ELSEVIER

EFFECTS OF UNILATERAL OVARIECTOMY ON FOLLICULAR DEVELOPMENT AND OVULATION IN CATTLE

M. Mohan and R. Rajamahendran[1]

Department of Animal Science, University of British Columbia
Vancouver, B.C. Canada V6T 1Z4

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ABSTRACT

In a study of 4 cyclic dry cows (Trial I) and 6 cyclic puberal heifers (Trial II), unilateral ovariectomy increased the number of ovulatory follicles, did not alter the hormone profile, cycle length or the number of follicular waves. Ovarian follicular development in all 4 cows was monitored daily using transrectal ultrasonography until the day of ovulation, during which period daily blood samples were also taken from the tail vein for determination of plasma FSH, LH and P_4 concentrations. Unilateral ovariectomy was performed on the day after ovulation and ovarian activity was again monitored daily (ultrasonography and blood sampling for FSH, LH and P_4) for 2 consecutive cycles (8 cycles in all). Estrus in all 6 heifers was synchronized using 2 injections of $PGF_{2\alpha}$ given 12 d apart. Similarly, ovarian activity in the 6 puberal heifers was monitored daily using ultrasonography and blood sampling for 1 complete control cycle. Following estrus and ovulation the left ovary was removed in all the animals, and thereafter 1 complete cycle was followed. Mean cycle length, FSH, LH and P_4 concentrations before and after unilateral ovariectomy were compared using paired sample t-test. The results show that unilateral ovariectomy neither altered the cycle length nor the number of follicular waves in the cows, but it increased the number of ovulatory follicles (2 follicles developed and ovulated in 6 of the 8 cycles). The mean diameter of the largest follicle was 16.1±0.9 mm and the second largest 12.5±0.9 mm. No significant ($P > 0.05$) differences were observed in FSH (0.72±0.09 vs 0.71±0.07), LH (0.42±0.1 vs 0.37±0.07) and P_4 (2.8±0.6 vs 2.6±0.4) levels before and after unilateral ovariectomy. Of the 6 heifers, 5 had 2 waves and 1 heifer had 3 waves of follicular growth during the control cycle, and this pattern did not change after the procedure. Mean cycle length (20.7±0.9 vs 21±0.9) did not differ before and after unilateral ovariectomy, and 4 of the 6 heifers ovulated twin follicles following ovariectomy. The mean diameter of the largest follicle was 14.5±0.7 mm and second largest measured 12.1±0.8 mm. No significant ($P > 0.05$) differences were observed in FSH (0.16±0.09 vs 0.21±0.07), LH (0.11±0.1 vs 0.15±0.07) and P_4 levels (3.6±0.26 vs 3.8±0.29) before and after unilateral ovariectomy. Based on these results, we conclude that unilateral ovariectomy is an ideal method for obtaining twin ovulations in cows and heifers

Key words: cattle, unilateral ovariectomy, follicular development, ovulation, follicle stimulating hormone

[1] Correspondence and reprint requests.

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INTRODUCTION

In monotocous species like cattle antral follicular growth and development is characterized by 2 or 3 successive waves of follicular development (37). Each of these waves consists of a recruitment phase followed by a selection phase during which 1 follicle is selected, enters the dominance phase, and either regresses or ovulates at the end of the cycle. Follicle stimulating hormone (FSH) plays a pivotal role in the recruitment and selection phases and determines the number of ovulatory follicles. An association is known to exist between the surges of FSH and the emergence of follicular waves in cattle (1). A priming dose of FSH given early in the cycle has been shown to increase the number of follicles that develop to the ovulatory size (27). Ovarian secretions (estradiol 17-β & inhibin) are known to regulate the output of FSH from the pituitary (11). A significant increase in serum FSH and luteinizing hormone (LH) occurs following bilateral ovariectomy in cattle (29). Accordingly, this study was based on the premise that unilateral ovariectomy would reduce the negative feedback effects of ovarian secretions on pituitary output of FSH to an extent that is sufficient for the selection, growth and ovulation of more than 1 follicle. Therefore, the objectives of this study were to investigate the effects of unilateral ovariectomy on plasma gonadotropin levels, follicular dynamics, ovulation rates and progesterone profiles.

MATERIALS AND METHODS

Trial I

Four dry cyclic Holstein cows were used. The animals were obtained from the Agriculture Canada Research Station, Agassiz, BC, and were housed at the University of British Columbia Dairy Teaching and Research Unit and cared for according to the guidelines of the Canadian Council of Animal Care. The cows were fed a standard ration of alfalfa cubes (approximately 16% crude protein) and timothy hay (13%). Ovarian activity in the cows was monitored daily using transrectal ultrasonography until the day of standing estrus. The day of estrus was also confirmed by ultrasonic detection of a large ovulatory follicle and by palpation per rectum for uterine turgidity. Unilateral ovariectomy was performed 1 d after ovulation using an ecraser (colpotomy) under epidural anesthesia (7). Following ovariectomy, ovarian follicular development in the retained ovary was monitored for 2 complete cycles. On Day 7 of the third cycle after ovariectomy the retained ovary was surgically removed in 3 cows to assess the number of ovulations. The retained ovary was not removed from the fourth cow since it was decided to retain her for breeding, while the other three were to be culled.

Trial II

Six cyclic pubertal heifers were used in the trial. The heifers were cared for as mentioned before. The estrous cycles in all heifers were synchronized with 2 injections of $PGF_{2\alpha}$ (Lutalyse; Upjohn, Kalamazoo, MI, USA) given 12 d apart. Following ovulation, ovarian follicular development in the heifers was monitored daily using transrectal ultrasonography for 1 complete cycle (control cycle) until subsequent ovulation. A laporotomy was performed under paravertebral anesthesia and the left ovary in all the heifers was removed using an ecraser. After unilateral ovariectomy, the heifers were given a period of rest and their estrous cycles were synchronized using $PGF_{2\alpha}$ injections. Following estrus and ovulation, ovarian follicular development in the retained ovary was monitored for 1 complete cycle.

Blood Sampling and Analysis for Plasma FSH, LH and P_4

Blood samples (7ml) were collected daily through a tail vessel puncture from all cows (Trial I) and heifers (Trial II) before and after unilateral ovariectomy just before each ultrasound examination for determining plasma FSH, LH and P_4 concentrations. Plasma P_4 concentrations were measured using a commercially available solid-phase radioimmunoassay kit (Coat-A-Count, Diagnostic Products Corp., Los Angels, CA). This kit has been previously validated in our laboratory for the measurement of P_4 in cow's milk and plasma (31). Quantification of plasma FSH was done by a double antibody radioimmunoassay validated by Rawlings et al. (33). All samples were measured in a single assay. Plasma LH concentration was assayed by the method of Sanford (36). Mean cycle length, plasma P_4, FSH and LH data before and after ULO in both cows and heifers were compared using paired sample t- test.

RESULTS

Ovarian Follicular Dynamics During the Control Cycle in Cows and Heifers

One of the four cows had a complete control cycle as she was in estrus at the beginning of the experiment. This cow had 2 follicular waves during her control cycle. At the time of estrus 3 of the 4 cows had 1 preovulatory follicle while the fourth cow had 2 preovulatory follicles. All heifers in Trial II had a mean cycle length of 20.7 ± 0.9 d before unilateral ovariectomy. Of the 6 heifers, 5 had 2 waves of follicular growth, while 1 cow had 3 follicular waves. All 6 heifers had 1 preovulatory follicle that developed and ovulated at the end of the cycle (Table 1). A representative pattern of follicular development and P_4 profile observed in cows and heifers prior to unilateral ovariectomy are shown in Figure 1.

Ovarian Follicular Dynamics Following Unilateral Ovariectomy in Cows and Heifers

Representative patterns of follicular growth and development observed in 6 of 8 cycles (Trial I) and in 4 of 6 cycles after unilateral ovariectomy (Trial II) are shown in Figure 2. All cows had a mean cycle length of 21 d after unilateral ovariectomy and follicular wave patterns remained the same in all the animals following the procedure. The number of ovulatory follicles increased in the interovulatory intervals after ovariectomy in cows as well as heifers.

Co-dominant follicles developed and ovulated in 6 of the 8 cycles followed in Trial I. An altered size distribution was observed between the 2 preovulatory follicles. The mean diameter of the largest follicle was 16.1±0.9 mm and the second largest 12.5±0.9 mm (Table 1). Following unilateral ovariectomy in the heifers, no alterations were observed in the mean cycle length (21±0.9 d). In heifers, of the 6 cycles (1 cycle per animal) observed (Trial II) after unilateral ovariectomy, twin ovulations were recorded in 4 cycles. The mean diameter of the largest preovulatory follicle was 14.5±0.7 mm and second largest 12.1±0.8 mm. The follicular wave pattern essentially remained the same in all heifers after unilateral ovariectomy. Removal of the retained ovary on Day 7 of the third cycle in Trial I revealed more than one ovulation in 2 cows that was evidenced by the presence of more than 1 corpora lutea (CL; Figures 3 and 4). The third cow had a single ovulation. The retained ovary was not removed from the fourth cow and twin corpora lutea resulting from twin ovulations were confirmed using ultrasonography.

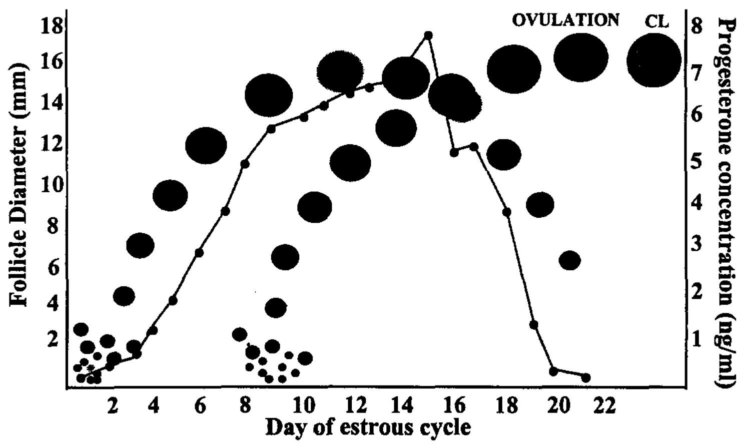

Figure 1. A representative pattern of follicular dynamics and progesterone profile observed during Trial I and II before unilateral ovariectomy.

Plasma FSH and LH Concentrations before and after Unilateral Ovariectomy in Heifers and Cows

Even though the cows did not have a complete control cycle, all cows had a minimum interval of 9 d between the start of the experiment and day of estrus. Hence mean FSH and LH concentrations in the samples taken during the last 7 d before they were observed in estrus served as the control levels, and were compared with concentrations during the last 7 d of the cycles after unilateral ovariectomy. No significant differences in mean plasma FSH (0.72±0.09 vs 0.71±0.07 ng/ml) and LH (0.42±0.1 vs 0.37±0.07 ng/ml) were observed in the cows (Table 1). Plasma FSH and LH in all heifers before unilateral ovariectomy were 0.16±0.02 and 0.11±0.03 ng/ml, respectively, and did not differ significantly from values after unilateral ovariectomy (0.21±0.03 and 0.15±0.04 ng/ml; Table 1).

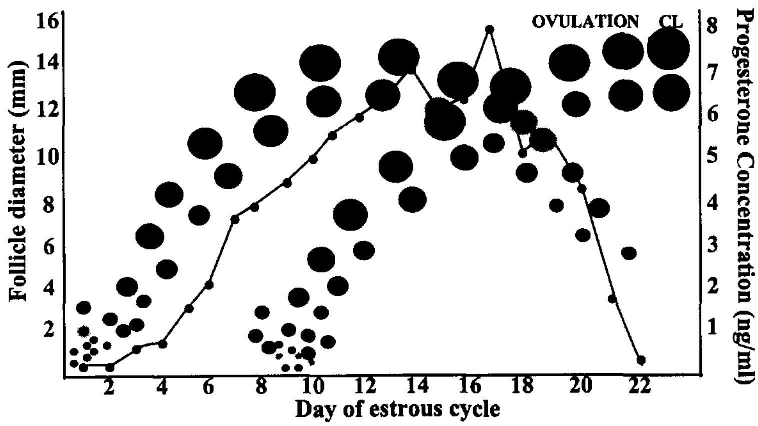

Figure 2. A representative pattern of follicular dynamics and progesterone profile observed during Trial I and II after unilateral ovariectomy.

Plasma P_4 Concentrations Before and after Unilateral Ovariectomy in Cows and Heifers

Mean plasma P_4 concentrations for all cows and heifers before unilateral ovariectomy were 2.8±0.6 and 3.6±0.26 ng/ml, respectively; after ovariectomy they were 2.6±0.4 and 3.8±0.29 ng/ml, respectively, in cows and heifers (Table 1). A slight increase in P_4 concentration was observed in the heifers after unilateral ovariectomy on account of 2 CL arising from twin ovulations that occurred after the rest period but was not statistically significant enough to be different from control levels.

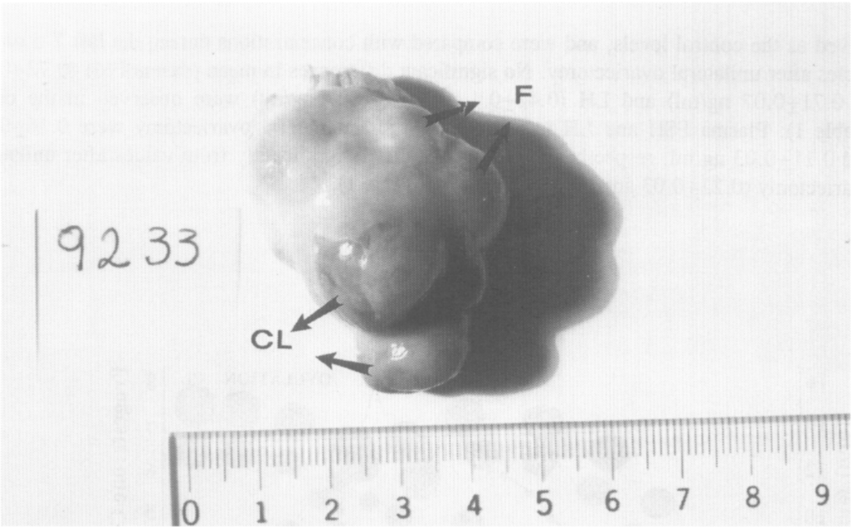

Figure 3. Ovary of a cow (# 9923) removed 7 days post estrus after the second experimental cycle following unilateral ovariectomy. Two corpora lutea (CL) measuring 18 and 17 mm in diameter along with two large follicles (F) measuring 15 and 10 mm in diameter.

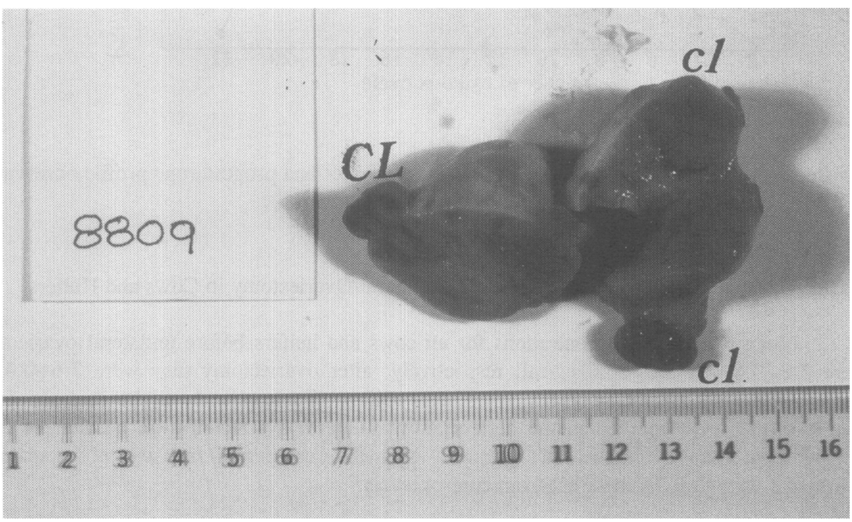

Figure 4. Cut section of the retained ovary (Cow # 8809) that was removed 7 days after the second experimental cycle following unilateral ovariectomy. The growth, development and ovulation of three follicles is evidenced by the presence of three corpora lutea measuring 15, 10 and 7mm in diameter, two of them sectioned and the third intact.

Table 1. Mean cycle length, plasma FSH, LH and P_4 concentrations before and after unilateral ovariectomy (Trials I and II)

	Trial I Cows (n= 4)		Trial II Heifers (n=6)	
Comparisons	Before ULO	After ULO	Before ULO	After ULO
Cycle length (Days)	21	21	20.7 ± 0.9	21 ± 0.9
Follicular waves	2	2	2 (n=5), 3 (n=1)	2 (n=5), 3 (n=1)
Ovulatory follicles	1 (3/4), 2 (1/4)	2 (6/8 cycles)	1 (6/6 cycles)	2 (4/6 cycles)
P_4 conc (ng/ml)	2.8 ± 0.6	2.6 ± 0.4*	3.6 ± 0.26	3.8 ± 0.29*
FSH conc (ng/ml)	0.72 ± 0.09	0.71 ± 0.07*	0.16 ± 0.02	0.21 ± 0.03*
LH conc (ng/ml)	0.42 ± 0.1	0.37 ± 0.07*	0.11 ± 0.03	0.15 ± 0.04*

* Not Significant (P>0.05)
ULO= Unilateral ovariectomy

DISCUSSION

Some 210 years ago, John Hunter (17) first demonstrated that litter size in swine remained unaltered following unilateral ovariectomy. Later, similar studies performed in various livestock and laboratory species during the estrous cycle showed that compensatory ovulation by the retained ovary occurs thereby maintaining species-specific litter size (21,15,26,5,22). Although polytocous livestock species have responded with a compensatory increase in the ovulation rate, no such reports have been available for monoovular species like cattle, leaving open the question as to how would cattle respond to such a procedure. In this study involving cattle, unilateral ovariectomy resulted in the doubling of the normal ovulatory quota, further adding credibility to a hoary old question as to whether or not twin ovulations could be induced in cattle following unilateral ovariectomy (35). During the 21-d estrous cycle in cattle, antral follicular growth and development is characterized by 2 or 3 successive waves of follicular development (37). Each of these waves consists of a recruitment phase followed by a selection phase from which 1 follicle is selected and enters the dominance phase, which is characterized by the growth of 1 large (> 10 mm) follicle (13). In our present study unilateral ovariectomy neither altered the interovulatory interval nor the number of follicular waves in the 2 trials. But following unilateral ovariectomy, an altered pattern of follicular growth and development was observed in both trials.

Earlier, Saiduddin et al. (35) had observed an altered size distribution of follicles following unilateral ovariectomy on Day 8 of the estrous cycle in cattle. At the time of estrus the largest follicle was smaller and the second largest follicle was larger in unilaterally ovariectomized than in intact animals. Unfortunately, their study suffered from the limitation that individual follicles could not be followed over time until ovulation. Real-time ultrasonography in addition to being a noninvasive method also allows for repeated examination of the ovaries, and follicles can be followed over long periods of time. In the light of our results, co-dominant follicles developed and either regressed or ovulated during each wave, and this pattern was observed in 6 of the 8 cycles in Trial I and 4 of 6 cycles in Trial II. Following unilateral ovariectomy, altered size distributions and patterns of follicular growth and development were observed in both trials. The largest ovulatory follicle in the retained ovary following ovariectomy was smaller in diameter than its counterpart during the control cycle. The second largest follicle, however, was smaller still. The morphological basis for the increased ovulation rate by the retained ovary is still not clear, and is believed to be due to a decrease in the amount of follicular atresia (16) and/or through an increase in the selection of developing follicles (24).

Actively growing follicles are dependant upon gonadotropin support for their growth and development. This study was based on the precept that unilateral ovariectomy would lower the inhibitory effects of ovarian secretions on pituitary output of FSH to an extent that is sufficient for the selection, growth and ovulation of more than 1 follicle. A transient increase in serum FSH following unilateral ovariectomy may stimulate additional follicular growth and the concomitant ovarian hypertrophy (3,10,39,34,14). In the present study, no significant differences ($P > 0.05$) were observed in serum gonadotropin (FSH & LH) levels before and after unilateral ovariectomy in Trial I (cows). In Trial II the mean plasma FSH and LH levels of heifers were comparatively lower than in the cows. However, there was a slight increase in FSH and LH levels after ovariectomy, but this increase was not statistically significant. This suggests that a relatively small increase in circulating FSH concentration might be sufficient to increase the ovulation rate by increasing the number of developing follicles exposed to their individual "threshold" concentration of FSH, thereby avoiding atresia and allowing for full maturation followed by ovulation (18). Because blood samples were not collected frequently, this transient increase in FSH following unilateral ovariectomy could have gone undetected.

A large body of evidence now sugggests the involvement of a neuroendocrine mechanism wherein the ovarian nerves play a modulatory role on the response of the remaining ovary to gonadotropins thereby, regulating compensatory contralateral ovarian hypertrophy (14,12,4). Ovarian innervation is provided by two neural pathways: the vagus nerve and the noradrenergic system. The superior ovarian nerve, which runs along the free border of the superior ligament of the ovary, is the main pathway of noradrenergic innervation to the ovary (23). Noradrenergic denervation of the ovary produced either by local treament of 6-hydroxydopamine on the remaining ovary or through section of the superior ovarian nerve blocked compensatory hypertrophy (14, 4). Treatment of hemiovariectomized rats with an ovarian autograft using guanethidine, a drug that inhibits the release of norepinephrine from the nerve endings blocked compensatory ovulation but not compensatory ovarian hypertrophy (2). These results indicate the likely involvement of adrenergic neural elements of the ovary in the development of compensatory ovulation and ovarian hypertrophy by the remaining ovary following unilateral ovariectomy.

An increase in the plasma concentrations of P_4 has been reported following hCG induced ovulation of the first (Day 7) or second (Day 14) wave dominant follicle during the estrous cycle in cattle (32). On the other hand, equivalent elevations in plasma P_4 were not observed (32) when twin CL resulted through the administraton of hCG on the day of estrus (Day 0). In the study reported here, some of the heifers had twin CL as a result of twin ovulations following induced estrus immediately after the rest period. However, mean plasma P_4 concentrations after unilateral ovariectomy although slightly higher than the levels prior to ovariectomy in Trial II (heifers) were not significantly different. There was also no significant effect of unilateral ovariectomy on plasma concentrations of P_4 in Trial I before and after ovariectomy. Since in our study, the formation of twin CL was spontaneous, a lack of correlation observed between P_4 concentrations and CL is similar to the findings of Rajamahendran et al. (28). A higher metabolic clearance of the hormone was the probable reason for the failure to observe elevated concentrations of P_4 following unilateral ovariectomy (30).

Increasing productivity in cattle has been one of the major goals of livestock research. Reproductive potential is low in cattle since most of the bovine females release only a single oocyte per estrous cycle and, thereby, produce 1 progeny each year. Ovulation of additional oocytes at natural estrus in cattle would provide a simple method for increasing productivity. An increased incidence of twin or multiple births has been reported following the administration of exogenous gonadotropins (PMS; 38) or FSH (8), through the introduction of a second embryo into the uterus of an already bred cow (6) or through immunoneutralization against the follicular peptide, inhibin (25). Given the limited capacity of the bovine uterus a modest increase in ovulation rate to 2 would be ideal. Absence of an effective methodology to regulate the ovulation rate is the major limiting factor to achieving increased productivity. Since we observed twin follicular growth and ovulations in both trials after unilateral ovariectomy the possibility of using this ovariectomy procedure to induce twinning in cattle seems promising.

A positive relationship has been shown (9) to exist between twinning in cattle and increased concentrations of IGF-I (insulin like growth factor- I) in both serum and follicular fluid. In their study, Echternkamp et al. (9) reported that cattle selected for twin births had a 46% higher concentration of IGF-I than cattle not selected for twinning. From these findings a possible role for intraovarian peptides like IGF-I in regulating folliculogenesis following unilateral ovariectomy in cattle cannot be overruled. The basis for expected increases in plasma IGF-I concentrations in cattle following unilateral ovariectomy is not clear. Even if twin pregnancies are not desired or do not result the availability of 2 oocytes following twin ovulations would increase the chances of fertilization. Twinning has not been attractive to the dairy farmers due to higher incidences of freemartinism. The problem of freemartinism can be overcome with refinement of new technologies (20) in semen separation procedures, so that twin calves of the same sex can be obtained.

To summarize, unilateral ovariectomy had caused no effect on cycle length, follicular wave pattern or on plasma gonadotropin (FSH & LH) and P_4 concentrations. The development and ovulation of co-dominant follicles following unilateral ovariectomy in 70% of the cycles monitored was the most important finding in this study. As of now it is not clear how long or for how many cycles twin follicular development and ovulation will continue. Since the growth and development of co-dominant follicles followed by ovulation was recorded for up to 3 cycles in Trial I following unilateral ovariectomy, a greater possibility for this pattern to continue in subsequent cycles can be envisioned. Hence, we conclude that unilateral ovariectomy is an ideal method for obtaining twin ovulations in cattle. However, further enquiry using more frequent plasma sampling will be needed to address the hypothesis that increasing concentrations of plasma FSH following unilateral ovariectomy is responsible for the development and ovulation of twin follicles. Whether intraovarian peptides like IGF-I are involved in regulating folliculogenesis following unilateral ovariectomy awaits determination.

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