New Zealand Society of Animal Production online archive

This paper is from the New Zealand Society for Animal Production online archive. NZSAP holds a regular annual conference in June or July each year for the presentation of technical and applied topics in animal production. NZSAP plays an important role as a forum fostering research in all areas of animal production including production systems, nutrition, meat science, animal welfare, wool science, animal breeding and genetics.

An invitation is extended to all those involved in the field of animal production to apply for membership of the New Zealand Society of Animal Production at our website www.nzsap.org.nz

View All Proceedings | Next Conference | Join NZSAP

The New Zealand Society of Animal Production in publishing the conference proceedings is engaged in disseminating information, not rendering professional advice or services. The views expressed herein do not necessarily represent the views of the New Zealand Society of Animal Production and the New Zealand Society of Animal Production expressly disclaims any form of liability with respect to anything done or omitted to be done in reliance upon the contents of these proceedings.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

You are free to:

Share— copy and redistribute the material in any medium or format

Under the following terms:

Attribution — You must give appropriate credit, provide a link to the license, and indicate if changes were made. You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.

NonCommercial — You may not use the material for commercial purposes.

NoDerivatives — If you remix, transform, or build upon the material, you may not distribute the modified material.

http://creativecommons.org.nz/licences/licences-explained/

Superovulation in ewes: are follicle numbers a useful predictor of ovulation rate?

W.H. McMILLAN AND D.R.H.HALL

MAF Technology, Whatawhata Research Centre, P.B. 3089, Hamilton, New Zealand.

ABSTRACT

A CIDR was inserted into 72 ewes which were either early in the cycle (E; days 1-3), at mid cycle (M; days 7-9) or late in the cycle (L; days 13-15). Half of the ewes received either 0 or 300 IU of pregnant mares' serum gonadotrophin (PMSG) at the start of superovulation treatment. M ewes had two thirds as many ovarian surface follicles > 1.5 mm diameter compared to E and L ewes (8.8 vs 13.6 vs 13.0; P<0.001). M ewes had approximately two thirds as many ovulations present 8 days later compared to E and L ewes (5.1 vs 7.7 vs 7.7; P<0.05). For ewes receiving 300 IU of PMSG as well as FSH, there was a significant regression of follicle number on ovulation rate (b=0.73 ± 0.16 P<0.01). Collectively, these results highlight the importance of ovarian surface follicle numbers as a significant source of variation in superovulation response.

Keywords Superovulation, ovarian follicles, PMSG, FSH, oestrous cycle, CL.

INTRODUCTION

The ovary of adult ewes contains between 12,000 and 86,000 primordial follicles (Cahill *et al.*, 1979). The number of normal follicles growing in the ovary of sheep during the oestrous cycle varies between 230 and 420 (Lahlou-Kassi and Mariana, 1984). The number of follicles >1 mm in diameter with an antrum present on the ovary during the cycle in sheep is 5-24 (McNatty,1982). However, the number that finally ovulates at any one time is usually 1 or 2. Little is known of the processes that control the recruitment, selection, growth, dominance and finally atresia or ovulation of ovarian follicles. This information is crucial to our understanding and manipulation of ovarian activity.

In sheep, some authors report waves of ovarian follicular growth (Smeaton and Robertson, 1971; Brand and De Jong, 1973) but other evidence is at variance with this view (Turnbull *et al.*, 1977; Lahlou-Kassi and Mariana, 1984). In cattle, there is increasing evidence that follicular development is dependent on stage of the oestrous cycle and occurs in waves (Boland *et al.*, 1990) but this view is not universal (Spicer and Echternkamp, 1986 review). Furthermore, in cattle, the timing of superovulation treatment at a stage of the cycle when follicle numbers are high has lead to an increase in the number of ovulations (Grasso *et al.*, 1989). We postulated that if sheep could be treated for superovulation at a stage of the cycle when surface ovarian follicle populations were high, then high numbers of ovulations could be achieved. The objectives of this study were, therefore, to describe the relationship between ovarian surface follicle numbers and superovulation response in adult ewes during the breeding season.

MATERIALS AND METHODS

One hundred adult Romney ewes were pretreated with a progesterone containing controlled internal drug release device CIDR (Carter Holt Harvey, Hamilton, New Zealand) for 12 days to synchronise heats. Following heat recording 72 ewes were chosen such that 24 ewes were each at an early (E ewes; day 1-3), mid (M ewes; day 7-9) or late (L ewes; day 13-15) stage of this synchronised cycle (day of heat detection = day 0). All ewes were then treated with a second CIDR device to resynchronise heats for superovulation. This CIDR device was replaced after 8 days with a new CIDR for 4 days, a procedure expected to improve superovulation responses (Thompson *et al.*, 1990). Harnessed vasectomised rams were used to detect the onset of heats for 4 days from final CIDR withdrawal.

Two days after CIDR replacement, a randomly chosen half of the ewes at each stage of the cycle received a single injection containing either 0 or 300 IU of pregnant mares' serum gonadotrophin PMSG (Folligon, Intervet, Holland), in a 3x2 factorial design (N=72, n=12 ewes). At the same time as PMSG was administered, all ewes received the first of 6 bi-daily injections of 1.8 ml of ovine follicle stimulating hormone (FSH; Ovagen, Immuno Chemical Products, Auckland, New Zealand). In the following text ewes receiving 300 IU of PMSG in conjunction with FSH are designated as PMSG ewes and ewes receiving only FSH injections are designated as FSH ewes.

Commencing immediately following the first FSH injection, the ovaries of all ewes were examined with the aid of a laparoscope and calibrated manipulating probe and all ovarian surface follicles >1.5mm were counted. Laparoscopies were performed daily for 5 days but only the results of the first examination are presented in this paper. A final laparoscopy was performed 5 days after the final CIDR withdrawal and superovulation rate determined.

Data analysis was carried out using the GENSTAT package with logit transformations for binomial data. Means ± s.e.m are presented where appropriate.

RESULTS

Heats

Only three (5%) of the 69 ewes with complete records failed to show a heat within 4 days of final CIDR withdrawal. The remaining 66 ewes had a mean heat onset of 40 ± 1.9 h after CIDR withdrawal. Stage of cycle effects on this interval were small (E 41.3 ± 1.9h ; M 42.1 ± 1.9h ; L 38.8 ± 2.0h). In contrast, PMSG ewes had a 12 hour shorter interval to heat than FSH ewes (34.2 ± 1.6h vs 46.4 ± 1.6h ; P<0.001).

Follicle Numbers

The mean number of follicles per pair of ovaries at the first observation was 11.8 ± 0.5. Stage of cycle effects were large with M ewes having the fewest follicles (E 13.6 ± 0.8 ; M 8.8 ± 0.8 ; L 13.0 ± 0.8 ; P<0.001). PMSG ewes had fewer follicles than FSH ewes (10.6 ± 0.7 vs 12.9 ± 0.7 ; P<0.05).

Ovulation

The mean incidence of treated ewes ovulating was 85%. Stage of cycle effects were small (E 87% ; M 83% ; L 83%). More PMSG ewes ovulated compared to FSH ewes (97% vs 71% ; P<0.001).

Superovulation

The mean incidence of ewes superovulating (i.e. >3 ovulations) was 64%. Stage of cycle effects were not significant (E 66% ; M 57% ; L 70%). More PMSG ewes superovulated compared to FSH ewes (88% vs 39% ; P<0.001).

The mean superovulation rate was 7.2 ± 0.6. Stage of cycle effects were large with M ewes having the lowest superovulation rate (E 7.7 ± 1.1 ; M 5.1 ± 1.1 ; L 7.7 ± 1.1 ; P<0.05). PMSG ewes had a higher superovulation rate than FSH ewes (10.4 ± 0.9 vs 4.0 ± 0.9 ; P<0.001). This ovulation data is unadjusted for any differences between groups in number of follicles present on the ovary and interval from CIDR removal to the onset of heat (see next section).

Regression of Interval to Oestrus and Follicle Numbers on Superovulation Rate.

There was a negative relationship between the interval from final CIDR removal to the onset of oestrus and superovulation rate (b=-0.23 ± 0.06 ovulations per ewe per hour to onset, P<0.05). Thus, for PMSG and FSH ewes with a common interval from CIDR removal to the onset of oestrus (40 h in this case), the expected number of ovulations per ewe are 10.1 and 4.4.

Within FSH ewes, there was no relationship between the number of follicles at the start of superovulation treatment and superovulation rate. For PMSG ewes, increasing follicle numbers were associated with an increasing superovulation rate (b=0.73 ± 0.16 ; P<0.01) (Fig 1). Thus, for E, M, and L ewes with the same number of follicles on the ovary surface (12 in this case), the expected numbers of ovulations following PMSG/FSH treatment are 10.9, 9.9, and 14.7 (P<0.05) compared to 12.1, 8.7 and 15.0 when no account is taken

of follicle number differences.

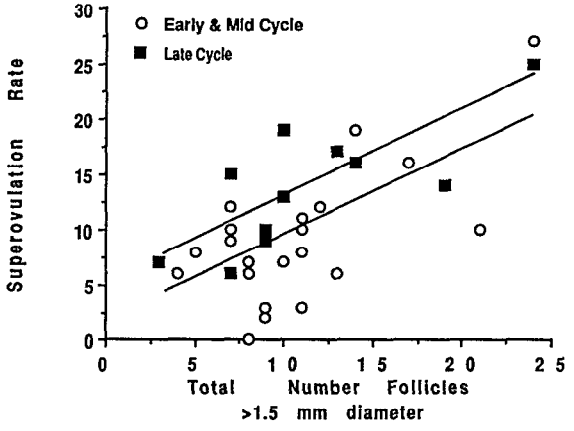

FIG 1 Regression of ovarian surface follicle numbers > 1.5 mm diameter at the commencement of superovulation treatment on superovulation rate per ewe treated with 10.8 ml of Ovagen FSH and 300 IU PMSG (r squared = 50%).

The probability of achieving a superovulation rate of between 5 and 15 from a given number of ovarian follicles in L ewes is illustrated in Figure 2. With 15 surface follicles, we can see that the probability of an individual ewe giving a superovulation rate of 10 is about 85% compared to over 95% for a superovulation rate of 5.

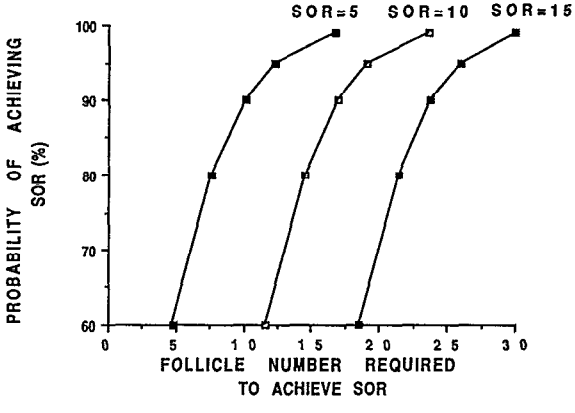

FIG 2 Relationship between number of ovarian surface follicles >1.5 mm diameter on the ovary surface of an individual late cycle ewe and the probability of achieving a superovulation rate of either 5, 10 or 15.

DISCUSSION

These results demonstrate a clear relationship between stage of the cycle at the insertion of a CIDR device (i.e. the interval since last heat) and ovarian surface follicle status some 10 days later. Thus, ewes which were at mid cycle at CIDR insertion had only two thirds the number of follicles > 1.5mm diameter that were present on the ovaries of late and early cycle ewes. In this study, 'cycle' lengths at first ovarian examination ranged from 11 days for E ewes (i.e. 1 day prior to CIDR insertion plus 10 days with a CIDR inserted) to 25 days for L ewes (15 days plus 10 days).

There are very few reports on the follicle status of ewes at known stages of either natural or progestagen augmented cycles. In unstimulated Merino ewes, there appears to be no relationship between stage of cycle and follicle populations (Turnbull *et al.*, 1977). Perhaps Merino ewes are different from Romney ewes in this regard. Other results from our laboratory indicate that Merino ewes differ from Romneys ewes in their superovulation response to the same treatment (superovulation rate of 10 vs 8, W.H. McMillan, unpublished data).

Interestingly, the mean number of follicles in the present study was similar to the 12.3 reported following the sectioning of ovaries and counting of follicles >2 mm diameter during the natural cycle in Texel ewes (Brand and De Jong, 1973). Furthermore, these workers found that mid cycle ewes had about 60% as many follicles >2 mm diameter compared to early and late cycle ewes. Our results confirm this previously overlooked result. The correspondence of the results in this previous study and ours suggests that ovarian surface follicle counts may be an acceptable method for estimating the total number of ovarian follicles >2 mm diameter in ovaries. *In vivo* and *in vitro* estimates are required to confirm this.

The close association between the number of follicles on the ovarian surface at the commencement of superovulation treatment and superovulation rate some 4-5 days later accounted for over half of the variation in superovulation rate in the present study. These results are consistent with those in the mouse where the largest number of ovulations are recorded when the largest number of growing follicles are present on the ovary

(Gates, 1971). By contrast, in unstimulated ewes during the oestrous cycle, there appears to be no relationship between ovarian populations and ovulation rate (Turnbull *et al.*, 1978).

In unstimulated breeds of ewes with a naturally high ovulation rate, attempts to account for variation in ovulation rate through variation in ovarian follicle numbers has generally been unsuccessful (Driancourt *et al.*, 1986). This is similar to the case in the present study with ewes stimulated with only FSH. It may be that the range in ovulation rate under these conditions is too narrow . However, when superovulation rate is sufficiently high and variable , as occurred with ewes stimulated by PMSG and FSH, the relationship becomes more apparent.

This relationship between follicle numbers and superovulation rate can be used to advantage in multiple ovulation and embryo transfer (MOET) programmes in several ways. Firstly, ewes can be programmed to begin synchronisation at a stage of the cycle when high numbers of responsive follicles can be expected some 10 days later. The current findings indicate that ewes in the late stages of the cycle best fit this criterion. Secondly, ewes can be screened for possible poor responders prior to the commencement of gonadotrophin treatment, by counting the follicles. In this regard our results indicate that a minimum of 5, 12, and 18 follicles need to be present in late cycle ewes to give an 80% probability of at least 5, 10 and 15 ovulations respectively. For a 95% probability, the minimum follicle counts are 10, 17 and 24 respectively. This can save the costs associated with stimulation as well as avoid unnecessary surgery. The effects of stage of cycle on embryo quality and viability need to be determined before this approach can be fully endorsed.

Including PMSG with FSH in superovulation treatments had multiple outcomes in the present study. Firstly, ewes came into heat earlier. This result was unexpected since ewes treated during the breeding season with a CIDR device and PMSG had similar intervals to the onset of heat as ewes treated with only CIDR devices (Smith *et al.*, 1988). The combination of PMSG with FSH as in the present study probably influences follicle steriod production and this may have some effect on neural centres involved in oestrous behaviour. Earlier oestrus may have benefits for the timing of CIDR withdrawal in companion recipient ewes if close synchrony of onset is required. The second benefit of PMSG was that more ewes ovulated. Thirdly, ovulation rates were higher in ovulating ewes when treated with PMSG.

The low superovulation rate in ewes treated with FSH alone in the present study was consistent with some of our previous work where superovulation rates were 6.1 without PMSG and 10.6 with PMSG (W.H. McMillan, unpublished data). Other work in Merino ewes has indicated a need for PMSG in conjunction with porcine FSH of pituitary origin (Ryan *et al.*, 1984).

We conclude that donor ewes treated with a CIDR device exhibit a pattern of follicle dynamics that can be exploited to enhance the efficiency of oocyte collection and superovulation in ewes. The commencement of synchronisation during the later stages of the cycle and the inclusion of a low dose of PMSG are important steps to capitalise on this opportunity. Studies on embryo quality and viability are required before this approach can be fully endorsed.

ACKNOWLEDGEMENTS

We acknowledge the assistance of Peter Evans and Libby Davies, Whatawhata Research Centre, with field work and Barbara Dow, Ruakura Biometrics Group, for biometrical assistance. In particular we thank Dr K.L. Macmillan, Ruakura Dairy Research Corporation, for his encouragement of this approach to studying superovulation.

REFERENCES

Boland, M.P.; Murphy, M.G.; Roche, J.F. 1990 The use of ultrasound to monitor ovarian function in farm animals. *AgBiotech News and Information* **2**: 841-844.

Brand, A.; De Jong, W.H.R. 1973 Qualitative and quantitative micro-morphological investigations of the tertiary follicle population during the oestrous cycle in sheep. *Journal of Reproduction and Fertility* **33**: 431-439.

Cahill, L.P.; Mariana, J.C.; Mauleon, P. 1979 Total follicular populations in ewes of high and low ovulation rates. *Journal of Reproduction and Fertility* **55**: 27-36.

Driancourt, M.A.; Gauld, I.K.; Terqui, M., Webb, R. 1986 Variations in patterns of follicle development in prolific breeds of sheep. *Journal of Reproduction and Fertility* **78**: 565-575.

Gates, A.H. 1971 Maximizing yield and developmental uniformity of eggs. In *Mammalian Embryology* Ed J.C. Daniel Jr. W.H. Freeman and Co., San Fransisco. pp. 64-75.

Grasso, F.; Guildbault, L.A.; Roy, G.L.; Matton, P.; Lussier, J.G. 1989 The influence of the presence of a dominant follicle at the time of initiation of a superovulatory treatment on superovulatory responses in cattle. *Theriogenology 31*: 1991.

Lahlou-Kassi, A.; Mariana, J.C. 1984 Ovarian follicular growth during the oestrous cycle in two reeds of ewe of different ovulation rate, the D'Man and the Timahdite. *Journal of Reproduction and Fertility 72*: 301-310.

McNatty, K.P. 1982 Ovarian follicular development from the onset of luteal regression in human and sheep. In *Follicular Maturation and Ovulation*, pp 1-18. Eds R. Rolland, E.V. van Hall, S.G. Hillier, K.P. McNatty, and J Schoemaker. Exerpta Medica, Amsterdam.

Ryan, J.P.; Bilton, R.J.; Hunton, J.R.; Maxwell, W.M.C. 1984 Superovulation of ewes with a combination of PMSG and FSH. In: Lindsay, D.R. and Pearce, D.T. (Eds), *Reproduction in sheep*. Australian Academy of Science, Canberra, 1984, pp 338-341.

Smeaton, T.C.; Robertson, H.A. 1971 Studies on the growth of and atresia of graffian follicles in the ovary of the sheep. *Journal of Reproduction and Fertility 25*: 243-252.

Smith, J.F.; Cruickshank, G.F.; McGowan, L.T.; Parr, J.; Mortimer, B.J. 1988 Seasonal changes in oestrus, ovulation and conception of Coopworth ewes treated with CIDRs and PMSG. *Proceedings of the New Zealand Society of Animal Production 48*: 99-102.

Spicer, L.J.; Echternkamp, S.E. 1986 Ovarian follicular growth, function and turnover in cattle: A review. *Journal of Animal Science 62*: 428-451.

Thompson, J.G.E. ;Simpson, A.C.; James, R.W. ; Tervit, R.W. 1990 The application of progesterone-containing CIDR devices to superovulated ewes. *Theriogenology 33*: 1297-1304.

Turnbull, K.E.; Braden, A.W.H.; Mattner, P.E. 1977 The pattern of follicular growth and atresia in the ovine ovary. *Australian Journal of Biological Sciences 30*: 229-241.

Turnbull, K.E.; Mattner, P.E.; George, J.M.; Scaramuzzi, R.J. 1978 The relation between patterns of ovarian follicle growth and ovulation rate in sheep. *Australian Journal of Biological Sciences 31*: 649-655.