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Animal Reproduction Science 66 (2001) 35–46

ANIMAL REPRODUCTION SCIENCE

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Progesterone metabolism in ovariectomised non-lactating Holstein–Friesian cows treated with progesterone with two levels of feed intake

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Received 25 June 2000; received in revised form 19 January 2001; accepted 22 January 2001

Abstract

The goal of this study was to measure the effects of level of feeding and the form of progesterone (P4) administration on the concentrations and yields of faecal P4 metabolites relative to differences in plasma P4 concentrations in non-lactating cows. Six non-lactating Holstein–Friesian cows were ovariectomised (OVX) and allocated to two groups: (i) P4 by subcutaneous injection (P4-s.c., $n = 3$); and (ii) P4 administration per vaginum (P4-p.v., $n = 3$). Each cow in the P4-s.c. group was injected subcutaneously once daily with 200 mg P4. Each cow in the P4-p.v. group had a CIDR device inserted for 11 days when it was removed and replaced with a second device for further 11 days. Cows were fed a ration containing lucerne (33%) and oaten (66%) chaff at a maintenance level (*M*) in two portions in the first period of the study, and at a half-maintenance (1/2*M*) level during the second period. Chromic oxide capsules (Cr_2O_3) were administered twice daily to allow faecal output (FO) to be estimated. Plasma P4 and faecal P4 metabolites (FP4M; 20-oxo-pregnanes, 20α- and 20β-OH-pregnanes) were measured during the treatment period. Daily FO declined after reducing the *M* diet to 1/2*M* (4.77 versus 2.61 kg; $P < 0.01$), whereas plasma P4 concentrations increased in the P4-s.c. group (4.2 versus 6.2 ng/ml; $P < 0.05$), but not in the P4-p.v. group (0.9 versus 1.0 ng/ml; $P > 0.2$). The mass of P4 released from a CIDR device during each 11-day period (*M* or 1/2*M*) was similar (0.66 versus 0.63 g). Faecal 20-oxo-pregnanes (20-oxo-) concentrations were not affected by day or level of feeding, whereas faecal 20α-OH (20α-) and 20β-OH (20β-) concentrations were increased with the 1/2*M* diet in the P4-s.c. group (4.3 versus 5.6 μg/g DM; 2.2 versus 5.6 μg/g DM, respectively; $P < 0.05$), but not in the P4-p.v. group (2.3 versus 2.7 μg/g DM; 1.7 versus 3.04 μg/g DM $P > 0.05$). These changes in concentration only partly compensated for the reduced FO with the 1/2*M* diets as daily yields of FP4M (20-oxo- and 20α-) were greater during the *M* diet period (20-oxo-: 6.9 versus 4.1 μg/g DM; 2.7 versus 1.5 μg/g DM, for P4-s.c. and

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PII: S0378-4320(01)00087-2

P4-p.v. groups, respectively; $P < 0.05$, 20α-: 19.9 versus 13.6 μg/g DM; 10.9 versus 6.6 μg/g DM for P4-s.c. and P4-p.v. groups, respectively; $P < 0.05$). The level of feeding and the route of P4 administration had key roles in controlling P4 concentrations in blood and daily FP4M yield.

Keywords: Progesterone; Faecal progesterone metabolites; Dairy cow

1. Introduction

Regulatory mechanisms controlling luteal function and blood P4 concentration in cattle during the postpartum period have received close attention during recent decades. Progesterone (P4) has an important role in normal fertilisation, embryo transport and embryo survival (Garcia-Winder et al., 1986; Ashworth et al., 1989; Diskin and Niswender, 1989; Mee et al., 1991; Taylor and Rajamahendran, 1994). Most of the research that has addressed the regulation of circulating concentrations of P4 and oestradiol has focused on secretion from the ovary. Several studies (Wise et al., 1982; Hooper et al., 1986; Rabiee et al., 1997a,b) have shown that P4 concentrations in the ovarian vein are 1000 to 10,000 times higher than peripheral blood. However, little is known whether changes in plasma concentration are related solely to differences in production rate or, even if there are alterations in the rate of catabolism of P4. The P4 clearance rate from the body is perceived to have an influence on circulating concentration, which in turn may influence the production rate (Parr, 1992).

In vivo studies have suggested that splanchnic tissues are the primary sites of degradation of P4 (Little et al., 1966; Bedford et al., 1974). In vitro studies have also demonstrated that the cytochrome enzyme complexes (mixed function oxidase) in liver microsomes may be involved in P4 and oestradiol metabolism (Estabrook et al., 1975; Cheng and Schnkman, 1984; Payne et al., 1991; Kaddouri et al., 1992).

Several studies have assessed aspects of ovarian function and steroid metabolism, particularly pathways involved in the biosynthesis, interconversion and metabolism of steroid hormones. One approach in assessing P4 metabolism has been to use radioactively labelled steroids in domestic and non-domestic species to determine the route of excretion and the type of metabolic end products of steroids in urine and faeces (Schwarzenberger et al., 1996a). Injecting a P4 isotope and measuring the rate of metabolism in dairy cows, particularly if they are grazing, is problematical. Another approach has been to measure arterio–venous differences across the liver (Parr et al., 1993; Freetly and Ferrell, 1994; Quintal-Franco et al., 1995) but this involves complicated surgical procedures. The most recent data have demonstrated that faecal P4 changes reflect plasma and milk profiles and therefore luteal and/or placental function (Schwarzenberger et al., 1996b, 1997; Palme et al., 1996). Therefore, the analysis of faecal P4 metabolites has emerged as an appropriate method for monitoring ovarian function and reproductive performance in general, as already shown in cattle (Desaulniers et al., 1989; Larter et al., 1994; Schwarzenberger et al., 1996b).

The present study was conducted to develop a protocol for the investigation of P4 metabolism in lactating dairy cows. The objective of the study was to measure the effects of level of feeding and the form of P4 administration on plasma P4, and the associated changes in the concentrations and yields of faecal P4 metabolites in non-lactating dairy cows.

2. Materials and methods

2.1. Animals and experimental protocol

Six non-lactating multiparous Holstein–Friesian cows, aged 4–7 years old were ovariectomised (OVX) using an Ecraseur instrument and treated with an appropriate antibiotic for 3–4 days post-operatively. Plasma concentrations of P4 were monitored for 4 weeks after ovariectomy to confirm complete clearance of P4 from the body. These OVX cows were allocated randomly into two equal groups: P4 by subcutaneous injection (P4-s.c.), and P4 administration per vagina (P4-p.v.). To measure the effect of level of feed intake (FI) on P4 metabolites excreted into faeces, it was necessary to measure daily FI and faecal output (FO). The diet for each individual animal was calculated based on body weight.

Each cow in the P4-s.c. group was injected subcutaneously once daily for 23 days with 200 mg P4 in 4 ml sterilised corn oil. P4 (Sigma) was dissolved initially in benzyl alcohol (C_7H_8O, Sigma) and then diluted in corn oil to a concentration of 50 mg/ml.

Each P4-p.v. cow had a CIDR device (InterAg, Pty. Ltd., Hamilton, New Zealand) inserted into the vagina. This device contained 1.9 g P4 and was replaced with a new device after 11 days for further 11 days. Each used device was rinsed with fresh tap water following withdrawal, before being wiped with a tissue and then air-dried. They were packed and stored frozen until the residual drug content of devices was measured (Rathbone et al., 1998).

In the first 11-day period of the study, cows were fed twice daily with a maintenance (*M*) diet of lucerne (33%) and oaten (66%) chaff, offered on an individual basis. The diet was based on 2.5% of body weight. It contained 26% crude protein (CP), 8.9% MJ/kg metabolic energy (ME) and 27% crude fibre (CF). In the second period, the entire procedure (period one) was repeated, but cows were fed half of the feed relative to their requirements (1/2*M*). Body weight and body condition scores were monitored weekly.

To estimate FO, chromic oxide (Cr_2O_3) capsules (gelatine capsules containing 10.3 g Cr_2O_3) were administered at 08.00 and 16.00 h each day. Total FO was also collected at the time of capsule administration to confirm the data obtained by Cr_2O_3 measurements. Daily faecal samples were taken for a period of 25 days following a preliminary dosing period of 7 days.

2.2. Blood and faecal sampling and assays

Samples were taken at 08.00 and 16.00 h from a coccygeal vessel into vacutainer tubes (lithium heparin). Each sample was centrifuged within 10 min (3000 rpm for 15 min at 4°C) and plasma stored at −20°C until assayed for P4 by direct RIA using a commercial, solid phase, ^{125}I (Coat-A-Count®, Kit, Los Angeles, California, USA) kit. All samples were run in one assay. The intra-assay CV was 5.4% and the assay sensitivity was 0.08 ng/ml.

Concentrations of blood glucose and urea were measured daily during the period of study. Plasma glucose was measured using the method of Farrance (1987) with Trace reagents (Trace Scientific, Melbourne, Australia). Plasma urea was measured as described by Talke and Schubert (1965) again using Trace reagents. Measurements were conducted with an autoanalyser (Cobas Mira, Roch Diagnostic Systems, Switzerland).

Faecal samples were collected directly from the rectum into 25 ml polycarbonate containers and immediately stored at −20°C until assayed. A 0.5 g sample was extracted with methanol as described by Schwarzenberger et al. (1996b). Faecal extracts were analysed by EIAs for immunoreactive P4 metabolites. Briefly, the group-specific antibodies used in the EIAs were raised in rabbits. The assays included 20-oxo-pregnanes (20-oxo-) (antibody: 5α-pregnane-3β-ol-20-one 3HS:BSA; Schwarzenberger et al., 1996b), 20α-OH-pregnanes (5β-pregnane-3α, 20α-diol 3HS:BSA; common name pregnanediol; Schwarzenberger et al., 1993), and 20β-OH-pregnanes (antibody: 4-pregnene-20β-ol-3-one 3CMO:BSA; Schwarzenberger et al., 1991). Significant cross-reactivities in these assays resulted with five-reduced P4 metabolites. Results were designated as measurements of pregnanes. These are the principal metabolites of P4 excreted into the faeces of cattle (Palme et al., 1996, 1997; Schwarzenberger et al., 1996a, 1997). Three assays were used in this study to measure the entire range of faecal pregnanes. The intra- and inter-assay coefficients of variation for these assays were similar to those described previously and ranged between 10 and 15%, respectively and the assay sensitivity was 7 ng/ml.

A faecal sample was taken from each cow (blank) before routine Cr_2O_3 administration. Gelatine capsules were administered to each cow by means of an applicator. Faecal samples were taken in aluminium containers at the same time over the period of study. Morning and afternoon samples were combined and analysed for Cr_2O_3. Faecal samples were weighed and oven-dried at 100°C for 3 days and then ground. Cr_2O_3 concentrations were measured using an X-ray fluorescence spectrometry method (Norrish and Hutton, 1977). Morning and afternoon FOs (wet) were also collected in individual bins and recorded at the time of Cr_2O_3 administration and faecal sampling. Estimated FO was used to measure the excretion rate of P4 metabolites through the faeces.

2.3. Residual drug content of CIDR

A Soxhlet extraction technique was used to determine the residual amount of P4 in used CIDR devices (Rathbone et al., 1998).

2.4. Data analyses

The results were analysed after excluding the first 3 days of observations. The effect of time (day) and diet on plasma P4 concentration and interactions between a.m. and p.m. observations, diet and time were analysed using GLM with repeated measures analysis included in the model in SPSS version 9.0 (SPSS, 1998). Analyses of variance (ANOVA) were also used to determine the variation among cows. The data for each treatment group were analysed separately.

3. Results

Concentrations of plasma P4 and FP4M in combination with the results of residual content of P4 in CIDR devices (in P4-p.v. group) were used as an indicator of P4 clearance rates and also to investigate the effect of level of feeding on the metabolic rate.

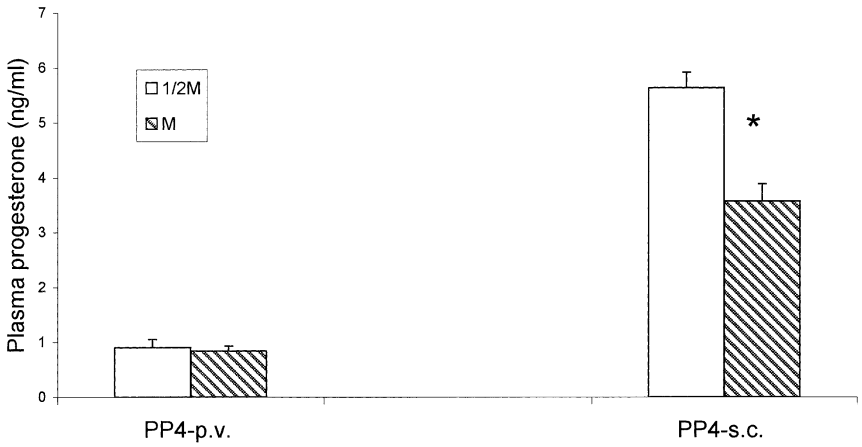

Fig. 1. Plasma progesterone concentrations in two groups of OVX cows administered P4 per vagina (P4-p.v.) or subcutaneously (P4-s.c.).

3.1. Feed intake and faecal output

Daily FO declined significantly after reducing the *M* diet to 1/2*M* in each group ($P = 0.001$, Table 1). FO/FI ratio changed from 43 to 44% with the diet change in the P4-p.v. group and from 39 to 45% ($P < 0.05$) in the P4-s.c. group (Table 1).

3.2. Plasma concentrations of progesterone

The level of feeding had a significant effect on plasma P4 concentrations in the P4-s.c., but not in the P4-p.v. group (Table 1, Fig. 1). The plasma P4 concentrations were greater during the 1/2*M* period for the cows in the P4-s.c. group ($P < 0.001$, Table 1, Fig. 1), but not for those in the P4-p.v. group ($P = 0.32$, Table 1, Fig. 1). Plasma P4 was not affected by day, time of sampling (a.m. versus p.m.) or any of the interactions ($P > 0.05$). Average plasma P4 concentrations among individual P4-p.v. cows ranged from 0.72 to 1.02 ng/ml in the *M* period and from 0.63 to 1.13 ng/ml in the 1/2*M* period ($P < 0.05$); and from 3.0 to 4.1 ng/ml in the *M* period, and from 5.3 to 6.2 ng/ml in the 1/2*M* period ($P > 0.05$) among the P4-s.c. cows.

3.3. Faecal concentration of progesterone metabolites (20-oxo-, 20α- and 20β-OH-pregnanes)

The concentrations of 20-oxo-, 20α-, 20β- and total FP4M were not affected by level of feeding in the P4-p.v. group ($P = 0.30$, Table 1, Fig. 2) but the concentrations of 20α-, 20β-OH and total FP4M were greater in the 1/2*M* period in the P4-s.c. group ($P = 0.05$, Table 1). Differences in average total FP4M concentrations among cows ranged from 4.0 to 5.3 μg/g in the *M* period and 5.60 to 7.6 μg/g in the 1/2*M* period in the P4-p.v. group

Table 1
Mean (±S.E.) of FI, FO, FO/FI ratio, plasma progesterone and faecal progesterone metabolites concentrations and yields for two groups of ovariectomised cows administrated progesterone per vagina (P4-p.v.) or subcutaneously (P4-s.c.)[a]

Trt	Diet	Feed intake (kg DM per day)	*P* value	Faecal output (kg DM per day)	*P* value	FO/FI (%)	*P* value	P4 (ng/ml)	*P* value	Total FP4M concentration (μg/g)	*P* value	Total FP4M yield (mg)	*P* value
P4-p.v.	*M*	11.62 ± 1.3	0.001	4.91 ± 0.18	0.001	43.0 ± 4.0	0.50	0.84 ± 0.09	0.32	4.50 ± 0.41	0.30	22.02 ± 1.33	0.03
	1/2*M*	5.80 ± 0.62		2.56 ± 0.19		44.0 ± 2.0		0.91 ± 0.15		6.48 ± 0.60		15.77 ± 0.18	
P4-s.c.	*M*	11.87 ± 0.43	0.001	4.64 ± 0.10	0.001	39.0 ± 2.0	0.05	3.57 ± 0.32	0.001	7.97 ± 1.20	0.05	37.03 ± 5.76	0.13
	1/2*M*	5.91 ± 0.24		2.65 ± 0.07		45.0 ± 1.0		5.63 ± 0.28		13.44 ± 2.19		32.13 ± 4.51	

[a] *M*: maintenance; 1/2*M*: half-maintenance; FI: feed intake; FO: faecal output; FP4M: faecal progesterone metabolites.

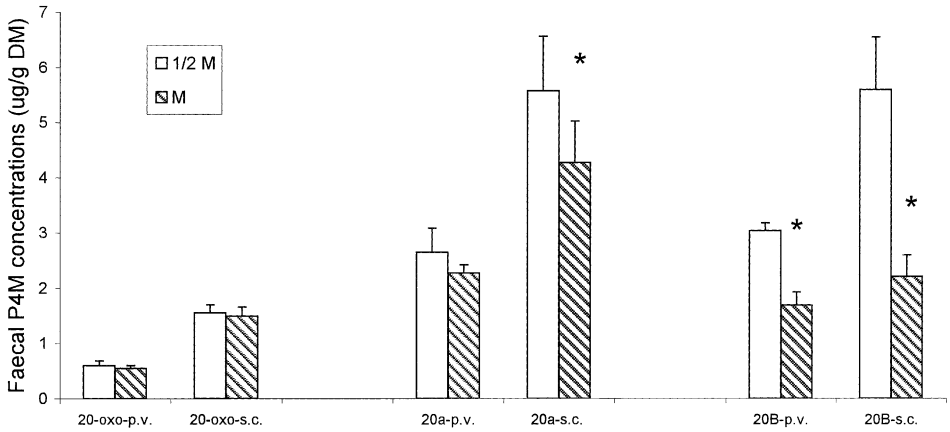

Fig. 2. Faecal progesterone metabolites (20-oxo-pregnanes, 20α- and 20β-OH) concentrations in two groups of OVX cows administered P4 per vagina (P4-p.v.) or subcutaneously (P4-s.c.).

($P < 0.05$). In the P4-s.c. group, these differences among cows varied from 5.6 to 9.4 μg/g in the *M* period, and 9.1 to 15.1 μg/g in the 1/2*M* period ($P < 0.05$). The concentrations of faecal P4 metabolites increased from 12 h after the insertion of a CIDR device in the P4-p.v. group and reached a steady state by the third day after CIDR insertion. The FP4M concentrations in the P4-s.c. group increased gradually to reach a steady state by the fourth day and remained at that level during the period of sampling (e.g. 20-oxo- concentrations, Fig. 3).

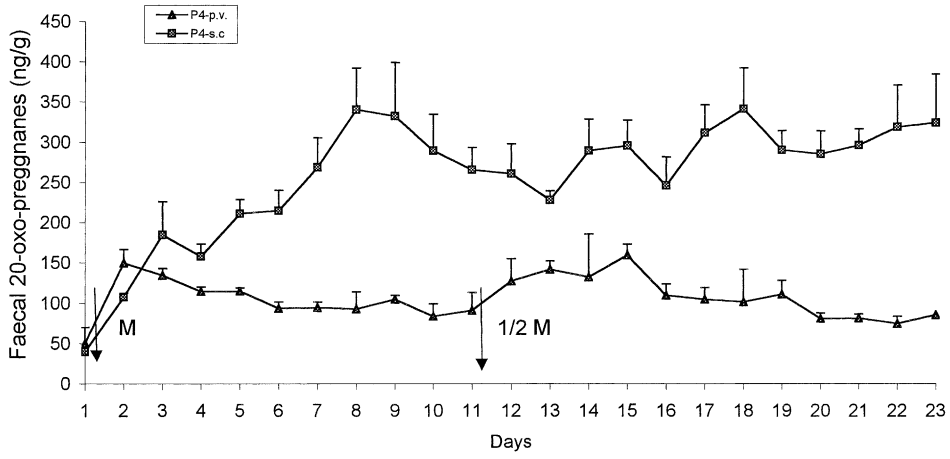

Fig. 3. Faecal concentrations of 20-oxo-pregnanes in two groups of OVX cows administered P4 per vagina (P4-p.v.) or subcutaneously (P4-s.c.).

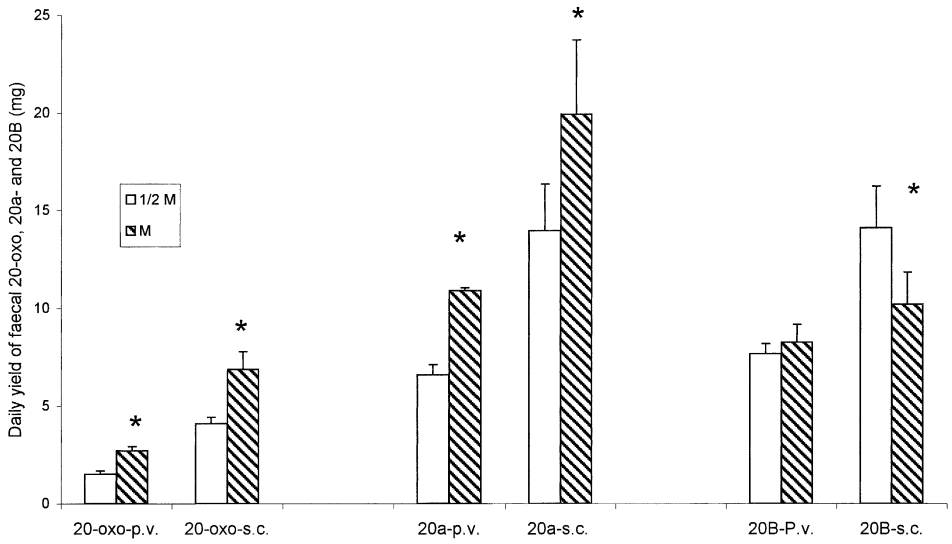

Fig. 4. Daily yield of faecal progesterone metabolites (20-oxo-pregnanes, 20α- and 20β-OH) in two groups of OVX cows administered P4 per vagina (P4-p.v.) or subcutaneously (P4-s.c.).

3.4. Daily yield of faecal progesterone metabolites

Daily FO and P4 metabolite concentrations were used to calculate daily P4 yield using the formula:

$$\text{Daily FP4MY (DM)} = \text{daily FO (kg DM)} \times \text{daily FP4M (ng/g DM)}$$

where P4MY is P4 metabolite yields; FO the faecal output; FP4M the faecal P4 metabolite concentrations; and total daily yield of P4 metabolites in faeces was calculated by adding daily yield of 20-oxo-, 20α- and 20β- for the *M* and 1/2*M* periods separately.

Daily yields of faecal 20-oxo-, 20α-, 20β- and total FP4M were affected by the level of FI (Table 1, $P < 0.05$), except for 20β- in the P4-p.v. group, and total FP4M in the P4-s.c. group (Table 1, Fig. 4). Daily yield of 20-oxo- changed during the sampling period in the P4-p.v. group ($P < 0.05$). The interaction between level of feeding and day had no effect on daily yield of FP4M in either group ($P > 0.05$). Differences among cows for the average daily yield of total FP4M were significant among P4-s.c. cows in both periods ($M = 26$–45 mg; $1/2M = 23$–39; $P < 0.05$). The differences in the average daily yield of total FP4M among cows in the P4-p.v. group were less and ranged from 21 to 25 mg in the *M* period ($P < 0.05$) and 15 to 16 mg in the 1/2*M* period ($P > 0.05$).

3.5. Residual drug content of CIDRs

The amount of P4 released over a 11-day period from individual CIDR devices varied from 0.54 to 0.75 g. The average delivery rate of P4 did not differ during either period (0.66 ± 0.06 versus 0.63 ± 0.05 g for the *M* and 1/2*M* periods, respectively).

3.6. Blood metabolites

The average glucose concentration was 4.66 mM and this was not affected by feeding level or form of P4 administration; neither did it vary among days ($P > 0.05$). There was a slight effect of diet on plasma urea concentration (4.09 versus 4.93 mM; $P = 0.09$).

4. Discussion

The reduced dietary intake increased the plasma P4 concentration only in the group of cows injected with P4. There is little information regarding the effect of FI on P4 metabolism in dairy cattle. Vasconcelos et al. (1998) demonstrated that feeding cows four times versus two times per day maintained greater plasma P4 concentrations. Other studies in sheep (Williams and Cumming, 1982; Parr et al., 1987, 1993; Parr, 1992; Adams et al., 1994) support observations in the present study that there is a negative relationship between the level of feeding and plasma P4 concentrations when the P4 is administered subcutaneously. There are several ways that the level of feeding may influence P4 metabolism. First, an increase in P4 clearance rate may result from greater degradative metabolism by the liver (Thomford and Dziuk, 1986; Thomas et al., 1987; Smith et al., 1990); and secondly, there may be an increase in hepatic blood flow (Parr, 1992; Parr et al., 1993). The level of feeding may have also influenced enterohepatic re-circulation of P4.

Our data suggest that the level of FI did not affect plasma P4 in OVX cows treated with CIDR devices when the cows housed inside a building. Parr et al. (1993) found plasma P4 in OVX sheep treated with CIDR devices (300 mg) to decrease 40% when they were fed ad libitum, compared with those fed half the diet of mainly lucerne chaff. However, when P4 solution was infused into the jugular vein of sheep (820 μg), there was no difference in plasma P4 associated with *M* and 1/2*M* intakes of the same diet. This is in agreement with observations with the P4-p.v. cows in the present study, but contrasts findings with the subcutaneous P4 administration. Findings reported by Williams and Cumming (1982), and Parr (1992), Parr et al. (1987, 1993) highlighted that P4 concentrations were significantly higher with a 1/2*M* diet compared with a 2*M* diet, but that there were either small or no differences between *M* and 2*M* diets.

Rabiee et al. (1999) have previously shown that environmental factors such as nutrition and housing can affect the profile of P4 release from a CIDR device. Low concentrations of plasma P4 in the P4-p.v. group compared with those in the P4-s.c. group suggest that the P4 assay was not sufficiently sensitive to detect the difference in plasma P4 between the two periods. Feed restriction was not as severe as in other studies with sheep and doses of P4 were also different between two groups in the present study. Level of feed restriction and the dose of administered P4 appear to have been contributed to a different outcome in the present study compared with previous studies in sheep.

The level of feeding did not affect the concentrations of FP4M in the P4-p.v. group, but increased faecal concentrations of 20α- and 20β- in the P4-s.c. group in the present study. The level of FI increased daily yield of 20-oxo- and 20α- in both groups, 20β- in the P4-s.c. group and total FP4M in the P4-p.v. group. It is the volume of faeces which mainly dictated the total daily excretion rate of P4 metabolites in the faeces. Adams et al. (1994) reported that the final amount of radioactivity excreted in the faeces of nutritionally

restricted and supplemented sheep was similar, but excretion was slower in the restricted ewes. The recovery of radioisotopes may simply not reflect the actual amount of FP4M in the faeces. Adams et al. (1994) also found that daily peaks of oestradiol excretion coincided with enhanced defecation about the time animals were fed, and the concentration of radioactivity remained greater in restricted than supplemented ewes. This indicates that the level of feeding has a determining role in controlling P4 metabolism and would suggest that the blood concentration of P4 does not necessarily indicate the rate of ovarian steroidogensis.

Diet composition, digestibility and level of feeding may have influenced the quantity of different P4 metabolites in the faeces. Antibodies against 20-oxo-, 20α- and 20β- are able to detect most metabolites with different degrees of specificity, but not every single metabolite. The physical form of faeces and their rate of passage may have also altered the re-absorbtion rate of some P4 metabolites. The significant elevation of plasma P4 in P4-s.c. group may support this explanation. Slow passage rate of faeces during the period of the 1/2*M* diet may also have altered the quantity of different P4 metabolites in the faeces due to gut microbial transformation. Arts et al. (1992) showed that a high level of feeding in rats was associated with an increase in the amount of oestrogen excreted in the faeces. The enhanced oestrogen excretion rate resulted in lower plasma oestrogen to produce a result similar to the study in sheep (Arts et al., 1992; Adams et al., 1994). The yield of P4 metabolites excreted by the P4-s.c. and P4-p.v. groups during both periods, *M* and 1/2*M* were similar, but the plasma P4 did not change significantly in P4-p.v. group. The level of feeding and the route of P4 administration had a key role in controlling plasma P4 and FP4M levels in the OVX cows utilised in the present study.

5. Conclusion

The level of FI and the route of P4 administration influenced plasma P4 concentrations as well as the yields and concentrations of faecal P4 metabolites in non-lactating OVX dairy cows. The level of feeding had a significant effect on plasma P4 in the P4-s.c. cows, but not those in the P4-p.v. group. The effect of level of FI on the concentrations of FP4M (20-oxo-, 20α- and 20β-) varied between treatment groups. The excretion rates of FP4M were higher during the period of *M* diet than 1/2*M* diet. Since the delivery rate of P4 was relatively constant during the trial within each group, the mechanism whereby the level of FI was able to control concentrations and daily yields of faecal P4 remain to be established. Further studies are required to understand P4 metabolism in dairy cows.

Acknowledgements

This project was funded by DRDC (UM-065) and conducted at the University of Melbourne. Thanks are given to A. Aichinger and E. Leitner (University of Veterinary Medicine, Vienna) for analysing faecal P4 metabolites.

References

Adams, N.R., Abordi, J.A., Briegel, J.R., Sanders, M.R., 1994. Effect of diet on the clearance of estradiol-17β in the ewe. Biol. Reprod. 51, 668–674.

Arts, C.J.M., Govers, C.A.R.L., Van Den Berg, H., Van't Veer, P., Blankenstein, M.A., Thijssen, J.H.H., 1992. Effect of wheat bran on secretion of radioactively labelled estradiol-17β and estrone–glucuronide injected intravenously in male rats. J. Steroid Biochem. Mol. Biol. 42, 103–111.

Ashworth, C.J., Sales, D.I., Wilmuth, I., 1989. Evidence of an association between the survival of embryos and preovulatory plasma progesterone concentrations in the ewe. J. Reprod. Fertil. 87, 23–32.

Bedford, C.A., Harrison, F.A., Heap, R.B., 1974. Splanchnic, uterine, ovarian and adrenal uptake of progesterone and 20α-dihydroprogesterone in the pregnant and non-pregnant sheep. J. Endocrinol. 62, 277–290.

Cheng, K.C., Schnkman, J.B., 1984. Metabolism of progesterone and oestradiol by microsomes and purified cytochrome P-4560 RLM3 and RLM5. Drug Metabol. Disposit. 12, 222–234.

Desaulniers, D.M., Goff, A.K., Betteridge, K.J., Rowell, J.E., Flood, P.F., 1989. Reproductive hormone concentrations in faeces during the oestorus cycle and pregnancy in cattle (*Bos taurus*) and Muskoxen (*Ovibos moschatus*). Can. J. Zool. 67, 1148–1154.

Diskin, M.G., Niswender, G.D., 1989. Effect of progesterone supplementation on pregnancy and embryo survival in ewes. J. Anim. Sci. 67, 1559–1563.

Estabrook, R.W., Martinez-Zedillo, G., Young, S., Peterson, J.A., McCarthy, J., 1975. The interaction of steroids with liver microsomal cytochrome P-450: a general hypothesis. J. Steroid Biochem. 6, 419–425.

Farrance, I., 1987. Plasma glucose methods: a review. Clin. Biochem. Rev. 8, 55–68.

Freetly, H.C., Ferrell, C.L., 1994. Net uptakes of oestradiol-17β and progesterone across the portal-drained viscera and the liver of ewes. J. Endocrinol. 141, 353–358.

Garcia-Winder, M., Lewis, P.E., Deaver, D.R., Smith, V.G., Lewis, G.S., Inskeep, E.K., 1986. Endocrine profiles associated with lifespan of induced corpora lutea in postpartum beef cows. J. Anim. Sci. 62, 1353–1362.

Hooper, S.B., Walker, D.W., Thorburn, G.D., 1986. Cannulation of the utero-ovarian vein in intact ewes: hormone concentration and blood levels during the oestrous cycle and early pregnancy. Acta Endocrinol. 112, 253–262.

Kaddouri, M., Brasset, N., Alvinerie, M., Eechoutte, C., Bonfils, C., Derancourt, J., Galtier, P., 1992. Ontogenic development of liver progesterone metabolism in female sheep. Contribution of cytochrome P4502B and P4503A subfamilies. J. Steroid Biochem. Mol. Biol. 42, 499–508.

Larter, N.C., Rajamahendran, R., Sivakumaran, K., 1994. Immunoreactive faecal progestins as indicators of reproductive status. Vet. Rec. 134, 474–475.

Little, B., Tait, J.F., Tait, S.A.S., Erlenmeyer, F., 1966. The metabolic clearance rate of progesterone in males and ovariectomised females. J. Clin. Invest. 45, 901–912.

Mee, M.O., Stevenson, J.S., Minton, J.E., 1991. First postpartum luteal function in dairy cows after ovulation induced by progesterone and gondotropin-releasing hormone. J. Dairy Sci. 74, 1573–1581.

Norrish, K., Hutton, J.T., 1977. Plant analyses by X-ray spectrometry. I. Low atomic number elements, sodium to calcium. X-ray Specterom. 6, 12–17.

Palme, R., Fischer, P., Schildorfer, H., Ismail, M.N., 1996. Excretion of infused 14C-steroid hormones via faeces and urine in domestic livestock. Anim. Reprod. Sci. 43, 43–63.

Palme, R., Möstl, E., Brem, G., Shellander, K., Bamberg, E., 1997. Faecal metabolites of infused ^{14}C-progesterone in domestic livestock. Reprod. Dom. Anim. 32, 199–206.

Parr, R.A., Davis, I.F., Fairclough, R.J., Miles, M.A., 1987. Overfeeding during early pregnancy reduces peripheral progesterone concentration and pregnancy rate in sheep. J. Reprod. Fertil. 80, 317–320.

Parr, R.A., 1992. Nutrition–progesterone interactions during early pregnancy in sheep. Reprod. Fertil. Dev. 4, 297–300.

Parr, R.A., Davis, I.F., Miles, M.A., Squires, T.J., 1993. Liver blood flow and metabolic clearance rate of progesterone in sheep. Res. Vet. Sci. 55, 311–316.

Payne, E., Smith, J.F., Cope, B.C., McGoawn, L.T., 1991. Studies on the role of liver cytochrome P-450 and oestradiol metabolism in the effects of nutrition and phenobarbital on ovulation rate in the ewe. Reprod. Fertil. Dev. 3, 725–736.

Quintal-Franco, J.A., Wehman, M.E., Melvin, E.J., Lindsey, B.R., Peters, K.E., Bergfeld, E.G.M., Zanella, E.L., Kojima, F.N., Melson, B.E., Kinder, J.E., 1995. Concentrations of progesterone in carotid, jugular, hepatic or portal vessels during the luteal phase of the estrous cycle of heifers. Society for the Study of Reproductive, Biol. Reprod. 52 (Suppl. 1) (Abstract 277).

Rabiee, A.R., Macmillan, K.L., Rathbone, M.J., 1999. Effect of feeding management of progesterone release from CIDR devices in ovariectmised dairy cows. Proc. Aust. Soc. Reprod. Biol. 30, 120.

Rabiee, A.R., Lean, I.J., Gooden, J.G., Miller, B.G., Scaramuzzi, R.J., 1997a. An evaluation of transovarian uptake of metabolites using arterio–venous difference methods in dairy cows. Anim. Reprod. Sci. 48, 9–25.

Rabiee, A.R., Lean, I.J., Gooden, J.G., Miller, B.G., 1997b. Short-term studies on ovarian metabolism in the ewe. Anim. Reprod. Sci. 47, 43–58.

Rathbone, M.J., Bunt, C.R., Burggraaf, S., Burke, C.R., Macmillan, K.L., 1998. Optimisation of a controlled release intravaginal drug delivery system containing progesterone for the control of the estrus in cattle. In: Proceedings of the International Symposium on Controlled Release of Bioactive Materials, Vol. 25, pp. 249–250.

Schwarzenberger, F., Möstl, E., Bamberg, E., Pammer, J., Schmehlik, O., 1991. Concentration of progestagens and oestrogens in the faeces of pregnant Lipizzan, Trotter and Thoroughbred mares. J. Reprod. Fertil. Suppl. 44, 489–499.

Schwarzenberger, F., Francke, R., Göltenboth, R., 1993. Concentrations of faecal immunoreactive progestagen metabolites during the oestrous cycle and pregnancy in the black rhinoceros (*Diceros bicornis michaeli*). J. Reprod. Fertil. 98, 285–291.

Schwarzenberger, F., Möstl, E., Palme, R., Bamberg, E., 1996a. Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. Anim. Reprod. Sci. 42, 515–526.

Schwarzenberger, F., Son, C.H., Pretting, R., Arbeiter, K., 1996b. Use of group-specific antibodies to detect faecal progesterone metabolites during the oestrous cycle of cows. Theriogenology 46, 23–32.

Schwarzenberger, F., Palme, R., Bamberg, E., Möstl, E., 1997. A review of faecal progesterone metabolite analysis for non-invasive monitoring of reproductive function in mammals. Inter. J. Mammal. Biol. 62 (Suppl. II), 214–221.

Smith, J.F., Payne, E., Peterson, A.J., McGowan, L.T., Cope, B., McLaughlin, R., 1990. Effects of phenobarbital, dietary protein intake, and ewe liveweight on ovulation rate and concentrations of plasma FSH and hepatic microsomal enzymes. Reprod. Fertil. Dev. 2, 623–632.

SPSS, 1998. SPSS User Guide: SPSS Inc.

Talke, H., Schubert, G.E., 1965. Enzymatishe hammstoffbestimmung in blut und serum im optishen test nach warburg. Klin Wochschr 43, 174–175.

Taylor, C., Rajamahendran, R., 1994. Effect of mid-luteal phase progesterone levels on the first wave dominant follicle in cattle. Can. J. Anim. Sci. 74, 281–285.

Thomford, P.J., Dziuk, P.J., 1986. The influence of dose of phenobarbital and interval to measurement of concentration of liver enzymes in barrows and gilts. J. Anim. Sci. 63, 1184–1190.

Thomas, D.L., Thomford, P.J., Crickman, J.G., Cobb, A.R., Dziuk, P.J., 1987. Effects of plane of nutrition and phenobarbital during the pre-mating period on reproduction in ewes fed differentially during the summer and mated in the fall. J. Anim. Sci. 64, 1144–1152.

Vasconcelos, J.L.M., Bungert, K.A., Tsai, S.J., Wechsler, F.S., Wiltbank, M.C., 1998. Acute reduction in serum progesterone concentrations due to feed intake in pregnant lactating dairy cows. J. Dairy Sci. 81 (Suppl. 1), 226.

Williams, A.H., Cumming, I.A., 1982. Inverse relationship between concentration of progesterone and nutrition in ewes. J. Agric. Sci. Camb. 98, 517–522.

Wise, T.H., Caton, D., Thatcher, W.W., Barron, D.H., Fields, M.J., 1982. Ovarian function during the estrous cycle of the cow: ovarian blood flow and progesterone release rate. J. Anim. Sci. 55, 627–638.