Control and induction of ovulation in cattle

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Summary. The control and induction of ovulation in cattle are discussed with particular reference to use of progesterone-impregnated coils in heifers and beef cows. Progesterone treatment for 14 days was required to obtain precise onset of oestrus. With 7, 9 or 12 days of progesterone treatment a luteolytic agent in the form of a prostaglandin (PG) or oestradiol benzoate had to be used. Fertility was normal after treatment durations of 7, 9 or 12 days, but fertility after 14-day treatment requires further testing. The progesterone coil was not effective in maintaining luteal-phase levels of progesterone throughout a 12-day treatment and increasing the concentration of progesterone in the coil from 4 to 20% was not effective in elevating the progesterone concentrations in blood. When progesterone concentrations dropped below approximately 1·5 ng/ml the basal level of LH began to rise before removal of the coil.

A 2-fold rise in the basal level of LH was observed following the removal of the progesterone coil. This early rise in LH was absent in cows which did not ovulate after they were given a 12-day progesterone treatment and GnRH 24–36 h after removal of coils to induce the main LH peak. Absence of this early rise suggests that frequency and amplitude of episodic LH release were inadequate in the post-partum anovulatory period. Ovariectomy in the early post-partum period was not followed by an abrupt LH release.

Introduction

Beef cows suckling calves return to oestrus within 30–120 days following parturition. The length of this post-partum anoestrus is affected by breed, plane of nutrition and age, but primarily by the suckling stimulus (Inskeep & Lishman, 1979). Increasing the calf crop by 5 % and advancing the calving date by 6 days could increase the estimated annual output of beef cattle in the United States of America by $797 million (Gerrits, Blosser, Purchase, Terrill & Warwick, 1979). Thus, small improvements in reproductive performance could result in large economic gains.

Attempts to synchronize oestrus in beef cows suckling calves have been hampered by the problem of prolonged post-partum anoestrus (Hafs, Manns & Drew, 1975; Roche, 1976a). Various methods have been used to shorten post-partum anoestrus and to control oestrus. The use of prostaglandin (PG) F-2α requires the presence of a corpus luteum, while the use of progesterone alone or in conjuction with pregnant mares' serum gonadotrophin (PMSG), gonadotrophin-releasing hormone (GnRH), and the temporary or early weaning of calves does not. Since cows with active and inactive ovaries are likely to be present in a herd of cows, a successful regimen must be capable of inducing and controlling a fertile ovulation in both circumstances. Development of such methods requires a better understanding of the endocrine control of follicular growth and ovulation. This paper deals with methods to control and induce ovulation in post-partum cattle.

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Control of ovulation

The aim in controlling ovulation in cattle is to have over 90% of treated animals ovulating within a 24–60-h period, so that one or two inseminations at pre-arranged times can be used. This means that treatments must synchronize the regression of the corpus luteum or delay periovulatory endocrine events until the corpora lutea have regressed. The synchronous termination of the luteal phase can be brought about by injection of luteolytic doses of PGF-2α or one of its analogues. This approach is not effective in beef cows suckling calves because 30–50% of the cows are in anoestrus (Roche, 1976a; Chupin, Pelot, Alonso de Miguel & Thimonier, 1976). The alternative method of delaying periovulatory events by administration of progesterone or synthetic progestagens is preferred because it facilitates the induction of ovulation (Roche, 1976a; Mulvehill & Sreenan, 1977; Petit, M'Baye & Palin, 1979). A major problem in using progesterone to control oestrus is the large daily dose of 20–30 mg required to suppress ovulation. One method of administering sufficient progesterone for this purpose is the use of a progesterone-releasing intravaginal coil (PRID; Abbott Laboratories, U.S.A.), which is inserted into the vagina by means of a speculum (Roche, 1976b). A second problem is low fertility at the synchronized oestrus following the 18–21 days of progesterone administration. This duration of progesterone treatment is necessary to give precise control of ovulation when progesterone or progestagens are used without a luteolytic agent. However, limiting the duration of treatment to between 9 and 12 days (Wiltbank & Kasson, 1968; Mauléon, 1974; Roche, 1974) overcomes the problems of low fertility.

Progesterone treatment for 12 days

Progesterone has no effect on the lifespan of the corpus luteum after Day 3 of the oestrous cycle (Ginther, 1970). A luteolytic agent is required to obtain precise onset of oestrus after 9–12 days of progestagen treatment. The luteolytic agent initially used was 5 mg oestradiol valerate (Wiltbank & Kasson, 1968) injected at the start of treatment. Even when oestradiol benzoate was used at the start of a 12-day treatment, the onset of the synchronized oestrus was delayed in 10–20% of treated animals (Roche, 1974; Roche, 1976c). In heifers this delay in onset of oestrus is related to progesterone concentrations at the time of removal of the PRID. Animals with progesterone concentrations greater than 2 ng/ml had a delayed onset of oestrus when compared to animals with lower levels (Mauer, Webel & Brown, 1975; Roche & Gosling, 1977). An experiment (J. F. Roche, unpublished) was carried out on lactating dairy cows, 5 or more weeks *post partum*, to determine whether the delay in onset of oestrus after coil removal was due to poor control of luteal function or to variation in follicular growth. Injection of PMSG at the time of coil removal did not reduce the variation in onset of oestrus (Table 1) indicating that delayed follicular growth was unlikely to be responsible for the variation in onset of oestrus. There was a significant correlation, however, between the level of progesterone in milk 24 h after coil removal and subsequent onset of oestrus ($P < 0.01$). Cows in oestrus within 2 days of coil removal had a milk progesterone concentration of 1.1 ± 0.19 ng/ml, compared with 4.96 ± 1.07 ng/ml for cows in oestrus on Day 4 and 14.3 ± 4.1 ng/ml for those in oestrus between Days 5 and 7 after coil removal. It can therefore be concluded that the major reason for variation in onset of oestrus in cattle following the 12-day progesterone treatment is poor control of luteal function.

The stage of the oestrous cycle at the start of a 12-day progesterone treatment affects the subsequent response. Animals treated between Days 0 and 5 of the oestrous cycle are most likely to have a delayed oestrus (Mauléon, 1974; Roche, 1974; Wiltbank & Gonzalez-Padilla, 1975). However, Woods, First & Pope (1967) reported that progesterone shortened the oestrous cycle in animals treated between Days 0 and 3. Ginther (1970) showed that progesterone was less effective in shortening the oestrous cycle as the interval between injection and the previous oestrus increased and no effect occurred when progesterone was given after Day 3. Oestrogens

Table 1. Onset of oestrus in lactating dairy cows and heifers following a 12-day treatment with progesterone coils with or without a 10 mg oestradiol benzoate capsule present on the coil or an injection of 750 i.u. PMSG at the time of coil removal

	No. of animals	No. observed in oestrus*	Onset of oestrus (days after removal)		
			2–3	4–6	6–16
Coil only					
Heifers	44	40	29	11	—
Dairy cows	102	94	69	22	3
Coil + PMSG					
Heifers	45	35	25	8	2
Dairy cows	89	70	54	13	3
Coil + oestradiol benzoate					
Heifers	42	37	35	2	—
Dairy cows	93	82	74	8	—
Coil + oestradiol benzoate + PMSG					
Heifers	43	37	32	4	1
Dairy cows	84	70	43	22	5

* Within 16 days of coil removal.

can shorten the oestrous cycle (Wiltbank, 1966; Lemon, 1975) and oestradiol or oestradiol–progesterone combinations can function as a luteolytic complex at the start of a 9–12-day progesterone treatment. The need to use progesterone in the luteolytic complex is determined by the speed of the initial rise in progesterone. It is important to get the concentration of progesterone close to mid-luteal phase concentrations (3–6 ng/ml) as soon as possible to prevent the oestrogen inducing ovulation in animals in the follicular phase (Roche, 1974) and to produce a maximum luteolytic effect in animals that have recently ovulated (Ginther, 1970). Therefore when progestagen implants are used extra progesterone is required at the start of treatment (Roche, 1974; Wiltbank & Gonzalez-Padilla, 1975), but with the Silastic coil the initial release rate is sufficient (Mauer *et al.*, 1975; Roche & Gosling, 1977).

If a treatment period of less than 12 days is used, the onset of oestrus is more variable. Significantly more heifers and dairy cows were observed in oestrus within 3 days of coil removal after a 12-day treatment compared to a 9-day treatment period (Roche, 1978). Fertility in animals bred at oestrus after 9 or 12 days of treatment was similar to that obtained in control cows.

Progesterone treatment for 14 days

Some countries will not allow routine use of oestrogens in cattle, and there is a need to develop alternative treatments. It has been shown that there is an interaction between the requirement for oestrogen and the length of progesterone treatment (Roche, 1978). When the treatment period is extended to 14 days oestrogen is not required to produce synchronized oestrus. However, in field trials using dairy cows, fertility following 14 days of progesterone treatment was significantly lower than fertility in control cows or in cows which had had a 12-day progesterone treatment with oestrogen given at the start of treatment. This lower fertility was due mainly to a low conception rate following a single insemination 56 h after treatment (table 2). Other work with dairy cows and heifers (Ellicott, Thompson & Hill, 1977; O'Farrell, 1978) has shown no reduction in fertility following one or two fixed-time inseminations after 12- or 14-day treatment periods. Fertility following a 14-day treatment period appears to be normal, particularly when two inseminations at 56 and 72 h have been used. However, this treatment regimen needs to be tested in large-scale experiments.

Table 2. Calving rate in control or treated dairy cows following 12 or 14 days of progesterone treatment, with insemination at 56 h or 56 and 74 h after removal of coils

	A.I. at oestrus	12-day treatment		14-day treatment	
	(controls)	56 h	56 + 74 h	56 h	56 + 74 h
No. inseminated	121	79	72	82	72
No. calved	68	42	36	25	31
% calved	56	53	50	30	43

Progesterone in combination with prostaglandin

An alternative treatment is to use a 7- or 9-day treatment period with progesterone and an injection of PGF-2α, or one of its analogues, at the end, or 1 or 2 days before the end, of the progesterone treatment. This eliminates the need for oestrogen and it is claimed that by giving the prostaglandin 1 or 2 days before the end of the progesterone treatment, the onset of oestrus is more precise (Thimonier, Chupin & Pelot, 1975; Hansel & Beal, 1979). Fertility following the 7-day progesterone treatment with prostaglandins given at the end (Roche, 1976d) or 1 day before coil removal (Hansel & Beal, 1979) is normal. Thus, there are advantages in using a progesterone–PGF-2α treatment in comparison to other treatment regimens.

The optimum concentration of progesterone in blood during a synchronization treatment has not been clearly defined. The importance of this question has become apparent since it has been demonstrated that progesterone has a negative feedback effect on basal LH levels in the ewe (Hauger, Karsch & Foster, 1977). A concentration of progesterone in blood sufficient to block oestrus may not suppress the LH and FSH concentrations to the basal values found during the luteal phase of the oestrous cycle.

Concentration of progesterone while coil was in vagina

In attempts to evaluate the relationships between progesterone and LH during a synchronized oestrous cycle, two experiments have been carried out with heifers. In the first experiment, 40 heifers, in the luteal phase (Days 8–10) or follicular phase of the oestrous cycle (Days 17–18), received intravaginal coils containing either 2% or 6·75% progesterone for 7 days. All animals received an injection of PGF-2α the day before the coils were removed. The blood concentrations of progesterone and LH before insertions of coils, and during treatment are shown in Text-fig. 1. In the second experiment, 24 of the heifers, following a normal oestrous cycle, were allocated at random to the following treatments for 12 days: (i) 4% progesterone coil with oestrogen capsule; (ii) 4% progesterone coil + injection of PG at coil insertion; (iii) as in (ii) but 20% coil used. Progesterone concentrations at time of coil insertion, during and after treatment are shown in Text-fig. 2.

The coil did not maintain luteal-phase concentrations of progesterone throughout the treatment period (Text-figs 1 and 2). Increasing the concentration of progesterone from 4 to 20% in Exp. 2 did not result in a significant increase in blood concentrations of progesterone, indicating that this is not an effective way to increase blood concentrations during the last half of a 12-day treatment. Decreasing the percentage of progesterone from 6·75 to 2% in Exp. 1 did result in a significant decrease in progesterone concentrations in heifers treated for a 7-day period during the follicular, but not the luteal phase of the cycle. It is also apparent that the shorter the duration of treatment, the higher the concentrations of progesterone in blood during treatment (Text-figs 1 and 2). Therefore, a 7-day treatment period has the advantage of maintaining blood levels of progesterone at normal luteal-phase levels during the treatment period.

In Exp. 1, declining concentrations of progesterone in follicular-phase heifers given 2%
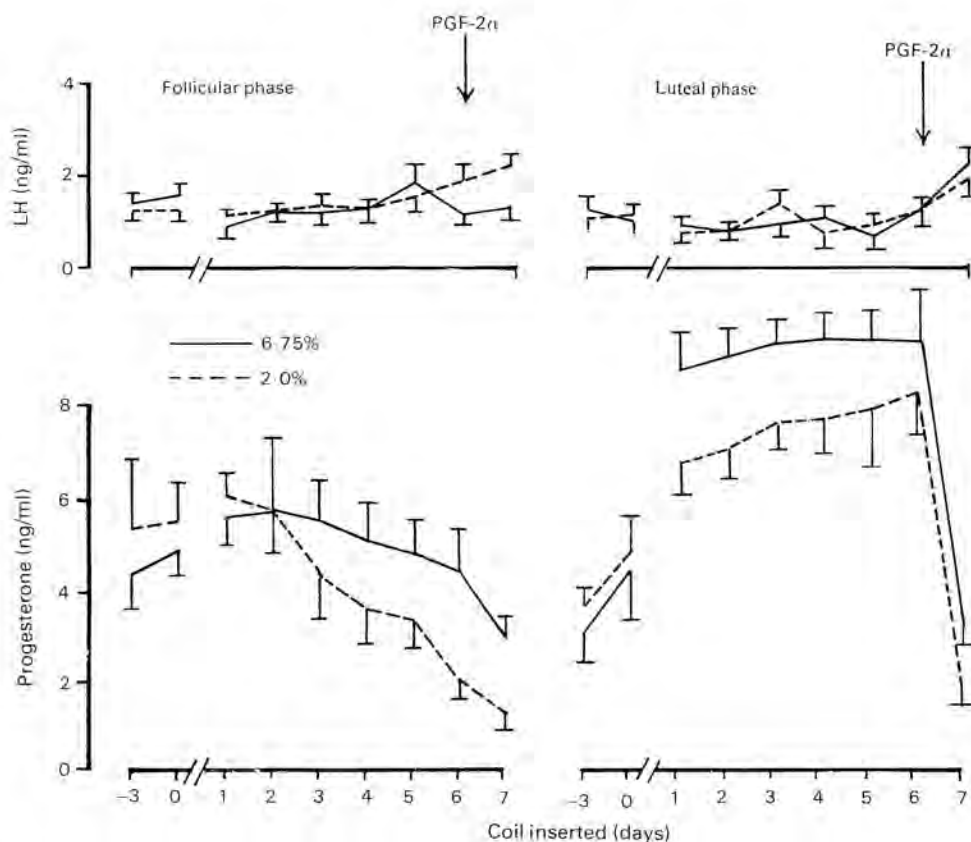
Text-fig. 1. Mean concentrations ($\pm$ s.e.m.) of progesterone and LH in serum of follicular or luteal-phase heifers before and during a 7-day treatment with progesterone coils. Coils contained 2 or 6·75% progesterone and all animals were injected with PGF-2α 1 day before removal of coils. There were 10 heifers per treatment.

progesterone coils were associated with increases in daily LH concentrations and an earlier onset of LH and FSH peaks after treatment. This suggests that progesterone, as in the ewe (Hauger *et al.*, 1977), has a negative-feedback effect on basal LH concentrations in heifers. The time course of the decline in progesterone and increase in LH concentrations following removal of coils is shown in Table 3. Within 4 h of removal of coils, progesterone concentrations had dropped by 58% while LH concentrations had increased by 86%. The optimum concentration of progesterone required to control oestrus may be that which maintains plasma LH at concentrations found during the luteal phase of the oestrous cycle, rather than the lower concentrations of progesterone required to block oestrus during treatment. The inter-relationships between ovarian steroids, pituitary gonadotrophins and follicular growth need to be more clearly defined so that the correct concentration of progesterone to maintain normal pituitary–ovarian function during a synchronizing treatment can be determined. Presently available delivery systems may have to be refined in order to maintain higher levels of progesterone in blood during the second half of a 12- or 14-day treatment period.

Conclusions

Because of the ensuing low fertility, the 18–21-day progestagen treatments have been superseded by treatment periods varying from 7 to 14 days. With a 14-day treatment period, a
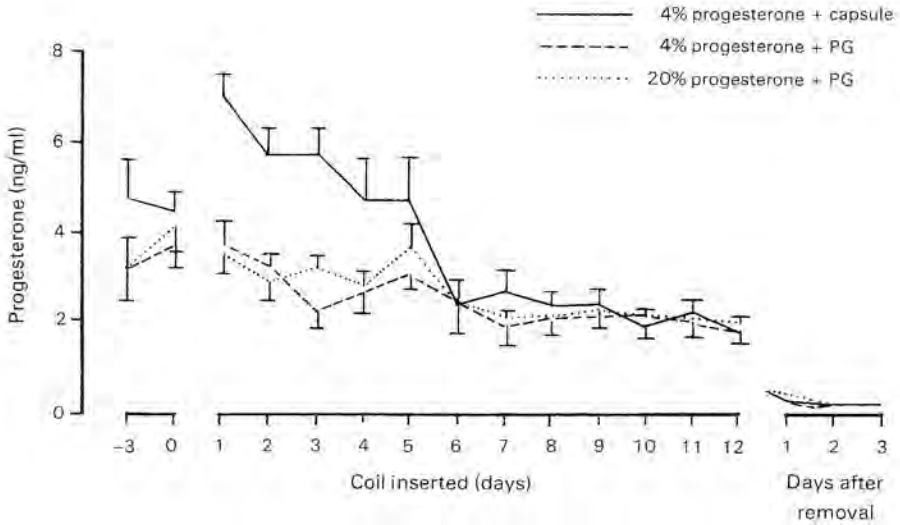
Text-fig. 2. Mean concentrations of progesterone ($\pm$ s.e.m.) before, during and after a 12-day treatment with progesterone. There were 8 heifers per treatment. Coils contained 4 or 20% progesterone and heifers in two treatments received an injection of PGF-2α at time of insertion of coils while the animals in the other group received 4% coils and a gelatin capsule containing 10 mg oestradiol benzoate.

Table 3. Time course of decline in progesterone and increase in LH concentrations (ng/ml) in heifers (10/treatment) after removal of progesterone coils following a 12-day treatment period

	4% Coil + capsule		4% Coil + PG		20% Coil + PG	
	Progesterone	LH	Progesterone	LH	Progesterone	LH
Days before coil removal						
1	2·2 ± 0·3	1·8 ± 0·3	1·9 ± 0·2	1·7 ± 0·3	2·0 ± 0·2	1·8 ± 0·2
0	1·7 ± 0·2	2·1 ± 0·3	1·8 ± 0·3	2·3 ± 0·5	1·9 ± 0·2	2·2 ± 0·4
Hours after coil removal						
4	0·7 ± 0·1	4·2 ± 0·8	0·7 ± 0·1	4·8 ± 1·3	0·8 ± 0·1	3·4 ± 0·5
8	0·7 ± 0·1	3·9 ± 0·8	0·7 ± 0·2	3·3 ± 0·8	0·6 ± 0·1	3·5 ± 0·8
12	0·5 ± 0·1	2·9 ± 0·8	0·5 ± 0·2	3·0 ± 0·8	0·5 ± 0·1	3·3 ± 0·8
16	0·6 ± 0·1	4·7 ± 1·0	0·6 ± 0·2	3·9 ± 0·8	0·5 ± 0·1	3·9 ± 0·7

Values are mean ± s.e.m.

luteolytic agent is not required in heifers to get a precise onset of oestrus. Fertility appears normal in most trials but this treatment regimen has not been as extensively tested as a 12-day treatment period. With a 12-day period, a luteolytic agent is required and an injection of 5 mg oestradiol benzoate or a gelatin capsule containing 10 mg oestradiol adhered to the coil is used. With a 7-day treatment period, an injection of prostaglandin is given at the end or 1 day before the end of treatment. This results in a precise onset of oestrus, particularly when prostaglandin is given 1 day before end of treatment, and fertility is normal (Roche, 1976d; Hansel & Beal, 1979). The 7-day treatment with a progesterone coil also has the advantage of maintaining high levels of progesterone, but the coil is not an effective method of maintaining luteal-phase levels of progesterone for more than 5–7 days. Preliminary evidence on the interaction between concentration of progesterone in blood and basal LH suggests that low progesterone levels may be associated with an increase in basal LH concentrations towards the end of treatment. A re-evaluation of presently available delivery systems for administering progesterone is required.

Induction of ovulation in beef cows

The factors responsible for initiation of follicular growth in cattle after parturition are not clearly understood. It is generally accepted that basal levels of gonadotrophins are required to maintain follicular growth. During the oestrous cycle of cattle at least 2 or 3 waves of follicular growth occur (Rajakoski, 1960; Ireland, Coulson & Murphree, 1979). This indicates that follicular growth may be continual despite high levels of plasma steroids. The factors that initiate growth and development of the follicle in the post-partum period are obscure. A study of 60 adult milked dairy cows has shown that the average size of the largest follicle increased from 9·6 mm at Day 7 *post partum* to 11·3 mm at Day 14 and 13·0 mm at Day 30. There were no differences in size of follicles between cows milked twice daily and suckling cows at Day 30 *post partum* (Wagner & Hansel, 1969). However, suckling delayed the appearance of follicles greater than 10 mm at 9–16 days after parturition (Wagner & Oxenreider, 1971).

More information is available on concentrations of hormones in blood during the post-partum period in beef cows. There is an increase in basal LH concentrations due to an increase in amplitude and frequency of episodic LH pulses beginning about 4 weeks before ovulation (Humphreys, 1977; Lamming, 1978). An LH peak occurs which may be responsible for a small transient rise in progesterone (Corah, Quealy, Dunn & Kaltenbach, 1974; Arije, Wiltbank & Hopwood, 1974; Lamming, 1978). This transient rise in progesterone lasts for 2–4 days and is generally followed by a normal ovulatory LH peak and a functional corpus luteum.

In spite of the lack of detailed factual information on initiation of growth and development of the follicle in the post-partum period, many attempts have been made to induce ovulation by hormonal therapy. The original impetus for this came from Casida, Meyer, McShan & Wisnicky (1943), who showed that the ovary of the post-partum cow was sensitive to exogenous gonadotrophins before first ovulation. Early attempts to induce ovulation in the post-partum cow using steroids gave rise to confusing results, with some authors (Foote, Hauser & Casida, 1960; Fosgate, Cameron & McLeod, 1962) reporting a delay in first ovulation following progesterone administration, while others (Ulberg & Lindley, 1960; Foote & Hunter, 1964; Saiduddin, Quevedo & Foote, 1968) reported that progestagen, particularly in combination with oestrogen, advanced the time of ovulation. Fertility was generally variable or low. Mulvehill & Sreenan (1977) reported that injection of 750 i.u. PMSG at the end of a 9-day progesterone treatment was an effective method of inducing a fertile ovulation in beef cows. This small dose of PMSG resulted in 15% of twin births with the greatest frequency in cows treated after 60 days *post partum*.

An alternative approach reported to result in an earlier post-partum ovulation is to use a single injection of GnRH in dairy cows (Schams, Höfer, Hoffmann, Ender & Karg, 1973; Britt, Kittock & Harrison, 1974) or two injections approximately 10 days apart in beef cows (Webb, Lamming, Haynes, Hafs & Manns, 1977). However, Manns & Richardson (1976) and Radford, Nancarrow & Mattner (1978) reported that neither GnRH nor oestradiol benzoate were effective in inducing ovulation in the post-partum period.

Use of GnRH or oestradiol to induce ovulation

Post-partum beef cows were injected with 100 µg GnRH or 400 µg oestradiol benzoate at Day 15 and again on Day 30 after calving and the number of animals with LH peaks (Mawhinney, Roche & Gosling, 1979) was determined by taking blood samples every hour for 30 h after injection. Daily blood samples for progesterone assay (Gosling, Parker & Fottrell, 1975) were also taken from 10 to 60 days after calving. Following treatment, 7/20 cows had an LH surge after oestradiol benzoate and 6/22 had an LH surge after GnRH compared to a 100% response obtained in follicular-phase cows. Both compounds failed to induce consistently an LH surge or ovulation. These results suggest that the hypothalamo–hypophysial axis was not capable of normal response to GnRH or oestradiol within the first 30 days of calving.

Use of progesterone coil to induce ovulation

Although effectiveness of progesterone treatment on induction of ovulation in the post-partum cow is not clear, there are sufficient data (Roche, 1976a; Mulvehill & Sreenan, 1977) to suggest that it has some beneficial effects. In a series of 4 experiments (S. Mawhinney & J. F. Roche, unpublished) we examined the effect of a 12-day progesterone treatment followed by injections of LH and FSH, GnRH or oestradiol benzoate on ovulation. Mainly first calving Hereford cross beef cows suckling calves were used. In all experiments, animals were grouped according to lactation number and post-partum interval and randomized within the various treatments. Ovarian activity before and after treatment was assessed by measurement of progesterone (Gosling *et al.*, 1975) in blood samples collected three times weekly. Animals with 3 consecutive values above 1·5 ng/ml were deemed to have ovulated. To determine whether LH peaks had occurred, jugular vein blood samples were obtained from a cannula every 4 h for 48 h beginning 24 h after removal of coils. LH was measured as previously described (Mawhinney *et al.*, 1979). Animal numbers in some experiments were small because some cows had reached normal reproductive activity before treatment and were excluded.

Experiment 1. Twenty-six anoestrous cows were randomly allocated to a control group or to a group receiving progesterone coils containing 6·75% progesterone for 12 days and had a 10 mg oestradiol benzoate capsule attached. The 12-day progesterone treatment did not affect the number of anoestrous cows ovulating (11/15) within a 4-week period after treatment when compared with the number of control cows ovulating (10/14).

Experiment 2. In this experiment 18 cows were allocated at random to 3 treatments: (i) control, (ii) progesterone coils and capsule as in Exp. 1, and (iii) as in (ii) plus 10 injections of 1 mg LH (NIH-LH-B9) and 1 mg FSH (NIH-FSH-B1) every 6 h beginning 60 h before removal of the coils. The number of cows that showed oestrus and that ovulated after the 3 treatments were 0/7, 1/6 and 4/5, and 1/7, 4/6 and 5/5 respectively. Large supplies of bovine LH and FSH were unavailable and so there were too few animals treated with LH and FSH for statistical analysis to be meaningful. However, the results suggest that silent ovulations were more frequent after the progesterone treatment than after progesterone–gonadotrophin treatment, and that the latter treatment resulted in more cows showing overt oestrus after removal of the coils than in the case of cows treated with coils alone.

Experiment 3. Cows ranging from 15 to 50 days *post partum* were allocated at random to the following treatments: (i) control, (ii) 6·75% progesterone coils, and (iii) 6·75% progesterone coils and an intramuscular injection of 400 µg oestradiol benzoate at time of removal of coils. Based on progesterone concentrations in plasma, all animals were acyclic at the start of the experiment. Following removal of coils, LH peaks were observed in 1/9 cows receiving the coils and in 3/9 cows receiving coils and oestradiol benzoate; 3/4 of the cows with LH peaks ovulated. Failure of the animals to have an ovulatory LH peak was the major cause for the low number of treated cows that ovulated after removal of the coils. This suggested inadequate follicular development during and after progesterone treatment.

Experiment 4. In this experiment, 14 anoestrous cows received progesterone coils for 12 days (6·75% progesterone) and were then allocated at random to receive (i) no further treatment, (ii) injection of 400 µg oestradiol benzoate at time of coil removal, and (iii) injection of 100µg LH-releasing hormone (GnRH) 36 h after removal of the coils. The number of cows that had LH peaks after treatment were 1/3, 3/5 and 6/6 for the 3 treatments, respectively. However, only 6/9 cows that had LH peaks in response to oestradiol benzoate or GnRH ovulated. Again, these results suggest that induction of an LH peak following progesterone is not always an effective method of increasing the number of animals ovulating.

The results of these experiments suggest that the progesterone coil on its own is only partly effective in inducing ovulation in the post-partum beef cow suckling calves (17/33 ovulated after coil removal in the 4 experiments). Failure of cows to ovulate after progesterone treatment can be attributed to a failure to have an LH peak or to ovulate in response to an induced LH peak.

Characteristics of the LH peak in cattle

The foregoing observations on failure of progesterone treatment to induce ovulation prompts the question as to what are the characteristics of the LH peak required to induce ovulation in cattle consistently. The characteristics of the events leading up to the LH peak and the LH peak itself can be observed (Text-fig. 3) for animals given 6·75% progesterone coils for 7 days from Days 17 or 18 of the cycle. During the 7-day treatment with progesterone, basal blood concentrations of LH doubled within 4 h and remained high up to the time of the LH peak. Following the peak, LH concentrations returned to base-line values, similar to those found before and during progesterone treatment. This 2-fold rise in LH in heifers is similar to that reported recently in the ewe (Hauger *et al.*, 1977). The importance of this rise in LH was not appreciated until concentrations of LH before and after the LH peak in the post-partum cow were examined in detail. In those cows in which an LH peak and ovulation occurred (indicated by a rise in progesterone), the concentrations of LH before the peak were significantly higher ($4·8 \pm 0·3$ ng/ml) than after the peak ($2·8 \pm 0·3$ ng/ml). Values were similar before ($2·6 \pm 0·1$ ng/ml) and after ($2·1 \pm 0·1$ ng/ml) the peak in cows that had no progesterone rise (Text-fig. 4). The absence of a rise in LH before the main peak appears to be associated with the failure to ovulate and this could indicate inadequate follicular stimulation. This rise in LH before the peak probably reflects increased amplitude and/or frequency of episodic LH secretion.
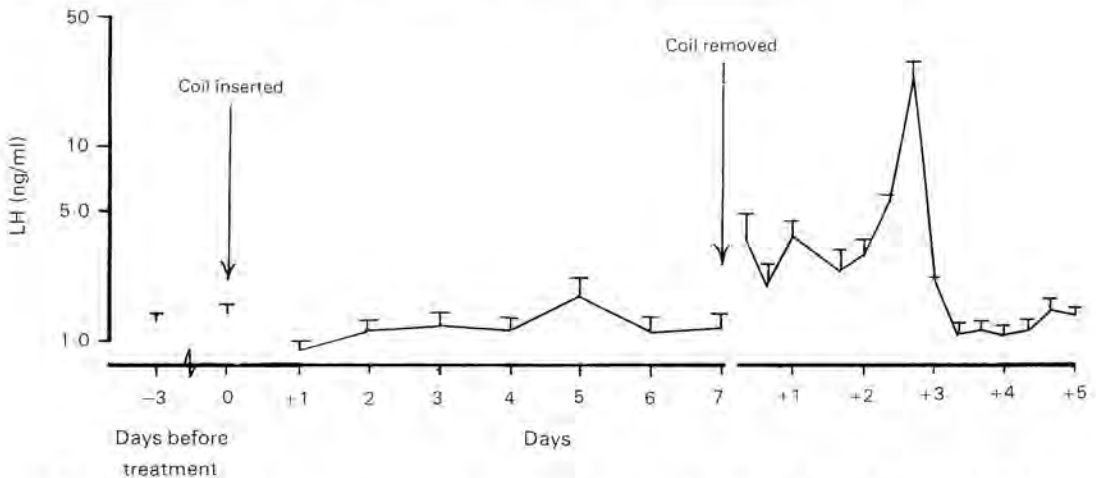
Text-fig. 3. Mean concentrations ($\pm$s.e.m.) of LH in serum of heifers before, during and after removal of progesterone coils. The 10 heifers were treated for 7 days from Days 17 and 18 after oestrus with coils containing 6·75% progesterone and injected with PGF-2α 1 day before removal of the coils.

Control of LH secretion in the post-partum cows

The absence of the early rise in LH described leads to the question of the nature of the control mechanisms governing LH secretion in the post-partum period. Ovariectomy of cyclic heifers results in an increase in LH (Hobson & Hansel, 1972; Beck, Smith, Seguin & Convey, 1976), suggesting that ovarian steroids have a negative feedback effect on LH. Replacement therapy showed that both progesterone and oestradiol were required to maintain LH at concentrations similar to those found in intact cyclic heifers (Beck *et al.*, 1976). Several workers have reported that the pituitary of the post-partum beef cow is refractory to GnRH. It is not clear whether the low levels of LH in the post-partum period are due to negative feedback from the ovary or to refractoriness of the hypothalamo–hypophysial system. To test the hypothesis that
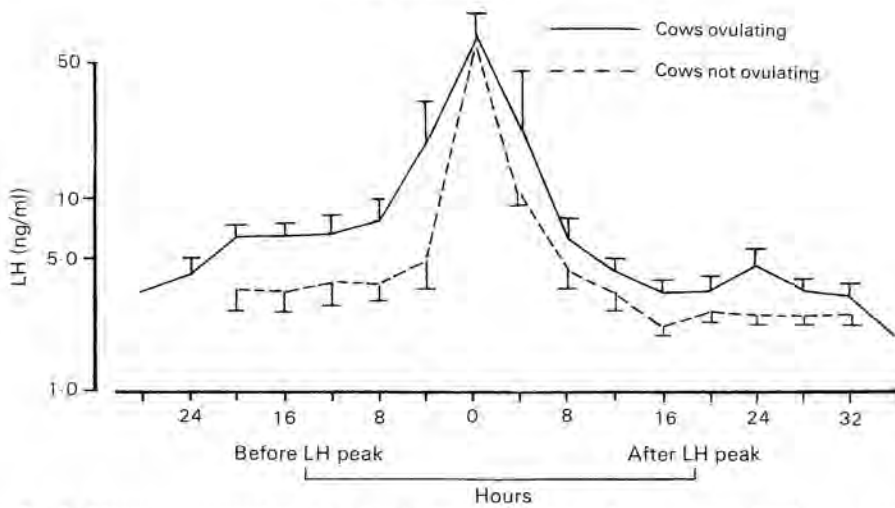
Text-fig. 4. Mean concentrations ($\pm$ s.e.m.) of LH in plasma of post-partum beef cows that did or did not ovulate following removal of progesterone coils and injection with 100 µg GnRH or 400 µg oestradiol benzoate. There were 6 animals in each group.

secretions from the ovary were having a negative feedback effect on LH release, post-partum cows were ovariectomized at Days 25, 45 and 65 *post partum* (S. Mawhinney & J. F. Roche, unpublished). The linear regression of LH values against time was determined for each animal and the mean slopes and constants of the regression lines were compared between groups of animals ovariectomized at different times (Table 4). In contrast to the abrupt increase in concentrations of LH in ovariectomized heifers (Hobson & Hansel, 1972; Beck *et al.*, 1976), there was only a gradual increase in plasma LH concentrations for cows at the 3 different post-partum intervals. The mean constants and slopes were similar, showing no differences in LH secretion following ovariectomy at Days 25, 45 and 65 *post partum*. These results suggest that the ovary, through negative feedback effects, is not responsible for maintaining the low levels of LH in post-partum cows shortly after calving.

Table 4. The mean constants and slopes of the regression lines of LH against post-partum interval in beef cows ovariectomized at different post-partum intervals

Post-partum interval at ovariectomy	No. of animals	Constant	Slope
Group 1			
Day 25	9	2·1	0·100
Day 45	9	2·1	0·060
Day 65	9	1·9	0·060
s.e.m.		0·42	0·024
Group 2			
Intact	2	1·65	−0·006
Day 5	3	0·98	0·019
s.e.m.		0·29	0·018

Conclusions

The gradual but small increase in LH secretion after ovariectomy of the post-partum beef cow, the variable period of refractoriness of the hypothalamo–hypophysial axis to release LH in response to GnRH or oestradiol (Webb *et al.*, 1977; Radford *et al.*, 1978; Mawhinney *et al.*,

1979), and the decreased LH release *in vitro* from pituitary extracts of suckling cows (Carruthers, Convey & Hafs, 1980), all suggest that the endocrine cause of anoestrus in the beef cow lies with the hypothalamo–hypophysial axis. Suckling does not affect basal FSH, prolactin, oestradiol or glucocorticoid concentrations (Humphreys, 1977; Carruthers *et al.*, 1980). Pituitary concentrations of LH and FSH and hypothalamic concentrations of GnRH are not altered by suckling (Carruthers *et al.*, 1980), although the frequency and amplitude of episodic LH release were reduced. In addition, pretreatment with progesterone and subsequent injection of GnRH resulted in all cows having LH peaks but not all cows ovulated. In those cows that had an LH peak without ovulation, the rise in LH before the main peak was not observed. This absence could relate to a decreased frequency and amplitude of episodic LH release, indicating that GnRH synthesis and release may be inadequate during the anovulatory period. The key to understanding the endocrine cause of anovulation in the beef cow may well depend on a greater understanding of the intricacies of the brain, the physiology of GnRH synthesis and release from the hypothalamus, and the factors that modulate the effects of GnRH on the pituitary gland.

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