Effect of acute treatment with progesterone on the timing and synchrony of ovulation in *Bos indicus* heifers treated with a norgestomet implant for 17 days

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The aim of the present study was to develop a treatment protocol for the precise synchronization of oestrus that would avoid the development of persistent dominant ovarian follicles. *Bos indicus* heifers, in which oestrous cycles had been presynchronized, were allocated randomly, according to the day of their oestrous cycle, to one of five treatment groups. All heifers received a subcutaneous ear implant containing 3 mg of norgestomet for 17 days starting on day 0 and an injection of an analogue of prostaglandin $F_{2\alpha}$ on days 0 and 4. Heifers in group 1 (control group; $n = 7$) received no other treatment, while heifers in groups 2 ($n = 8$), 3 ($n = 7$), 4 ($n = 7$), and 5 ($n = 7$) received a single progesterone-releasing controlled internal drug release device (CIDR) for 24 h on days 10, 12, 14 and 16, respectively. Treatment with a single CIDR delayed the mean time of ovulation and the day of emergence of the ovulatory follicle in heifers treated on days 14 and 16 compared with control heifers ($P < 0.05$). There was less variation in the interval to ovulation in heifers treated on day 10 compared with other treated heifers ($P < 0.05$). The variation among heifers in the day of emergence of the ovulatory follicle and the age of the ovulatory follicle at ovulation was less for all groups treated with a CIDR than for the control group ($P < 0.05$). The duration of dominance and variation in the duration of dominance of the ovulatory follicle was less in heifers treated with a CIDR device on days 10 and 16 than for control heifers ($P < 0.05$). Mean age (days from emergence to ovulation) of the ovulatory follicle did not differ among treatment groups ($P > 0.05$). Concentrations of LH and oestradiol decreased coincident with increased concentrations of progesterone on the days of CIDR treatment in treated compared with control heifers ($P < 0.02$) but increased again after removal of the CIDR. A smaller proportion of follicles in the growing phase of follicular development at the time of CIDR treatment become atretic compared with follicles that had reached a plateau phase of follicular growth (14.3% (1/7) versus 90.5% (19/21), respectively; $P < 0.001$). It was concluded that acute treatment with progesterone can influence the growth pattern of ovarian follicular development. However, the effect varies with the stage of ovarian follicular development. Short term treatment with progesterone 7 days before the end of a 17 day period of norgestomet treatment resulted in precise synchrony of ovulation without the ovulation of a persistent dominant ovarian follicle.

Introduction

Progestogens are commonly used to synchronize oestrus in cattle by synchronizing the end of the progestational phase of the oestrous cycle (Odde, 1990; Wright and Malmo, 1992). However, while progestin-based systems of synchronizing oestrus are widely used and provide good control of the

oestrous cycles of cattle, fertility at the synchronized oestrus has been variable (Miksch *et al.*, 1978; Spitzer *et al.*, 1978; Rentfrow *et al.*, 1987; Brown *et al.*, 1988; Favero *et al.*, 1988; Odde, 1990). The reduction in fertility associated with the use of progestogens, in concentrations normally used to synchronize oestrus, has been attributed to abnormal oocyte development (Kinder *et al.*, 1996).

When progestogens are administered in the absence of a functional corpus luteum at doses that are used commercially to synchronize oestrus, there is an increase in the frequency of release of LH from the anterior pituitary gland, the increase in

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peripheral concentrations of oestradiol is prolonged and ovarian follicles that are persistent develop, all of which are associated with a reduction in fertility at the synchronized oestrus (Savio *et al.*, 1993; Cooperative Regional Research Project, 1996; Kinder *et al.*, 1996). Treatments designed to prevent either the development of persistent follicles (Sanchez *et al.*, 1993; Wehrman *et al.*, 1993; Cooperative Regional Research Project, 1996) or induce recruitment of a new dominant follicle (Schmitt *et al.*, 1996) have resulted in an improvement in fertility at the synchronized oestrus. Fertility is not compromised when the duration of dominance of the ovulatory follicle is limited to 4 days or fewer (Mihm *et al.*, 1994). Effective methods to synchronize oestrus should, therefore, synchronize either ovarian follicular development, the time of individual luteal regression, or the end of a period of progesterone treatment. In addition, treatment should prevent prolonged and increased peripheral concentrations of oestradiol or an increase in the frequency of LH secretion and avoid prolonged periods of dominance of the ovulatory follicle.

Acute treatment with progesterone during a period of progesterone treatment results in atresia of persistent ovarian follicles and improves fertility at the synchronized oestrus in *Bos taurus* cattle (Anderson and Day, 1994). However, the effects of acute treatment with progesterone in *Bos indicus* heifers and the timing of such treatment relative to the end of the period of progesterone treatment on the synchrony of ovulation and its influence on ovarian follicular development have not been determined. The objective of the present study was to synchronize the time of ovulation in heifers without the development of a persistent dominant ovarian follicle and to determine the optimum time for short-term treatment with progesterone relative to the end of the period of progesterone treatment, that would result in the best synchrony of ovulation. Our aim was to develop a protocol for synchronizing behavioural oestrus that would result in precise synchrony of ovulation with normal fertility.

Materials and Methods

Animals and treatments

The study was conducted in spring. Serial ultrasound examination was used to confirm cyclic ovarian activity in 36, 2-year-old *Bos indicus* heifers (7/8 to 15/16 Brahman; mean weight $\pm$ SEM: 347.4 ± 7.2 kg). The stage of the oestrous cycle was then synchronized with a s.c. silicone implant containing 3 mg of norgestomet (17 α -acetoxy-11 β -methyl-19-norpreg-4-en-3,20 dione; Crestar[®], Intervet (Aust), Castle Hill, NSW), for 9 days and an i.m. injection of an analogue of prostaglandin F_{2 α} (PGF_{2 α} ; 15 mg Prosolvin[®], Intervet (Aust), Castle Hill, NSW) on the day of implant insertion and removal. Ten days after the implants had been removed (day 0), heifers were allocated randomly, according to the day of their oestrous cycle (days 2 to 5, $n = 7$; day 6, $n = 9$; day 7, $n = 12$; and day 8, $n = 8$; respectively), to one of five treatment groups. All heifers received a single s.c. norgestomet ear implant (3 mg) on day 0 that was removed 17 days later and were injected with 15 mg PGF_{2 α} analogue on days 0 and 4 to induce regression of corpora lutea. A preliminary study had confirmed that a 3 mg

Fig. 1. Diagrammatic representation of treatment protocol. The open rectangles represent a single subcutaneous norgestomet implant inserted into heifers from days 0 to 17. A progesterone-releasing controlled internal drug releasing device (shaded rectangles) was inserted on day 10 (P10), day 12 (P12), day 14 (P14) or day 16 (P16) and removed the following day. An injection of an analogue of prostaglandin F_{2 α} (PG) was given to all heifers on days 0 and 4. Implants were removed from all animals on day 17.

norgestomet ear implant would maintain *Bos indicus* heifers in anoestrus for at least 21 days (J. Cavalieri, unpublished). Heifers in the control group (control, $n = 7$), received no other treatment. A progesterone-releasing controlled internal drug release (CIDR) device was inserted into the vagina of the remaining heifers for 24.2 ± 0.06 h (mean $\pm$ SEM) either on day 10 (P10, $n = 8$), day 12 (P12, $n = 7$), day 14 (P14, $n = 7$) or day 16 (P16, $n = 7$; Fig. 1).

Ultrasonography

The ovaries of heifers were examined using transrectal ultrasonography (Aloka 210 DX: 7.5 Mhz probe), by the same operator, on alternate days from days 0 to 10, then once a day until signs of behavioural oestrus were detected. The ovaries of heifers were then examined every 6 h until ovulation was detected. The stage of growth of ovarian follicles at the time of treatment with progesterone was categorized as either in the growing phase (when a follicle was 5–9 mm in diameter and still increasing in size) or as in the plateau phase (when there had been no increase in the size of the dominant follicle for 2 consecutive days or the dominant follicle was ≥ 10 mm in diameter). The mean maximum diameter of dominant follicles in *Bos indicus* heifers has been reported as 10 mm (Rhodes *et al.*, 1994). A new wave of follicular development always began before regression of the dominant follicle had been detected.

The day of emergence of the ovulatory follicle was defined, retrospectively, as the day on which the ovulatory follicle was first detected at a size of > 5 mm in diameter. The age of the ovulatory follicle was defined as the time interval (days) between the day of emergence of the ovulatory follicle and the time of ovulation. The first day of dominance of the ovulatory follicle was defined using the criteria described by Mihm *et al.* (1994), that is, the first day that (1) subordinate follicles stop increasing in diameter, (2) the ovulatory follicle was ≥ 8.5 mm, or (3) the diameter of the ovulatory follicle was > 2 mm larger than any subordinate follicles (not essential). The duration of

dominance of the ovulatory follicle (days) was defined as the interval between the first day of dominance and the day of ovulation minus one day.

Blood sampling and radioimmunoassays

Concentrations of progesterone in plasma were measured in unextracted plasma samples by radioimmunoassay using a modification of the Danazol method (McGinley and Casey, 1979) described by Jolly (1992). The sensitivity of the assay (90% zero-binding) was 0.03 ng ml^{-1} and intra- and inter-assay coefficients of variation at the approximate mid-point of the standard curve (1.0 ng ml^{-1}) were 13.1% and 16.1%, respectively.

Concentrations of plasma LH were measured by double-antibody radioimmunoassay, using a modification of the method described by Niswender *et al.* (1969) that has been validated in our laboratory (Rhodes *et al.*, 1995a). Purified bovine LH (USDA-bLH-B-6, AFP-11743-B) was provided by D. Bolt (USDA Animal Hormone Program, Beltsville, MD, USA). The antiserum used was NIDDK-anti-oLH-1 (AFP-192279), which was supplied by NIDDK, Bethesda, MD. The sensitivity of this assay (90% of zero-binding) was 0.2 ng ml^{-1} . Intra- and interassay coefficients of variation were 13.5% and 16.5%, respectively.

Concentrations of plasma oestradiol were determined by radioimmunoassay in the Physiology Laboratory of the University of Nebraska, using antiserum to oestradiol (lot no. 022367) provided by N. R. Mason (Lilly Research Laboratories, Indianapolis, IN) and oestradiol, (Sigma Chemical Co., St. Louis, MO) as standard. The procedure for this assay has been reported and validated by Kojima *et al.* (1992). The sensitivity of this assay (90% of zero binding) was 0.07 pg ml^{-1} . Intra- and interassay coefficients of variation were 13.4% and 16.4%, respectively.

Statistical analyses

Initial data analysis suggested highly variable non-normal distribution of data. Therefore, bootstrap estimates of location (mean) and spread (SD) were used for the variables, age of follicle (number of days between emergence and ovulation of follicle), day of emergence and duration of dominance of the ovulatory follicle, and time elapsing from implant removal to ovulation. The bootstrap is a recently developed technique for making certain types of statistical inferences (Efron and Tibshirani, 1993). Bias corrected and accelerated estimates were used to calculate both estimates and confidence intervals (CIs; Efron and Tibshirani, 1993) using the bootstrap library provided in Statlab (Splus, StatSci Division, MathSoft Inc., Seattle, Washington, 1995). Splus 3.3 for Windows was used to run the library.

For the purposes of comparisons of means and SD among treatment groups, the 90% CIs can be used as follows. Non-overlapping 90% CIs represent a significant difference at approximately the 1% level of significance. This makes no allowance for multiple comparisons and is hence equivalent to performing multiple *t* tests. Multiple comparisons using the Bonferroni method were catered for by increasing the level of

1% by approximately the number of tests used. Thus, if each group is to be compared with the control (that is, four comparisons), then the level of significance would be marginally less than 5%.

Endocrine concentrations were log transformed (base 10) to achieve constant variance over the sampling period. Changes in plasma concentrations of progesterone, LH, and oestradiol over time were analysed for effects of group, day and group-by-day interaction using multivariate repeated measures analysis of variance (Lindsey, 1993). If the group effect or the day-by-group effect was significant ($P < 0.05$), means of individual treatment groups were compared with the control group using Student's *t* test. The proportion of either growing (5–9 mm in diameter) or plateau phase ovarian follicles, present at the start of treatment with progesterone, that became atretic and did not ovulate was compared using a two-tailed Fisher's exact test (Bradley, 1968).

Results

One animal in the P16 group failed to ovulate within 10 days of implant removal and data from this animal were excluded from analyses pertaining to follicular growth, but were included in the analyses of endocrine changes.

Endocrine changes

For the purpose of statistical analysis, changes in concentrations of hormones over time were divided into two time periods, days 0–10 and days 10–18. Changes in concentrations of oestradiol, LH and progesterone for each treatment group from days 0 to 18 are shown (Fig. 2).

Days 0 to 10. There was a significant effect of day ($P < 0.001$), but no group ($P > 0.270$), or group-by-day interaction ($P > 0.280$) on plasma concentrations of progesterone, oestradiol and LH between days 0–10. Mean ($\pm$ SEM) concentrations of progesterone declined ($P < 0.001$) from day 0 ($3.023 \pm 0.267 \text{ ng ml}^{-1}$) to day 2 ($0.525 \pm 0.065 \text{ ng ml}^{-1}$) and remained at basal concentrations in all groups until day 10. Concentrations of LH initially increased ($P < 0.001$) from day 0 to day 2, decreased ($P < 0.001$) from day 2 to day 6 and then increased ($P < 0.001$) again by day 10. Concentrations of oestradiol increased ($P < 0.001$) from day 0 to day 10.

Days 10 to 18. A significant group-by-day interaction ($P < 0.001$) was detected for plasma concentrations of progesterone between days 10 and 18. Insertion of a CIDR device for 24 h resulted in increased concentrations of progesterone in the treated animals compared with the control animals during the period of CIDR treatment ($P < 0.001$). Mean concentrations of progesterone in the plasma of treated heifers at the time of removal of the CIDR ranged between 4.196 and 4.879 ng ml^{-1} , while mean concentrations of progesterone in plasma of the control heifers were $< 0.250 \text{ ng ml}^{-1}$ on each of the days CIDRs were in place. Concentrations of progesterone decreased to mean basal values of $< 0.7 \text{ ng ml}^{-1}$ in each of the treated groups 24 h after removal of the CIDR. Luteal

regression did not occur in one heifer in group P10 until day 14 and this contributed to the group effect ($P = 0.033$) detected between days 10 and 18.

A group-by-day interaction ($P < 0.001$) was detected for concentrations of LH between days 10 and 18. At the time of removal of the CIDR, mean concentrations of plasma LH in each group of treated heifers were lower than concentrations of LH in control heifers (control versus P10, P14, P16, $P < 0.01$; control versus P12, $P = 0.02$). Mean concentrations of LH in heifers in groups 2, 3, 4 and 5 at the time of CIDR removal were 53, 41, 61 and 45%, respectively for each group, lower than concentrations of LH in the control heifers. Mean concentrations of LH in animals in groups P10, P12 and P14 increased 24 h after removal of the CIDR and were not different from concentrations in the control heifers ($P > 0.150$). Although concentrations of LH in heifers in group P16 increased from day 17 to day 18, LH concentrations in these heifers on day 18 was still significantly lower ($P = 0.040$) than corresponding LH concentrations in the control heifers, due to the recording of a preovulatory LH surge in some of the control animals.

A group-by-day interaction ($P < 0.001$) was detected for concentrations of oestradiol between days 10 and 18. Mean circulating concentrations of oestradiol at the time of CIDR removal had decreased by 85%, 84%, 82%, 91%, respectively, for groups P10, P12, P14 and P16, when compared with the time of CIDR insertion. Concentrations of oestradiol were lower in all treatment groups at the time of CIDR removal than the respective concentrations of oestradiol in the control heifers on that day ($P < 0.001$). Concentrations of oestradiol in the plasma of treated heifers increased after removal of CIDR devices. The times required for mean concentrations of oestradiol to increase, after progesterone withdrawal, to within the 95% confidence interval of the mean concentration of oestradiol for the 2 days before treatment, were 5, 3, 4 and 6 days for groups P10, P12, P14 and P16, respectively.

Ovarian follicular development

During the 17 day period of norgestomet treatment, heifers from the control and P10 groups had two or three waves of follicular development, while heifers in groups P12–P16 had two, three or four waves of follicular development. Treatment with a CIDR device for 24 h delayed ovulation and the day of emergence of the ovulatory follicle only in animals treated within 3 days of implant removal (groups P14 and P16) ($P < 0.05$; Fig. 3). A greater variability in the time of ovulation was evident in the P12 and P16 animals compared with the control animals and in the P16 animals compared with P10, P12 and P14 heifers. Variability in the time of ovulation between the P10, P14 groups and the control group was similar ($P > 0.05$; Fig. 3). The day of emergence of the ovulatory follicle varied less among heifers in each of the four treatment groups compared with heifers in the control group ($P < 0.05$;

Fig. 3). There was a trend towards a reduction in the mean age of the ovulatory follicle in the treated animals compared with control animals, although the difference was never significant ($P > 0.0$; Fig. 4). The age of the ovulatory follicle, however, varied more among the control animals than among any of the animals in the other four treatment groups ($P < 0.05$, Fig. 4). The mean duration of dominance (days) of the ovulatory follicle was less in animals treated on day 10 and day 16, but also varied less in animals treated on days 10, 14 and 16 compared with the control animals ($P < 0.05$, Fig. 4). None of the ovarian follicles observed in animals in the P10 group at the beginning of treatment with progesterone could be retrospectively identified as the ovulatory follicle. However, in 57.1% (4/7), 28.6% (2/7) and 33.3% (2/6) of animals in groups 3, 4 and 5, respectively, the ovulatory follicle could retrospectively be identified as being present in the ovary at the time when treatment with progesterone began. The growth of the ovulatory follicle for each animal in each treatment group is shown (Fig. 5). The probability of causing atresia of ovarian follicles in animals treated with progesterone differed according to stage of follicular growth at the time of treatment with progesterone. A smaller proportion of follicles that were in the growing phase (5–9 mm in diameter) of follicular development became atretic as a result of treatment with progesterone when compared with follicles that had reached the plateau phase of follicular growth (14.3% (1/7) versus 90.5% (19/21), respectively; $P < 0.001$).

Discussion

The results of the present study are in agreement with the findings of others who have reported that acute treatment with progesterone can induce atresia of dominant ovarian follicles and the emergence of a new wave of follicular development (Rajamahendran and Manikkam, 1994; Anderson and Day, 1994). Data from the present study, however, indicate that the time when progesterone treatment is administered, relative to the end of a period of norgestomet treatment, will affect the timing and synchrony of ovulation. Treatment with progesterone, 7 days before (day 10) the removal of norgestomet implants, provided the best balance between achieving a precise time of ovulation and avoiding the ovulation of an aged oocyte. Mihm *et al.* (1994) demonstrated that a sequential reduction in pregnancy rates occurred as the duration of dominance of the largest follicle increased from 4 days to 8 days in heifers in which oestrus was synchronized with norgestomet, while no pregnancies occurred in heifers in which the duration of follicular dominance exceeded 10 days (Mihm *et al.*, 1994). In studies in which small doses of progestins were used, the extension of periods of follicular dominance resulted in a reduction in fertility (Savio *et al.*, 1993; Stock and Fortune, 1993; Cooperative Regional Research Project, 1996). Acute treatment with progesterone 7 days before ending

Fig. 2. Profiles of mean ($\pm$ SEM) concentrations of (a) progesterone, (b) LH and (c) oestradiol in heifers treated with a norgestomet implant for 17 days (starting on day 0) and an injection of an analogue of prostaglandin $F_{2\alpha}$ (PG) on days 0 and 4. Heifers in the control group ($\circ$) received no other treatment. Heifers in groups P10 ($\bullet$), P12 ($\blacksquare$), P14 ($\triangle$) and P16 (∇) were treated with a progesterone-releasing controlled internal drug releasing device for 24 h starting on days 10, 12, 14 and 16, respectively.

Fig. 3. Bootstrap estimates of location (mean) and spread (SD) ($\pm 90\%$ confidence interval) of the time interval from the end of norgestomet treatment to ovulation and day of emergence of the ovulatory follicle. Non-overlapping 90% confidence intervals differ ($P < 0.05$). Heifers were treated with a norgestomet implant for 17 days (starting on day 0) and an injection of an analogue of prostaglandin $F_{2\alpha}$ on days 0 and 4. Heifers in the control group received no other treatment. Heifers in groups P10, P12, P14 and P16 were treated with a progesterone-releasing controlled internal drug releasing device for 24 h starting on days 10, 12, 14 and 16, respectively.

norgestomet treatment offers the advantage of producing precise ovulation synchrony while at the same time preventing ovulation of persistent dominant follicles and would, therefore, be expected to result in normal fertility.

Variability of synchrony of ovulation occurred in *Bos indicus* cows when they were treated with a CIDR device for 10 days (day 0 to day 10) and then treated with an additional CIDR device for 48 h from day 8 to day 10 (Cavalieri *et al.*, 1997). It was concluded in that study that variation among animals in the timing of emergence of ovulatory follicles, follicular growth rates and maturation times for ovarian follicles after the administration of the atretogenic treatment could have contributed to the variability of ovulation synchrony that was observed. In addition, a longer period of progestin treatment

after the application of an atretogenic treatment might be needed to improve the synchrony of ovulation. The results of the study reported here confirm that acute treatment with progesterone at the end of a period of progestogen treatment does result in a variable pattern of ovulation synchrony and that increasing the time interval between progesterone treatment and extending the period of progestogen treatment to 7 days improves ovulation synchrony.

Two to three waves of ovarian follicular development were detected in the control animals even though regression of corpora lutea was induced early during the period of norgestomet treatment. This finding contrasts with previous studies in which the use of single s.c. norgestomet implants in *Bos taurus* cattle, in the absence of a corpus luteum, increased

Fig. 4. Bootstrap estimates of location (mean) and spread (SD) ($\pm 90\%$ confidence interval) of the age and duration of dominance of the ovulatory follicle. Non-overlapping 90% confidence intervals differ ($P < 0.05$). Heifers were treated with a norgestomet implant for 17 days (starting on day 0) and an injection of an analogue of prostaglandin $F_{2\alpha}$ on days 0 and 4. Heifers in the control group received no other treatment. Heifers in groups P10, P12, P14 and P16 were treated with a progesterone-releasing controlled internal drug releasing device for 24 h starting on days 10, 12, 14 and 16, respectively.

LH pulse frequency and prolonged the maintenance of dominant ovarian follicles (Savio *et al.*, 1993; Taylor *et al.*, 1993, 1994). In cows undergoing normal oestrous cycles, the presence of basal concentrations of gonadotrophins during the luteal phase of the oestrous cycle results in regular waves of ovarian follicular development (Fortune, 1993). It would appear that concentrations of circulating norgestomet were sufficient, in some of the heifers in the control group in the present study, to allow the continued periodic emergence of follicular waves during the implantation period. Differences in genotype, sensitivity to circulating concentrations of norgestomet, body-weight or the timing of insertion of the norgestomet implants might explain why dominant follicles were not maintained for the duration of progestogen treatment in the control heifers in the present study. Greater fertility and differing patterns of

release of norgestomet have been observed when norgestomet was administered in silicone as opposed to hydron implants (Kesler *et al.*, 1995), which might indicate that persistent dominant follicles are less common when silicone implants are used to administer norgestomet. Acute treatment with progesterone offers an additional means of reducing variability in the growth pattern of the ovulatory follicle in heifers implanted with a norgestomet implant.

The reduction in observed variability in the growth pattern of the ovulatory follicle in most heifers treated with progesterone was due to treatment rendering most follicles anovulatory. Acute treatment with progesterone, however, was more effective in follicles that had reached a plateau phase in growth at the time of treatment compared with follicles still in the growing phase. This finding indicates that the development

Fig. 5. Pattern of emergence and growth of the ovulatory follicle for each heifer in each treatment group, up until ovulation was detected, as recorded by transrectal ultrasonography. (■) indicates the presence of a progesterone-releasing controlled internal drug releasing device; (○) indicates time ovulation was detected. Heifers were treated with a norgestomet implant for 17 days (starting on day 0) and an injection of an analogue of prostaglandin $F_{2\alpha}$ on days 0 and 4. Heifers in the control group received no other treatment. Heifers in groups P10, P12, P14 and P16 were treated with a progesterone-releasing controlled internal drug releasing device for 24 h starting on days 10, 12, 14 and 16, respectively.

and maintenance of dominant follicles are less likely to be arrested by exogenous treatment with progesterone during the growing stages of ovarian follicular growth. Differences in the sensitivity of ovarian follicles to atresia resulting from treatment with progesterone may relate to the number of LH receptors present in a follicle and to the dependence of a follicle on LH for continued viability. Dominant, gonadotrophin-dependent, ovarian follicles are thought to be able to survive in an environment of reducing concentrations of FSH because of their increased sensitivity to FSH. LH receptors in granulosa cells, therefore, are transferring their gonadotrophic requirement from FSH to LH (Scaramuzzi *et al.*, 1993; Campbell *et al.*, 1995). This change, however, makes dominant follicles

critically dependent on LH for survival, and so the reduction in mean LH concentrations observed in the present study, coincident with progesterone treatment, may have deprived dominant follicles of sufficient aromatisable substrate and thereby, initiated atresia. During the early stages of growth, those follicles, that are less dependent on LH for their survival, would be less susceptible to acute treatments with progesterone which reduce the frequency of pulsatile LH secretion (Rajamahendran and Manikkam, 1994). Therefore, in cattle, in which oestrus has been synchronized with progestogens, acute treatment with progesterone is more likely to render anovulatory those follicles that had achieved a functional state of dominance at the time of treatment, as these follicles would be more susceptible to a reduction in LH secretion. This would explain why in the study described here, actively growing follicles were less susceptible to the atretogenic effects of treatment with progesterone.

Atresia of all dominant or growing follicles that were observed in the ovaries at the time of treatment with progesterone was achieved in the P10 heifers as a result of treatment. This atresia resulted in a more synchronous emergence of the ovulatory follicle in this group and may have contributed to the more precise timing of ovulation observed compared with heifers in the other progesterone-treated groups. One step towards improving ovulation synchrony might be to improve the efficiency of atretogenic treatments. The results of the present study suggest that this could be achieved by inducing the development of a persistent dominant follicle, before administration of the atretogenic treatment, by treatment with progesterone at concentrations that are lower than usually occur during a normal luteal phase of an oestrous cycle ($< 3 \text{ ng ml}^{-1}$), or by using concentrations of progestogens that are used commercially to synchronize oestrus in the absence of a corpus luteum (Sirois and Fortune, 1990; Adams *et al.*, 1992; Savio *et al.*, 1993; Stock and Fortune, 1993; Cooperative Regional Research Project, 1996).

The reduction in mean concentrations of LH in plasma 24 h after the beginning of treatment with progesterone is in agreement with previous findings in both *Bos indicus* cows (Cavalieri *et al.*, 1997), and *Bos taurus* heifers (Burke and Macmillan, 1995; Bergfeld *et al.*, 1996) treated with exogenous progesterone. The decline in mean concentrations of oestradiol during the period of progesterone treatment and subsequent increase after the removal of CIDR devices is probably due to changes in both the secretory pattern of LH and the disruption of the functional integrity of dominant follicles by progesterone treatment. Production of oestradiol is related to the frequency of LH pulses (Walters *et al.*, 1984; Rhodes *et al.*, 1995a). Thus, the reduction in mean concentrations of oestradiol is consistent with the reduction in mean concentrations of LH in the present study.

The increase in mean concentrations of LH in each group of heifers treated with progesterone after treatment had stopped was probably due to a reduction in progesterone-mediated negative feedback on LH secretion after the removal of the CIDR devices; progesterone is known to affect the pulsatile secretion of LH in a dose-dependent manner (Ireland and Roche, 1982; Roberson *et al.*, 1989; Bergfeld *et al.*, 1995, 1996). Mean concentrations of oestradiol, however, increased at a variable rate after the removal of CIDR devices with

concentrations of oestradiol taking between 3 and 6 days to increase to pretreatment values.

Other studies have shown that atresia of dominant follicles is associated with a reduced capacity for secretion of oestradiol (Sunderland *et al.*, 1994; Rhodes *et al.*, 1995b). Thus, our results are consistent with progesterone treatment causing a loss of functional dominance of ovarian follicles. The more rapid increase in oestradiol concentrations in, for example, the heifers in the P12 group was probably due to a smaller proportion of follicles being rendered anovulatory by the treatment. LH concentrations were reduced only for the first 24 h of CIDR treatment, yet atresia of dominant follicles was still observed in most heifers treated with progesterone. This result supports the contention of Anderson and Day (1994) that only a transient reduction in LH secretion is needed to induce atresia of dominant follicles. Whether there is a critical threshold for circulating concentrations of progesterone, or a critical duration for the presence of physiological concentrations of progesterone that is necessary before a dominant follicle is rendered anovulatory remains to be determined. In addition, whether the dose and duration of progesterone that is necessary to cause atresia varies with the stage of follicular development also requires further investigation.

In conclusion, the findings reported here indicate that acute treatment with progesterone during a period of norgestomet treatment can reduce variation in the day of emergence, and the age and duration of a dominant follicle, and, therefore, could be used to improve fertility in animals in which the stage of oestrous cycles has been synchronized with progestogens. However, extensive field trials are needed to test the potential of this treatment protocol. The timing and synchrony of ovulation were influenced by the timing of acute treatment with progesterone relative to the end of progestogen treatment. Treatment with progesterone, 7 days before the ending of a 17 day period of norgestomet treatment, resulted in precise synchrony of ovulation and prevented the ovulation of a persistent dominant ovarian follicle. Susceptibility of ovarian follicles to atresia during exogenous treatment with progesterone also varies with stage of follicular development and this will need to be considered when designing treatments to synchronize oestrus.

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