Effect of a progesterone-releasing intravaginal device on the milk progesterone levels, vaginal flora, milk yield and fertility of cyclic and non-cyclic dairy cows

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Summary. A progesterone-releasing intravaginal device (PRID) was inserted for 14 days into 10 lactating dairy cows at least 50 days after calving. Synchronization of oestrus was good and 5 of the cows conceived to a double insemination 48 and 72 h after PRID removal. In 9 cows vaginal infection was present when the PRID was removed but resolved spontaneously. The level of progesterone in the milk and the milk yield were similar to those in the 10 control animals. PRID treatment of 9 cows whose ovaries had remained inactive for at least 50 days after calving was followed by ovulation, as judged by the milk progesterone profile, in 8 animals after removal of the device.

Introduction

Christian & Casida (1948) showed that daily injections of progesterone suppressed oestrus and ovulation in heifers. Numerous investigators thereafter (e.g. Ulberg & Lindley, 1960; Wiltbank, Zimmerman, Ingalls & Rowden, 1965; Britt & Ulberg, 1972; Roche, 1974) found that fertility at the synchronized oestrus was depressed. However, progesterone treatment for shorter periods, together with oestradiol administration at the start of treatment, have resulted in fertility in cattle equivalent to that of controls (Wishart & Young, 1974; Sreenan, 1975; Roche, 1976a).

Progestagen treatment, often with oestrogen, can also induce oestrus and ovulation in cattle after calving (Foote & Hunter, 1964; Brown, Peterson & Foote, 1972; Britt, Morrow, Kittok & Seguin, 1974; Lavoie & Moody, 1974). Silastic implants have proved to be an easier method of application of progesterone than repeated injections (Wishart & Young, 1974) and intravaginal progesterone-releasing devices are even more convenient for maintaining blood progesterone levels without the need for repeated injections or surgical interference (Roche, 1976b). The present study was to provide information on the effect of such intravaginal devices on the progesterone levels in cyclic and anoestrous cattle, and the influence this method of application has on infection of the reproductive tract and milk yields of treated animals.

Materials and Methods

Lactating Friesian cows from 4 commercial dairy farms were used. Milk samples from the collecting jars were placed in 30 ml plastic pots, each containing a preservative tablet (Lactab, Mark II: Thompson and Capper Ltd, Liverpool), and stored at 4°C. Progesterone was measured by radioimmunoassay in unextracted aliquots of whole milk as described by Bulman & Lamming (1976) and Lamming & Bulman (1976). The antiserum was BF 465 No. 6 (Furr, 1973). The intra-assay coefficient of

variation between duplicate pairs was 8.9% and the inter-assay coefficient of variation was 10.3%. The limit of sensitivity, defined as twice the s.d. of the blank values, was 0.38 ng/ml (10 assays). When the progesterone concentration in the samples was indistinguishable from that in the blanks it has been given as <0.4 ng/ml.

The progesterone-releasing intravaginal device (PRID, Abbott Laboratories Ltd) is a spiral, conditioned-steel strip coated with a silicone rubber matrix containing 2.25 g micronized progesterone. The device is approximately 31 cm long, 3.45 cm wide and has a diameter of 4.6 cm with a total weight of about 47 g. It was inserted by means of a Perspex speculum thoroughly cleaned in a solution of 1.5% (w/v) chlorhexidine glucerate plus 1.5% cetrimide (Savlon: ICI Ltd) immediately before use. No oestradiol benzoate was provided at the start of treatment.

The contents of the anterior vagina were examined bacteriologically. The swabs (Sovereign Sterile Culture Swab: Sherwood Medical Industries, St Louis, Missouri, U.S.A.), collected by a 'guarded sterile' method, were inoculated onto 5% or 10% sheep blood agar (SBA), McConkey agar, malt agar, serum-dextrose agar (SDA), Farrell's agar (Farrell & Robertson, 1972), brilliant green agar and Edward's aesculin medium. All plates were incubated in air at 37°C except for one 5% SBA plate which was incubated under strict anaerobic conditions and the 10% SBA, Farrell's and SDA plates which were incubated in an atmosphere of 10% CO₂. The plates were examined after 24, 48 and 96 h and 7 days.

Experiment 1

This involved 20 cows from one farm which had all calved at least 50 days previously and which were experiencing normal oestrous cycles, as judged by milk progesterone analysis. After vaginal swabbing a PRID was inserted into 10 of the animals for 2 weeks. The speculum used was briefly inserted into the vagina of 5 of the 10 control cows. A vaginal swab was collected from all animals at the time of PRID removal and 7 and 14 days later. The treated cows were inseminated 48 and 72 h after PRID removal. Control cows were inseminated artificially or naturally at the next observed oestrus. Milk samples for progesterone assay were collected from each cow at the times shown in Table 1, and twice weekly until pregnancy was diagnosed by a high progesterone concentration 21–24 days after service. Milk yields of each animal were recorded weekly.

Experiment 2

Milk samples were collected twice weekly from all cows on 4 farms from parturition until a positive diagnosis of pregnancy was made from the milk progesterone profile. There was no rise in milk progesterone in 6% of the cows by 50 days *post partum* and 8 of these animals were fitted with a PRID for 14 days. Nine similar cows were left untreated as controls. Cows in both groups were artificially inseminated when oestrus was observed.

Results

Experiment 1

The milk progesterone levels in the control and PRID-bearing cows are shown in Table 1. During the 14-day PRID treatment, progesterone concentrations in milk were similar to those characteristic of the luteal phase of the oestrous cycle except for 3 isolated samples in which the concentrations were <2 ng/ml. Concentrations fell rapidly after removal of the PRID and were at characteristic oestrous values by 24 h (9 cows) and 72 h (10 cows). Seven animals were in oestrus within 3 days of PRID removal.

The total milk progesterone output from Days 1 to 21 was estimated for each cow by calculating the area under the curve of a graph of milk progesterone concentration against time. There was no significant difference ($P > 0.05$, Student's *t* test) in the mean ($\pm$ s.e.m.) unit area (9.9 ± 1.26 for the 10 treated cows, 12.9 ± 0.97 for 10 controls) or in the maximum level recorded for each cow (23.1 ± 2.66 ng/ml for treated cows, 23.8 ± 2.52 ng/ml for control cows).

Table 1. The effect of a PRID inserted between Days 0 and 14 on progesterone levels (ng/ml) in the milk of cyclic cows inseminated on Days 16 and 17

Group	Days relative to PRID insertion																	29
	-10	-5	1	3	5	7	9	11	13	15	17	19	21	23	25	27		
Treated cows																		
621*	1.5	3.7	20.7	22.6	18.7	21.7	30.2	24.5	9.9	<0.4	0.5	1.1	6.9	16.5	14.5	15.0	29.5	
629*	1.3	0.5	22.7	27.4	28.2	30.1	30.6	7.7	11.7	<0.4	0.9	3.4	14.8	24.0	38.2	15.0	24.0	
636*	23.5	35.2	14.7	11.5	8.5	13.2	12.7	27.5	9.2	<0.4	<0.4	<0.4	1.4	7.0	22.2	26.0	39.0	
640*	24.7	3.0	5.7	10.7	4.6	5.7	8.0	9.7	8.5	<0.4	<0.4	<0.4	<0.4	<0.4	6.0	14.2	37.7	
653*	20.2	27.3	18.6	8.7	8.1	7.4	9.0	14.7	9.5	0.5	0.7	1.0	2.7	9.5	17.5	7.5	8.2	
654	27.7	17.6	5.0	13.5	14.5	28.0	20.5	11.5	19.5	13.2	0.6	<0.4	1.0	5.5	20.5	10.3	15.0	
659	3.5	16.3	22.3	26.3	23.8	18.0	14.1	12.3	12.0	<0.4	<0.4	<0.4	0.5	8.0	19.7	14.5	5.5	
622	3.3	15.1	22.6	21.2	9.1	10.6	1.6	9.9	7.6	0.5	0.7	2.4	15.2	26.0	26.7	14.0	0.5	
552	18.1	24.5	7.2	6.5	4.3	19.9	1.7	11.5	7.0	<0.4	<0.4	0.7	1.5	12.7	26.5	18.0	3.7	
652	17.2	17.7	18.8	21.2	34.2	22.2	18.5	1.5	16.5	0.7	<0.4	<0.4	1.0	0.4	16.5	14.7	16.0	
Control cows																		
231	21.8	16.7	0.5	3.0	4.2	17.7	21.2	22.7	18.0	6.0	17.0	0.7	<0.4†	<0.4	0.7	12.7	11.5	
328	25.5	30.9	6.0	12.3	16.2	28.0	13.5	27.5	36.3	1.5	<0.4†	<0.4	2.7	7.5	29.2	19.0	16.5	
464	1.1	0.5	16.0	14.9	13.5	29.0	32.7	8.0	3.7	<0.4†	1.0	4.5	12.8	20.2	19.2	19.0	25.5	
474	26.2	1.8	1.7	7.5	17.0	22.0	28.7	10.0	14.0	28.1	1.0	<0.4†	0.5	7.2	21.0	24.0	17.5	
542	18.0	24.5	1.3	0.7	3.7	18.2	17.5	15.0	12.2	21.7	28.5	22.0	12.0	5.5	1.5†	1.5	3.5	
548	14.0	26.5	2.2	0.6	<0.4	7.7	15.3	24.0	2.2	6.2	32.5	31.7	29.0	40.2	3.5	0.7†	6.5	
633	26.0	<0.4	<0.4	1.2	2.0	7.0	12.0	8.7	8.5	7.7	6.0	0.7†	0.5	8.7	27.7	17.0	10.0	
634	17.7	27.5	27.0	9.1	1.5	<0.4	<0.4	11.0	9.0	22.5	27.5	29.0	29.0	17.0	2.7†	<0.4	5.5	
645	26.0	30.3	29.2	17.5	1.4	<0.4	5.0	5.6	12.7	15.0	13.0	16.0	8.0	17.0	10.7	<0.4†	1.5	
650	2.5	4.2	9.7	20.0	19.5	23.2	31.7	15.5	5.5	<0.4†	2.0	17.7	29.0	27.7	22.7	26.7	17.5	

* These cows conceived to the fixed-time inseminations.

† Day of AI for control cows. No animals were served before Day 14.

Insemination 48 and 72 h after removal of the PRID resulted in conception in 5 of the treated cows. All 10 control cows exhibited oestrus between Days 1 and 21: 9 conceived to the first service (8 natural, 1 artificial). The first-service conception rate for the rest of the herd during this period was 9/14 (64%) for natural service and 15/21 (76%) for artificial insemination.

The results of the bacteriological examinations are shown in Table 2. Heavy growths of non-pathogenic organisms were obtained from 9 of the treated cows when the PRIDs were removed on Day 14, contrasting markedly with the results from the control group. Few organisms were seen in the swabs taken on Days 21 and 28. Swabs taken from inside the barrel and on the speculum plunger after the disinfection procedure contained small numbers of anthracoids. Large numbers of anthracoids were present on the outside of the speculum barrel.

Table 2. Results of bacteriological examination of the anterior vagina of cows before and after a 14-day PRID treatment (Days 0–14)

Group	Cow No.	Time of collection of vaginal swab				Fertile to first service
		Day 1	Day 14	Day 21	Day 28	
PRID treatment	621	Sterile	Sterile	Sterile	Sterile	Yes
	622	Sterile	<i>Corynebacterium bovis</i> ++ <i>Proteus</i> sp. ++ <i>E. coli</i> ++	Sterile	Sterile	No
	629	Sterile	<i>Enterococci</i> + <i>Enterococci</i> + <i>E. coli</i> +	Sterile	Sterile	Yes
	636	<i>Staphylococci</i> + <i>Mycoplasma bovis genitalium</i> +	<i>Enterococci</i> + <i>Proteus</i> sp. +	<i>E. coli</i> +	Sterile	Yes
	640	<i>Enterococci</i> +	<i>Proteus</i> sp. +++ <i>E. coli</i> +++	Sterile	Sterile	Yes
	652	Sterile	<i>Proteus</i> sp. +++	Sterile	Sterile	No
	653	Sterile	Anthracoids + <i>C. bovis</i> +++	Sterile	Sterile	Yes
	654	Sterile	<i>C. bovis</i> +++	Sterile	Sterile	No
	659	Sterile	<i>C. bovis</i> ++	Sterile	Sterile	No
	552	Sterile	<i>C. bovis</i> ++	Sterile	Sterile	No
Control (no treatment)	231	Anthracoids +	Sterile	Sterile	<i>E. coli</i> + <i>Enterococci</i> +	Yes
	542	Sterile	Sterile	Sterile	Sterile	Yes
	548	Sterile	Sterile	Sterile	Sterile	Yes
	645	Sterile	Sterile	Sterile	Sterile	Yes
	650	Sterile	<i>C. bovis</i> +	Sterile	Sterile	No
Control (speculum only inserted)	328	Sterile	Sterile	Sterile	Sterile	Yes
	464	Sterile	Unidentified enterobactericids +	Anthracoids +	Sterile	Yes
	474	Sterile	Sterile	Sterile	Sterile	Yes
	633	Sterile	<i>Enterococci</i> +	Sterile	Sterile	Yes
	634	Sterile	Sterile	Sterile	Sterile	Yes

+, ++, +++ indicates degree of infection.

Analysis of variance of the weekly milk yields from 9 weeks before until 12 weeks after insertion of the PRID revealed no significant differences between the treated and control animals, nor a significant 'group × time' interaction.

Experiment 2

The effect of a PRID on the progesterone output of 8 non-cyclic cows varied (Table 3). Of the 7 which had low progesterone levels after removal of the PRID, 4 (Nos 3, 5, 7 and 8) came into oestrus and were inseminated within 3 days. None conceived at that time but 3 of the 4 conceived at the second service. Cow 4 conceived to an insemination 11 days after PRID removal. Cow 6 was the only animal which did not have an oestrous cycle after PRID removal.

Table 3. The effect of a PRID, inserted between Days 1 and 14, on progesterone levels (ng/ml) in the milk of non-cyclic cows*

Cow no.	Days relative to PRID insertion															
	-17	-14	-11	-8	-5	-2	2	5	8	11	14	17	20	23	26	29
1	0.7	3.7	1.2	1.0	1.9	3.5	11.7	17.7	10.0	5.4	3.0	1.7	0.7	<0.4	17.1	—
2	4.0	1.2	5.0	<0.4	2.4	2.2	17.8	16.5	12.2	19.0	<0.4	<0.4	<0.4	1.0	9.0	10.5
3	3.1	2.1	2.2	1.2	0.5	0.7	—	2.6	3.2	—	1.5	<0.4†	4.7	6.5	17.5	13.2
4	<0.4	0.6	0.5	—	<0.4	<0.4	0.4	7.6	26.0	28.0	—	34.5	14.5	0.6†	<0.4	4.7
5	<0.4	<0.4	<0.4	0.5	<0.4	—	4.5	5.2	3.0	1.2	—	0.7†	<0.4	0.7	5.4	—
6	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	5.5	1.4	2.1	—	5.2	0.7	1.0	1.5	0.6	0.7
7	1.5	1.0	0.6	0.5	0.7	0.5	—	5.6	3.1	—	2.7	<0.4†	2.1	11.2	9.7	25.6
8	<0.4	<0.4	—	0.8	1.0	0.5	4.1	—	2.4	2.5	0.7	<0.4†	7.8	11.1	—	14.2

* Milk samples were generally collected twice weekly regardless of treatment. The progesterone value given is that from the day of collection nearest to the day shown above. The blanks occur when no samples were collected.

† Day of AI.

Table 4. The fertility of non-cyclic cows treated with a PRID for 14 days

Cow no.	Days with low progesterone before treatment†	Days from PRID removal to AI	Services/conception	Days from start of treatment to conception	Days from calving to conception
Treated cows					
1	55	31	2	55	98
2	81	25	1	38	119
3	87	2	2	34	121
4	39	11	1	24	63
5	50	3	2	38	90
6	46	47	1	61	106
7	60	2	>6	—	—
8	50	2	2	35	85
Mean $\pm$ s.e.m. (N)	58.5 (8)	15.4 (8)	1.6 $\pm$ 0.20 (7)	40.7 $\pm$ 4.85 (7)	97.4 $\pm$ 7.70 (7)
Control cows*					
9	56	15	3	64	120
10	127	-1	3	36	163
11	57	17	2	63	120
12	63	23	1	23	86
13	53	24	4	81	134
14	51	19	2	39	90
15	53	16	1	16	69
16	109	20	>1	—	—
17	61	16	2	40	101
Mean $\pm$ s.e.m. (N)	70.0 (9)	17.3 (9)	2.3 $\pm$ 0.36 (8)	45.3 $\pm$ 7.84 (8)	110.4 $\pm$ 10.63 (8)

* In these cows the start of progesterone secretion was equated with the insertion of the PRID in the experimental animals.

The fertility of the treated animals was compared with that of the 9 untreated cows which had also shown a delay in the resumption of ovarian activity after parturition (Table 4). A progesterone concentration >3 ng/ml followed by normal ovarian activity in the control cows was equated with the time of PRID insertion in the treated cows. Cows 7 and 16 remained barren, probably because of their age, and were excluded from the calculations. The treated cows required fewer services per conception and had a shorter period between calving and conception (see Table 4), but these differences were not significant.

Discussion

The PRIDs used were designed to release about 70 mg progesterone/day and the presence of a PRID in the cyclic cows resulted in plasma progesterone concentrations high enough to suppress oestrus and ovulation. The varied milk progesterone pattern in the non-cyclic cows could have been due to variations in the rate of progesterone metabolism or to the surface area of the PRID in contact with the vaginal wall.

A satisfactory degree of synchronization was found for the induced oestrus in cyclic and non-cyclic cows and a conception rate of 50% was obtained in Exp. 1 when inseminations were at 48 and 72 h. These timings were chosen as being a more convenient commercial possibility, although Roche (1975) and Ellicot & Thompson (1976) showed that normal fertility could be achieved with a single insemination 56 h after PRID removal. None of the 4 cows in Exp. 2 conceived to insemination when in oestrus immediately after PRID removal, but conceptions were satisfactory (5/8) at the second ovulation.

A small rise of plasma or milk progesterone frequently precedes the first ovulation after calving in milked and suckled cows (Lamming & Bulman, 1976; Webb, Lamming, Haynes, Hafs & Manns, 1977). Progestagen treatment during the post-partum period can induce earlier ovulation (Foote & Hunter, 1964; Britt *et al.*, 1974), and Roche (1976c) found higher conception rates in suckling cattle which were synchronized with progesterone than with prostaglandin. In the present study 8/9 acyclic cows ovulated after PRID removal, perhaps indicating a priming effect of progesterone on the hypothalamic-pituitary-ovarian axis but this view needs further investigation.

Hale & Symington (1969) found that progestagen-impregnated vaginal tampons were coated with foul-smelling mucus when withdrawn, and we have also noticed (unpublished) that a vaginal reaction could occur following PRID insertion. The numerous organisms recovered in the present experiment are among those characteristically present on the skin and in the faeces of cattle. They were presumably introduced into the vagina when the PRID was inserted; the cavity formed by the PRID could result in the accumulation of secretions in which the organisms can multiply. A minor degree of contamination, e.g. in the control animals, could have occurred during collection of the swabs. The use of a disposable speculum for the PRID insertions would eliminate the risk of transfer of specific pathogens between cows. Metritis is unlikely to result from insemination shortly after treatment with a PRID because the cervical canal is closed except at oestrus and the vaginal infections in the treated cattle disappeared quickly. Rowson, Lamming & Fry (1953) have shown that during oestrus, when the reproductive tract is under the influence of oestrogens, the bovine uterus is highly resistant to ascending non-specific infection. In a similar experiment Ahern & Jennings (1976) found that the treatment of ewes with progestagen-impregnated sponges increased the bacterial content of the vagina but did not apparently reduce fertility.

During the period of treatment with the PRID, progesterone concentrations in the milk remained at normal levels and therefore the risk of exogenous progesterone affecting human consumers seems remote.

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