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OVUM PRODUCTION: ACTION OF VARIOUS GONADOTROPHINS IN SHEEP AND GOATS

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INTRODUCTION

The success of investigations on viable mammalian ova is largely determined by their availability. These must be at a suitable stage of cleavage and produced at a predetermined time usually from a limited number of donors. From the variable results experienced at this Institute in efforts at obtaining suitable ova, the need for a comparison of the response of some small live-stock breeds to various readily procurable gonadotrophins was evident. The Merino ewe has been studied extensively; however, our earlier attempts to recover ova from goats were hampered by apparent discrepancies in their response to gonadotrophin treatments.

This study was undertaken during the time of the year when the majority of ewes were normally in anoestrus. As early as 1933, Cole & Miller demonstrated that ovulation may be induced in anoestrous ewes by a single injection of pregnant mare serum (PMS), without the appearance of behavioural oestrus. The necessity for pretreatment with progesterone to obtain superovulation accompanied by oestrus has been amply demonstrated (Robinson, 1952; Dutt, 1952; Pursel & Graham, 1962). Robinson (1962) found that 20 mg progesterone injected 48-hourly gave results equivalent to those obtained after daily injection. Attempts to decrease the injection frequency further have been accompanied by less satisfactory results (Robinson, 1962; Lishman & Hunter, 1961; Gordon, 1963). The variability of onset of oestrus was found to be least when the final progesterone injection was given in the morning (Lamond & Bindon, 1962.)

The relatively unpredictable rate of ovulation following the use of PMS is familiar to most workers, but this readily available source of potent gonadotrophin should not be discarded lightly. The administration of human chorionic gonadotrophin (HCG) after PMS to control ovulation, has proved disappointing, as behavioural oestrus appears to be suppressed in a large proportion of ewes (Braden, Lamond & Radford, 1960; Southcott, Braden & Moule, 1962).

HCG alone has been demonstrated to be more active than PMS, but even more unreliable (Robinson, 1962). Little work on the simultaneous injection of combinations of various gonadotrophins has been reported. A mixture of the ratio 80 FSH: 20 LH was found by Brown & Billewicz (1962), to be potent and highly synergistic in immature mice. The combinations of PMS and HCG used by Lamond (1962) in sheep proved unsatisfactory in many respects.

Recently Moore & Shelton (1962a), used a potent horse anterior pituitary extract (HAP) with appreciable success in an effort to utilize the egg transfer technique for the rapid propagation of the polled Merino gene. They obtained a mean of 11·9 ovulations in cycling ewes.

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In the present trial the responses of two breeds of goats are compared with those of Merino ewes. The responses to PMS alone, a PMS/HCG combination and the HAP preparation of Moore & Shelton are also compared.

PROCEDURE

Animals

Groups of ewes were treated from August, 1962, to January, 1963. During this period the majority of local sheep and goats are usually in anoestrus. The total of 45 treated ewes comprised 15 Merino, 15 Angora and 15 Boergoat ewes, the latter being a hardy type of goat common in Africa. The ewes of each breed were randomized into groups of five each. In each breed a group was given one of the three gonadotrophin treatments.

Prior to and throughout treatment the ewes were confined in small pens which contained a maximum of six animals. All ewes were on a standard ration of dry lucern hay supplemented with a small quantity of green lucern and concentrate mixture for at least six months before treatment. Generally an equal number of each breed received the same treatment schedules simultaneously.

Treatment schedules

Five intramuscular injections of 20 mg progesterone in ethyl oleate were administered at 48 hour intervals to all ewes between 8 to 9 a.m. Twenty-four hours after the last progesterone injection gonadotrophin was administered.

The three gonadotrophins used were as follows:—

PMS—a single subcutaneous injection of 750 i.u. pregnant mare serum (" PMS 1500 "—B.W.).

PMS/HCG—a single subcutaneous injection of 500 i.u. PMS and a simultaneous intramuscular injection of 250 i.u. human chorionic gonadotrophin (" Luthormone " —B.W.).

HAP—Horse anterior pituitary gonadotrophin was prepared from frozen pituitaries according to Moore & Shelton (1962a). The dried powder was reconstituted at a concentration of 5 mg/ml and stored at −20° C until used. Each animal received a total of 90 mg in six subcutaneous injections over three days. An additional 10 mg progesterone was given 24 hours after the commencement of HAP treatment, as it was found in preliminary experiments that a large proportion of ewes exhibited oestrus during the third day of treatment.

Therefore, fifteen ewes, five of each breed, received each of three treatment schedules.

Observations

Rams of the same breed as the ewes and which had yielded high quality semen on electrical ejaculation, were introduced daily for a short period during the 10 days of progesterone treatment. Thereafter they were introduced twice daily, at 8 a.m. and 4 p.m., for the following two weeks. The behaviour of each ewe to the ram was carefully observed and in cases of doubt the particular ewe was confined individually with the ram. New rams were not introduced as it has been our experience, particularly with goats, that an oestrous ewe may refuse a strange male. Service was allowed daily while the ewe remained in oestrus. Sterile teaser rams were used on eight Boergoats acting as potential recipients for ova.

All 45 ewes were subjected to midventral laparotomy under intravenous pentobarbitone anaesthesia between 50 to 80 hours after the first positive oestrus observation. The number of young corpora lutea, degree of luteinization, and all visible intact follicles larger than 5 mm in diameter were recorded. When doubt on the size of follicles existed, sterilized calipers were used.

Recovery of ova

Seitz-filtered homologous serum was prepared according to Averill (1958). The technique employed for *in vivo* flushing of the fallopian tubes was similar to that described by Hunter, Adams & Rowson (1955), except that a 25 cm length of 1 mm bore polythene tubing was inserted 1 cm into the ampulla and allowed to drain directly into a watchglass. The fallopian tube from each ovary exhibiting ovulation was flushed with 3 ml serum which was then examined under a stereoscopic microscope mounted in a cabinet equipped with ultraviolet light. The cleavage stage of all ova was confirmed under 50× magnification.

The procedure to be used for recovery of ova in this experiment was first checked on five Merino ewes following natural oestrus as ovum reception and passage down the fallopian tube would not be disturbed. There were six ovulations and six eight-cell ova were recovered. Four ova were transferred singly to recipient ewes resulting in the birth of three lambs.

Results

Ovarian Morphology

Rate of ovulation

Merino ewes were the most responsive breed to PMS and HAP, yielding at least twice the number of ovulations as goats, but PMS/HCG in sheep gave a relatively poor response. Overall ovulation rate was poorest with PMS (Table 1), which also gave the most variable results. The results with PMS in Merino ewes are higher

TABLE 1.—*Mean rate of ovulation following hormonal treatment*

Breed	PMS	PMS/HCG	HAP	Total mean
Merino	8·2±8·6	4·2±3·7	9·0±2·7	7·1
Angora	1·0±0·7	3·6±2·7	4·4±5·0	3·0
Boergoat	3·0±2·9	6·0±2·6	5·4±6·8	4·8
Total mean	4·1±5·8	4·6±2·9	6·3±5·3	—

than is usually experienced mainly due to one animal with 22 ovulations. The ovulation rate of Angora ewes was consistently poorer than in the other two breeds and particularly so with PMS.

The smaller dosage of PMS supplemented by 250 i.u. HCG greatly increased the ovulation rate in both caprine breeds, but this position was reversed in the ovine group. Generally PMS/HCG resulted in the least variable ovulation rate.

HAP yielded results superior to those obtained with PMS and PMS/HCG in sheep ($P<0·05$). When compared with PMS/HCG in goats, however, there was no difference in the mean rate of ovulation but the results with HAP were highly variable.

Four of the 45 ewes had failed to ovulate when examined. Two of these were Angora and Boergoat ewes following HAP stimulation. One Angora ewe failed with PMS alone and a Merino ewe after PMS/HCG.

Luteinization of ruptured follicles was clearly more advanced in the PMS/HCG treated groups and least in HAP stimulated ewes. Angora ewes were strikingly deficient in this respect and Merino ewes invariably more advanced than all goats.

Persistent follicles

Several unruptured follicles larger than 5 mm in diameter were encountered in the majority of animals. The total pre-ovulatory follicular growth would be represented at laparotomy by ovulation points and large intact follicles. The means of this "total follicular growth" and the percentage which ovulated together with the remaining follicles are presented in Table 2 which demonstrates the extent to which

TABLE 2.—*Total follicular response, percentage ovulation and persistent follicles*

Breed	PMS			PMS/HCG			HAP		
	Total follicles	Ovulation	Persistent follicles	Total follicles	Ovulation	Persistent follicles	Total follicles	Ovulation	Persistent follicles
		%			%			%	
Merino..	10·3	76	2·6	8·8	48	4·6	12·2	74	3·2
Angora..	2·6	39	1·6	5·2	69	1·6	16·0	28	11·6
Boergoat	6·4	47	3·4	6·5	92	0·5	11·8	46	6·4

variations in the ovulation rate were due to ovulatory efficiency or to sensitivity of the ovary to the various gonadotrophins. In sheep HCG supplementation of PMS appeared to interfere seriously with ovulation, but markedly enhanced ovulation in both goat breeds ($P<0·01$). An excellent follicular growth response was obtained following HAP stimulation in all breeds. However, in goats, especially the Angora, the majority of follicles failed to ovulate.

No previous information appears to be available on the regression of persistent follicles after oestrus. From the data in Table 3 it is clear that little change occurs from 50 to 70 hours after the onset of oestrus, but thereafter regression is rapid ($P<0·01$).

All breeds exhibited slightly more ovulations and follicles on the right ovary, though the means difference was only 2·6 per cent but significant at the 5 per cent level.

TABLE 3.—*The presence of persistent follicles at intervals following oestrus onset*

Interval following oestrus	Mean number of follicles				
	Merino	Angora	Boergoat	Mean	No. of animals
50–60 hr.....	4·3	7·9	3·5	5·2	24
61–70 hr.....	4·7	2·5	5·5	4·2	7
71–80 hr.....	2·2	1·2	2·5	2·0	12

Recovery and Fertilization of Ova

The percentage of potential ova recovered was poorest with Merino ewes (Table 4) and declined markedly between 50 to 80 hours after the onset of oestrus. Uterine ova were recovered from three of five Merino ewes where tubal flushing had failed or yielded only a small proportion of the potential ova. These results contrasted unfavourably with the 100 per cent recovery in the preliminary experiment on ewes following natural oestrus, and clearly confirm accelerated tubal transport of ova in superovulated Merino ewes (Averill, 1958).

In goats there was no evidence of accelerated passage of ova through the fallopian tube. Further, in relation to oestrous onset, cleavage appeared to be slower particularly in the Angora breed of goats. This phenomenon complicated concurrent work on transfer of ova, as none of the 40 Angora ova recovered had reached the eight cell stage. Two uterine transfers of four-blastomere ova failed, but that of a third four-blastomere Angora ovum transferred to the fallopian tube of a Boergoat was successful.

A very high proportion (47 per cent) of all ova recovered had failed to cleave, and fertilization was presumed to have failed. The influence of the number of follicles on

TABLE 4.—*Influence of the time between oestrous onset and laparotomy on ovum recovery and cleavage*

Oestrus laparotomy interval	Breed	No. of animals	Total ovulations	Ova recovered	Blastomere stage of ova				
					1	2	4	6–8	16
50–60 hr..	Merino....	5	53	62%	18	9	3	3	
	Angora....	6	26	96%	17	3	5		
	Boergoat...	2	9	89%	5	2	1		
61–70 hr..	Merino....	2	10	10%				1	
	Angora....	2	3	33%		1			
	Boergoat...	2	19	84%	5	2	9		
71–80 hr..	Merino....	5	34	3%					1
	Angora....	5	16	88%	4	1	9		
	Boergoat...	2	12	33%		1	1	2	

fertilization and recovery of ova, and also the ovulation rate are reflected in Table 5. Since Moore & Shelton (1962b) found a marked decrease in fertilization when more

TABLE 5.—*Influence of the number of follicles on the rate of ovulation, recovery, and fertilization*

Range of follicles	Number of animals	Ovulations, mean	Potential Ova recovered	Ova fertilized (recovered)
			%	%
0–3.....	13	2·9	70	77
4–6.....	5	6·8	38	62
>7.....	6	8·3	86	54

than 20 corpora lutea were present, no ewes with more than this number of ovulation points are included in this table. There was a sharp increase in the number of corpora lutea present when more than three follicles were present in the ovaries ($P<0{\cdot}01$). The proportion of uncleaved ova is also seen to rise progressively with increased follicles, but the recovery of ova does not appear to be affected.

The proportion of cleaved ova was high in the PMS and PMS/HCG groups, but low with HAP (Table 6). This variation appears to be correlated with the number of persistent follicles in the ovaries. Again animals with an excessive number of ovulations were excluded from this table to eliminate this variable.

TABLE 6.—*Influence of gonadotrophin on fertilization*

Gonadotrophin	Number of animals	Mean number of follicles	No. of unfertilized ova	No. of fertilized ova
PMS	9	2·5	5	15 (75%)
PMS/HCG	8	2·2	6	20 (77%)
HAP	6	7·1	20	20 (50%)

OESTRUS PHENOMENA

Occurrence of oestrus

Only two of the 45 ewes failed to exhibit signs of oestrus within five days following gonadotrophin treatment. Both had been subjected to HAP treatment. The one, a Merino, exhibited silent heat with nine ovulations, and the other, an Angora, had no ovulations but three large follicles.

The degree of oestrous synchronization is presented in Table 7, from which it is clear that the most predictable occurrence follows PMS/HCG stimulation. The widest distribution was obtained with HAP, but two ewes so treated failed to exhibit behavioural oestrus. Nevertheless, 84 per cent of all ewes commenced oestrus on the second or third day after gonadotrophin treatment. Boergoats tended to take longer to respond than any other breed.

TABLE 7.—*Days after gonadotrophin injection on which onset of oestrus was exhibited*

Treatment	Breed	2	3	4	5	No. oestrus
PMS	Merino	3	1	1		
	Angora	1	4			
	Boergoat	2	3			
PMS/HCG	Merino	4	1			
	Angora	5				
	Boergoat	1	4			
HAP	Merino	3	1			1
	Angora	2	2			1
	Boergoat	1	1	1	2	

HCG supplementation of PMS appeared to reduce the interval between treatment and time of onset of oestrus appreciably in Merino and Angora ewes.

The duration of the induced oestrus (Table 8) tended to be rather longer than normal with PMS, but was reduced by a mean of 10 hours with PMS/HCG treatment. Oestrus following HAP treatment was also short in Merino and Boergoat ewes, but longer in the Angora breed. No marked deviations from natural oestrus were recorded.

TABLE 8.—*Duration of oestrus in hours*

Breed	PMS	PMS/HCG	HAP	Mean
Merino	38	24	24	28·7
Angora	34	31	33	32·7
Boergoat	34	22	24	26·7
Mean	35·3	25·7	27	—

Oestrus and ovarian morphology

The number of ovulations and follicles in relation to the interval between the gonadotrophin injection and onset of oestrus is presented in Table 9. The 15 HAP-treated ewes are excluded from this table as their treatment extended over three days. It is clear that the number of follicles present in the ovaries of ewes exhibiting oestrus within 52 hours, is larger than the number in ewes showing oestrus later ($P<0·02$). Ewes exhibiting their first positive oestrous test in the morning appeared to have many more ovulations than those commencing oestrus during the day.

TABLE 9.—*The gonadotrophin-oestrus onset interval in relation to the number of ovulations and follicles*

Interval (hr)	No. ewes	Ovulations	Follicles
40	12	4·6	3·3
52	4	1·8	3·2
64	10	5·1	1·4
75	3	2·3	1·7

No influence of the number of ovulations and follicles on oestrous duration could be found. Twenty-two animals exhibiting oestrus for less than 30 hours had a mean of 4·6 and 4·0 ovulations and follicles, respectively and for 21 ewes exhibiting oestrus for longer than 30 hours the corresponding figures were 4·6 and 4·1.

DISCUSSION

The variable results obtained with PMS were not unexpected, as the dose-response curve of this placental gonadotrophin is too steep in the desirable range of 2 to 4 follicles (Lamond, 1962). Studies on the variation of the FSH and LH content of the pituitaries of ewes have suggested a similar pattern of release of both gonadotrophins during the oestrous cycle (Santolucito, Clegg & Cole, 1960; Robertson & Hutchinson, 1962). Though these workers' investigations did not include the day prior to oestrus, their results do encourage studies involving the simultaneous

administration of these hormones. As Lamond (1962) pointed out, however, excessive LH prior to oestrus may lead to premature luteinization of follicles and result in suppression of oestrus by progesterone. In the present work, HCG did not suppress oestrus although the duration was considerably shorter than usual. Evidence of interference with ovulation was found in sheep, but HCG supplementation markedly potentiated follicular growth and ovulation in goats and resulted in superior synchronization of oestrus.

The pituitary of sheep has an extraordinarily high LH content when compared with other domestic animals, and in all probability this rich endogenous source together with the injected preparation resulted in excessive luteinizing activity in the blood. In goats on the other hand, the number of follicles which ovulated and the extent of luteinization were proportional to the luteotrophic activity of the particular gonadotrophin adminstered. Robinson (1962) did not detect any differences in the degree of luteinization with different types of gonadotrophins in sheep. Corpus luteum growth was markedly deficient in goats following HAP injection. An investigation of HCG supplementation of HAP therapy in goats is clearly indicated.

Variation in the rate of ovulation among the three breeds appears to be related to the " depth " of anoestrus, as the mean rate was directly proportional to the length of the breeding season exhibited locally by each breed. Subsequent work has in fact shown that the response of Angora ewes to PMS is comparable to that in Merino ewes during the peak of the breeding season.

The high sensitivity of the Angora ovary to preparations with relatively pure FSH-like action has been demonstrated. However, the failure of the majority of follicles to ovulate and the almost absence of luteinization three days after oestrus was in remarkable contrast to the findings in sheep. This constitutes indirect evidence of deficient endogenous LH function in this breed, an aberration which may be related to the problem of gestational failure common in South African Angora ewes. The growth of the corpus luteum during the natural oestrous cycle is markedly retarded in habitually aborting Angora ewes (van Rensburg—unpublished). The previous reproductive performance of the ewes used in this experiment is unknown.

The recorded characteristics of Boergoat ewes are generally intermediate between Angora and Merino ewes. Reproductive failure due to inadequate hormonal function has not been recorded in this breed.

Wastage of ova in this experiment was predominantly due to apparent failure of fertilization (47 per cent of 104 ova). The factors responsible are presumably associated with a hormonal imbalance due to exogenous administration and endogenous production from excessively stimulated ovaries. Evidence supporting the latter hypothesis is seen in Table 5, where the percentage of fertilized ova is seen to decrease rapidly with the presence of increasing numbers of follicles. This presumably hyperoestrogenic state would last for 70 hours, whereafter regression of the follicles was found to be rapid. Fertilization rate was very much higher with the PMS/HCG treatment and this combination may therefore represent a closer approach to the natural endogenous ratio of gonadotrophin. The physiological actions of PMS and HCG have been reviewed by Robinson (1962) and Lamond (1962).

The successful recovery of ova from goats contrasted with the disappointing recoveries from Merino ewes. This difference was undoubtedly due to accelerated tubal transport of ova in the Merino. Our poor results with these ewes approximate the 57 per cent recovery achieved by Averill (1958) in his comprehensive work. The apparent retarded tubal passage of goat ova necessitates considerable revision of

recovery intervals accepted for sheep when ova in a more advanced stage of cleavage are required. It is unlikely that goat ova enter the uterus at a lesser stage of cleavage than in sheep, as transfers of four-blastomere ova to the uterus failed, but were successful when placed in the fallopian tube.

As far as predicting the yield of ova is concerned, it is disappointing that the only behavioural characteristic which appears to be associated with ovarian activity is the hastening of oestrous onset in the presence of excessive follicles. Highest yields were obtained in this experiment with HAP in Merino ewes and in goats with PMS/HCG.

Summary

Following progesterone pretreatment a total of 45 anoestrous ewes consisting of equal numbers of Merino, Angora, and Boergoat ewes was treated with gonadotrophin according to three different schedules. Detailed observations were made on behavioural response and, at laparotomy, on ovarian morphology and ovum recovery.

Merino ewes showed the highest overall ovulation rate (7·1) followed by Boergoat (4·8) and Angora ewes (3·0). Pregnant mare serum (PMS) was highly variable in sheep and resulted in poor responses in goats. A horse anterior pituitary gonadotrophin (HAP) resulted in variable but marked follicular growth in all breeds; however, in goats the majority of follicles failed to ovulate. PMS combined with simultaneous human chorionic gonadotrophin (PMS/HCG) suppressed ovulation to some extent in sheep but markedly potentiated follicular growth and ovulation in goats and was the most predictable of the three gonadotrophins. A small but significant difference of total follicular growth in favour of the right ovary was found.

Large persistent follicles were encountered in most animals and increased proportionally with the number of ovulations. These follicles persisted up to 70 hours after oestrous onset, whereafter regression was rapid. Excessive follicles were accompanied by an increased incidence of unfertilized ova. Recovery of cleaved ova was also seriously hampered by accelerated tubal transport in Merino ewes, but no such phenomenon was encountered in goat ewes, which in fact exhibited evidence of retarded tubal transport of ova in relation to oestrous onset.

Oestrus commenced in 84 per cent of all animals on the second or third day following gonadotrophin treatment and was longer than usual with PMS and considerably shortened by PMS/HCG. Excessive persistent follicles hastened the onset of oestrus but the numbers of follicles and ovulations had no effect on oestrous duration.

Indirect evidence that the endogenous luteinizing hormone contribution is optimal in sheep, marginal in Boergoats and deficient in Angora goats is discussed and a relationship to the high incidence of gestational failure in Angora ewes suggested.

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