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Plasma Levels of Prostaglandin $F_{2\alpha}$ Metabolite and Progesterone in Repeat Breeder Heifers

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Albihn, A., M. Shamsuddin, H. Qunshan and H. Kindahl: Plasma levels of prostaglandin $F_{2\alpha}$ metabolite and progesterone in repeat breeder heifers. Acta vet. scand. 1991, 32, 361–371. – A detailed clinical-endocrine investigation was performed in 6 repear breeder heifers (RBH) with the aim being to ascertain whether endocrine asynchronism exists at luteal regression and during early pregnancy. The heifers were first studied during an open cycle and then after insemination when 3 heifers became pregnant. Circulating plasma levels of $PGF_{2\alpha}$ metabolite were measured every 2nd h, while progesterone (P_4) levels were measured every 6th h. The oestrous period and intervals between the onset of oestrus and ovulation were relatively longer, compared with what is normally seen in heifers. Plasma levels of P_4 at the onset of oestrus were higher than normal, but it was concluded that the plasma levels of $PGF_{2\alpha}$ metabolite and P_4 in RBH at luteal regression and early pregnancy were normal.

oestrous cycle; early pregnancy; endocrine asynchronism.

Introduction

Repeat breeding is one of the most important causes of reproductive failure in cattle. Approximately 10 per cent of the cows and heifers in Sweden have to be culled every year due to reproductive failures (*Anon.* 1987–88).

Repeat breeding can result from fertilization failure or embryonic mortality. Most of the embryonic losses take place between 8 and 18 days after service (*Roche* 1981, *Sreenan & Diskin* 1985). Embryos collected during days 7–17 from the repeat breeding heifers (RBH) often show retarded development and signs of degeneration (*Gustafsson & Plöen* 1985, *Albihn et al.* 1991a).

Early embryonic death may be the result of a spontaneously occurring asynchrony between the embryonic and maternal environments (*Maurer & Echternkamp* 1982, *Wilmuth et al.* 1985, *Pope* 1988, *Albihn et al.* 1991b). Retarded growth and degeneration of the bovine embryo have been experimentally induced by transferring day 7 embryos to day 4 recipients (*Albihn et al.* 1991b).

Previous studies have indicated that a hormonal imbalance exists in the repeat breeding animals, which has been termed "endocrine asynchrony". *Gustafsson et al.* (1986) found significant differences between RBH and virgin heifers (VH) in concentrations of LH, progesterone (P_4) and $PGF_{2\alpha}$ metabolite during oestrus and 7 days thereafter. The interval from the onset of oestrus to the preovulatory LH peak was longer, and its total release was lower in RBH as compared with VH. The mean duration of oestrus was significantly longer in the RBH group. Also, the P_4 level was higher during oestrus and lower during metoestrus, with progress being less accentuated after oestrus in the RBH than in the VH. The number of $PGF_{2\alpha}$ metabolite

peaks found during oestrus was higher in the RBH than in the VH.

The role of $PGF_{2\alpha}$ as a luteolytic agent in the cyclic sheep and cow has long been established (*McCracken et al.* 1972, *Kindahl et al.* 1976a,b). Recognition of a conceptus by the maternal endocrine system is essential to the establishment and maintenance of pregnancy. In the absence of a sufficiently developed embryo, endometrial $PGF_{2\alpha}$ secretion induces luteal regression (*Goding* 1974, *Peterson et al.* 1975, *Thatcher et al.* 1984, *Heap et al.* 1988).

Cows showing high serum (*Henrichs et al.* 1971, *Maurer & Echternkamp* 1982, 1985) and milk (*Lee & Ax* 1984, *Lamming et al.* 1989) P_4 levels shortly after corpus luteum formation tended to have higher pregnancy rates than cows with lower P_4 levels. Supplementation of P_4 after AI in repeat breeders or in connection with embryo transfer in asynchronous recipients has had variable success in increasing pregnancy rates (*Johnsson et al.* 1958, *Garrett et al.* 1988, *Fox et al.* 1988). For a review of the effects of P_4 on bovine embryo survival, see *Diskin & Sreenan (*1985).

In this study the peripheral plasma concentrations of $PGF_{2\alpha}$ metabolite and progesterone were measured during the late luteal and follicular phases of the oestrous cycle as well as during the early pregnancy period in the RBH.

Materials and methods

Animals

The RBH in this study is defined as a clinically and gynaecological healthy animal that failed to conceive after being bred 3 or more times to a fertile bull or artificially inseminated with fertile semen from a bull with proven fertility. This definition of the RBH allies to a generally accepted nomenclature. In addition, the RBH generally shows regular insemination intervals (18–24 days). Six repeat breeding Swedish Red and White heifers between 19 and 21 months of age with a history of several unsuccessful services were selected for this study (see Table 1). The heifers were bought from a number of dairy farms, all members of the local AI associations. The heifers were housed indoors and fed according to Swedish standards. They were checked by rectal palpation and by real-time ultrasonography, using a 5 MHZ rectal transducer (Aloka SSD-210, Aloka Co. Ltd., Japan) for breeding soundness. The animals were also examined for bovine virus diarrhoea (BVDV) (*Carlson et al.* 1989) and found to be uninfected. No detectable serum antibodies were found using an indirect enzyme linked immunosorbent assay (ELISA) (*Juntti et al.* 1987). The trials were started not earlier than 2 months after arrival of the animals at the clinic. The heifers were first studied during luteolysis in an open cycle (experiment I). They were then inseminated and sampled again during the corresponding period (experiment II). After the experiments, the heifers were slaughtered. Their genital organs were then removed within 10 min and subjected to macroscopical post-mortem examination. The uterine horns of the animals not showing oestrus at the end of the inseminated cycle were carefully flushed (*Albihn et al.* 1991a) using Dulbecos phosphate buffered saline supplemented with 2 % heat-treated foetal calf serum. The recovered embryos were measured and evaluated under a stereomicroscope (Wild M8, Switzerland) at a magnification of 20–40 x.

Oestrous synchronization

Oestrous synchronization was performed by intramuscular injection of 2 doses of 500 µg cloprostenol (Estrumate®, Cooper Animal Health, UK) 10 days apart. One spontane-

ous oestrus was allowed before the blood sampling was started. After completion of exp. I, the heifers were synchronized again with a single cloprostenol injection. Inseminations were made during the 1st spontaneous oestrus.

Oestrous detection and blood sampling

The heifers were checked for oestrus twice daily, by clinical examination and by teasing with a bull. The 1st day of oestrus was considered to be day 0 of the oestrous cycle. Oestrus, ovulation and CL development were confirmed by combining rectal palpation, ultrasonography and a P_4 assay of peripheral plasma, collected once daily from 18th day after 2nd induced oestrus until the beginning of intensive blood sampling. In both experiments, intensive blood collection was started after the 1st spontaneous oestrus. Blood was collected every 2nd h by jugular venopuncture in evacuated tubes containing heparin, (Vacutainer® System, Becton-Dickinson, Rutherford, USA). The content of 15-keto-13,14-dihydro-$PGF_{2\alpha}$ was determined in all samples while a P_4 assays was conducted on 4 samples a day (*Kindahl et al.* 1976b, *Granström & Kindahl* 1982). This collection schedule was followed until standing oestrus or, in exp. II, until pregnancy was confirmed, i.e. to heartbeats of the embryo were visible using ultrasonography. The plasma was immediately separated by centrifugation (3000 rpm) and stored at –20°C prior to the radioimmunoassay. In exp. II, the heifers were inseminated during their 1st spontaneous heat with frozen semen from either of 2 bulls known to be highly fertile. The blood samples were collected as described above.

Data analysis

The number of $PGF_{2\alpha}$ pulses, time of their occurrence, intervals between pulses, amount of $PGF_{2\alpha}$ metabolite released during a single pulse and total production of $PGF_{2\alpha}$ metabolite were calculated during luteal regression and early pregnancy. The patterns of P_4 release during the late luteal and follicular phases as well as during early pregnancy are shown graphically.

Peaks in the $PGF_{2\alpha}$ metabolite were identified following the method described by *Zarco et al.* (1984). Briefly, the mean and standard deviation (SD) of all samples taken during both experiments were calculated for each heifer, and values greater than 3 SDs above the mean were considered to represent a significant elevation i.e. "a peak". The values corresponding to points identified as peaks were then eliminated, and the calculation was repeated until no new peaks were detected (usually 2 to 6 times). All values of $PGF_{2\alpha}$ metabolite identified as peaks by this sequential method were considered to represent a significant synthesis and release of $PGF_{2\alpha}$. Areas (mm^2) under the peaks were determined by a Digiplan electronic integrator (Kontron Messgeräte GmbH, Germany).

Results

The clinical findings of the experimental animals are summarized in Table 1. The interoestrous interval observed at the clinic varied from 18 to 23 days.

Clinical examination of heifer D did not reveal any pathological findings other than an udder that was enlarged during several oestrous periods and occasionally produced a milk like secretion. During the luteal phase she also had an unusually large udder. Otherwise, her oestrous cycle appeared normal.

Heifer E was not included in exp. II because of unsuccessful synchronization of oestrous cycle with other animals. In this heifer at post-mortem examination 6 small (10–13

Table 1. Clinical findings in 6 experimental repeat breeder heifers.

Parameters	Heifer					
	A	B	C	D	E	F
Age (month)	19	19	20	20	21	19
No. of services at farm	5	5	3	4	10	3+2[b)]
Duration of oestrus (h)[a)]	24	56	36	24	–	36
Oestrus-ovulation interval (h)[a)]	36	56	48	24	–	–
No. of AI[a)]	1	3	2	1	0	2
Pregnant[a)]	–	+	+	+	–	–

a) in exp. II
b) with a bull

mm) CL (day 10) were found in 1 ovary. No other abnormalities were detected.

After arrival in the clinic, gynaecological examination of heifer F revealed that she had a cystic CL on her right ovary and suspected adhesions on both ovaries. During the follicular phase this heifer continued to show very low P_4 values for 5–6 days, although the level of this hormone slowly rose. Prostaglandin $F_{2\alpha}$ metabolite levels did not differ from those measured in the other heifers. During 7 observed cycles at the clinic she developed thick-walled cystic structures that became luteolysed within ordinary cyclus interval. During exp. II, this heifer was inseminated twice during a 36 oestrous period; ultrasonography revealed that she did not ovulate; instead she developed a cystlike structure again, as described above. Heifer F was excluded from the endocrinological results after confirming a massive ovaro-bursal adhesion at post mortem examination, which prevented the heifer from fulfilling the criteria set for repeat breeders in this study.

No other pathological changes were detected in the genital tract of the heifers at post-mortem examination.

Plasma levels of P_4 and $PGF_{2\alpha}$ metabolite in the 5 heifers (A-E) are shown in Figs. 1–5.

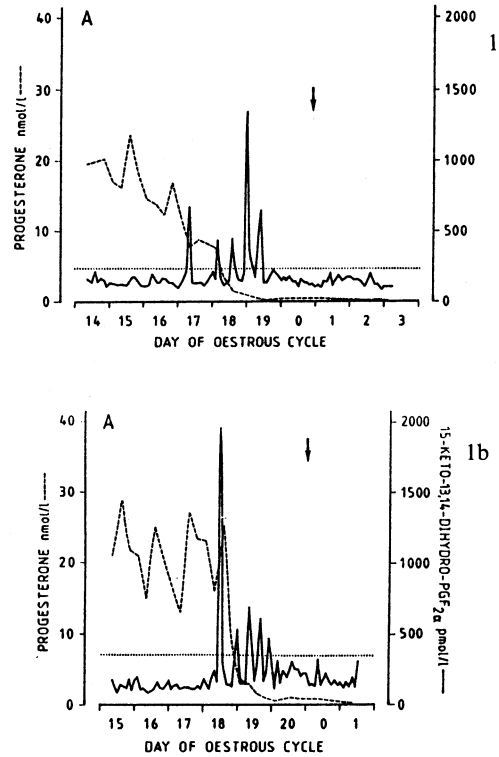

Figure 1a-b. Peripheral plasma levels of progesterone (P_4) and prostaglandin $F_{2\alpha}$ metabolite during open (exp. 1, Fig. 1a) and inseminated cycles (exp. 2, Fig. 1b) for heifer A. Dotted horisontal line indicates kurtosis; arrow indicates first observation of standing oestrus.

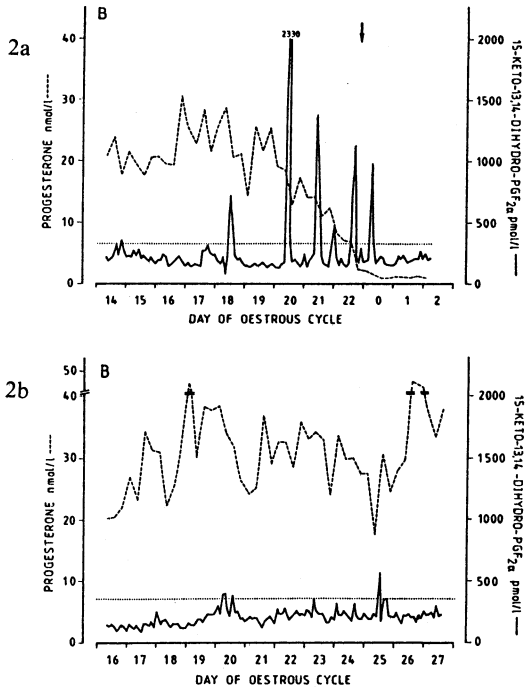

Figure 2a-b. Peripheral plasma levels of progesterone (P_4) and prostaglandin $F_{2\alpha}$ metabolite during open (exp. 1, Fig. 2a) and inseminated cycles (exp. 2, Fig. 2b) for heifer B. Dotted horisontal line indicates kurtosis; arrow indicates first observation of standing oestrus.

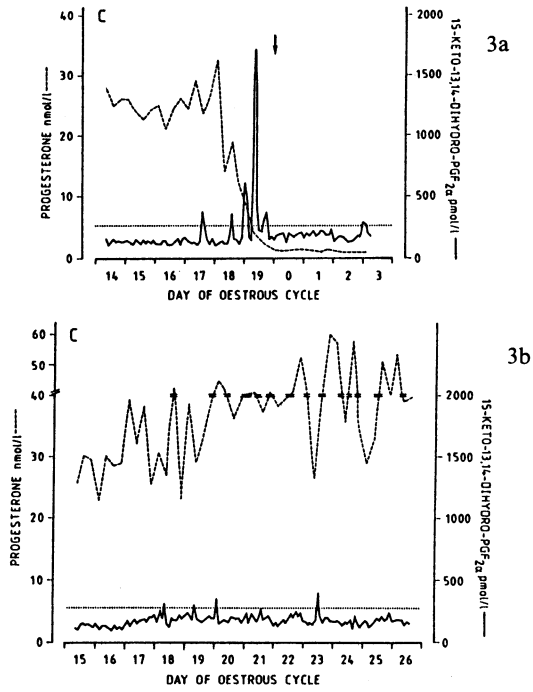

Figure 3a-b. Peripheral plasma levels of progesterone (P_4) and prostaglandin $F_{2\alpha}$ metabolite during open (exp. 1, Fig. 3a) and inseminated cycles (exp. 2, Fig. 3b) for heifer C. Dotted horisontal line indicates kurtosis; arrow indicates first observation of standing oestrus.

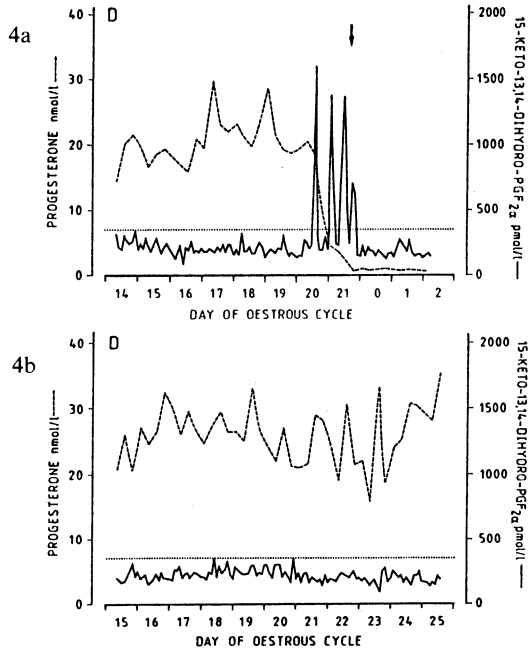

Figure 4a-b. Peripheral plasma levels of progesterone (P_4) and prostaglandin $F_{2\alpha}$ metabolite during open (exp. 1, Fig. 4a) and inseminated cycles (exp. 2, Fig. 4b) for heifer D. Dotted horisontal line indicates kurtosis; arrow indicates first observation of standing oestrus.

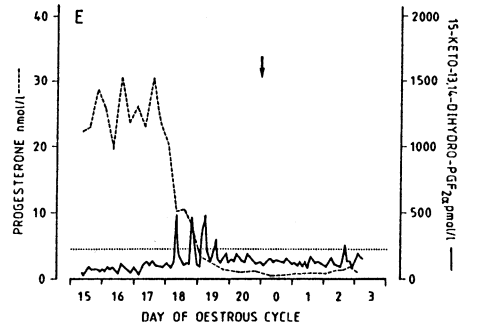

Figure 5. Peripheral plasma levels of progesterone (P_4) and prostaglandin $F_{2\alpha}$ metabolite during open cycle (exp. 1) for heifer E. Dotted horisontal line indicates kurtosis; arrow indicates first observation of standing oestrus.

Plasma P_4 values revealed that luteolysis began 30–92 h ($\bar{X} \pm SD = 59 \pm 23$) before the onset of oestrus and was completed within 30–60 h ($\bar{X} \pm SD = 44 \pm 11$) (Table 2). The number of significant peaks of the $PGF_{2\alpha}$ metabolite varied from 4 to 6 among heifers ($\bar{X} \pm SD = 5 \pm 1$) with mean intervals of 9 ± 3 to 23 ± 14 h. The mean release of $PGF_{2\alpha}$ metabolite during a single peak varied from 44 ± 26 to 245 ± 101 mm^2 while the total release ranged from 174 to 1139 mm^2.

During exp. II 3 out of 4 heifers (A-D) became pregnant after 1–3 inseminations during a single oestrous period (Table 2). Both ultrasonography and postmortem examination (day 27–30) revealed live embryos of normal size and appearance.

As also shown in Table 2, the number of $PGF_{2\alpha}$ metabolite peaks in pregnant heifers varied from 1 to 4. The mean release of $PGF_{2\alpha}$ during a single peak was $\leq 13 \pm 13$ mm^2, and the total release was ≤ 40 mm^2. The total release was 0–6 % of that measured during non-pregnant cycle.

Discussion

The prolongation of the oestrous period (24–56 h) in the RBH, corresponds to that observed in other studies (*Gustafsson et al.* 1986, *Albihn* 1991). *Gustafsson et al.* (1986) found a significant difference in the length of the oestrous period between RBH and VH groups ($\bar{X}$ = 31.5 vs $\bar{X}$ = 23.8 h). In this study ovulation took place 24–56 h ($\bar{X}$ = 41 h) after the onset of standing oestrus. In a study of VH, an interval of 26–38 h ($\bar{X}$ = 32.3) was detected between onset of oestrus and ovulation (*Larsson* 1987). The delayed ovulation in RBHs could be due to an asynchronism in the hormonal release pattern during oestrus and ovulation. *Gustafsson et al.* 1986) reported a delayed LH peak

Table 2. Summary of the endocrine events in conjunction with luteolysis and early pregnancy in repeat breeder heifers.

Heifer	$PGF_{2\alpha}$ metabolite parameters									Time of completion of luteolysis (h)	P4 value at onset of heat (nmol/l)
	No. of peaks	Intervals (h) between peak no.					Basal level (pmol/l)	Mean release during single pulses (mm^2 peak area)	Total release (mm^2 peak area)		
		1–2	2–3	3–4	4–5	5–6					
EXPERIMENT I											
A	5	20	10	10	10	–	142	92 ± 128	462	42	0.4
B	6	48	24	12	16	16	193	199 ± 132	1139	60	2.2
C	5	28	10	8	10	–	155	100 ± 137	501	46	1.2
D	4	12	10	6	–	–	199	245 ± 101	979	30	1.2
E	4	12	10	8	–	–	133	44 ± 26	174	42	1.0
EXPERIMENT II											
A	5	12	8	8	6	–	165	100 ± 75	498	24	0.8
B	3	6	118	–	–	–	196	13 ± 13	40	p	p
C	4	24	18	80	–	–	173	8 ± 8	30	p	p
D	1	–	–	–	–	–	215	0	0	p	p

p = pregnant

in RBH. In the present investigation the LH pattern was not determined. However, ovulation seems to occur at a rather fixed time after the preovulatory LH peak (*Henrichs et al.* 1970, *Gustafsson et al.* 1986, *Larsson* 1987). Farmers are generally recommended to inseminate about 12 h after the onset of standing oestrus. The prolonged oestrous period in the RBHs probably leads to a situation in which inseminations are performed too early in relation to ovulation in the original herd, since the life span of frozen and thawed semen in the genital tract is limited.

In this study 3 out of 4 heifers become pregnant after a variable number of inseminations (1–3). In other studies with repeat breeding animals, a pregnancy rate of around 30 % has been reported (*Tanabe & Almqvist* 1953, *Maurer & Echternkamp* 1985, *Gustafsson & Larsson* 1985). Even better results were obtained in repeat breeding animals after an additional AI under controlled conditions (*de Kruif* 1977, *Refsdal* 1979, *O'Farrell et al.* 1983, *Albihn* 1991). Pregnancy rates in the present investigation were probably high because of strict observed ovulation and provided additional AI when necessary. However the number of animals was also low. It is clear that management is one important cause of the RBH syndrome and that additional inseminations in the herd for some animals can increase pregnancy rates.

To measure pulsatile hormonal release, as prostaglandin $F_{2\alpha}$, it is desirable to collect blood samples very frequently, preferably continuously (*Basu & Kindahl* 1987a). In this study we selected a sampling interval of 2 h, and the samples were collected by venopuncture. Although we detected 4 to 6 peaks of $PGF_{2\alpha}$ metabolite in all heifers, not all these peaks, were directly associated with luteolysis as indicated by the fact that P_4 levels did not decrease. Nevertheless, the 1st peak probably initiated the pulsatile release of $PGF_{2\alpha}$, as is the case in nearly all heifers.

From a study in the ewe (*Zaco et al.* 1984), where blood samples also were collected at 2 h intervals to follow luteolysis, it was also found that some peaks were not associated with luteolysis. In the present study the interval between 1st and 2nd peaks was longer than the remaining intervals, concurring with the findings of *Basu & Kindahl* (1987a). The decline in P_4 concentrations that occurs after $PGF_{2\alpha}$ release is followed by an increase in the frequency and amplitude of $PGF_{2\alpha}$ pulses (*Thorburn et al.* 1973, *Kindahl et al.* 1976b, *Rothchild* 1981). *Basu & Kindahl* (1987a) considered a peak to have occurred if, in succession of 3 consecutive samples, levels remained above the line of skewness. In their study blood samples were collected continuously, and each sample represented a period of 3.7 min. In the present study, a peak could not be defined in this way because of longer intervals between the samples.

In 2 heifers, elevated levels of $PGF_{2\alpha}$ metabolite were detected around day 2 to 3 of the cycle. These peaks were not included when calculating the total release of $PGF_{2\alpha}$. The significance of these peaks is not yet clear. They might be the results of endometrial changes concomitant with post-oestrous bleeding (*Kindahl et al.* 1976a,b).

The key to obtaining maternal recognition of pregnancy in the bovine is to lengthen the life span of the cyclic corpus luteum by blocking production or effects of the endometrial luteolytic substance $PGF_{2\alpha}$ (*Kindahl et al.* 1976a, *Harvey et al.* 1984, *Thatcher et al.* 1984, *Basu & Kindahl* 1987a,b). In the present study, 3 pregnant RBHs each produced between 1 and 4 low $PGF_{2\alpha}$ metabolite peaks. These elevated values were lower than those measured when the heifers were not pregnant (0–6 % of total release). In addition the inter-pulse intervals were longer and irregular in the pregnant animals. Luteal function was maintained as indicated by elevated plasma P_4 values ($>$ 8 nmol/l). Similar small pulses of $PGF_{2\alpha}$ metabolite have been reported in pregnant heifers of normal fertility (*Basu & Kindahl* 1987a). That slightly higher metabolite levels were found in the pregnant heifers and in the inseminated non-pregnant heifer than in the non-inseminated heifers is in accordance with *Basu & Kindahl* (1987a). The frequent pulsatile release of $PGF_{2\alpha}$, leading to luteolysis in non-pregnant RBH, and the few irregular pulses of $PGF_{2\alpha}$, which had no effect on luteal function in pregnant RBH, found in this study are comparable to those reported from VH.

Several studies have been conducted to evaluate the possible negative influence of lower milk or serum progesterone levels, occasionally observed in repeat breeders, on early embryonic development (*Henrichs et al.* 1971, *Maurer & Echternkamp* 1982, 1985, *Lee & Ax* 1984, *Lamming et al.* 1989). In addition significant differences in the amounts of nuclear progesterone receptors (PRn) in the endometrium on day 15 have been observed between RBH and VH (*Stanchev et al.* 1991). The amount of PRn in endometric holding embryonic structures was significantly higher in VH than in pregnant RBH. However, no statistical differences were found between their plasma progesterone levels. Also, PRn concentrations were higher in the uterine horn holding an elongated, morphologically normal embryo than in horns with small embryos, regardless of recipient group.

The higher than usual plasma P_4 levels at oestrus ($<$ 0.4 nmol/l) found in this study are similar to those reported from RBH in other studies (*Gustafsson et al.* 1986, *Albihn et al.* 1990a). This finding may reflect impaired fertilization and early embryonic development since the higher level may alter

the transport time through the oviduct and change the milieu in the oviduct and uterus (*Henrichs et al.* 1971, *Crisman et al.* 1980).

Conclusion

The result of this study show that management is one important factor involved in the problem of repeat breeding. With the exception of tendencies towards higher than usual plasma P_4 levels at oestrus, the levels and release patterns of $PGF_{2\alpha}$ and P_4 in connection with luteal regression and early pregnancy in RBH were comparable to those in VH. However, the number of animals was small, and individual variability was pronounced.

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Sammanfattning

Plasma nivåer av prostaglandin $F_{2\alpha}$ metabolit och progesteron hos omlöparkvigor.

Till försöket användes 6 kvigor som tidigare inseminerats 3–10 ggr. och därefter löpt om med normalt brunstintervall. Djuren studerades kliniskt och endokrinologiskt under 2 sexualcykler, varav den ena efter insemination.

Plasmanivåer av prostaglandin $F_{2\alpha}$ metabolit mättes varannan timme och progesteron (P_4) var sjätte timme under luteolysen eller under motsvarande tid efter insemination.

Brunsten och intervallet från brunstens början till ägglossningen var längre än vad som vanligen ses hos kvigor, de 3 kvigorna som blev dräktiga inseminerades 1–3 ggr. med 24 timmars intervall tills ägglossningen konstaterats. Plasmanivån av P_4 vid brunstens början var högre än vad som vanligen ses hos kvigor, däremot bedömmdes nivån av prostaglandin $F_{2\alpha}$ metabolit och P_4 under luteolys och tidig dräktighet att ej skilja sig från vad som vanligen ses hos nötkreatur. Skötseln av djuren diskuteras som en orsak till omlöparsyndromet.

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