THE SYNCHRONISATION OF OESTRUS IN SHEEP. 2. DOSE EFFECT OF PROSTAGLANDIN IN THE DOUBLE INJECTION REGIME

Receipt of MS 20-04-1979

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(Key words:Synchronisation, oestrus, sheep, prostaglandin, progesterone)(Sleutelwoorde:Sinkronisasie, estrus, skape, prostaglandien, progesteroon)

OPSOMMING: SINKRONISASH VAN ESTRUS BY SKAPE, 2. DIE EFFEK VAN PROSTAGLANDIEN DOSIS IN DIE DUB-BELE INSPUTTINGS TEGNIEK

Aangesien die corpus luteum van die ooi net gedurende dae 4 tot 14 van die estrus siklus gevoelig is vir die luteolitiese effek van prostaglandien, is dit noodsaaklik om twee inspuitings met 8 tot 14 dae tussenposes toe te dien ten einde alle ooie te sinkroniseer. Twee inspuitings elk bestaande uit $31,25\,\mu$ g; $62,5\,\mu$ g; $125\,\mu$ g of $250\,\mu$ g cloprostenol is met 'n tussenpose van 10 dae toegedien. 'n Verhoging van die dosis is gevolg deur 'n betekenisvolle verbetering in die aantal ooie wat estrus getoon het (50,0 persent: 56,3 persent; 81,3 persent en 100,0 persent). Te oordeel aan die verandering in die serum progesteroon konsentrasies, het die hoër dosisse ($125\,\mu$ g en $250\,\mu$ g) cloprostenol 'n meer doeltreffende luteoliese veroorsaak, terwyl die laer dosisse ($31,25\,\mu$ g en $62,5\,\mu$ g) dikwels onvolledige luteoliese tot gevolg gehad het.

SUMMARY.

In order to overcome the refractory period of the corpus luteum to prostaglandin (day 15 of the cycle through oestrus to day 3 of the next cycle), two injections each consisting of either $31,25\,\mu g$; $62,5\,\mu g$; $125\,\mu g$ or $250\,\mu g$ of cloprostenol were injected at a 10-day interval. An increase in the dosage was followed by a significant increase in oestrous response (50,0 per cent; 56,3 per cent; 81,3 per cent and 100,0 per cent). Higher dosages ($125\,\mu g$ and $250\,\mu g$) of cloprostenol caused more rapid and more complete luteolysis as reflected by the decrease in the plasma progesterone concentration, while lower dosages ($31,25\,\mu g$ and $62,5\,\mu g$) often fialed to induce complete luteolysis.

The corpus luteum of the ewe is responsive to cloprostenol treatment only between days 4 and 14 of the oestrous cycle (Greyling, 1978). Thus, to overcome the insensitive period of the ovary to prostaglandin, a double injection of cloprostenol was given 8 to 14 days apart with $100\mu g$ or $125\mu g$ cloprostenol (Fairnie, Cumming & Martin, 1976; Haresign, 1976). The minimum effective dose of cloprostenol sufficient to induce luteolysis and a consequent decrease in serum progesterone concentration, when employing the double injection regime, was investigated in this experiment.

Procedure

The experiment was conducted during February (1978) using 64 mature S.A. Mutton Merino ewes in which the stage of the oestrous cycle had previously been recorded. The ewes were divided into four groups

of 16 ewes such that when the first injection of cloprostenol was administered, each group consisted of two sheep which had reached day 2, 4, 6, 8, 10, 12, 14 or 16 of the oestrous cycle (day of oestrus = day 0). The four groups each received two intramuscular injections of the following doses cloprostenol ("Estrumate" - ICI 80996) at a 10-day interval:

| 1 | : | 31,25µg |
|---|---|---------|
| 2 | : | 62,5µg |
| 3 | : | 125µg |
| 4 | : | 250µg |
| | 3 | 3 : |

Following the second injection of cloprostenol the ewes were regularly tested (08h00 and 16h00) for oestrus using vasectomised rams and blood samples (10ml) were collected (at eight-hour intervals) for a period of 96 hours or until the ewes showed oestrus. The serum was recovered and stored at -20 °C until assayed for serum progesterone concentration by the RIA technique of Yousefnejadian, Florensa, Collins & Sommerville (1972), as modified by Faure (1975).

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Table 1

The oestrous response, the interval to onset of oestrus (hours) and serum progesterone concentrations in ewes receiving two injections of 31,25 µg, 62,5 µg, 125 µg or 250 µg cloprostenol 10 days apart

| | Group 1 (31,25µg) | Group 2 (62,5µg) | Group 3 (125µg) | Group 4 (250µg) |
|-------------------------------------|-------------------------|----------------------------|----------------------|-----------------------------|
| Number of Ewes | 16 | 16 | 16 | 16 |
| Number of Ewes showing oestrus (%) | 8 (50,0) <u>a</u> | 9 (56,25) <mark>a</mark> – | 13 (81,25) <u>b</u> | 16 (100,0) <u>b</u> |
| Interval between Second Injection | | | | |
| and onset of Oestrus (h) | $39,6 \pm 21,17$ | $41,56 \pm 4,71^{a}$ | 45,38± 9,91 <u>a</u> | 44,31 ± 7,59 ^a - |
| Range (h) | 8 - 72 | 35 - 48 | 32 - 72 | 32 - 56 |
| Mean serum progesterone concen- | | | | |
| tration at onset of oestrus (ng/ml) | $1,44 \pm 0,57^{a}_{-}$ | 1,45 ± 0,53 ^a | 1,31± 0,33 <u>a</u> | 0,96 ± 0,46 ^a |
| a b | | | | |

 $\frac{a}{b}$ Within the body of the table, figures having the same superscript are not significantly different from each other.

Results

The results (Table 1) indicate that when the dose of cloprostenol exceeded 62,5µg this was accompanied by a significant increase in the oestrous response. The higher dosage (250 ug) also produced the most favourable response in terms of both synchronisation of oestrus and proportion of ewes exhibiting oestrus. There was a great variation in the oestrous response by ewes in Group 1 (31,25 μ g) where only 50 per cent showed oestrus within the 96 hour observation period, compared to Group 4 ($250\mu g$) where all the ewes responded within 56 hours (P < 0.05). Furthermore, the day of the oestrous cycle on which cloprostenol was first administered did not influence the oestrous response of the ewes to this prostaglandin analogue. The interval between the second injection and the onset of oestrus, did not differ significantly between the treatment groups.

According to changes in the serum progesterone concentrations (Fig. 1 and 2) it is obvious that $250\mu g$ of cloprostenol caused complete and rapid luteolysis in all the ewes (Fig. 2), while the lower dosages often resulted in only a temporary decrease in circulating progesterone (Fig. 1). For the sake of comparison, the rates of decrease in serum progesterone concentration of all the ewes which responded are presented in Figure 2. Although the injection of $250\mu g$ cloprostenol caused the most rapid decrease in serum progesterone concentration, the differences in the rates of decrease, between groups, were not significant. The mean serum progesterone concentrations at the onset of oestrus did not differ significantly between groups.

Discussion

The results presented here demonstrate that oestrus can be synchronised very efficiently by two injections of $250 \mu g$ cloprostenol given at a 10-day interval. Lower doses were often insufficient to induce complete luteolysis. In those ewes which did not respond fully to the luteolytic effect of cloprostenol and

failed to exhibit oestrus an initial decline in serum progesterone level following cloprostenol injection was followed by a gradual increase in serum progesterone concentration which indicated that luteal function recovered. Similar results have been reported by Thorburn and Nicol (1971). The occurrence of this phenomenon increased in frequency as the dose of cloprostenol decreased and was apparently not related to the stage of the cycle at which treatment commenced.

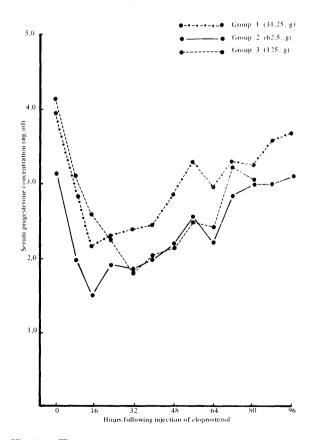


Fig. 1 The mean serum progesterone concentrations of those ewes failing to show oestrus when cloprostenol was administered according to the double injection regime, but using various dose levels

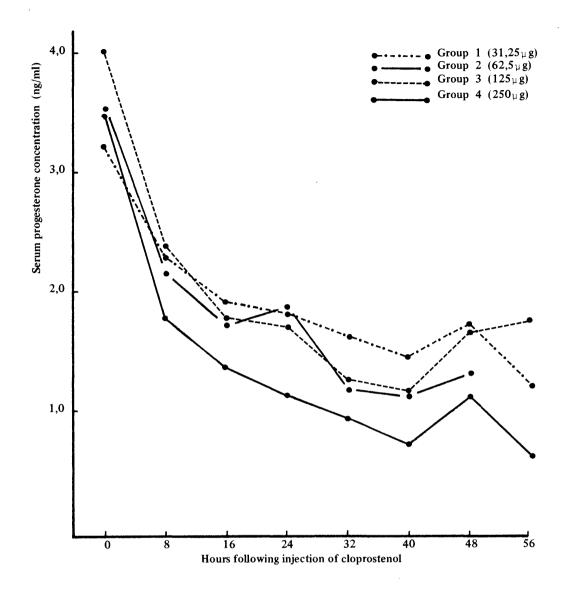


Fig. 2 Serum progesterone concentrations of all ewes responding to the double injection treatments

References

- FAIRNIE, I.J., CUMMING, I.A. & MARTIN, E.R. 1976. Use of prostaglandin analogue ICI 80996, to synchronise ovulation in sheep in an artificial insemination programme. *Proc. Aust. Soc. Anim. Prod.* 11, 133.
- FAURE, A.S. 1975. Vroeë embrionale verlies weens wanvoeding by Merinoskape. M.Sc. (Landbou), Universiteit van Stellenbosch.
- GREYLING, J.P.C. 1978. Control of ovulation in cycling ewes with a prostaglandin $F_{2\alpha}$ analogue. M.Sc. (Agric). Thesis, University of Stellenbosch.
- HARESIGN, W. 1976. Controlled breeding in sheep using the prostaglandin analogue ICI 80996. Proc. Brit. Soc. Anim. Prod. 22, 137.
- THORBURN, G.D. & NICOL, D.H. 1971. Regression of the ovine corpus luteum after infusion of prostaglandin F_{2∝} into the ovarian artery and uterine vein. J. Endocr. 51, 785.
- YOUSEFNEJADIAN, E., FLORENSA, E., COLLINS, W.P. & SOMMERVILLE, I.F. 1972. Radioimmuno-assay of plasma progesterone. J. Steroid Biochem. 3, 893.