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Timing of multiple ovulations in the ewe after treatment with FSH or PMSG with and without GnRH

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Summary. Ovulation rate, median time to first ovulation, median time of all ovulations and median time from first to last ovulation were studied by repeated laparoscopy in Merino ewes. Treatments with FSH or PMSG significantly affected ovulation rate (8·4 ± 0·81 and 7·3 ± 1·21 respectively, $P < 0·05$) and in median time of all ovulations (60 and 54 h respectively after progestagen sponge removal, $P < 0·05$). Differences in the median time to first ovulation (60 and 48 h) and median time from first to last ovulation (6 and 6 h) for the respective treatments were not significant. The synchrony of ovulation after both treatments was adversely affected by (1) the occurrence of premature ovulations before the onset of superovulation, (2) variability in the time of commencement of superovulation, and (3) variability in the time from first to last ovulation.

Administration of GnRH synchronized the timing of ovulation with both gonadotrophin treatments. This synchrony was due to a reduction in the period during which superovulation began and in the interval from first to last ovulation. The median time of all ovulations was significantly less with FSH + GnRH than with PMSG + GnRH (45 and 48 h after progestagen sponge removal, respectively, $P < 0·05$). Administration of GnRH at 16, 20 or 24 h after progestagen sponge removal significantly affected all traits examined except ovulation rate. Administration at 20 and 24 h produced an equally good synchrony of ovulation which was better than that obtained at 16 h.

We suggest that the use of GnRH in embryo collection programmes appears justified and is likely to improve embryo yields due to improved rates of fertilization.

Introduction

The efficient collection of embryos is a limitation to the exploitation of embryo transfer technology in the sheep. Although gonadotrophin treatment for multiple ovulation is frequently being re-assessed (Ryan, Bilton, Hunton & Maxwell, 1984; Evans, Holland, Nottle, Sharpe & Armstrong, 1984), there is little information on the synchrony of ovulation following such treatment, despite the probability that poor synchrony is a likely cause of fertilization failure and hence embryo yield.

Gonadotrophin-releasing hormone (GnRH) is of potential value in embryo collection programmes as a means of synchronizing multiple ovulations but the efficacy of the treatment has not been thoroughly examined in the sheep. Quirke, Jennings, Hanrahan & Gosling (1979) reported that when 50 μg GnRH were given 24 h after progestagen sponge removal ovulation occurred in 44–46% of ewes within 24 h and in all ewes within 34 h. Nancarrow, Murray, Boland, Sutton & Hazelton (1984) administered 50 μg GnRH to ewes treated with 1500 i.u. PMSG and found an increase in ovulation rate, fertilization rate and synchrony of ovulation.

The aims of the present study were to examine (1) the timing of multiple ovulations after treatment with FSH or PMSG and (2) the effect of GnRH administration on the synchrony of ovulation after gonadotrophin treatment.

Materials and Methods

This study was conducted during the mid-breeding season (March) of 1985, using 5-year-old Merino ewes located at Turretfield Research Centre, South Australia (138°44′E, 34°38′S).

Method of superovulation. Ewes were treated for 12 days with a 60 mg progestagen sponge (Upjohn Pty Ltd, Sydney, New South Wales) and either 15 mg FSH of pig origin (Burns Biotec, Omaha, Nebraska) or 1200 i.u. PMSG (Livestock Laboratories Pty Ltd, Melbourne, Victoria). FSH was administered (i.m.) as 6 injections, about 12 h apart, with the first injection given 48 h before sponge removal. PMSG was administered (i.m.) in a single injection, 24 h before sponge removal.

GnRH was obtained from Ayerst Laboratories Pty Ltd (Parramatta, New South Wales; Gonadorelin, Exp. 1) and Bachem Ag (Bubendorf, Switzerland; luteinizing hormone-releasing hormone, Exp. 2).

Experimental procedure. In Exp. 1 the timing of ovulation after treatment with FSH or PMSG with and without GnRH was examined. Treatments were (1) FSH (N = 20), (2) FSH + GnRH (25 or 100 μg i.v., N = 30), (3) PMSG (N = 20) or (4) PMSG + GnRH (100 μg i.v., N = 20). GnRH was administered 24 h after sponge removal. The optimal time of GnRH administration after sponge removal for synchrony of the timing of ovulation was examined in Exp. 2. In this study all ewes received FSH and 50 μg GnRH administered (i.v.) at 16 h (Treatment 1, N = 12), 20 h (Treatment 2, N = 13) or 24 h (Treatment 3, N = 12) after sponge removal.

Observations. Ovaries were examined by repeated laparoscopy and the number of corpora lutea was recorded. Observations began at 30 h (Exp. 1) or 24 h (Exp. 2) after sponge removal and were then made every 3 h in ewes treated with GnRH and every 6 h in other ewes until all preovulatory follicles had ovulated. Laparoscopy ceased by 54 h after sponge removal in the GnRH treatments and by 72 h in the non-GnRH treatments. A random sample of ewes from each treatment was re-examined at about 144 h to check accuracy of these recordings.

The timing of GnRH administration was such that (1) the interval from administration to initial laparoscopy and (2) the interval between successive observations were similar for all sheep. Ewes given GnRH were sedated with 0·2 ml 2% xylazine (i.m.) (Bayer Australia Ltd, Botany, New South Wales) whilst other ewes remained unsedated. Preliminary observations indicated that xylazine administration does not affect timing of ovulation when GnRH is used but may delay or prevent ovulation in the absence of GnRH.

Effects of stress associated with repeated laparoscopy. The effects of stress were assessed by replicating the PMSG and PMSG + GnRH treatments of Exp. 1 in an additional group of ewes; ewes in these replicates were returned to paddocks after treatment and not subjected to repeated laparoscopy. Ovaries were examined 72 and 144 h after sponge removal. Ovulation rate and the number of ovulations occurring between 72 and 144 h were determined and compared with data from Exp. 1.

Statistical analyses. Comparisons were made in Exp. 1 between FSH and PMSG treatments (data collected on a 6-h observation period) and between FSH + GnRH and PMSG + GnRH treatments (data collected on a 3-h observation period). Medians were compared using the Mann–Whitney and Kolmogorov–Smirnov tests (Siegel, 1956). Medians in Exp. 2 were compared using the Kruskal–Wallis test (Siegel, 1956). The effect of treatments on the mean number of ovulations was tested for significance using the statistical package for social sciences (Nie, Hull, Jenkins, Steinbrenner & Bent, 1975).

Results

Ewes with >3 ovulations were defined as having superovulated in response to the gonadotrophin treatments. Many ewes ovulated one or occasionally two follicles up to 24 h before the characteristic superovulatory response; these ovulations are referred to as premature ovulations. No new corpora lutea were observed at 144 h compared with the penultimate observation.

Experiment 1

Three ewes were eliminated from the study, one in the FSH treatment due to loss of the sponge and one in each of the PMSG and PMSG + GnRH treatments due to failure to ovulate. Data are presented in Tables 1 and 2 and Figs 1 and 2.

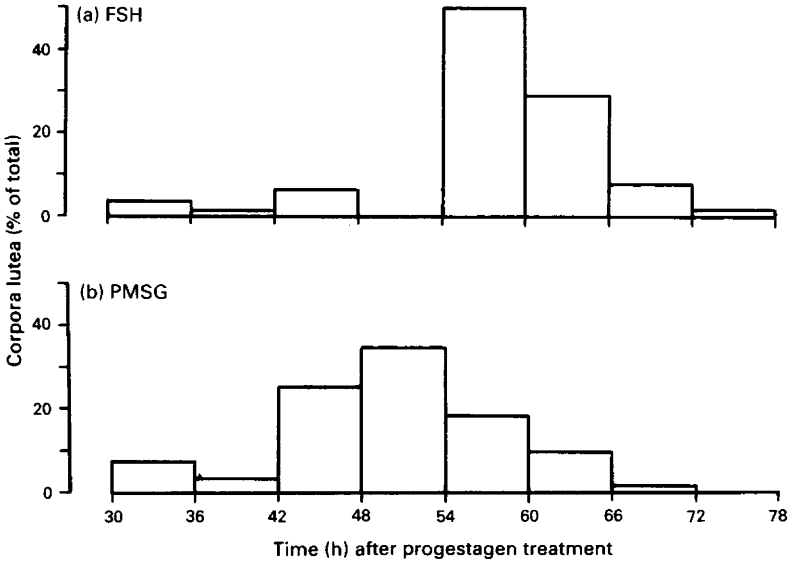

Fig. 1. Number of new corpora lutea observed in successive 6-h periods after removal of progestagen sponge in ewes treated with (a) FSH or (b) PMSG.

FSH and PMSG treatments. There were no significant differences between treatments in ovulation rate, median time to first ovulation or in median time from first to last ovulation (excluding premature ovulations in the latter trait) (Table 1). However, the median time of all ovulations was significantly less with PMSG than with FSH (54 and 60 h respectively, $P<0{\cdot}05$). Superovulation also occurred over a longer period in PMSG-treated ewes; 78% of the ovulations occurred between 42 and 60 h after sponge removal whereas 79% of the FSH-stimulated ovulations occurred between 54 and 66 h (Fig. 1).

Premature ovulations were observed in 7/17 and 4/14 ewes which superovulated in the FSH and PMSG treatments respectively (Table 2). However, these ovulations accounted for only 4·9% and 5·7% of the total ovulations of the respective treatments (Fig. 1).

Table 1. Ovulatory performance, as estimated by repeated laparoscopy, of ewes treated with FSH ± GnRH or PMSG ± GnRH

Exp.	Treatment (N)	Time of GnRH injection* (h)	Frequency of observation	Proportion of ewes superovulated	Mean ovulation rate† (±s.e.m.)	Median (range) time (h)		
						First ovulation	All ovulations	First–last ovulation‡
1 (March)	FSH (19)	—	6 h	17/19	$8{\cdot}4 \pm 0{\cdot}81^{a}$	60 (⩽36–72)	60 (⩽36–78)^{a}	6 (6–18)
	PMSG (19)	—	6 h	14/19	$7{\cdot}3 \pm 1{\cdot}21^{ab}$	48 (⩽36–60)	54 (⩽36–72)^{b}	6 (6–24)
	FSH + GnRH (30)	24	3 h	24/30	$7{\cdot}4 \pm 0{\cdot}78^{ab}$	45 (⩽36–48)	45 (⩽36–54)^{a}	6 (3–12)
	PMSG + GnRH (19)	24	3 h	16/19	$5{\cdot}5 \pm 0{\cdot}55^{b}$	45 (⩽36–51)	48 (⩽36–54)^{b}	6 (3–9)
2 (March)	FSH + GnRH (12)	16	3 h	10/12	$5{\cdot}3 \pm 0{\cdot}97$	35 (⩽33–39)^{a}	39 (⩽33–39)^{a}	9 (6–15)^{a}
	FSH + GnRH (13)	20	3 h	12/13	$6{\cdot}6 \pm 0{\cdot}87$	42 (⩽33–42)^{b}	42 (⩽33–48)^{b}	6 (3–12)^{b}
	FSH + GnRH (12)	24	3 h	10/12	$4{\cdot}5 \pm 0{\cdot}73$	45 (⩽33–48)^{c}	45 (⩽33–48)^{c}	6 (3–6)^{b}

* In relation to time of progestagen sponge removal.
† Includes all ewes.
‡ Excludes premature ovulations and is a measure of time taken for superovulation.
Within each column and between rows, means with different superscripts are significantly different, $P < 0{\cdot}05$.

Table 2. Number of ewes assessed to ovulate for the first time and in parentheses the number of ewes assessed as having begun to superovulate at different times after progestagen sponge removal (a, 6-h interval; b, 3-h interval)

		Time (h) after progestagen sponge removal											
		30	33	36	39	42	45	48	51	54	60	66	72
(a)	FSH (Exp. 1)	0	—	6	—	1	—	1 (1)	—	0	6 (9)	4 (6)	1 (1)
	PMSG (Exp. 1)	0	—	2 (1)	—	3	—	5 (3)	—	5 (5)	4 (3)	0 (2)	0
(b)	FSH+GnRH (Exp. 1)	0	0	7 (3)	2 (1)	1 (1)	17 (12)	3 (7)	0				
	PMSG+GnRH (Exp. 1)	0	0	5	0	1	3 (1)	8 (14)	2 (1)				
	FSH+GnRH (Exp. 2)												
	Treatment 1	1 (1)	1	6 (3)	4 (6)	0	0	0	0				
	Treatment 2	2	0	0	3 (5)	8 (7)	0	0	0				
	Treatment 3	2 (1)	0	1	0	1 (1)	7 (6)	7 (1)	1				

FSH + GnRH and PMSG + GnRH treatments. There was no significant difference in the ovulatory response of FSH-treated ewes treated with 25 or 100 μg GnRH. The results have therefore been combined.

There were no significant differences between treatments in ovulation rate, median time to first ovulation or in median time from first to last ovulation (Table 1). However, median time of all ovulations was significantly less with FSH + GnRH than with PMSG + GnRH (45 and 48 h respectively, $P < 0{\cdot}05$). A high degree of synchrony in the timing of ovulation was obtained with both treatments (Fig. 2).

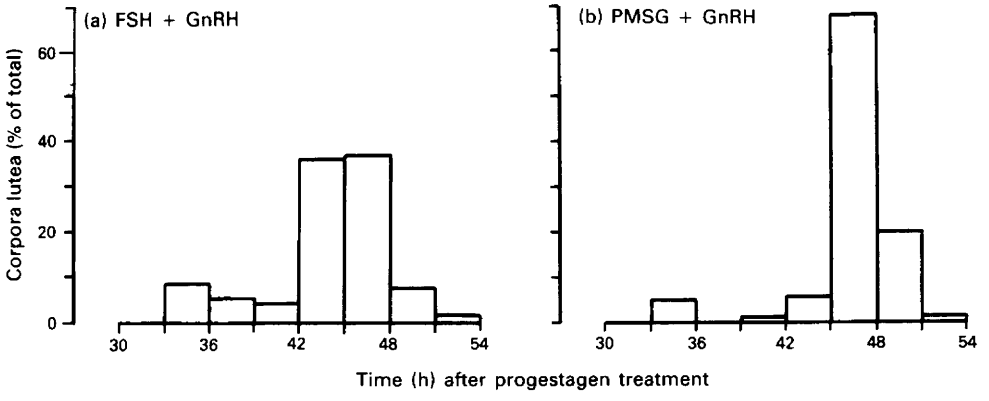

Fig. 2. Number of new corpora lutea observed in successive 3-h periods after removal of progestagen sponge in ewes treated with (a) FSH + GnRH or (b) PMSG + GnRH.

Premature ovulations were observed in 6/24 and 6/16 ewes which superovulated in the FSH + GnRH and PMSG + GnRH treatments respectively (Table 2) and these ovulations accounted for 18·3% and 11·5% of the total ovulations of the respective treatments.

Experiment 2

Data on superovulation following GnRH treatment at different times after sponge removal are presented in Tables 1 and 2.

There were significant ($P < 0{\cdot}05$) effects of treatment on median time to first ovulation, median time of all ovulations and in the median time from first to last ovulation (Table 1). Median time to first ovulation and of all ovulations increased as the time between sponge removal and GnRH administration increased. The median time from first to last ovulation was longer in ewes treated with GnRH at 16 h after sponge removal compared with treatment at 20 and 24 h.

Premature ovulations were observed in 1/10 and 2/10 ewes which superovulated in Treatments 1, 2 and 3 respectively (Table 2). These ovulations accounted for only 3·2%, 3·5% and 3·6% of the total ovulations in the respective treatments.

Effects of stress

There was no significant effect of repeated laparoscopy (2–9 observations per sheep) on ovulation rate. PMSG-treated ewes that underwent repeated laparoscopy had a mean ovulation

rate of 7·3 ± 1·21 (N = 19) compared with 8·9 ± 1·03 (N = 12) in control ewes that underwent laparoscopy on only two occasions. Comparable figures for ewes treated with PMSG + GnRH were 5·5 ± 0·55 (N = 19) and 4·8 ± 1·09 (n = 11). However, 12·6% (13/103) of the ovulations in the control ewes occurred later than 72 h after sponge removal whereas no ovulations occurred in this period in ewes that underwent repeated laparoscopy ($\chi^2_1 = 17{\cdot}2$, $P < 0{\cdot}001$).

Discussion

The time of ovulation in relation to sponge withdrawal is known to be advanced by treatment with both PMSG (Killeen & Moore, 1970) and FSH (Evans & Armstrong, 1984). In the present study, ewes treated with PMSG ovulated earlier than those treated with FSH (Table 1) but the timing of ovulation was less synchronous in the former treatment (Fig. 1). In FSH-treated ewes most ovulations occurred between 54 and 60 h after sponge removal, confirming similar data of Evans *et al.* (1984). However, there was probably sufficient spread in the time of ovulation in both treatments to contribute to fertilization failure depending on the timing and frequency of insemination.

Several factors contributed to the spread in the timing of ovulation. Firstly, premature ovulations were observed in all treatments although their contribution to total ovulations was small (e.g. 11·1% and 10·7% in FSH and PMSG treatments respectively). Secondly, some ewes required 2 or more 6-h observation periods to complete superovulation. A total of 4/17 and 5/14 ewes in the FSH and PMSG treatments respectively fitted this category. Thirdly, there was a lack of synchrony in the time at which ewes began to superovulate. This was greater in the PMSG treatment (36–66 h) than in the FSH treatment (48–72 h) (Table 2).

Administration of GnRH to ewes treated with FSH or PMSG synchronized the timing of ovulation (Fig. 2). This synchrony was due to a reduction in both the time from first to last ovulation and in the time at which superovulation began (Table 2). A similar degree of synchrony was obtained when GnRH was administered at 20 h compared with 24 h after sponge removal. Premature ovulations were observed in ewes treated with GnRH but the causes of these ovulations are not known.

The findings of this study are relevant to the timing of insemination following gonadotrophin treatment. Intrauterine insemination of superovulated ewes 48 h after sponge removal is favoured by some workers (Evans *et al.*, 1984; Evans & Armstrong, 1984; Armstrong & Evans, 1984). Insemination into the uterus at 54 or 60 h is reported to result in a reduced fertilization rate (Evans *et al.*, 1984) in FSH-treated ewes. These times correspond with the occurrence of superovulation, indicating that insemination at the time of ovulation may be detrimental. Our study indicates that insemination before 48 h may be warranted, particularly with PMSG treatment, since superovulation began 42–48 h after sponge removal. Good fertilization rates have been reported in ewes treated with FSH (64·1% and 88·5% of ova) and PMSG (86·7% of ova) and inseminated with fresh semen 24 h after sponge removal (Ryan *et al.*, 1984). Nevertheless, the difference in this timing of insemination and the onset of superovulation in our study (18 h and 30 h for PMSG and FSH treatments respectively) appears unnecessarily long and insemination closer to the time of superovulation may be worthwhile.

The probability of fertilization failure due to asynchrony in the timing of ovulation after GnRH treatment is likely to be substantially reduced, assuming equal viability of ova produced by the various treatments. However, the optimal time of insemination in ewes treated with GnRH has not been examined. Nancarrow *et al.* (1984) reported that, in ewes treated with PMSG and inseminated into the uterus with fresh semen 24 h after sponge removal, 75·9% of the ova were fertilized when GnRH was used compared with 49·8% without GnRH. Comparable figures of 73·7% and 53·3% were obtained in ewes inseminated with frozen–thawed semen 48 h after sponge removal (S. K. Walker & G. M. Warnes, unpublished data). Determinations of the optimal time of insemination and optimal sperm dose rate in ewes treated with gonadotrophins and GnRH are required.

Ovulation rates were reduced by GnRH treatment in this study (Table 1), but the reasons for this reduction are not known. However, Nancarrow *et al.* (1984) reported an increase in ovulation rate and embryo yields (from 1·9 to 4·2 per ewe) with the use of GnRH. A similar increase in embryo yield from 2·8 to 4·2 per ewe has been obtained with GnRH treatment in this laboratory (S. K. Walker & G. M. Warnes, unpublished data) despite a reduction in ovulation rate. It is postulated that the improvement in embryo yield is due to an enhanced fertilization rate resulting from an improved synchrony in the timing of ovulation.

The stress experienced by the ewes as an inevitable consequence of repeated laparoscopy may have had an effect on the time course of ovulation. However, it was assessed to be small and unlikely to invalidate our overall conclusions. We have confirmed that the synchrony of ovulation after commonly used gonadotrophin treatments is imprecise and could be an important source of fertilization failure. Inclusion of GnRH in treatment protocols has been shown to result in a highly synchronized timing of ovulation, irrespective of the gonadotrophin treatment. This synchrony is likely to contribute to improved embryo yields due to improved rates of fertilization of ova. The use of GnRH in oocyte or embryo collection programmes can therefore be recommended, particularly when fixed time insemination is used. This recommendation is subject to further assessment of the effect of GnRH treatment on embryo quality.

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