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EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$ AND ITS ANALOGS ON PLASMA ESTRADIOL 17-β AND PROGESTERONE LEVELS IN DOE RABBITS

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ABSTRACT : To assess the effect of prostaglandin $F_{2\alpha}$and two of its analogs on plasma estradiol-17β and progesterone levels in the immediate post partum period, 19 primiparous New Zealand White does were injected on day 29 of pregnancy with prostaglandin $F_{2\alpha}$ (Lutalyse: ®Upjohn; 4 does), cloprostenol (Estrumate: ®Mobay Corp.; 5 does), fenprostalene (Bovilene: ®Syntex Animal Health; 4 does), or sterile saline as a control (6 does). Treatment with prostaglandin $F_{2\alpha}$ and the two analogs shortened the gestation period by more than one day when compared to the saline treated group ($p<0.05$) but there were no effects of treatment on the number of kits born, number of kits born alive or the mean birth weight of the kits. Treatment with prostaglandin $F_{2\alpha}$ and analogs lowered the plasma estradiol-17β and progesterone levels immediately following treatment. After kindling, the prostaglandin-treated does had a dramatic increase in plasma estradiol peaking at three days and returning to post-treatment levels about day five. The treated does had low plasma progesterone levels with minor fluctuations until about six days post-partum when a slow rise began. It is speculated that the elevated estradiol and lowered progesterone levels could might have beneficial effects on prolactin related activities in the immediate post-partum period.

RESUME : Effets de la prostaglandine $F_{2\alpha}$ et de ses analogues sur les taux d'oestradiol 17-β et de progestérone chez la lapine. Dix-neuf lapines néo-zélandaises primipares ont été utilisées pour évaluer l'effet de la prostaglandine $F_{2\alpha}$ et de deux de ses analogues pendant la période immédiatement post-partum. La prostaglandine $F_{2\alpha}$ (Lutalyse : ®Upjohn, 4 lapines), le cloprostenol (Estrumate : ®Mobay Corp., 5 lapines) le fenprostalène (Bovilene : ®Syntex Animal Health, 4 lapines) et une solution saline stérile (lot contrôle, 6 lapines) ont été injectés à ces lapines, au 29ème jour de gestation. Le traitement par la prostaglandine $F_{2\alpha}$ et ses 2 analogues comparés à la solution saline, raccourcit la durée de la gestation de plus d'un jour ($P<0,05$) mais n'a pas de conséquences sur le nombre total de lapereaux nés, le nombre de lapereaux nés vivants ou le poids moyen des lapereaux à la naissance. La prostaglandine $F_{2\alpha}$ et ses 2 analogues diminuent, immédiatement après le traitement, les taux d'oestradiol 17-β et de progestérone sanguins. Au troisième jour après la mise bas, on observe un pic important du taux d'oestradiol chez les lapines traitées par la prostaglandine, qui revient le cinquième jour au niveau précédent le traitement. Les lapines traitées ont un taux de progestérone faible avec de petites fluctuations jusqu'à environ de sixième jour post-partum, puis une légère augmentation s'amorce. On peut supposer que les taux, élevé d'oestradiol et faible de progestérone, ont un effet bénéfique sur l'activité de la prolactine pendant la période post-partum.

INTRODUCTION

Prostaglandin $F_{2\alpha}$ and its metabolite13,14-dihydro-PGF-2α applied systemically result in declines in progesterone indicating luteolysis (KEHL and CARLSON, 1981). As discussed by numerous authors, e.g. MCNITT *et al.* (1997), exogenous prostaglandin $F_{2\alpha}$ can be used to induce kindling. REBOLLAR *et al.* (1989) found that induction of the previous parturition increased the fertility of the does in the subsequent litter. They attributed this phenomenon to a more rapid fall in the level of plasma progesterone leading to an increased secretion of GnRH, thus FSH and LH, resulting in a larger crop of follicles which could develop for the next cycle.

This study was carried out to evaluate the effects of prostaglandin $F_{2\alpha}$ and two of its analogs on plasma estradiol-17β and progesterone levels in the immediate post-partum period.

MATERIALS AND METHODS

Animals.

Primiparous New Zealand White does from the production herd of the Small Farm Family Resource Development Center were used for this study. Management of the does was similar to that normally used for commercial meat rabbits in the southern United States as previously described by MCNITT *et al.* (1997). The rabbits were housed in suspended, single deck, all wire cages (760 x 760 x 460 mm) inside a building with opened side panels that provided protection from rain and sun. Fans and a tube distribution system were used for air circulation when ambient temperatures exceeded about 23°C. The 16L:8D light cycle normally used in the rabbitry was maintained with automatic timers throughout the trial. Water was continuously available from automatic valves. A commercial, alfalfa-based, pelleted rabbit ration with a guaranteed minimum analysis of 18% crude protein and 2% crude fat and a maximum of 17% crude fiber was used.

To start the experiment, 28 does aged five to seven months at a weight of about 4,000 g were bred to four commercial New Zealand White bucks within a two hour period. As described by JENKINS and MCNITT (1998), on day 14, the does were manually palpated and 22 (78.6%) were found to be pregnant. Of these, 19 produced live litters and were used for hormone assays.

The does were randomly assigned to one of four treatment groups: saline, prostaglandin $F_{2\alpha}$ (Lutalyse : ®Upjohn), cloprostenol (Estrumate: ®Mobay Corp.) and fenprostalene (Bovilene: ®Syntex Animal Health)

Table 1. Treatments and number of animals used

Treatment	Abbreviation	Dosage/ rabbit	Route of administration	Nb of does
Prostaglandin $F_{2\alpha}$	LUT	5 mg	Intramuscular	4
Cloprostenol	CLO	35 µg	Intramuscular	5
Fenprostalene	FEN	35 µg	Subcutaneous	4
Saline	SAL	1 ml	Intramuscular	6

(Table 1) and injected on day 29 of pregnancy. Blood samples (about 1.5 ml/rabbit) were collected daily in the morning for 14 days starting 24 hr after drug administration. Blood was collected in prechilled heparinized tubes from the marginal ear vein. Plasma levels of estradiol-17β and progesterone were measured by radioimmunoassay.

Sample preparation.

The blood was centrifuged for 15 min at 1600g, 4°C and the plasma was collected and stored at -20°C until extraction of steroids. Twenty five µl aliquots were taken into glass culture tubes for extraction with hexane:ethyl acetate mixture (70:30 v/v). Extraction was done twice using 2.5 ml of the extraction solvent each time. The pooled organic phase was evaporated under a stream of air. The sides of the tubes were washed with 0.75 ml of absolute methanol, evaporated in stream of air, and stored at -20°C until assayed. Steroid concentration standards for the radioimmunoassay were prepared by evaporating known quantities of unlabeled estradiol or progesterone solutions in absolute methanol.

Extraction efficiencies, tested by adding a known amount of labeled steroid to a fixed volume of plasma, were 88.4 ± 3.4% and 86.6 ± 1.3% for estradiol and progesterone, respectively.

Radioimmunoassay.

[^{3}H]-steroids (17β-estradiol and progesterone) were obtained from DuPont New England Nuclear. Antibodies and standards were acquired from Wien Laboratories. The radioimmunoassay procedure was a modification of the procedure outlined by LIN *et al.* (1987). The procedure was first validated for use in our laboratory with rabbit blood. To measure the concentration of estradiol or progesterone, 25 µl of plasma was used. Preliminary experiments were carried out in which plasma was incubated at 4° C or room temperature for varying lengths of time to determine the optimum incubation time and temperature (Table 2). Based on the results of these trials, incubation was carried out at 4°C for 60 min. Bound and free tracer were separated using 0.5 ml cold dextran coated charcoal. Radioactivity was counted using a liquid scintillation counter (Beckman LS 6000SC).

The intra- and inter-assay coefficients of variation for the RIA were, respectively, 10.8 and 11.4% for estradiol and 7.3 and 11.1% for progesterone. For each of the steroids standards of 10 pg could be distinguished from the buffer blank.

Data analysis.

Post-kindling plasma hormone levels were analyzed by repeated-measures analysis of variance, using the SAS GLM MIXED procedure as described by LITTELL *et al.* (1996; 1998). Preliminary analyses indicated all correlation coefficients between the repeated measures were near zero, so all further analyses were carried out using univariate techniques.

RESULTS AND DISCUSSION

Effects on kindling

In agreement with previous work (MCNITT *et al.* 1997) treatment with prostaglandin $F_{2\alpha}$ and the two analogs shortened the gestation period when compared to the saline treated group ($p<0.05$). Does treated with saline (SAL) kindled after 3.67 ± 0.33 days whereas does treated with lutalyse (LUT) kindled after 2.25 ± 25 days, those treated with cloprostenol (CLO) kindled after 2.20 ± 0.20 days, and those treated with fenprostalene (FEN) kindled after 2.00 ± 0.00 days. There were no

Table 2 : Effects of incubation temperature and time on [^{3}H] estradiol-17β and [^{3}H] progesterone binding

Steroid	Temp.	Time (min.) 15	30	45	60	90	120
					% bound[1]		
Estradiol-17β	4°C	37.2 ± 1.1	42.6 ± 0.8	45.0 ± 1.2^b	46.8 ± 0.8^b	48.8 ± 1.5^b	50.4 ± 0.7
	RT[2]	39.4 ± 1.2	42.1 ± 0.6	41.6 ± 0.6^a	41.5 ± 0.7^a	42.0 ± 0.6^a	42.9 ± 0.8
Progesterone	4°C	39.4 ± 2.4	41.2 ± 1.0	43.0 ± 1.9^b	44.8 ± 1.3^b	45.5 ± 2.1	50.9 ± 5.6
	RT	33.2 ± 2.1	35.0 ± 2.3	35.6 ± 1.9^a	36.1 ± 1.9^a	37.5 ± 1.9	38.3 ± 2.9

[1] Within steroid, values in the same column with differing superscripts are different ($P<0.05$).
[2] RT= Room temperature (about 22°C)

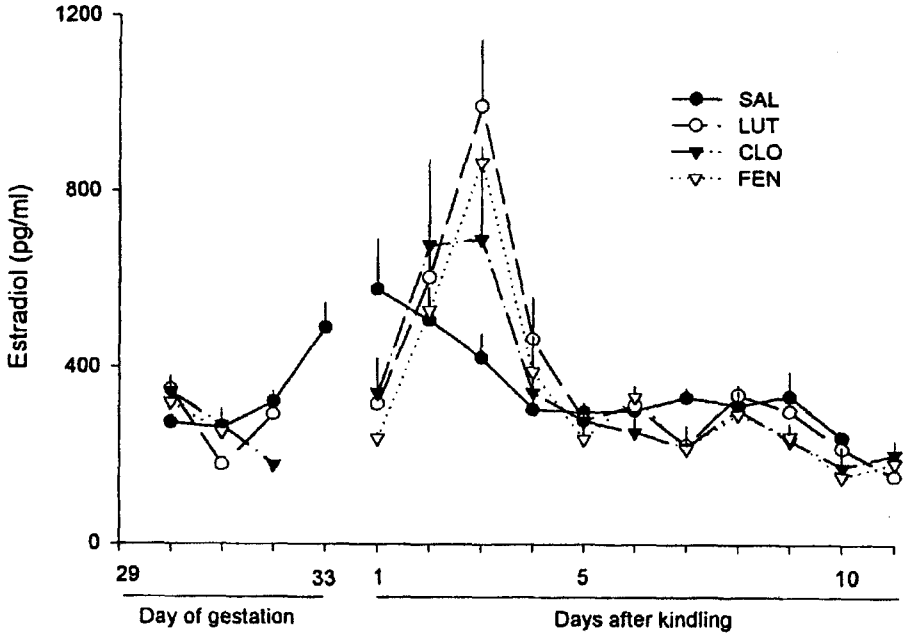

Figure 1 : The effects on plasma estradiol level of injection on day 29 of pregnancy with saline (SAL), lutalyse (LUT), cloprostenol (CLO) or fenprostalene (FEN)

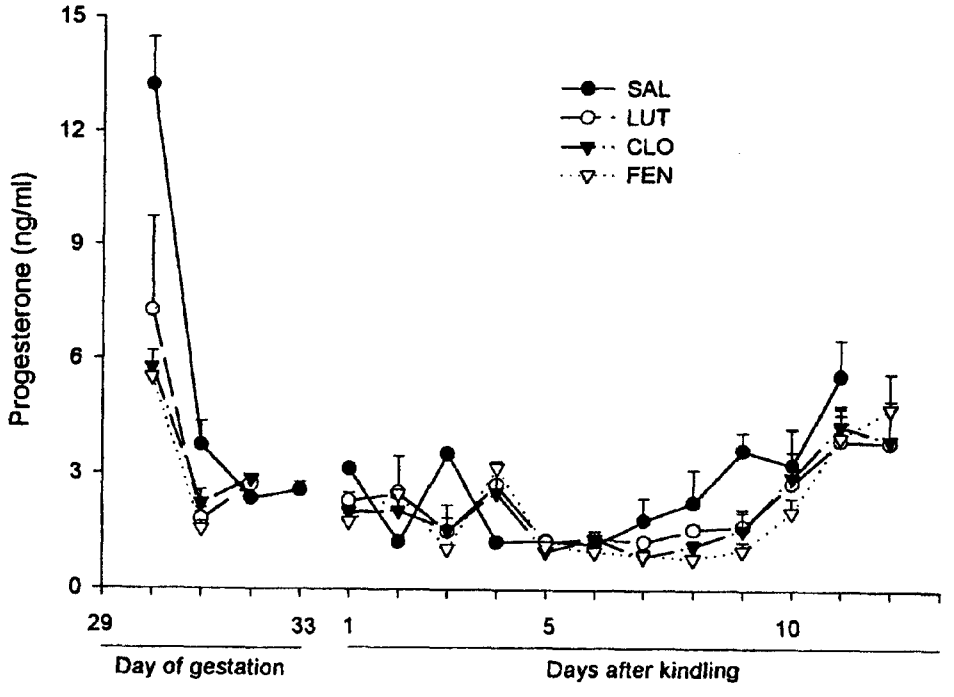

Figure 2 : The effect on plasma progesterone levels of injection on day 29 of pregnanc with saline (SAL), lutalyse (LUT), cloprostenol (CLO) or fenprostalene (FEN)

differences among the treated does. As shown in Table 3, there were no effects of treatment on the number of kits born, number of kits born alive, the mean birth weight of the kits or litter three week weight.

Table 3 : Production results for does treated with prostaglandin $F_{2\alpha}$ and its analogs (least square means ± standard error).

Treatment[1]	LUT	CLO	FEN	SAL
Total kits born (n)	8.5 ± 1.2	7.2 ± 1.0	5.5 ± 1.2	6.5 ± 1.0
Kits born live (n)	8.0 ± 1.1	6.8 ± 1.0	5.5 ± 1.1	6.3 ± 0.9
Kits born dead (n)	0.5 ± 0.3	0.4 ± 0.3	0.0 ± 0.3	0.2 ± 0.3
Mean kit weight (g)	47.7 ± 5.3	50.7 ± 4.7	49.0 ± 5.3	56.2 ± 4.3
Litter 3-week wt. (g)	1620 ± 271	1647 ± 271	1515 ± 313	1373 ± 242

[1] LUT = prostaglandin $F_{2\alpha}$ (Lutalyse: ®Upjohn); CLO= cloprostenol (Estrumate: ®Mobay Corp.); FEN= fenprostalene (Bovilene: ®Syntex Animal Health) and SAL= 0.9% sterile saline

Effects on plasma estradiol and progesterone levels

The effects of $PGF_{2\alpha}$ and its analogs on estradiol and progesterone production are presented in Figures 1 and 2. Since the rabbits kindled on different days, statistical analysis was performed for estradiol and progesterone levels post-kindling using day of kindling as day 0.

Treatment with prostaglandin $F_{2\alpha}$ and analogs lowered the plasma estradiol level following treatment (Figure 1). REBOLLAR *et al.* (1997) reported a similar pattern although in that study, the estradiol levels of the uninjected controls also dropped, though not as sharply as the $PGF_{2\alpha}$ treated does. MUNSEL *et al.* (1982) using untreated does found a sharp drop the day before parturition with a return to pre-parturient levels within two days. In the present work, there was an increase in the plasma estradiol levels at the time of parturition for the SAL does. This gradually declined until reaching pre-kindling levels about four days post kindling. The prostaglandin-treated does all showed a steep increase in plasma estradiol post-kindling peaking at three days and returning to post-treatment levels about day five post-kindling. After four or five days post-kindling, the estradiol levels remained fairly constant throughout the remainder of the study. A number of workers (e.g. VERMOUTH and DIAS, 1974; GUDELSKY *et al.* 1981; SOAJE and DEIS, 1994) have shown that, in pregnant or post-partum rats, estrogen stimulates prolactin secretion whereas progesterone is inhibitory. Because of the effect of estradiol increasing prolactin activity, the increased post-partum estradiol levels of the $PGF_{2\alpha}$ treated does could have important implications for mothering behavior and milk production; especially in the first few days post-partum. However, the litter three week weights shown in Table 3 indicate no significant differences between the SAL and the treated does. The lack of significance may have been due to the small sample sizes although similar results were reported by UBILLA and RODRIGUEZ (1989). Since the effect appears in the first week of lactation, the differences may be less apparent by three weeks of age. However, PARTRIDGE *et al.* (1986) weighed kits at day 1 and at day 7 and found no effect of treatment on the growth rates of the kits in that early period.

Comparison of estradiol levels between each of the treatments (LUT, CLO, FEN) versus the control (SAL) indicate significant differences on some of the sampling days. The values for LUT and FEN groups were different from the SAL group on days 1, 3 and 7. The CLO group differed from the SAL group only on day 7. At all other days there was no significant difference between the treated and SAL groups. The day effects and the day x treatment interaction were important sources of variation ($P<0.05$). However, when data for LUT, CLO and FEN only were considered, the day x treatment interaction was not significant, indicating that the response profile is not different between these groups. The implication is that the response profile of the SAL group was different from the treated groups. Contrasts between the LUT, CLO and FEN groups only showed no differences among the plasma estradiol levels at each of the sampling days following kindling.

In agreement with REBOLLAR *et al.* (1997), treatment with $PGF_{2\alpha}$ and its analogs caused a sharp drop in plasma progesterone levels immediately after administration (Figure 2). A similar drop was seen for the SAL group but it occurred two days later than with the treated does. The treated does had low plasma progesterone levels with minor fluctuations until about six days post-partum when a slow rise began. A similar rise was not reported by MUNSEL *et al.* (1982). In the present study however, following normal practice for this rabbitry, 18 of the does were remated seven days post-partum which may have influenced the results. Plasma progesterone levels of the saline treated does fluctuated for the first four days post-partum then, at day 6, began to rise somewhat faster than the levels of the treated does. In agreement with UBILLA *et al.* (1992), prior treatment with prostaglandins did not affect conception rates of the does that were rebred. The pregnancy rates, determined by manual palpation at 14 days, were LUT 50%, CLO 60%, Fen 50% and SAL 20%. Based on χ^2 analysis, the differences between the SAL and treated does were not significant. The experiment was carried out during the summer when the daily maximum temperatures regularly exceed 30°C (MCNITT and MOODY, 1990) which was probably the cause for the extremely poor pregnancy rates for all groups.

Comparing the progesterone level of the treatments with the SAL group indicated less day-to-day variability for the treated groups as well as important differences ($P<0.05$) for some of the sampling days. The values for

FEN does were different from SAL on days 1, 3, 4, 8 and 9. The LUT and CLO groups differed from SAL only on days 1 and 9. On all other days following kindling, there was no significant difference between the treated and SAL groups. On the days where differences were noted the values for the treated groups were lower than that of SAL except that of the FEN group on day 3. On day 3 the value for the FEN group was higher than the SAL group. As with the effects of elevated estradiol increasing prolactin activity, the lower levels of progesterone in the post-partum period may mean less inhibition of prolactin.

The day x treatment interaction was not significant. Contrasts between the treatment groups (CLO vs. FEN, CLO vs. LUT, and LUT vs. FEN) indicate that on day 1 the values for CLO and FEN groups were different, and on day 8 there was a difference between the three comparison groups. There were no differences among the groups on other days.

In summary, prostaglandin $F_{2\alpha}$ and two of its analogs applied on day 29 of pregnancy shortened the gestation period, but had no effect on the production traits measured. There were no consistent differences among the effects of the three treatments on pregnancy nor on hormone levels. Plasma progesterone and estradiol 17β levels dropped immediately after treatment. In the post-partum period, the estradiol 17β levels rose to about day three and then dropped to a pretreatment level by day five. The progesterone levels remained at a low level until about day six when a slow rise began. It is suggested that the elevated estradiol 17β and lowered progesterone levels could have positive effects on prolactin related activities of the lactating does.

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