ELSEVIER

THE REPEATABILITY OF SUPEROVULATORY RESPONSE AND EMBRYO RECOVERY IN SHEEP

F Bari,[1a] M Khalid,[2] B Wolf,[1] W Haresign,[1b] A Murray[3] and B Merrell[3]

[1]Institute of Rural Studies, University of Wales Aberystwyth, Llanbadarn Campus, Aberystwyth Ceredigion SY23 3AL, UK
[2]Department of Farm Animal and Equine Medicine and Surgery
The Royal Veterinary College, Hawkshead Lane, North Mymms
Hatfield, Hertfordshire, AL9 7TA
[3]ADAS Redesdale, Rochester, Otterburn, Northumberland NE19 1SB, UK

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ABSTRACT

Over an 8-year period, a total of 328 Scottish Blackface donor ewes were involved in a MOET program. They were synchronized with fluorogestone acetate sponges and superovulated with ovine FSH. After the onset of estrus, ewes were hand-mated and laparoscopic artificial insemination was performed with fresh semen 44-46 h after sponge removal. Embryos were recovered semi-laparoscopically on either Day 5 or Day 6 after insemination. Of the 328 donor ewes used, 222 ewes were supervoulated only once, while the remaining ewes were superovulated either twice (73 ewes), 3 times (26 ewes) or 4 times (7 ewes) at yearly intervals to generate a maximum of 474 records for subsequent analysis. There was no significant change in either mean ovulation rate or the mean number of embryos recovered per donor ewe at successive treatments. However, significant ($P < 0.05$ at least) effects of both year and donor ewe age existed for superovulatory response and number of embryos recovered, though only the effect of year was significant ($P < 0.001$) for percentage embryo recovery. Repeatability was significant ($P < 0.05$ at least) for both superovulatory response ($r = 0.55$, s.e. 0.055) and number of embryos recovered ($r = 0.38$, SE 0.074), but not for percentage embryo recovery ($r = 0.04$, SE 0.102).

Key words: superovulatory response, repeatability, sheep

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[a]Current address: Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

[b]Correspondence and reprint requests. Tel: 01970 621644, e-mail: wih@aber.ac.uk

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INTRODUCTION

The ability repeatedly superovulate selected high-genetic-merit donor ewes, with predictably high mean responses and minimal variation in ovulation rate and embryo yield, could accelerate rates of genetic improvement compared to a single collection, and this would benefit the commercial exploitation of MOET.

Previous studies involving repeated superovulatory treatments at yearly or six-month intervals demonstrated satisfactory responses, without any significant fall in responsiveness (13,14). Subsequent work, involving superovulation repeated up to 5 times during the breeding and non breeding seasons, showed that, although overall mean responses were satisfactory, there was a progressive reduction in the proportion of ewes superovulating and a significant drop in mean ovulation rate, especially between first and second treatment (1,9,11,17). However, most of these studies used PMSG, HAP or pFSH, and all used relatively small group sizes.

The overall success of repeated MOET procedures depends not only on the ovulation rate achieved but also the embryo recovery rate over successive collections. Most of the above-mentioned studies used a surgical recovery method that resulted in a significant decline in embryo recovery rates and a decline in the proportion of ewes being flushed due to post operative scar tissue formation and adhesions (1,9,16,17). Although the work of McKelvey et al. (11), involving a laparoscopic flushing method, showed a decline in recovery rates over successive flushes that was not statistically significant, the study was based on group sizes that were too small to yield reliable and robust results

Variation among ewes in superovulatory treatment is a consistent problem with MOET, irrespective of the hormone used to induce superovulation, choice of mating system, priming dose of progestagen or the type of flushing system used (2). However, it is not clear whether this variation is a function of the individual animal and its genetic makeup, or whether it represents random environmental variation. In none of the studies reported to date has the repeatability of superovulatory response been estimated. Indeed, such information is even scarce in cattle (12). Without this information it is impossible to determine whether superovulatory treatments should be repeated using the same standard protocol in situations where the initial treatments resulted in poor superovulatory responses.

An ongoing MOET program for breed improvement purposes, in which a proportion of the ewes were flushed over successive breeding seasons, provided an opportunity to determine the repeatability of superovulatory responses and embryo recovery rates to a standard treatment protocol

MATERIALS AND METHODS

This study involved results collected over an 8-year period from a MOET program at ADAS Redesdale in which approximately 60 Scottish Blackface donor ewes were subjected to treatment each year. Because of the criteria used to select ewes into the nucleus flock in the initial years, many of the donors were aged ewes and needed to be culled after only 1 or 2 years. Thereafter, an increasing number of nucleus-bred replacements were introduced and were flushed for the first time at 18-months of age. Among the total of 328 donor ewes used over the 8-year period, 224 were treated only once, while the remaining 106 ewes were treated between 2 and 4 times at annual intervals (Table 1). Although there was a total of 474 records for superovulatory responses, the responses were insufficient to justify embryo collection only on 469 occasions. This explains why there were slightly fewer records of embryo collection than ovulation rate for ewes used three times, and why therefore there was a corresponding small increase in the numbers flushed relative to those generating records of ovulation rate for ewes used once and twice.

Table 1. Number of Scottish Blackface ewes used within the MOET program, and the numbers of records for superovulatory response and embryo recovery generated, over an 8-year period.

	Number of times individual ewes were used*				
	1	2	3	4	Total
Ovulation rate					
Number ewes	222	73	26	7	328
Number records	222	146	78	28	474
Embryo recovery					
Number ewes	224	74	23	7	328
Number records	224	148	69	28	469

*Differences in the numbers of ewes/records available for parameter measurements are due to not flushing some ewes with ≤3 ovulations (see text).

Donor ewes used in the experimental program were kept outside under traditional hill flock management at ADAS Redesdale, both before and after the MOET treatment, and were housed only during the period extending from FSH injections until just after flushing. During housing, they were kept on a maintenance diet of hay, with ad libitum access to water. In each year the donor ewes were synchronized by the insertion of an intra-vaginal sponge containing 30 or 45 mg fluorogestone acetate (Chronogest®, Intervet Laboratories plc, Cambridge, UK) on Day 0 of the program and left in situ for 12 days. From Day 10 of the treatment schedule, donor ewes were subjected to twice daily injections of 1.25 mL of ovine FSH (Ovagen®, Immunological Products Ltd., Aukland, New Zealand) for four consecutive days. Sponges were removed at the time of the sixth FSH dose. After the onset of estrus, donor ewes were hand-mated with their rams at 4 h intervals, at a ewe:ram ratio of 5:1. Laparoscopic intrauterine AI was also performed with fresh diluted semen (30×10^6 motile sperm/horn) from the same rams 44 to 46 h after sponge removal.

Ovulation rate was counted and embryos were recovered semi-laparoscopically according to the method described earlier (2), on either Day 5 or Day 6 after insemination. Essentially, this technique involved introducing the Foley catheter into the uterus laparoscopically and then exteriorising the tip of the uterine horn through a 1 cm midline incision to allow introduction of the flushing medium. Any donor ewe with an ovulation rate of ≤3 was deemed not to have superovulated and, if not scheduled for culling at the end of the MOET program that year, was not flushed but left in lamb. Consequently, the numbers of ewes and records for ovulation rate and embryo recovery that were available for analysis were slightly different (see Table 1).

The data were analyzed using the reduced maximum likelihood (REML) procedures of Genstat (10), fitting the fixed effects of year of treatment and donor ewe age, with ewe effects fitted as a random term. Interactions of year of treatment x donor ewe age were not significant and were therefore not included in the model. Repeatabilities of the estimates for superovulatory response and embryo recovery were calculated from the estimates of the components of variance between- and within-ewes. The incidence of superovulation was compared using chi-squared analysis.

The data for both ovulation rate and numbers of embryos recovered were approximately normally distributed (Figure 1), and transformation to a logarithmic scale had no significant effect on the distribution of the residuals or the estimates of repeatability. Consequently, and to aid understanding, the results presented are primarily those from analyses of the untransformed data.

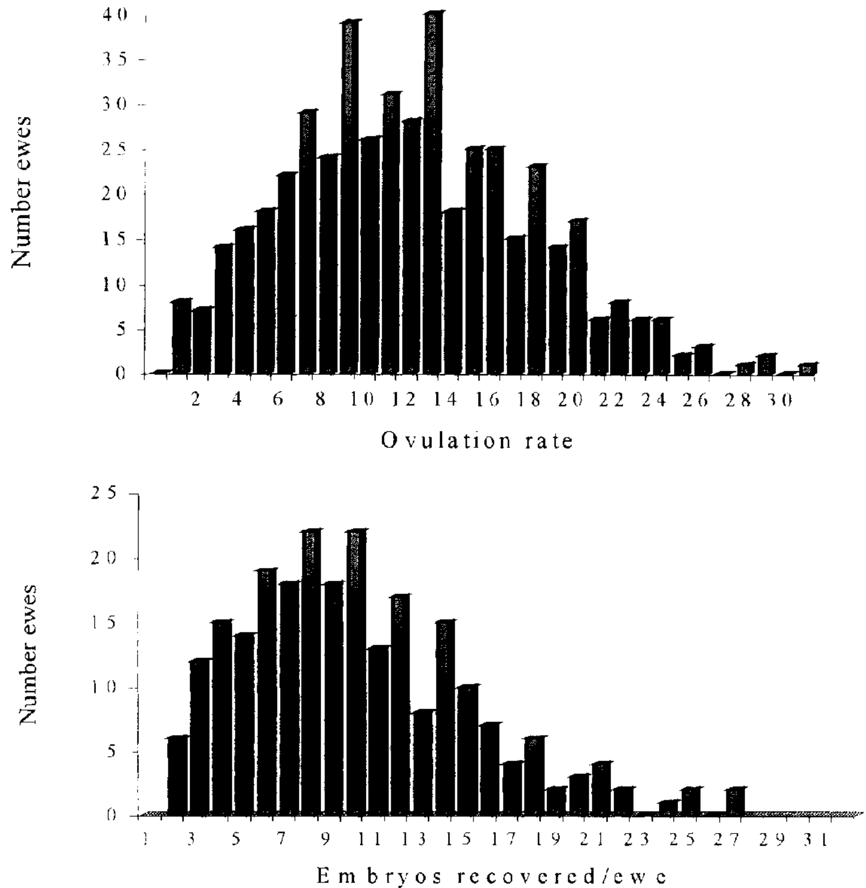

Figure 1. Distribution of ovulation rate and embryo recovery rate for ewes used in the MOET program

RESULTS

Over the full 8-year period of the study, all treated donor ewes came into heat. The incidence of superovulation was also very high; only in 15 of a maximum number of 474 possible opportunities

(3.1%) was an ovulation rate of ≤3 recorded. However, many of the ewes with 3 ovulations were flushed

The fixed effects of year of treatment and donor ewe age on mean superovulatory response, number of embryos recovered/donor ewe and embryo recovery percentage are presented in Table 2 Both year of treatment and ewe age had a significant ($P < 0.05$ at least) effect on ovulation rate and embryo yield, but only year of treatment had a significant effect on embryo recovery percentage. There was no obvious or predictable pattern to the changes in ovulation rate or number of embryos/donor ewe with year. However, in the case of embryo recovery percentage, refinements to the flushing procedure were introduced in 1997 and this significantly improved embryo recovery.

In the case of donor ewe age, older ewes had a significantly higher superovulatory response ($P < 0.001$) and, because embryo recovery percentage remained unaffected, a higher number of embryos were recovered per donor ewe ($P < 0.05$) than for young ewes.

There was no evidence for any change in mean ovulation rate or embryo yield when treatments were conducted at annual intervals (Table 3) Furthermore, the range in ovulation rate and the incidence of superovulatory responses were unaffected by repeat treatments No data are given for the fourth treatment because only 7 ewes were involved. However, all ewes superovulated and yielded embryos, and the actual mean responses were slightly higher for both parameters (14 14 and 11 14, respectively)

The number of ewes excluded from repeated laparoscopic flushing due to complications arising from flushing in the previous year was very low There were only two ewes excluded from the second flush due to adhesions of the uterus and/or ovary to omental fat Minor difficulties were encountered in two more ewes, one at the second flush and one at the third flush However, these did not interfere with the success of the flushing process. Consequently, from the total 106 ewes potentially available for flushing for a second or further time, problems were encountered in only 4 of them, of which 2 were significant enough to preclude flushing.

Repeatability was significant for both ovulation rate and number of embryos recovered/donor ewe (Table 4) The estimates (and their standard errors) derived from the actual and the $\log_{10}$ transformed data were not significantly different from each other The repeatability of ovulation rate was greater than that for number of embryos recovered/donor ewe However, the repeatability estimate for embryo recovery percentage was not significantly different from zero

DISCUSSION

Between 92% and 97% of ewes superovulated over successive treatments at annual intervals in this study, indicating that there was no refractoriness to Ovagen®, as has been suggested for PMSG (15). Despite a consistently high degree of variation in superovulatory response, the mean ovulation rate and number of embryos recovered/donor ewe were very similar for successive flushes in the same animals. This indicates that MOET can be conducted in successive years without compromising its likely outcome. Earlier studies using either PMSG or HAP also indicated similar responses over successive years, although the overall mean ovulation rates were lower than in the present experiment (13,14).

Table 2. The influence of the fixed effects of year of superovulatory treatment and donor ewe age on mean ovulation rate, number of embryos recovered/donor ewe and embryo recovery percentage from Scottish Blackface ewes in a MOET program.

	Year of study							
	1992	1993	1994	1995	1996	1997	1998	1999
Ovulation rate	14.2	11.8	8.6	12.8	12.1	10.1	12.6	13.0
				SED 1.02 (P < 0.001)				
Embryos recovered/ donor ewe	9.8	7.7	6.8	8.7	8.0	8.5	10.1	9.8
				SED 0.92 (P < 0.001)				
Embryo recovery %	71.6	69.5	73.8	68.0	63.6	80.8	82.2	79.6
				SED 4.74 (P < 0.001)				

	Donor ewe age					
	1	2	3	4	5	6
Ovulation rate	10.4	9.8	11.3	12.7	12.9	14.4
			SED 1.21 (P < 0.001)			
Embryos recovered/ donor ewe	7.4	7.3	8.7	10.0	9.5	9.0
			SED 4.74 (P < 0.05)			
Embryo recovery %	77.7	70.7	74.3	77.4	73.4	68.4
			SED 4.42 (NS)			

Table 3. Responses to repeated superovulatory treatments conducted at annual intervals on mean ovulation rate and embryo recovery in Scottish Blackface ewes.

	First treatment	Second treatment	Third treatment	SED
Ovulation rate	12.2	11.6	12.9	0.69 (NS)
Range in ovulation rate	1 to 28	3 to 26	2 to 24	
Number (%) ewes superovulating*	97/106 (91.6%)	103/106 (97.2%)	31/33 (93.9%)	NS
Embryos recovered/ donor ewe	8.9	8.7	8.8	0.65 (NS)
Embryo recovery %	70.1	74.5	69.5	3.03 (NS)

*Ovulation rate >3

Table 4. Repeatability estimates for ovulation rate, number of embryos recovered per donor ewe and embryo recovery percentage, with and without log_{10} transformation of the data, for Scottish Blackface ewes subjected to MOET procedures at annual intervals

	Ovulation rate	Log ovulation rate	Embryos recovered/ewe	Log embryos recovered/ewe	Embryo recovery %
Repeatability	0.55	0 55	0.38	0 35	0 04
SE	0.055	0.055	0.074	0.077	0.102

The results for repeated superovulation across years within the present study differ from results in studies in which superovulation was induced two, three or five times during the same breeding season. In these studies there was a progressive reduction in the number of ewes superovulating (1,9) as well as a significantly lower ovulation rate at the second treatment (1,9,11,17) These authors suggested that refractoriness of the ovary, or damage and adhesions from surgical recovery, might have had detrimental effects on subsequent ovulation rate when superovulation is repeated at such short intervals However, no such reduction was reported in another study when MOET was repeated at 2- to 6-month intervals (5) There is continuous growth of primordial follicles during each estrous cycle, and it takes about 40 days for follicles to grow from the point of entering the antral follicle pool to ovulatory status. Furthermore, it has also been reported that there are some 20 to 30 gonadotropin-responsive follicles in the ovary during each normal estrous cycle in sheep (18). If this is true, then repeated superovulatory treatments at intervals as short as bi-monthly should not, in theory, produce significant reductions in response within MOET programs.

The overall success of MOET depends not only on ovulation rate but also on the ability to flush ewes repeatedly without a significant drop in embryo recovery rate This cannot be achieved with surgical flushing because of adhesion formation (1,17). Alternative, less invasive methods are therefore required Attempts to recover embryos through the cervix have not been particularly successful in ewes (3,6,7) However, laparoscopic methods, as used in the present study, enable ewes to be repeatedly flushed without compromising embryo recovery rate Indeed, only 2% of ewes were not suitable for flushing on a second or subsequent occasion because of adhesion formation from a previous flush. This is in direct contrast to the much poorer results after a full surgical flushing procedure (1,9,17)

The mean embryo recovery rates in the present study (70, 75 and 70% for the first second and third flush) are comparable to those (53, 76 and 66%) reported for a full laparoscopic procedure that did not involve any exteriorisation of the uterine horn (11) However, exteriorisation of the tip of the uterine horn through a small incision on the mid-line to permit introduction of flushing medium is technically easier to perform and, on the basis of the current study, can also be performed without any major problems of adhesion formation. Refinements to the procedure (2) introduced in 1997 increased embryo recovery by around 12%, resulting in embryo recovery rates comparable to the full surgical approach and better than those reported for the full laparoscopic method (11).

The reason for the significant between-year variation in the present study is unclear There were no obvious differences in flock management before or during the MOET program, and the body condition scores of the ewes did not vary between years Furthermore, this effect cannot be due to the confounding influences of using different animals in different years, since similar differences in response between years were apparent in those animals that were used repeatedly over successive years The influence of donor ewe age was as expected, with more mature ewes having higher ovulation rates and numbers of embryos/donor ewe.

When more than one measurement of the character can be made on each individual, the phenotypic variance can be partitioned into variance within individuals and among individuals (4). The variation within individuals is due to temporary differences of environment between successive treatments and the between-individual component is due to genetic and environmental effects that influence the individual permanently. In the present experiment, there was significant repeatability in superovulatory response (0.55) and mean number of embryos collected per donor ewe (0.38) over sequential treatments, but not for embryo recovery % (0.04). However, it should be noted that this accounted for only 30% of the variation (r^2) in superovulatory response and 14% of the variation in number of embryos recovered per donor ewe. A similar repeatability (0.37) for superovulatory responses has been observed for cattle over two successive treatments with PMSG (8). Previous studies (2) indicated that ovulation rate is the single most important determinant of embryo yield, with a tight relationship between the two parameters, and that was apparent within the current data set. However, since embryo yield depends on a number of factors additional to ovulation rate (e.g., collection efficiency, embryo searching efficiency) that are independent of the superovulatory response and donor animal, the environmental influence on number of embryos recovered/ewe would be expected to be greater than for superovulatory response. This is consistent with the lower repeatability value for the number of embryos recovered compared to superovulatory response.

In conclusion, the results of the present experiment confirm the large degree of variation between ewes in superovulatory response and embryo yield, even when using ovine FSH for superovulation and a semi-laparoscopic method for embryo recovery. There was no evidence for a reduction in responses with successive treatments conducted at annual intervals. Because most variation among ewes in both ovulation rate and number of embryos recovered per donor ewe was due to non-repeatable effects, a poor response in an individual animal at the first attempt does not necessarily preclude a satisfactory response at a subsequent treatment.

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