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EFFECT OF REMOVAL OF THE OVARY CONTAINING THE LARGEST FOLLICLE BEFORE OVULATION ON SUBSEQUENT FUNCTION OF THE REMAINING OVARY IN BRAHMAN COWS

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SUMMARY

Effects of removal of the ovary containing the largest follicle before ovulation (presumably pre-ovulatory follicle) on subsequent function of the remaining ovary were evaluated in 9 Brahman cows exhibiting normal estrous cycles. An epididymectomized bull equipped with chin-ball marker was used to aid in detection of estrus. Ovarian follicles were evaluated daily by ultrasonography from day 0 (day of estrus) of the first estrous cycle until the second estrus(after the ovary with the largest follicle was removed). Following the third observed estrus after unilateral ovariectomy, the remaining ovary was scanned daily for a complete estrous cycle. Blood samples were collected daily during the 2 scanning periods. Before unilateral ovariectomy, 7 out of 9 cows had the corpus luteum (CL) and largest follicle on the same ovary. Number of follicular waves, estrous cycle length, number of follicles at emergence of the first, second and ovulatory wave and day when progesterone peak occurred were similar whether the cows had one or two ovaries. However when cows had two ovaries, large-sized follicles (13.5±0.5mm) were larger ($P<0.005$) and serum progesterone concentration (10.5±1.5ng/ml) was higher ($P<0.005$) than when cows had one ovary (10.5±0.5mm and 6.3±1.5ng/ml, respectively). There was a positive correlation between size of the largest follicle and peak serum progesterone concentration ($r=0.55$; $P<0.05$).

Keywords: Compensatory hypertrophy, follicle, unilateral ovariectomy, ovary

INTRODUCTION

The ovary that contains the dominant follicle and the CL appears to secrete more progesterone and have bigger large-sized follicles than an ovary that has a CL contralateral to the dominant follicle. This suggests an ovarian compensatory hpertrophy which might depend on the removed ovary. Unilateral ovariectomy is reported to be followed by compensatory ovarian follicular hypertrophy in multiple

and in single ovulating species (Hatai, 1913; Greenwald, 1960; Saiduddin *et al.*, 1970; Bo *et al.*, 1995). Staigmiller *et al.* (1974) reported a compensatory hypertrophy of luteal tissue in unilateral ovariectomized gilts and this hypertrophy was due to an increase in luteal tissue rather than to an increase in progesterone secretion per gram of tissue. However, in cows the results have been inconsistent. Saiduddin *et al.* (1970) reported compensatory hypertrophy, while no evidence of hypertrophy was reported by Short *et al.* (1970). However, Staigmiller and England (1982) reported that hypertrophy occurred in cattle only if the largest follicle was removed.

Ginther *et al.* (1989) suggested that ovarian follicular interactions were mediated through systemic rather than local pathways. However, more recent information indicate that ovarian interactions may occur via both local and systemic pathways (Lazar and Maracek, 1994; King *et al.*, 1995). Lazar and Maracek (1994) suggested a possible paracrine interaction between the corpus luteum (CL) and the development of small follicles. Furthermore, the dominant follicle suppressed follicular development in the same ovary, but stimulated the number of medium and large follicles on the contralateral ovary, an effect which might occur through systemic circulation (Lazar and Maracek, 1994). In addition, King *et al.* (1995) suggested that possible local ovarian interactions affect compensatory ovarian hypertrophy in unilateral ovariectomized gilts. Results from these studies suggest that removal of the ovary containing the dominant follicle might affect follicular and endocrine patterns in the remaining ovary.

The objective of this study was to evaluate the effects of removal of the ovary containing the pre-ovulatory follicle on subsequent estrous cycle length, follicular wave dynamics, number of follicles at wave emergence, and progesterone secretion by the remaining ovary.

MATERIALS AND METHODS

Twelve multiparous Brahman cows exhibiting normal estrous cycles and having body condition scores (BCS) of 5.2±0.08 [1=thin, emaciated and 9=fat, obese; as described by Godfrey *et al.* (1988)] were maintained on Coastal bermuda grass hay *ad libitum* and supplemented with a ration containing corn (1.88kg $cow^{-1,d-1}$) and soybean meal (0.32 kg $cow^{-1,d-1}$). Cows were observed for estrous behavior twice daily for at least 30 min at each period. An epididymectomized bull equipped with a chin-ball marker was placed with the cows to aid in the detection of estrus. When estrous behavior was detected (day 0), ultrasonography (Aloka 210, linear array transducer and a 5 MHZ rectal probe) was performed daily throughout the first estrous cycle (Figure 1) until the largest follicle of the second follicular wave reached 8 mm in diameter, prostaglandin F_2 (PGF_2 ; Lutalyse, Upjohn Co; Kalamazoo, MI) was injected intramuscularly (25mg) to the cows and the ovary cotaining the largest follicle was removed before ovulation by paralumbar laparotomy as described by Irvin *et al.* (1988). It is important to point out that 7 of 9 cows had the CL and the largest follicle on the same ovary at the time of PGF_2 injection. After unilateral ovariectomy cows were observed for two estrous cycles. Following the observance of

the third estrus, the remaining ovary was scanned daily for a complete estrous cycle (Figure 1). Comparisons were performed between estrous cycles 1 and 5.

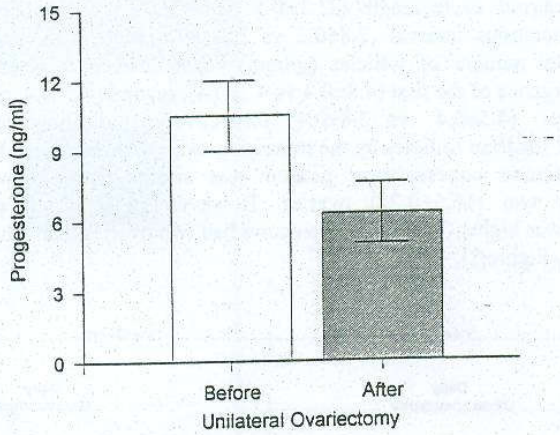

Figure 1. Procedures showing day of prostaglandin F2 (PGF2) injection and day of unilateral ovariectomy as well as estrous cycles in which ovarian ultrasongraphy was performed daily (Comparison was performed between estrous cycles 1 and 5).

Each ovary was scanned in more than one plane and at least two images per ovary were recorded using a Sony UP 850 graphics printer. Follicular antrum diameters were measured with a caliper to the nearest 0.1mm on the printed image. Measurements were corrected for a 10% distortion in area produced by the printer (Quirk *et al.*, 1986). Follicles were classified as small (4mm), medium (4.1-8mm) and large (8.1mm). Three out of the twelve cows were excluded because two of them had very short estrous cycles and the third one went for 26 days without ovulation.

Blood samples were collected daily via tail venipuncture during both scanning periods. Samples were maintained at 5°C for 24hr, then the serum was harvested and stored at –20°C until progesterone concentrations were assayed by a single antibody technique (Villeneuve *et al.*, 1988) using RIA procedures. The antibody was #337 anti progesterone-11-BSA (G.D. Niswender, Colorado State University, Fort Collins, CO). Intra and inter-assay CV were 16 and 23% respectively.

Data were analyzed using SAS GLM procedures for analysis of variance and differences before and after unilateral ovariectomy were determined by least square means methods using the PDIFF option (SAS, 1985). Correlation coefficients were calculated for the number of ovaries and size of the largest follicle and between size of the largest follicle and peak progesterone concentration (SAS, 1985).

RESULTS

After unilateral ovariectomy cows exhibited estrous behavior followed by ovulation between 3 and 7 days after ovariectomy. Presence of one or two ovaries did not influence estrous cycle length (21.1±0.5 vs 21.3±0.5d) or number of follicular waves per interstrous interval (2.8±0.2 vs 2.8±0.2). There were no differences (P>0.10) in total number of follicles (≥4mm) when cows had one or two ovaries during the emergence of the first (4.8±0.4 vs 4.2±0.4), second (4.5±0.4 vs 4.3±0.4) or ovulatory wave (4.5±0.4 vs 3.8±0.4) respectively, indicating compensatory hypertrophy of ovarian follicles by the remaining ovary. Furthermore, day on which serum progesterone concentration peaked was similar when cows had one (15.6±0.7d) or two (16.5±0.7d) ovaries. However, peak serum progesterone concentration was higher (P<0.005) when cows had two ovaries than when cows had only one ovary (Figure 2).

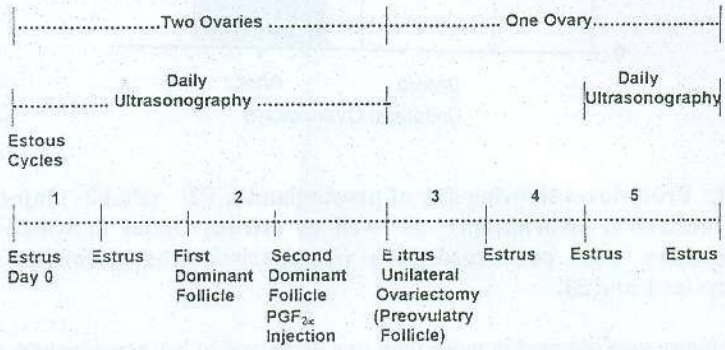

Figure 2. Serum peak progesterone concentrations as affected (P<0.005) by unilateral ovariectomy in cycling Brahman cows (Before and after unilateral ovariectomy, n = 9; SEM = 0.58).

The largest follicle that developed during the estrous cycle before unilateral ovariectomy, was larger (P<0.005) than the largest follicle developed after unilateral ovariectomy (13.5± 0.5 v. 10.7±0.5mm). There was also a positive correlation between size of the largest follicle and peak serum progesterone concentration (r=0.55; P<0.06).

DISCUSSION

Total number of follicles at wave emergence was similar when cows had one or two ovaries. The increase in follicular number of the remaining ovary suggests that an ovarian follicular compensatory hypertrophy had occurred. Compensatory ovarian follicular hypertrophy after unilateral ovariectomy has been reported in cattle (Saiduddin *et al.*, 1970) and in other species such as sheep (Sundaram and Stob,

1967), rats (Hatai, 1913), hamsters (Greenwald, 1960), and pigs (Brinkley *et al.*, 1964). However, in cattle it has also been reported that ovarian follicular compensatory hypertrophy only occurred if the ovary containing the largest follicle was removed (Staigmiller and England, 1982). Gonadotropins are known to be involved in the regulation of follicular development (Ireland, 1987; Tonetta and DeZerega, 1989; Adms, *et al.*, 1992b) and the compensatory hypertrophy, observed after unilateral ovairectomy, may be caused by a partial removal of the steroid negative feedback mechanism and subsequent increases in gonadotropin secretion (Howland and Skinner, 1973). Therefore, ovarian follicular compensatory hypertrophy observed in this study may be due to removal of the suppressive effects of the dominant follicle and increases in gonadotropin secretion. In addition, many non-steroidal intra-ovarian factors have been reported to be involved in follicular dynamics (Ackland *et al.*, 1992), and the possibility exists that non-steroidal intra-ovarian factors could be involved in ovarian follicular compensatory hypertrophy.

Different laboratories, using different approaches have demonstrated a positive local relationship between the CL and follicles during the estrous cycle (Matton *et al.*, 1981; Maurasse *et al.*, 1985 and Pierson and Ginther, 1987). The diameter of the dominant follicle was also found to be greater when the CL was on the same ovary as the dominant follicle (Staigmiller and England, 1982), suggesting possible reciprocal interactions between the CL and the dominant follicle when present on the same ovary, and these interactions may influence both follicular and luteal function. Roberts *et al.* (1984) reported that in anestrous ewes induced to ovulate, the largest follicle secretes less progesterone than in estrous cycling ewes receiving the same treatment and having a large follicle of similar size (7mm), indicating that the ovulatory follicle could influence progesterone secretion. Lower progesterone secretion when cows had one *vs.* two ovaries could have been the result of smaller ovulatory follicles, which after ovulation secreted less progesterone. This is supported by the positive correlation found in this study between size of the largest follicle and progesterone secretion. On the other hand, a confounding but also possible explanation for a bigger pre-ovulatory large-sized follicles before unilateral ovariectomy is the effect of PGF_2 injection. Villeneuve *et al.* (1988) reported that administration of PGF_2 to beef cows during the early postpartum period increased the diameter of the largest follicles on both ovaries. The lack of PGF_2 injection after unilateral ovairectomy in the present study could have made the difference in follicular size.

Estrous behavior exhibited shortly after unilateral ovariectomy may have been due to the removal of the dominant follicle and the absence of a negative feedback, resulting in the development of a new follicular wave associated with increasing levels of estradiol. Adams *et al.* (1992a) reported that cauterization of the dominant follicle or its suppression with steroid-free bovine follicular fluid during the growing phase resulted in premature FSH release and emergence of a new follicular wave. Furthermore, ablation of follicles larger than 5 mm in heifers of unknown stages of the estrous cycle also resulted in emergence of a new follicular wave (Bo *et al.*, 1995). Similarity in estrous cycle length before and after unilateral ovariectomy agrees with results reported in gilts (King *et al.*, 1995). This may be an indication that

unilateral ovariectomy does not influence PGF_2 patterns and/ or interactions between the CL and PGF_2 , and requires further investigation. Dynamics of follicular waves were similar when cows had one or two ovaries. This might be an indication that even if there are changes in ovarian follicular numbers in the remaining ovary, there are no changes in the relationships between dominant and subordinate follicles that would affect the dynamics of the follicular waves.

Results from this study suggest that removal of the ovary containing both the CL and the largest follicle before ovulation may affect follicular size and progesterone secretion of the remaining ovary.

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أثر إزالة المبيض المحتوى على الحويصلة الناضجة قبل التبويض علي وظائف المبيض المتبقي في أبقار البراهمان.

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أجريت هذه التجربة علي ٩ أبقار براهمان تشبع بشكل طبيعي بمحطة أوفرتون التابعة لجامعة تكساس. تم تتبع عمليات النمو الحويصلي للمبايض باستخدام جهاز الموجات فوق الصوتية ابتداء من اليوم صفر (يوم الشياع) حتي الدخول في شياع تال وظهور حويصلة كبيرة في أحد المبايض وإزالة هذا المبيض جراحيا. وكانت هذه الأبقار تترك بعد الجراحة لمدة ٣ دورات شياع متتالية ومرئية ثم بعد الشياع الثالث تم تتبع حالة المبيض المتبقي يوميا بالموجات فوق الصوتية لمدة دورة شياع كاملة. وكانت تؤخذ عينات من الدم يوميا في كلا المرحلتين (مرحلة وجود المبيضين – ومرحلة وجود مبيض واحد) لتقدير تركيز هرمون البروجيستيرون.

وأظهرت النتائج أنه قبل إزالة المبيض وجد في سبع أبقار من التسع المستخدمة يتواجد الجسم الأصفر والحويصلة الناضجة معا علي نفس المبيض. وسواء كان بالبقرة مبيضين أو مبيض واحد لم تظهر فروقا معنوية ($P<0.10$) في عدد موجات النمو الحويصلي في دورة الشياع أو طول دورة الشياع أو عدد الحويصلات الموجودة علي المبايض عند بداية أول موجة أو ثاني موجة أو موجة التبويض. كذلك لم تظهر فروق معنوية بين الحالتين (مبيض واحد أو مبيضين) في وقت أو يوم ظهور أعلي تركيز لهرمون البروجيستيرون. ومن جانب أخر ففي حالة تواجد المبيضين كان قطر الحويصلة الناضجة أكبر (١٣,٥±٠,٥مم). وكان تركيز البروجيستيرون في السيرم أعلي (١٠,٥±١,٥ نانوجرام/مل) عنه في حالة تواجد مبيض واحد (١٠,٥±٠,٥مم، ٦,٣±١,٥ نانوجرام/مل – علي التوالي) وكان هناك تلازم معنوي ($r=0.55$; $P<0.06$) بين حجم الحويصلة الناضجة وتركيز البروجيستيرون في السيرم.

واستنتج من هذا البحث أن إزالة المبيض المحتوي علي الحويصلة المتوقع لها التبويض ينتج عنه زيادة تعويضية في حجم الحويصلة الناضجة في المبيض المتبقي مصحوبة بزيادة في مستوي البروجيستيرون في الدم. بينما لم يتأثر طول دورة الشياع أو عدد موجات النمو الحويضلية بإزالة أحد المبيضين.