Relationship between the fertility of superovulated ewes and the preovulatory E_2 surge

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The influence of the preovulatory oestradiol-17ß (E_2) surge on yield of viable embryos was studied in 28 oestrus synchronised SAMM ewes. Ewes were superovulated either with FSHo (88 units in 6 divided doses) or PMSG (1200 IU). Blood samples for E_2 were drawn at 4-h intervals for 48 h after progestagen sponge withdrawal. Each ewe was mated six times by natural service. Embryos were recovered on the sixth day after oestrus. Ewes which received FSHo had significantly higher ($p < 0.05$) ovulation rates and total ovarian responses ($p < 0.01$) than those treated with PMSG. Higher recovery rates ($p < 0.01$), fertilization rates ($p < 0.01$), percentage ($p < 0.01$) and yield ($p < 0.05$) of viable embryos were also obtained for FSHo than for PMSG treatment. Although the timing of the E_2 surge in relation to sponge withdrawal varied with the superovulatory treatment, no relationship between peculiarities of the surge and embryo characteristics could be established.

Die invloed van die voorovulasiese vrystelling van estradiol-17ß (E_2) op die opbrengs van lewensvatbare embrios is bestudeer by 28 estrusgesinchroniseerde SAVM ooie. Superovulasie is deur middel van óf FSHo (88 eenhede in 6 verdeelde dosisse) of DMSG (1200 IE) bewerkstellig. Bloedmonsters vir E_2 ontleding is met vieruurlikse tussenposes vir 48 h na progesteroonsponsonttrekking verkry. Ooie is elk ses keer gedek (natuurlike dekking). Embrios is op die sesde dag na estrus herwin. FSHo het 'n betekenisvolle ($p < 0.05$) hoër ovulasietempo en totale ovariese respons ($p < 0.01$) teweeggebring, as DMSG. Hoër herwinningstempos ($p < 0.01$), bevrugtingstempos ($p < 0.01$), persentasie ($p < 0.01$) en opbrengs ($p < 0.05$) van lewensvatbare embrios is ook waargeneem waar FSH toegedien is. Alhoewel die tyd van E_2 vrystelling, met betrekking tot sponsonttrekking, deur superovulasiebehandeling beïnvloed is, kon geen verwantskap tussen kenmerke van die vrystelling en embrio-eienskappe vasgestel word nie.

Keywords: Fertility, superovulated ewes, preovulatory E_2

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Introduction

The considerable amounts of E_2 produced by ovulatory follicles (Campbell *et al.*, 1990; Baird *et al.*,1991) are directly responsible for oestrus and also trigger the pre-ovulatory LH surge which, in turn, precipitates ovulation. Although the E_2 surge has important functions, excessive secretion in superovulated animals may detrimentally influence the yield of viable embryos (Jabbour & Evans, 1991). Negating the effects of high preovulatory E_2 levels in superovulated ewes can improve their fertility (Lishman *et al.*, 1996), but it is unclear what aspect(s) of preovulatory E_2 adversely affect fertility. Clarification is required as to whether it is the magnitude or duration of the preovulatory E_2 surge, the time of mating relative to the release of E_2 or some other characteristic that reduces fertility. If a direct relationship between such characteristics and the fertility of superovulated ewes could be found, it would have considerable practical importance in the development of improved superovulation protocols. Techniques could then be developed and evaluated on their ability to remove or reduce the negative components. By improving the fertilization rates of such ewes, the yield of viable embryos might be increased.

Therefore, the following investigation was designed to determine:

(1) If any direct relationship exists between characteristics of the preovulatory E_2 surge and the fertility of superovulated ewes?

(2) Whether there are differences in the characteristics of the preovulatory E_2 release in ewes superovulated either with FSHo or PMSG?

Ewes superovulated with PMSG usually have a poor fertility (Bindon & Piper, 1982) in contrast to superovulation with FSHo. Differences in the characteristics of the preovulatory E_2 surge resulting from the use of such gonadotropin sources might then explain the observed differences in fertility.

Materials and Methods

Experimental design

Twenty eight mature South African Mutton Merino ewes were treated with 60 mg medoxyprogesterone acetate intravaginal sponges (Repromap, Upjohn) for 14 days to synchronize oestrus. The ewes were randomly divided into two equal groups one of which (Group 1) was superovulated with FSHo (Ovagen), administered on three successive days as two intramuscular injections per day (07:30 and 16:30), starting 48 h before sponge withdrawal. A decreasing dose regime of 22, 22, 13.75, 13.75, 8.25 and 8.25 units was used. Group 2 ewes received 1200 IU of PMSG (Fostim) as a single intramuscular injection 48 h before sponge withdrawal.

Characterization of the preovulatory E_2 surges

To enable characterization of the preovulatory E_2 surge profiles, 10 ml blood samples were collected by jugular venepuncture (into heparinized tubes) at 4-h intervals for 48 h after sponge withdrawal (13 samples per ewe). This sampling interval was chosen so as to avoid unduly stressing the exper-

imental animals. Plasma was stored at –20 °C until assayed for E_2 by RIA according to the method of Butcher *et al.* (1974). The sensitivity of the assay was 1.3 pg/ml (the value of twice the standard deviation for the blank sample) and the inter- and intra-assay coefficients of variation were 12% and 5%, respectively.

The characteristics of the preovulatory E_2 surge that were recorded were:

(1) the total area of the surge during the sampling period,
(2) the time from sponge withdrawal to, and the magnitude of the peak,
(3) the time from the peak to the end of the surge,
(4) the area of the surge before and also the area after the peak,
(5) the time from sponge withdrawal to oestrus/mating,
(6) the E_2 level at mating,
(7) the time from mating to the end of the surge,
(8) the area of the surge before mating,
(9) the area of the surge after mating.

Area measurements provided a measure of exposure to E_2 (concentration/time). Sponge withdrawal was considered as the starting point of the surge and the end was regarded as the point where E_2 levels dropped below 3 pg/ml or when blood sampling ceased.

The effects of these characteristics on the yield of viable embryos were examined.

Management of experimental animals and mating procedures

The ewes were penned for the duration of the experiment and were fed kikuyu silage. Sponges were removed at 02:00 to negate the effects of high midday temperatures on mating. After sponge withdrawal, a single ram was drawn from a pool of five rams and placed with the ewes for a period of 1 h, after which he was replaced by another ram. This ensured that no single ram was overworked. Ewes that came into oestrus were allowed to mate twice with the ram, within a period of no more than 15 min, after which the ewe was removed from the pen. One hour later, the ewe was introduced to another penned ram and again allowed to mate twice. This procedure was then repeated again, 1 h later, with a third ram. In each case, a period of no more than 15 min was allowed for mating to occur twice. Thus, each ewe was mated six times over three hours.

Recovery and assessment of embryos

Ewes were slaughtered on the sixth day after oestrus, the reproductive tract removed and the uterine horns flushed to recover embryos and unfertilized ova. Dulbecco's PBS containing 1% foetal calf serum and 0.1% gentamycin was used as the flushing medium. Approximately 10 ml of flushing medium was used to flush each horn. The recovered embryos were then assessed for normality under a light microscope at 45× magnification. Any oocytes/embryos at the 1-cell stage were regarded as unfertilized ova. The ovulation rate, total ovarian response and the percentage of unovulated follicles were determined.

Statistical analysis

Data for the time to onset of oestrus, total ovarian response, number of ovulations, yield of viable embryos and the preovulatory E_2 peak corrected for the total ovarian response, were analysed by analysis of variance (ANOVA). Data for responses expressed in proportions, such as fertility, were analysed by logistic analysis. All data were analysed using Genstat (Version 5.1.3, 1988, Lawes Agricultural Trust, Rothamstead Experimental Station, U.K.).

Results

Oestrus and ovulatory responses

Two of the ewes in Group 1 lost the progestagen sponges and were excluded from the experiment. Only 50% (6/12) of the ewes treated with FSHo exhibited behavioural oestrus after sponge withdrawal (Table 1), and the higher percentage among PMSG-treated ewes was not significant. Ewes that did not exhibit oestrus were also excluded from the experiment. Significant differences in the ovulation rate ($p < 0.05$) and the total ovarian response ($p < 0.01$) were observed between the two treatments (Table 1). The mean number of ovulations was 10.0 ± 1.6 and 5.5 ± 1.2 in Groups I and II respectively. The differences were significant ($p < 0.05$). The total ovarian response (ovulations plus unovulated follicles) were more ($p < 0.01$) in Group I than Group II (15.0 ± 1.8 vs. 7.5 ± 1.3).

Recovery rates and embryo characteristics

Less than 50% of the embryos were recovered in ewes superovulated with PMSG (Table 2), while the percentage of embryos recovered and judged to be fertile and viable, were higher ($p < 0.01$) in FSHo-treated ewes than PMSG-treated ewes. The fertility rate of ewes treated with FSHo was nearly 51% greater than that of PMSG-treated ewes. This was largely due to the fact that only three of the 11 PMSG-treated ewes that responded, produced fertile ova. The mean number

Table 1 Oestrus and ovulatory responses of ewes superovulated with FSHo or PMSG (values are means ± s.e.m)

	FSHo ($n = 6$)	PMSG ($n = 11$)	p
Incidence of oestrus (%)	6/12 (50.0)	11/14 (78.6)	N.S.
No. of ovulations	10.0 ± 1.6	5.5 ± 1.2	< 0.05
Total ovarian response[1]	15.0 ± 1.8	7.5 ± 1.3	< 0.01
% Follicles unovulated[2]	33.3 ± 5.0	26.8 ± 4.9	N.S.

[1] no. of ovulations + no. of large (> 5 mm in diameter) unovulated follicles

[2] (no. of large unovulated follicles)/(total ovarian response)

Table 2 Recovery rates and characteristics of embryos recovered from ewes superovulated with FSHo or PMSG (values are means ± s.e.m.)

	FSHo ($n = 6$)	PMSG ($n = 11$)	p
% Recovered[1]	73.3 ± 5.7	46.7 ± 6.4	< 0.01
% Fertile[2]	68.2 ± 7.0	17.9 ± 7.2	< 0.01
% Viable[2]	59.1 ± 7.4	17.9 ± 7.2	< 0.01
Yield of viable embryos	4.3 ± 1.2	0.5 ± 0.9	< 0.05

[1] (no. of recovered embryos)/(no. of ovulations)

[2] expressed as a percentage of the no. of embryos recovered

of viable embryos produced by FSHo-treated ewes was nearly nine times greater than that produced by ewes superovulated with PMSG (Table 2).

Characteristics of the preovulatory E_2 surges

From the results in Figure 1 it is clear that up to 24 h into the sampling period, the mean E_2 levels of PMSG-treated ewes were significantly higher than those of ewes treated with FSHo. Conversely, from 40 h after sponge withdrawal FSHo-treated ewes had significantly higher mean E_2 levels than those of PMSG-treated ewes.

E_2 levels of FSHo-treated ewes did not reach basal levels by the end of the sampling period (Figure 1). Therefore, no values for the time after the E_2 peak for E_2 to reach basal levels, the area of the surge after the E_2 peak, the time after mating for E_2 to reach basal levels and the area of the surge after mating could be obtained.

There was a strong, but non-significant tendency, for the corrected total area to be larger in the PMSG-treatment group than the FSHo-treatment group (Table 3). Although the corrected area of the surge before the E_2 peak was not significantly different between treatments, that before oestrus/mating was larger ($p < 0.05$) in PMSG-treated ewes than FSHo-treated ewes (Table 3). Similarly, no significant difference between treatments was observed for the corrected E_2 peak, but there was a strong, but non-significant, tendency for the corrected E_2 level at mating to be higher in PMSG-treated ewes than for FSHo-treated ewes, with E_2 levels at this time being nearly two-fold higher in PMSG-treated ewes (Table 3).

The preovulatory E_2 surge peaked significantly earlier ($p < 0.01$) in relation to the time of sponge withdrawal in PMSG-treated ewes than ewes superovulated with FSHo (Table 3). Thus oestrus/mating also occurred earlier ($p < 0.05$) after sponge withdrawal in PMSG-treated ewes than in ewes treated with FSHo (Table 3).

From the data in Table 3 it is apparent that ewes superovulated with PMSG tended to exhibit oestrus and thus mate just after the preovulatory E_2 surge had peaked. This was in contrast to ewes superovulated with FSHo, that exhibited oestrus and hence mated well before the peak of the preovulatory E_2 surge.

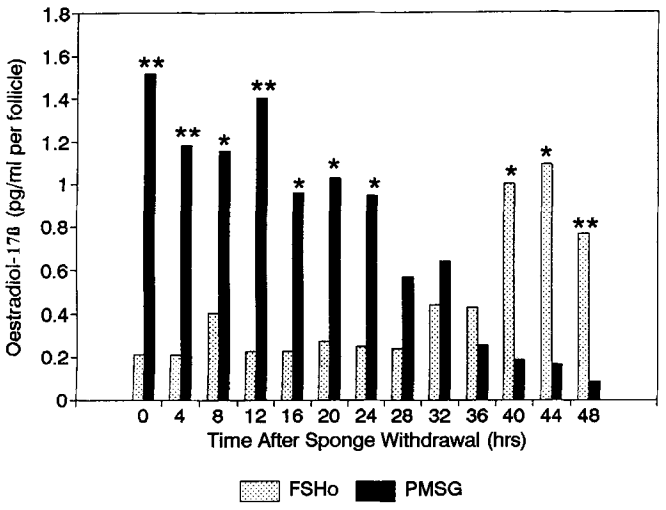

Figure 1 Mean E_2 levels, corrected for the total ovarian response, at various stages after sponge withdrawal in ewes superovulated with FSHo or PMSG.

Table 3 Characteristics of the preovulatory E_2 surge in ewes superovulated with FSHo or PMSG (values are means ± s.e.m.)

	FSHo ($n = 6$)	PMSG ($n = 11$)	p
Total area[1]	26.5 ± 9.6	44.7 ± 7.1	N.S.
Area before peak[1]	21.3 ± 8.1	21.0 ± 6.0	N.S.
Area before oestrus/mating[1]	14.5 ± 6.7	25.6 ± 5.0	< 0.05
Peak E_2[1]	1.7 ± 0.5	2.3 ± 0.3	N.S.
E_2 at oestrus/mating[1]	0.6 ± 0.3	1.1 ± 0.3	N.S.
Time to peak[2] (hours)	42.0 ± 4.3	15.3 ± 3.2	< 0.01
Time to oestrus/mating[2] (hours)	32.5 ± 2.9	22.1 ± 2.1	< 0.05
Time between oestrus/mating and peak[3] (hours)	10.8 ± 5.7	−5.5 ± 4.2	< 0.05

[1] corrected for the total ovarian response; units = pg/ml*hour*follicle^{-1}

[2] time = 0 at sponge withdrawal

[3] negative sign = oestrus/mating after peak

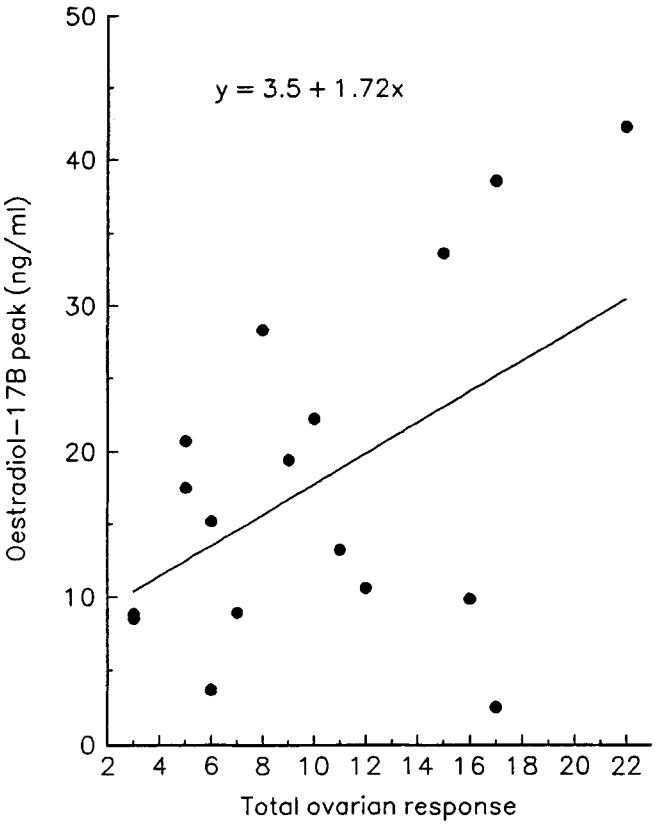

Figure 2 The relationship between the preovulatory E_2 peak and the total ovarian response in superovulated ewes.

The preovulatory E_2 surge and embryo characteristics

No relationships could be found between the characteristics of preovulatory E_2 surge and fertility, embryo viability or the yield of viable embryos. However, a significant ($p < 0.01$) relationship between the preovulatory E_2 peak and the total ovarian response was observed. A comparison of regression

analysis indicated that a single linear regression line could be fitted to both treatment groups (Figure 2). The adjusted regression coefficient was 60.4%.

Discussion

This investigation failed to show any direct relationship between the characteristics of the preovulatory E_2 surge and the fertility of superovulated ewes. However, this result must be treated with caution as only three of the 11 PMSG-treated ewes produced any fertile ova at all and there were only six observations for the FSH-treatment group. This was unfortunate as other observations in this study provided indirect evidence that such a relationship existed. The significant treatment differences in the proportion of embryos recovered, matured, fertile and viable, might be due, at least in part, to treatment differences in the characteristics of the preovulatory E_2 surge.

The results of this investigation confirm the contention (Jabbour & Evans, 1991) that PMSG is substantially more steroidogenic than FSHo. At sponge withdrawal, PMSG-treated ewes already had elevated E_2 levels. This was in contrast to FSHo-treated ewes, where E_2 levels began to rise markedly, only 40 h after sponge withdrawal. The result was that the preovulatory E_2 surges of PMSG-treated ewes reached peaks that were not significantly different from FSH-treated ewes, but that occurred 26.7 ± 5.3 h earlier. This reaffirms the findings of Moor *et al.* (1985) and Driancourt (1991) who found that follicles stimulated by PMSG are more steroidogenic than follicles stimulated by FSH. Further evidence that PMSG is more steroidogenic than FSHo comes from the observation of a strong tendency for the corrected total area of the surge to be higher in PMSG- than in FSH-treated ewes. Therefore, during the sampling period more E_2 was secreted per follicle stimulated by PMSG than per follicle stimulated by FSH.

Jabbour & Evans (1991) found that the preovulatory E_2 surge peaked 26.1 h earlier in PMSG-treated ewes than in FSHo-treated ewes. This observation is remarkably similar to the 26.7 ± 5.3 h time-difference observed in this investigation. Despite the large time difference to the peak in E_2, Jabbour & Evans (1991) did not observe any difference in the time of occurrence of the LH peaks of PMSG-treated and FSHo-treated ewes. The time to ovulation was also not significantly different between these two treatments. In a more extensive study concerning the time of ovulation in superovulated sheep, Walker *et al.* (1986) could also not detect a difference in the median time to the first ovulation, or in the median time of all ovulations, in ewes treated with PMSG or FSHp. This is peculiar in view of the fact that E_2 levels rose to a peak much earlier in PMSG-treated ewes. Rozell & Keisler (1990), in their studies with ovariectomized ewes, proposed that the rate of increase of preovulatory E_2 levels determined the time of the LH surge. In their study, they found that the higher the rate of increase of preovulatory E_2 levels, the sooner the LH surge was triggered. Clearly, this hypothesis is in conflict with the findings of Jabbour & Evans (1991) and Walker *et al.* (1986) who should have then observed ovulation to occur considerably earlier in PMSG-treated ewes than in FSH-treated ewes.

If indeed ovulation did not occur earlier in PMSG-treated ewes than in FSH-treated ewes in this study, as observed in the studies of Jabbour & Evans (1991) and Walker *et al.* (1986), then PMSG-treated ewes were mated too early in relation to ovulation. The rapidly increasing preovulatory E_2 levels of PMSG-treated ewes triggered these ewes into oestrus 10.4 ± 3.6 h earlier than in FSH-treated ewes. With mating being allowed only around the time of oestrus, the sperm ejaculated into PMSG-treated ewes would have to wait 10.4 ± 3.6 h longer than FSH-treated ewes for ovulation to occur. Sperm viability could have been compromised. Indeed, Dukelow & Riegle (1974) list the fertile life of sheep sperm to be approximately 14 h. This problem, together with the poor transport of sperm that has been observed in PMSG-treated ewes (Evans & Armstrong, 1984), could account for the poor fertility of PMSG-treated ewes in this investigation (mean fertility rate of PMSG-treated ewes = $17.9 \pm 7.2\%$). Six services in unstimulated ewes would not be expected to result in such a poor fertility rate.

The low percentage of ewes that exhibited oestrus and thus responded to the FSHo treatment, has also been observed in other ovine studies involving superovulation with FSHp (Eppleston *et al.*, 1984; Jabbour & Evans, 1991). This is peculiar since ewes in the breeding season would normally be expected to ovulate following treatment with progestagen sponges alone (Robinson, 1965). This lack of responsiveness may be due to insufficient LH activity in the FSH preparation. This would result in insufficient E_2 being produced to trigger oestrus and the preovulatory LH surge for ovulation. Such an hypothesis is supported by the findings of Ryan *et al.* (1984) and Jabbour & Evans (1991) who found that this problem could be overcome by treating FSH-stimulated ewes with PMSG, which has predominant LH-activity.

Finally, the finding of a relationship between the E_2 peak and the total ovarian response suggests that the RIA used in this investigation for the determination of E_2 levels was sound. This was particularly encouraging after the E_2 levels determined in this investigation again appeared to be lower than those observed by Jabbour & Evans (1991). The discovery of this relationship justified the correction of the characteristics of the preovulatory E_2 surge for the total ovarian response. Such a correction ensured that any treatment differences in surge characteristics were not due to differences in the total ovarian response.

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