The effect of stage of cycle, oestrous synchronisation method and large follicles on superovulatory response in Merino ewes

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ABSTRACT

Superovulatory response to a single injection of PMSG (300ui) and of oFSH (3.6mg Ovagen®) given at either day 11, 16 or 18 after ovulation and a 7 day CIDR-G® or 30mg FGA sponge treatment was studied in Merino ewes. Follicle populations just before the administration of exogenous gonadotrophin and total ovarian response to treatment at day 5 of the next cycle were determined using transrectal ultrasound and laparoscopy respectively. The stage of the cycle had no significant effect on follicle population at the time of superovulation treatment or superovulatory response. Compared to FGA sponges, CIDR-G treatment reduced the number of 4-5mm follicles (2.83 ± 0.89 vs. 1.0 ± 0.3, $P<0.05$), the incidence of ewes with follicles •4mm (100% vs. 50%, $P<0.05$) and tended to increase superovulatory response (6.3 ± 3.7 vs. 10.3 ± 3.5, $P<0.1$). Ewes were classified according to the absence (n=4) and the presence (n=17) of a large follicle (•4mm diameter) at the time of superovulation treatment. Ewes without a large follicle had higher superovulatory response (13.8 ± 2.7 vs. 5.0 ± 1.3, $P<0.05$) and tended to have lower numbers of anovulatory follicles (0.5 ± 0.7 vs. 1.8 ± 0.3, $P<0.1$). These results suggest that the presence of a large follicle at the time of exogenous gonadotrophin treatment reduces superovulatory response in ewes and that the incidence of such follicles may be reduced by the use of a 7 day CIDR-G treatment.

Keywords: superovulation; oestrous synchronisation; large follicles; sheep.

INTRODUCTION

Superovulation is a major limiting factor for embryo transfer programmes in ruminants. Traditional superovulation treatments often result in highly variable ovulation rates possibly due to between animal variation in the status of follicular development at the time of exogenous gonadotrophin treatment (Armstrong, 1993; Adams, 1994). Indeed, synchronisation of follicle wave emergence (in the absence of an actively dominant follicle) with the initiation of exogenous gonadotrophin treatments in cows, has resulted in increased superovulatory response (Nasser *et al.*, 1993).

In sheep, it has been observed that the number of follicles present at the time of superovulation is positively correlated to superovulatory response (McMillan and Hall, 1991). However, investigation into the effects of the stage of follicle development on superovulatory outcome has been limited, possibly by the lack of knowledge regarding the factors that control follicle dynamics, especially follicle recruitment and dominance. The few studies that have investigated these mechanisms have equivocal results. In one study it was found that ovulatory response to PMSG given either at the time of progestagen pessary removal or 36 hours after (postulated times before and after the selection of the dominant ovulatory follicle) were similar (Driancourt *et al.*, 1991). Also, ablation of the largest follicle (6mm) before PMSG treatment did not result in an increased ovulation rate. Other workers have found that ewes without a large follicle (•4mm) at the time of exogenous gonadotrophin treatment, had qualitatively better superovulatory responses (Rubianes *et al.*, 1995).

It has been shown that the emergence of the ovulatory follicle wave may occur as early as day 2 (day 0 = ovulation) with another wave of follicle activity on day 11 (Ravindra *et al.*, 1994). The aim of the present preliminary study was to synchronise oestrus in ewes so that an exogenous gonadotrophin treatment coincided with possible times of either follicle wave emergence or follicle dominance (ie the presence of large follicle •4mm). This study also examined the effects of flurogestone acetate (FGA) sponges and Controlled Internal Drug Release devices on follicle populations and superovulatory response.

MATERIALS AND METHODS

Animals and Treatments

Twenty one Merino ewes were allocated according to genotype to 4 treatment groups. All animals were given prostaglandin F2α (2 injections at least 9 days apart) to synchronise oestrus so that superovulation treatments could be imposed at specific stages of the cycle.

Ewes in Groups 1, 2 and 3 received a superovulation treatment at either 11(n=5), 16 (n=5) or 18 (n=5) days after ovulation, based on the assumption that ovulation occurs 48 hours after PGF2α injection. It was postulated that day 11 and 18 may coincide with times of follicle wave emergence while day 16 could correspond with the development of a large non-ovulatory follicle. Five days prior to superovulation treatment ewes in these groups were treated with flurogestone acetate (FGA) sponges (30mg chrono-gest®-Intervet International B. V., Holland) for 7 days. Superovulatory treatment consisted of a single injection of 3.6mg of ovine FSH (Ovagen® - Imuno-Chemical Prod-

ucts Ltd., New Zealand) and 300iu PMSG (Folligon® - Intervet International B. V., Holland) 48 hours before sponges were removed.

Group 4 (n=6) underwent identical treatment to that of the 'day 16' group but were treated with a CIDR-G® (0.3% progesterone- EAZI-breed, New Zealand) device instead of a FGA sponge. The superovulatory response of ewes receiving either an FGA sponge or a CIDR-G® device was compared.

All groups were run with vasectomised rams fitted with harnesses and crayons to detect oestrus, and ewes were examined daily. All ewes underwent transrectal ultrasonography on the day of superovulation treatment. Follicle numbers and sizes were observed and recorded. Ovulation rates and follicle scores were observed by laparoscopy 7 days after CIDR/sponge removal. Given that ovulation rate of more than 2 is rare in Merinos, ewes observed to have 3 or more corpora lutea at this time were regarded as having responded to the superovulation treatment.

To determine the effects of a large follicle on superovulatory response, ewes were retrospectively reclassified according to the presence or absence of a •4mm diameter follicle as observed on the ultrasound image at the time of superovulation.

Transrectal Ultrasound Technique

This technique provided an atraumatic method by which ovarian activity could be observed daily over an extended period of time. Two operators conducted scanning concurrently using an Aloka 210 with a 7.5-MHz transducer. The refined version of this technique involved restraining the ewe in a milking 'hammock' so that there was no contact with the ground. Fifteen millilitres of lubricant (vegetable oil) was injected into the rectum to act as a coupling medium. The transducer was then inserted into the rectum until the bladder and then the uterine horns were observed on the monitor. The transducer was rotated 90° clockwise and then 180° anti-clockwise across the tract until both ovaries had been scanned. Follicles were identified as solid black areas within the image of the ovary and those with antral diameters of •2 were measured using callipers. Corpora lutea were also recorded, being identified as 'mushroom' areas of grey.

Statistical Analysis

Treatment means were compared using analysis of variance (AOV) with live weight as a covariate. Data were transformed when necessary and the means presented are untransformed. The proportion of ewes responding to the superovulation treatment (ovulation rate •3) was analysed using a General Linear Model (GLM) with a Chi-square distribution. Correlation and regression analyses were used to determine relationships between ovulation rate and the number of small follicles.

RESULTS

There were no significant differences in ovulation rate between groups of ewes treated with exogenous gonadotrophin on days 11, 16 and 18 (Figure 1). Proportions of ewes superovulated were also similar with 73% (11/15) of ewes having 3 or more corpora lutea overall. Follicle populations on the day of superovulation for groups of ewes at day 11, 16 and 18 after ovulation were not significantly different. The mean number of 2-3mm, 4-5mm and •6mm follicles were 7.3 ± 0.9, 2.2 ± 0.4 and 0.2 ± 0.2 respectively. The mean diameter of the largest follicle was 4.5mm ± 0.3mm.

Ewes superovulated on day 16 and treated with CIDR-G tended to have higher superovulatory responses compared to ewes treated with FGA sponges ($P<0.1$, Figure 2). There was no significant difference between oestrous synchronisation treatments in the proportion of ewes that responded to superovulation treatment (overall 64%). The effect of oestrous synchronisation method on follicle populations at the time of superovulation treatment are presented in Table 1. Ewes treated with CIDR-G had significantly fewer 4-5mm follicles than FGA treated ewes ($P<0.5$) while the mean number of 2-3mm, •6mm follicles and the diameter of the largest follicle were similar between groups. In addition, the incidence of ewes with large follicles (•4mm) was significantly lower in the CIDR-G treated group compared to the FGA group ($P<0.05$).

Differences in the response to superovulatory treatment were observed when the animals were grouped ac-

FIGURE 1: Ovulation rate in ewes superovulated on day 11, 16 and 18 after ovulation.

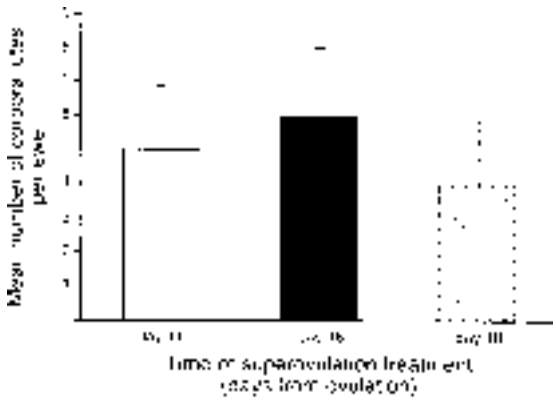

FIGURE 2: Ovulation rate in ewes treated for 7 days with either progestagen sponge or CIDR-G and superovulated 16 days after ovulation.

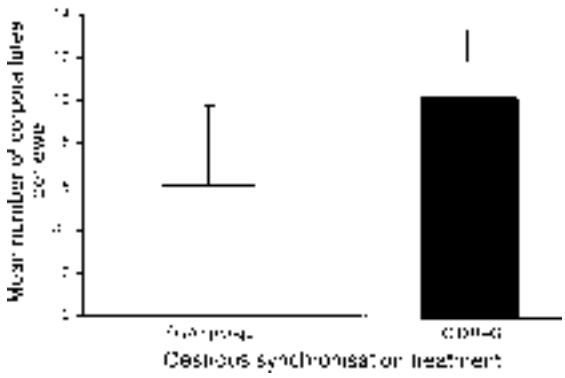

TABLE 1: The effect of the oestrous synchronisation method on the number of 4-5mm follicles and the incidence of ewes with large follicles at the time of superovulation treatment.

Parameter	Method of oestrous synchronisation	
	FGA sponge (n=5)	CIDR-G (n=6)
No. 2-3mm follicles	8.5 ± 1.65	11.9 ± 1.5
No. 4-5mm follicles	2.6 ± 0.4	1.0 ± 0.3*
No. follicles 6mm	0.2 ± 0.1	0.0 ± 0.1
Diameter of largest follicle	4.8 ± 0.4	3.8 ± 0.3
Incidence of ewes with large follicle (%)	5/5 (100)	3/6 (50)*

Mean ± s.e.m. *P<0.05 significant difference within rows

cording to the absence (n=4) or presence (n=17) of a large follicle at the time of superovulation treatment (Table 2). It should be noted that ¾ of the ewes without a •4mm follicle were from the CIDR-G group and were superovulated at day 16 of the oestrous cycle. The other ewe was from the group treated with progestagen sponges and was at the 'day 11' stage of the cycle.

A higher proportion of ewes without a large follicle at the time of superovulation treatment responded to treatment (P<0.05, Table 2). These ewes also had a higher ovulation rate in response to superovulation treatment compared to ewes with a large follicle. There was a tendency for ewes without a follicle •4mm at the time of superovulation treatment, to have more 2-3mm follicles at this time compared to ewes with a large follicle (11.25 ± 1.9 vs. 8.1 ± 0.9, P<0.1). When all ewes were included in the analysis, there was a significant correlation between the number of small follicles at superovulation treatment and the ovulation rate in response to this treatment (P<0.05, r=0.442).

TABLE 2: The effect of the absence of a large follicle at the time of exogenous gonadotrophin treatment on superovulatory response

Parameter	•4mm follicle at superovulation	
	Absent	Present
Proportion of ewes superovulating (%)	4/4 (100)	10/17 (58)*
Ovulation rate	13.8 ± 2.7	5.0 ± 1.3*
No. Retained follicles	0.5 ± 0.66	1.76 ± 0.32

Mean ± s.e.m. *P<0.05 significant difference within rows

DISCUSSION

In this study, ewes were treated so that superovulation treatments coincided with the postulated time of follicle wave emergence on days 2 and 11 and large growing pre-ovulatory follicle on day 16 (Ravindra *et al.,* 1994). The lack of difference between treatment groups in follicle populations at the time of exogenous gonadotrophin administration may indicate that follicle development is a continuum during the luteal phase (Driancourt *et al.,* 1991). On the other hand, recent findings of Ginther *et al.,* (1995) suggests that follicle development in ewes occurs in waves throughout the oestrous cycle. These workers not only demonstrated that follicle waves emerge approximately every 4 days, but also observed a high variability in the number of waves per cycle between individual ewes and in the emergence of the ovulatory follicle wave, a characteristic that may have prevailed in the present study.

A significant difference in superovulatory response of ewes treated with FGA sponges, or CIDR-G devices could not be demonstrated in the present experiment. This supports the results of Boland *et al.* (1988) who showed a slight but not significant increase in ovulation rates in ewes treated with CIDR devices for 12 days compared to ewes treated with 30mg FGA sponges. However, in the present study, there appears to be some evidence that FGA sponges and CIDR devices have different effects on follicle activity at the time of superovulation treatment. Ewes in the CIDR-G treatment group had fewer 4-5mm follicles and consequently a lower incidence of •4mm follicles on the day of superovulation. This may suggest that different types of progestagen have a differential effect on gonadotrophin activity (Boland *et al.,* 1983).

The results of this experiment suggest that ewes without a 'large' follicle at the time of superovulation respond better to exogenous gonadotrophin treatment (Table 2); a finding supported by Rubianes *et al.* (1995). However, the low numbers of ewes without a large follicle in this study suggest that further studies are required to confirm this effect. The equivocal nature of other studies (Driancourt *et al.,* 1991; Rubianes *et al.,* 1995) may be due to the variability in the size of a follicle at the start of dominance activity (Huhtinen *et al.,* 1992) or the inaccuracy of follicle size as an indicator of follicle dominance and health (Souza *et al.,* 1995). Bungartz and Niemann (1994) have indicated that, in cows, the number of small subordinate follicles as determined by a single ultrasound observation, can be used as a criterion for the presence or absence of a dominant follicle. In the present study, grouping of ewes according to the number of 2-3mm follicles (•10) at the time of superovulation did not reveal significantly different ovulation rates (data not shown). There was however, a significant correlation between these variables, a pattern previously reported by McMillan and Hall (1991).

In conclusion, the high variability of superovulatory response and the low numbers of animals involved in this study preclude any dogmatic statements. However, the trends of these data suggest that the presence of a large follicle at the time of superovulation treatment may limit superovulatory response in ewes. There are also some indications that the prevalence of large follicles may be influenced by the type of oestrous synchronisation device used; a 7 day CIDR-G treatment may suppress follicle activity more effectively than an FGA sponge. Further investigation to determine treatment which will reliably induce the emergence of a follicle wave to coincide with exogenous gonadotrophin administration, could yield considerable benefit to the success of superovulation and embryo transfer protocols in ewes.

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