tubes with anticoagulant (sodium fluoride and potassium oxalate), and centrifuged at 2000 X g for 15 minutes (4C). Plasma was extracted and stored at -20 C until analyzed for P4. Plasma P4 concetrations were determined by radioimmunoassay, as descibed by Kirby et al. (199) at the University of Missouri (10). Intrassay and interassay coefficients of variation were 7% and 6%, respectively, for the P4 assay.

All cows were fed in the same ratio: 25% corn silage, 25% alfalfa hay, and 50% concentrate including 17.6% crude protein and 0.765 mcal/lbs, as a net energy for lactation throughout the experiment under the same management in a barn with tied stalls.

Data was analyzed by two-sample t test statistic for number of follicular waves, length of cycle, and size and number of follicles in different classes. P4 data and CL measurements for differences between genetic lines were analyzed by repeated measures of analysis of variance procedure of SAS. Correlation of CL and P4 data from all cows were analyzed by Pearson correlation coefficiency, and CL and P4 data were normalized with the 21-day estrous cycle accepted as a model.

Results

Nine of the 17 estrous cycles investigated in this trial had lengths of 22.2±0.4 days with two follicular waves, the second wave resulting in a Graafian follicle 18.1±0.4 mm in size the day before estrus. Eight of the other cycles had lengths of 24.5±0.7 days with three follicular waves, the third ovulatory wave producing a Graafian follicle 16.0±0.9 mm in size. The average length of the 2-wave interovulatory period was significantly shorter (P<0.02) than that of the 3-wave interval. One selected and cow had an unusually long interestrus interval (29 days) and both plasma P4 level and CL started to regress on day 25. The dominant follicle arising from the third follicular wave in this cow was 21 mm the day before ovulation.

Since most cows were not monitored during estrus, except subestrus cows examined by ultrasound to confirm ovulation, the first day of the estrous cycle was accepted as an initiation of the first follicular wave. For the 2-wave intervals, the second wave began on day 11.3±0.4. For the 3-wave intervals, on the other hand, the second and third waves began on days 9.7±0.5 and 17.8±0.7, respectively (Table 1). There was no difference in the maximum diameters of first-wave dominant and second-wave dominant follicles of the two waves. Similarly, no differences were found in the maximum diameters of first-wave, second-wave and third-wave dominant follicles for the three-wave cycle. Likewise, there was no difference in the maximum diameters of first-wave dominant follicles for 2-wave and 3-wave cycles. In contrast, 2-wave-cycle Graafian follicles were significantly larger (P<0.05) than 3-wave cycle Graafian follicles (Table 1).

Table 1. Comparisons of ovarian characteristics (mean±S.E.M.) between 2-wave and 3-wave estrous cycles of dairy cows.

	2-wave cycle	3-wave cycle
No. of cows	9	8
Length of estrous cycle (day)	22.4±0.4*	24.5±.0.7*
Initiation of 1st wave (day)	1	1
Initiation of 2nd wave (day)	11.3±0.4	9.7±0.5
Initiation of 3rd wave (day)	–	17.8±0.7
Size of 1st anovulatory follicle (mm)	17.4±0.6	17.1±1.4
Size of 2nd anovulatory follicle (mm)	–	17.1±1.4
Size of Graafian follicles (mm)	18.1±0.4**	16.0±0.9**

* Difference is statistically significant, P<0.02

** Difference is statistically significant, P<0.05

Of the 17 cyles monitored, CLs were first recognized on day 1 (2 cows, day 0=estrus) day 2 (3 cows), day 3 (8 cows), day 4 (2 cows), and day 5 (1 cow). One cow's CL was first recognized on day 6 because this cow was not examined on days 4 and 5 due to rectal sensitivity and bleeding. Only one cow (select) was found with double CL in this trial. There was a positive correlation between the size of CL monitored by ultrasound and plasma P4 concentration. In order to assess the best correlation between CL data and P4 data, CL was measured in three different ways –length, the average of length and width, and product of length and width of CL on each day– and compared by using all data from all cows and the Pearson correlation coefficiency. The correlation between the product of length and width of CL and plasma P4 levels (r=0.618, P<0.01) was higher than the correlation between the diameter of CL and plasma P4 level (r=0.59, P<0.01) and the correlation between the average of length and width of CL and P4 concentration (r=0.58, P<0.01). On the other hand, a higher correlation was found for each of the comparison techniques by using mean numbers instead of raw data: correlation coefficiency for the products of lengths and widths, diameters and averages of lengths and widths of CL were r=0.92, P<0.01, r=0.89, P<0.01, and r=0.91, P<0.01, respectively.

In regard to comparison between the two genetic lines of dairy cows, no differences were found in terms of size of ovulatory follicles, first-wave dominant follicles and first-wave second largest follicles, plasma P4 levels and CL sizes, lengths of estrous cycles, number of follicular waves, total number of follicles ≥10 mm prior to ovulation, total number of Class 1, 2 and 3 follicles and the total amount of follicles during the monitored estrous cycle. Although the mean size of Graafian follicles and total number of Class 2 follicles in select cows slightly were higher than in controls, the differences were not statistically significant (P>0.05). The only important difference found was that genetically select cows had a significantly larger second largest follicle (P<0.05) than control cows during the ovulatory wave prior to estrus (Table 2).

Because the second anovulatory wave occurred in only seven cows, it was not compared. Likewise, plasma P4 concentration and CL growth and sizes did not differ between these distinct genetic lines during the monitored period (second or third cycle).

Discussion

It is not possible to agree completely with either Ginther et al, who found mostly (81%) 2-wave cycles, or Savio et al. (1988) and Sirois et al. (1988), who found mostly (80%) 3-wave cycles in dairy cows, because a lower number of animals were examined (14). On the other hand, the nine 2-wave cycles and eight 3-wave cycles found in this experiment are in agreement with previous studies indicating that cattle generally have two or three and occasionally one or four follicular waves during the estrous cycle. Moreover, intervals between follicular waves were found for both 2-wave and 3-wave cycles, and the time of first recognition of most of the CLs (day 3) agree with Ginther et al. (14, 15).

The correlation between CL and the plasma P4 level found in this study was lower than that found in previous

Table 2. Comparisons of follicular and estrous cycle characteristics (mean±S.E.M.) between genetically high-producing (select) and low-producing (control) dairy cows.

	Select	Control
No. of cows	8	9
Length of estrus (day)	23.0±0.9	23.5±0.5
No. of follicular waves	2.5±0.1	2.4±0.1
Size of Graafian follicle (mm)	18.1±0.6	16.4±0.8
Size of 2nd largest ovulatory follicle (mm)	14.0±1.2*	10.8±0.8*
Size of 1st wave dominant follicle (mm)	17.6±1.2	16.5±1.0
Size of 1st wave 2nd largest follicle (mm)	11.6±1.0	11.8±1.3
No.of follicles ≥ 10 mm before estrus	3.6±0.8	3.0±0.7
No.of Class I (2 to 5 mm) follicles	189.8±36.7	188.4±20.8
No.of Class II (6 to 9 mm) follicles	85.5±21.1	66.6±11.1
No.of Class III (≥10 mm) follicles	45.2±7.2	35.4±4.6
No.of follicles <10 mm	275.3±54.8	255.1±27.9
Total No. of follicles during monitored cycle	320.6±60.4	290.5±30.5

* Difference is statistically significant, P<0.05

studies (16, 17). However, a higher correlation (r=0.92) was found when average numbers were used from P4 data and multiplication of lengths and widths of CL rather than all data from each cow. Because during days 15 and 17 (day 0=estrus) CL sizes declined slower than P4 levels (correlation between CL sizes and P4 level was r=0.08, P=0.7 on day 16-17), and ultrasonography was less accurate for the detection of young and old CL, the overall correlation was not higher.

The Graafian follicle found significantly greater size (P<0.05) in the 2-wave cycle than in the 3-wave cycle is in agreement with Ginther et al. (14). On the other hand, no differences were found between the size of ovulatory and anovulatory follicles in 2- and 3-wave cycles. Moreover, there was no difference in the size of first-wave and second-wave dominant follicles when there was a third follicular wave. These results are not in agreement with previous studies which found the first-wave dominant follicle to be larger than the second-wave dominant follicle in the 3-wave cycle (18). Perhaps more examination is necessary to achieve an agreement. In the first part of this study; thus, ultrasonography was determined to be an effective tool for monitoring follicles and CL in dairy cows; however, measurements of either follicles or CL by ultrasound alone for only one day cannot indicate the stage of the bovine reproductive cycle without observation at regular intervals with the aid of known dates and signs of estrus, and mature CL.

Despite the higher mean number of class II follicles in select cows, there was no statistical difference; thus, no support was found for the idea that endogenous bST may cause more class II follicles. This was unexpected because cows treated with bST had more class II follicles as the first wave dominant follicle was compromised to suppress growth of subordinate follicles via reduction in energy balance (11). Differences may not have been recognized due to an insufficient number of animals, or because endogenous bST did not work as well as bST treatment probably because of an inadequate amount or different composition, or because the low energy balance was overcome somehow. Thus, more research is necessary to compare endogenous and exogenous bST with different dosages and different nutrition regimes.

There was no difference in the number of class III follicles between the two genetic lines. This supports Lucy et al. who found that the number of class 3 follicles was not altered by bST treatment in dairy cows (11). Furthermore, there was no difference in second largest follicles between the two genetic lines during the first follicular wave. In contrast, Lucy et al. declared that bST treatment resulted in a larger second-largest follicle during the first wave (11). Although Lucy et al. stated that bST treatment stimulated ovarian follicles less than 10 mm, no differences were found in this regard between two lines (19). Perhaps this disagreement is due to an inadequate amount of endogenous bST and/or different action from exogenous bST, or other reasons such as inadequate nutrition or insufficient data.

The maximum diameter of the second largest follicle during the ovulatory wave was significantly larger (P<0.05) in select cows, in accordance with Lucy et al., who stated that second-largest follicles were larger during the second follicular wave in both lactating and nonlactating cows treated with bST. Lucy et al. explained this result by suggesting that there may be extra stimulatory effects of bST on follicular growth in a low P4 milieu regardless of energy balance (11). The bST could directly affect the ovary via interactions with its own receptor, because messenger RNA for the bST receptor was discovered in the CL of cows. Since the liver has more bST receptors, it produces insulin-like growth factor I (IGF-I) in response to bST. The increased level of IGF-I in the blood stream stimulates the ovary because IGF-I receptors were found in follicular and luteal tissue; thus, bST indirectly affects the ovary via hepatic IGF-I. Moreover, bST may directly stimulate the ovary for IGF-I production causing stimulation of ovarian function in a paracrine manner; however, this potential has not been proven for the cow yet. In addition, it was indicated that ovarian granulosa cells produce IGF-I, and that IGF-I stimulates granulosa cell mitosis, steroidogenesis and increased numbers of LH receptors in follicles as an intraovarian regulator of follicular growth and differentiation. In regard to ovarian steroidogenesis, it was found by in vitro studies that an increased IGF-I level was not associated with increased follicular estrogen concentration; however, the increased IGF-I level was found to be related to increased P4 concentration in follicular fluid, thus supporting the regulation of P4 biosynthesis in vivo by IGF-I in follicular fluid. Moreover, IGF-I and FSH act synergistically to increase the number of granulosal cell LH receptors in addition to increasing granulosal cell mitosis and follicular estrogen and P4 production. Therefore, it was concluded that bST has an influence on ovarian function in dairy cows due probably to a combination of direct effects of bST and indirect effects of IGF-I on the ovary (10, 11).

The larger size of the second largest follicle in select cows during the ovulatory wave may indicate higher twinning rates in this genetic line, because it is well known that oocytes originating from large follicles are more developmentally capable than those from smaller follicles (20). This idea was also supported by Romagnoli et al., who found that the percentage of twin pregnancy rates is significantly higher (P=0.003) in select cows (8.1%) than control cows (4%) at multiparous calving (21). Moreover, it has been proven that natural twinning in cows selected for twin births is associated with an enhanced level of IGF-I in both blood and follicular fluid; thus, IGF-I from the ovary and/or systemic blood circulation has an effect on the regulation of folliculogenesis, and it mediates multiple ovulations in cattle as a genetic component (22). On the other hand, it has been concluded that treatment with bovine growth hormone-releasing factor does not enhance IGF-I concentrations in the follicle; however, the development of large follicles is affected, and so follicular fluid concentrations of IGF-I increase with follicular size (23, 24). Therefore, this higher twinning rate in select cows is most probably caused by higher endogenous bST, which results in secretion of IGF-I, because it is known that bST treatment affects folliculogenesis and ovulation, and increases the incidence of multiple births and the number of transferable embryos twofold in embryo transfer. In contrast, it has been stated that bST treatment does not increase multiple births (8).

No difference was found in terms of plasma P4 levels and luteal function during the monitored period between the two different genetic lines. This result is in disagreement with the previous studies which found that negative energy balance caused poor luteal function in the second and third estrous cycle in many high producing cows (8). However, several previous studies have indicated that bST treatment increases the size of the CL as well as the concentration of blood P4 concentrations, whereas others have not found any difference. High milk production leading to negative energy balance might adversely affect folliculogenesis because GnRH release is suppressed by ketones via increased lipolysis and by endorphins via appetite due to increased glucose utilization causing hypoglycemia and hypoinsulinemia (8). Likewise, hypoinsulinemia may adversely affect follicles' response to gonadotropin stimulation. However, no adverse effects were found in the two genetic lines in terms of follicular wave patterns and folliculogenesis. It is known that high-producing cows can attain acceptable reproductive efficiency if the nutrition ratio contains sufficient energy and protein. Presumably, in this trial, caloric deficiency might have been well balanced, or the system of the higher-producing cow might genetically have balanced the energy itself.

In conclusion, there is no difference in follicular wave patterns or follicular populations between genetically high and low producing dairy cows, except for the second largest follicle during the ovulatory wave. Hence, the larger second-largest follicle during the ovulatory wave in select cows indicates a higher twinning rate in this genetic line. Furthermore, the reason for the inability of endogenous bST to alter certain ovarian parameters which are increased by exogenous bST, and the reason why the negative energy balance of select cows does not detrimentally affect folliculogenesis are open to debate.

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