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ULTRASONOGRAPHIC EVALUATION FOR THE EFFECT OF POSTPARTAL OVARIAN ACTIVITY AND UTERINE INVOLUTION ON SUBSEQUENT REPRODUCTIVE PERFORMANCE OF DAIRY COWS

(With 5 Tables)

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استخدام الموجات فوق الصوتية لتقييم تاثير النشاط المبيضى و انكماش الرحم بعد الولادة على الكفاءة التناسلية التالية فى الابقار الحلابة

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أجريت هذه الدراسة لهدف اختبار النظرية التي تفترض أن وجود جر يبات سائدة ما بين الأسبوع الثاني والرابع بعد الولادة على المبيض المقابل لقرن الرحم الذي كان به الحمل السابق يحسن من الكفاءة التناسلية. وقد تم فحص عدد ٢٨ بقرة هولشتين-فرزيان بمزرعة كلية الطب البيطري جامعة أسيوط بالموجات فوق الصوتية وتصوير الرحم والمبايض عن طريق المستقيم مرتين أسبوعيا ما بين الأسبوع الثاني والرابع بعد الولادة. أظهرت الدراسة بان عدد الجريبات الصغيرة التى قطرها 5 ≥ ملليمتر كان قليلا على المبيض المقابل لقرن الرحم الذى كان به الحمل السابق (٠,٧ ± ٠,٠٨) بالمقارنة بالقرن الذي كان غير عشار (١,٠٤ ± ٠,٩) وكان الفرق معنويا ($P < ٠,٠٠١$) وكان عدد الحيوانات التى على مبايضها المقابلة للقرن الذى كان حاملا جريبات سائدة (٢٥%) فى حين على المبايض المعاكسة للقرن الذى كان عشار (٥٣,٦٩%) وكان الفرق معنوى ($P < ٠,٠٥$). كما لوحظ عدد الابقار التى على مبايضها المقابلة للقرن الذى كان حاملا اجسام صفراء اقل من التى عليها الاجسام الصفراء بالجهة المعاكسة للقرون الرحمية سابقة الحمل (١٤,٣%) و(٣٩,٣%) على التوالى. وكان اول شبق بعد الولادة وعدد التلقيحات لكل اخصاب فى المجموعة التى على مبايضها المقابلة للقرن الذى كان حاملا جريبات سائدة والمجموعة الاخرى التى بها جريبات سائدة على المبيض المعاكس هى على التوالى ٥٢,٧٣ ± ٦,٤ و ١,٩٦ ± ٠,٢٤ ،. ٧٩,٧٨ ± ٣,٤ و ١,٨٧ ± ٠,١١ لقد وجد ان الفترة من الولادة حتى الاخصاب تتاثر تاثيرا معنويا بسرعة انكماش الرحم. كما لوحظ ان طول الفترة بعد الولادة حتى يصبح الحيوان عشارا قصيرة بشكل واضح بالحيوانات التى بها نشاط مبيضى (سواء المبيض المقابل او الاخر) (عدد= ٢٢ حوالى ٩٧ يوم) عنها بالحيوانات التى ليس بها نشاط

(عـــدد= ٦ حوالـــى ١٢٢). علـــى المبيضـــين اثـــناء فـــترة الفحـــص وكان الفرق معنويا (P < ٠,٠١). هـــذة النـــتائج تنشـــأ سؤال شيق وهو: كيف تؤثر هذة الجريبات على الكفائة التناسلية للحيوان؟ هل لها تاثير على الرحم ام العكس؟.

SUMMARY

The objective of this study was to test the hypothesis that presence of dominant follicle in the ovary ipsilateral to the previously gravid uterine horn (PGH), between 2 and 4 weeks postpartum, improves subsequent reproductive performance. A total 28 Holstein-Fresian lactating cows in the farm of the Faculty of veterinary medicine Assiut university were examined using transrectal ultrasonography twice weekly between second and fourth week postpartum. The number of follicles (≥ 5 mm diameter) in the ovary ipsilateral to the PGH was fewer than that in the ovary contralateral to the PGH (0.70 ± 0.08 vs. 1.04 ± 0.09 , P < 0.001). There were fewer cows with a dominant follicle (≥ 10 mm diameter) in the ovary ipsilateral to the PGH compared with those with dominant follicles in the contralateral ovary (25.0 %, n= 7, vs. 53.69%, n= 15, P < 0.05). In addition, there were fewer cows with a corpus luteum in the ovary ipsilateral to the PGH compared with those having corpora lutea in the contralateral ovary (4/28, 14.3%) vs. (11/28, 39.3%, P < 0.001). Days to the 1st service and number of service per conception in the group which had dominant follicles in the ovary ipsilateral to the PGH (n= 7) and animals without such follicles (n= 21) were 52.73 ± 6.4, 1.97 ± 0.24 and 79.78 ± 3.4, 1.87 ± 0.11 respectively. Days open in the animals with ovarian activity in either ovaries (n= 22) were 97.04 ± 8.4 days and in animals without ovarian activity (n= 6) were 122.2 ± 7.8 days (P < 0.01). The calving to conception interval was shorter in those animals with a smaller diameter of the PGH (< 60 mm diameter was 95.8 ± 5.3 days and > 60 mm diameter was 122. 3± 1.09 days, P < 0.01). This observation raises an interesting question: How does this follicle affect subsequent fertility – does the follicle exert a local influence on the uterus, or vice versa?.

Key words: *Cow, ultrasonography, postpartum, ovarian activity, uterine involution, fertility.*

INTRODUCTION

An excessively long postpartum estrus interval results in huge economic losses, as well as increasing managerial problems. The relationship between reproductive performance and postpartum changes in the uterus or ovaries of cattle has been investigated by a number of authors (Studer and Morrow, 1978, Oltenacu *et. al,* 1983). However, little attention has been paid to the local relationship between the previously gravid uterine horn (PGH) and follicular growth in the ipsilateral ovary.

Previous results suggested that the occurrence of a large follicle, determined by palpation per rectum, in the ovary ipsilateral to the PGH on day 26 ± 3 postpartum, increased the likelihood of shorter calving to conception intervals (Bonnet *et. al,* 1993). However the accuracy of manual palpation in accurately identifying and measuring such structures, as well as determining the degree of uterine involution, is open to criticism due to the high level of subjectivity. The monitoring of follicular growth and uterine involution in cows can be improved by the use of transrectal ultrasonography (Pierson and Ginther, 1988, Savio *et. al,* 1990).

Greatest follicular activity after calving occurs initially in the ovary contralateral to the PGH (Lewis *et al.,* 1984, Guilbault *et al.,* 1987, Risco *et al.,* 1994). Kamimura *et al.* (1993) reported that only 18% of the first dominant follicles were identified in the ovary ipsilateral to the PGH. The negative influence of the previously gravid horn on the return of ovarian cyclicity in the ovary ipsilateral to the PGH declines with increasing time postpartum (Saiduddin *et al.,* 1967, Foote and Peterson 1968, Marion and Gier, 1968).

There are conflicting reports on the relationship between the return of ovarian cyclicity (as determined by the interval from calving to first rise in milk progesterone concentration) and calving to conception interval. Darwash *et al.* (1997) claimed that early return of cyclicity is associated with shorter calving to conception interval, whereas Smith and Wallace (1998) claimed the reverse. However, such studies are unable to determine in which ovary the return to cyclicity occurs in relation to the PGH.

The objective of this study was to evaluate the hypothesis that presence of large follicle (≥10 mm diameter) follicle in the ovary ipsilateral to the PGH, between 2 and 4 weeks postpartum, improves subsequent reproductive performance.

MATERIALS and METHODS

A total of 28 Holstein-Friesian clinically healthy dairy cows were included in this study. The cows were between 4 and 9 years old belonged to the farm of the Faculty of Veterinary Medicine, Assiut University. During the study, the animals were housed in fixed stall barns with grass green food (*Trifolium alexandrinum*) available ad libitum. Concentrate feed was supplied at 0.3 kg/L milk yield. The farm had policy of natural mating beginning from 45 days postpartum. All animals were hand bred at first post partum observed estrus by fertile bull.

The cows were examined twice per week beginning from second week postpartum to the fourth week. Calving date, date of examination and lactation number were recorded.

The PGH was determined transrectally as that which was longer and of greater diameter than the contralateral horn. The genital tract was scanned per rectum using ultrasound scanner with 6/8 MHz linear array transrectal probe (PIE MEDICAL 100 L. C., Holland) twice weekly for three weeks. Follicles were defined as nonechogenic (black) spherical structures with a clear demarcation between the follicular wall and the rest of the ovarian matrix. Corpora lutea were defined as grainy echogenic structures that had a well-defined border with the less echogenic ovarian stroma. The internal diameter of the largest follicular and luteal ovarian structures and the external diameter of the uterine horns at mid-point were measured using the internal calipers of the machine. When the image of the scanned structure was not spherical, the diameter was estimated by averaging two 90°measurments. In addition, follicles >5 mm diameter in each ovary were counted. Sonograms were recorded and printed by Sony video graphic printer (UP-890 MD, Australia).

Ovarian activity was defined as the presence of a follicle and/ or corpus luteum ≥ 10 mm diameter. Similar definitions have been used previously (Guilbault *et al.*, 1987, Risco *et al.*, 1994).

The data were analyzed for 2 periods of calving to examination (14 to 21 and 22 to 28 days). Results are expressed as mean ± SEM. Analysis performed using SPSS (Version 8.0, SPSS, Chicago, IL). P values of < 0.05 were regarded as significant.

RESULTS

Follicles ≥5 mm diameter were observed in both ovaries ipsilateral and contralateral to the PGH. The number of follicles ≥ 5 mm diameter in the ovary ipsilateral to the PGH was fewer than that in the ovary contralateral to the PGH (0.70 ± 0.08 vs. 1.04 ± 0.09 , $P < 0.001$) (Table 1). The difference in their numbers declined with increasing time from calving to examination (Table 1). There were significant correlation between the interval from calving to examination and the number of follicles ≥5 mm diameter ($r= 0.14$, $P < 0.05$) in the ovary ipsilateral to the PGH.

Fewer cows had a CL in the ovary ipsilateral to the PGH compared with the contralateral ovary (Table 2, $P < 0.001$). In addition, the cows had a follicle ≥ 10 mm diameter in the ovary ipsilateral to the PGH were fewer compared with those which had follicle in the contralateral ovary (Table 2, $P < 0.001$). In some corpora lutea there was a normal non echogenic lacuna (corpora lutea with cavities).

The proportion of cows with a follicle ≥ 10 mm diameter differed between the ovaries ipsilateral and contralateral to the PGH at 14 to 21 and 22 to 28 days from calving to examination (Table 2). There were significant correlation between the interval from calving to examination and the presence of cows possessing follicle ≥ 10 mm ($r= 0.13$, $P < 0.05$) in the ovary ipsilateral to the PGH.

The calving to 1st postpartum estrus interval was 52.73 ± 6.4 days in the first group which had dominant follicles in the ovary ipsilateral to the PGH ($n = 7$), while in the second group, the animals without such follicles ($n = 21$), was 91.31 ± 5.3 days the difference was significant ($P < 0.01$). The number of service per conception was 1.97 ± 0.24 in first group against 1.87 ± 0.11 in the second group, the difference was not significant (Table 3).

The calving to conception interval was shorter in those animals with a smaller diameter of the PGH ($P < 0.01$) (Table, 4).

Days open in the animals with ovarian activity in either ovaries at 2 periods after calving ($n = 22$) were 97.04 ± 8.4 days and in the animals without ovarian activity ($n = 6$) were 122.2 ± 7.8 days and the difference was significant ($P < 0.01$) (Table, 5).

Table 1: The number of follicles ≥5 mm diameter in the ovary ipsilateral or contralateral to the previously gravid uterine horn at 2 time periods after calving

Calving to examination period (days)	Number of cows	number of follicles (mean ± SEM)	
		Ipsilateral ovary	Contralateral ovary
14 to 21	12	0.56 ± 0.08^{ab} (0 – 2)	0.97 ± 0.09^{ab} (0 – 3)
22 to 28	16	0.84 ± 0.07^{a} (0 –3)	1.09 ± 0.08^{a} (0 – 4)
Total	28	0.70 ± 0.08^{ab} (0 – 3)	1.04 ± 0.09^{ab} (0 –4)

Values are significantly different between the ipsilateral and contralateral ovary within a calving to examination period
($^{ab}P < 0.001$, $^{a}P < 0.01$).

Table 2: Percent of animals possessing follicles ≥ 10 mm diameter and CL in the ovary ipsilateral or contralateral to the previously gravid uterine horn at 2 time periods after calving.

Calving to examination period (days)	Number of cows	Ipsilateral ovary		Contralateral ovary	
		% of cows having follicles ≥ 10 mm	% of cows having CL	% of cows having follicles ≥ 10 mm	% of cows having CL
14 to 21	12	16.7^{abc}	33.3	75.0^{abc}	33.3
22 to 28	16	31.3^{ab}	18.8^{ab}	43.8^{ab}	43.8^{ab}
Total	28	25.0^{abc}	25.0^{abc}	53.6^{abc}	39.3^{abc}

Values are significantly different between the ipsilateral and contralateral ovary within calving to examination period
($^{abc}P < 0.001$, $^{ab}P < 0.01$).

Table 3: The calving to 1st postpartum estrus interval (days) and number of service per conception In cows with or without dominant follicles in the ovary ipsilateral to the PGH

Criteria	Cows with dominant follicles (n = 7)	Cows without dominant follicles (n = 21)
Calving to 1st postpartum estrus interval (days)	52.73 ± 6.4[ab] (22 - 79)	91.31 ± 5.3[ab] (55 – 119)
Number of service per conception	1.97 ± 0.24 (1 – 3)	1.87 ± 0.11 (1 – 3)

Values are significantly different between the cows with and without dominant follicle in the ovary ipsilateral to the PGH ([ab]P < 0.01). values without small letters are not significantly differ.

Table 4: The calving to conception interval in relation to the previously gravid uterine horn (PGH) diameter at 28 day after calving.

Diameter of the PGH at 28 day p.p (mm).	Number of cows	Calving to conception interval
< 60 mm diameter	18	95.8 ± 5.3[a] (42 – 115)
> 60 mm diameter	10	122. 3± 1.09[a] (75 – 165)

Values are significantly different between the PGH diameter <60 mm and >60 mm diameter ([a]P < 0.01).

Table 5: Days open in animals with or without ovarian activity in either ovaries at 2 periods after calving

Calving to examination period (days)	Number of cows	Days open (days)	
		With ovarian activity (n)	Without ovarian activity (n)
14 to 21	12	100.5 ± 10.0[ab] (9)	146.7 ± 11.9[ab] (3)
22 to 28	16	93.58 ± 7.0 (13)	96.8 ± 5.9 (3)
Total	28	97.04 ± 8.4[a] (22)	122.2 ± 7.8[a] (6)

Values are significantly different between cows with and without ovarian activity ([ab]P < 0.01, [a]P < 0.05). values without small letters are not significantly differ.

DISCUSSION

The results of the present study showed that the presence of a follicle ≥ 10 mm diameter between 14 and 28 days after calving in the ovary ipsilateral to the PGH is associated with shorter calving to conception intervals.

This observation confirmed the study of Bonnet et al. (1993) on 66 cows by. They reported that cows with good subsequent reproductive performance had larger follicular diameter (6.1 ± 1.3 mm) on the ovary ipsilateral to the PGH, compared with a smaller follicle (2.7 ± 0.9 mm) in cows with poor performance. The shorter calving to conception interval associated with the presence of a larger follicle in the ovary ipsilateral to the PGH may be due to the influence of the follicle on the uterine endometrium and/ or myometrium. One hypothesis is that estradiol synthesiyed by a follicle ≥ 10 mm diameter has a benifical local effect on uterine function. Plasma estradiol concentrations are greater within the utero-ovarian vien draining the ovary containing the ovulatory follicle (Ireland *et al.*, 1984).

The current study shows that cows with ovarian activity, as defined by ultrasonographic presence of a CL and/ or follicle ≥ 10 mm diameter, had a shorter calving to conception interval compared with those lacking these structures. This observation supports Shanks et al. (1979), who reported shorter calving to conception intervals for cows that had an initial ovulation before, rather than after 41 days postpartum. Similarly, Darwash *et al.* (1997) reported a significant reduction in the calving to conception interval with shorter intervals to the first postpartum increase in milk progesterone concentration, although, Smith and Wallace (1998) observed the reverse.

A local effect of the uterus on ovarian function is an established concept, for example, in relation to the transfer of luteolysins (Ginther, 1968), and it has been implicated in the action of interferon (Spencer *et al.*, 1999). Furthermore, in the present study there was a positive correlation between postpartum interval and number of follicles in the ovary ipsilateral to the PGH. These observations would support a hypothesis of a declining influence of uterine-drived inhibitor on ovarian function as uterine involution progresses. Possible uterine factors could include inflammatory mediators such as PGF2α, which is produced by postpartum uterus in high concentrations during involution and in response to utereine infection (Lindell *et al.*, 1982). However, there are a large number of other inflammatory mediators, particularly cytokines,

that participate in folliculogenesis and ovulation (Terranova and Rice, 1997).

An alternative mechanism might be via immune or inflammatory cell cytokine release and/ or cell-cell communication in the ovary. Neutrophils readly migrate into the uterine lumen in response to a chemotactic stimulus at all stages of bovine reproductive cycle. Furthermonre, intrauterine and peripheral neutrophils function is modified by reproductive hormonal status, retained fetal membrane and uterine infection (Cai *et al.*, 1994, Subandrio and Noakes, 1997). Perhaps immune cells could migrate from the uterine horn, or a uterine-derived chemotactic substance could attract immune cells to the ipsilateral ovary, and so influene folliculogenesis. Immune cells have been detected in ovarian follicles and luteal tissue in association with a modulation of follicular and luteal activity (Brannstrom *et al.*, 1994, Gaytan *et al.*, 1998, Penny *et al.* 1999).

In this study it was found that, follicular activity was suppressed in the ovary ipsilateral to the PGH between 14 and 28 days postpartum. The presence of a follicle ≥10 mm diameter in the ovary ipsilateral to the PGH between 14 and 28 days postpartum was associated with shorter calving to conception intervals. It was concluded that, presence of dominant follicle in the ovary ipsilateral to the previously gravid uterine horn (PGH), between 2 and 4 weeks postpartum as well as rapid uterine involution , improve subsequent reproductive performance.

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