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THE CONCEPTION RATE OF MAP AND MAP-PMSG-TREATED KARAKUL EWES INSEMINATED WITH DILUTED SEMEN

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OPSOMMING: DIE EFFEK VAN VERDUNNING VAN SEMEN OP BESETTING NA KUNSMATIGE INSEMINASIE VAN MAP-DMS-BEHANDELDE KARAKOELOOIE

Ses groepe van 45 volwasse Karakoelooie is met 40 mg MAP sponse vir 15 dae behandel. Vyf van die groepe het 400 IE DMS twee dae voor sponsonttrekking ontvang. Al die ooie is gepaar 12 uur na aanvang van oestrus en weer 9 tot 10 uur later, òf kunsmatig òf deur middel van natuurlike paring. Een van die groepe is op 'n tydsbasis, 48 uur na sponsonttrekking, geïnsemineer. Die besetting was gemiddeld 57,8% na KI en 68,6% na natuurlike paring. Die getal spermatozoa geïnsemineer het nie besetting beïnvloed nie, maar die groter inseminaat volume het wel besetting benadeel. Die buise van 5 ooie in elke groep is 24 uur na eerste inseminasie gespoel en die getal spermatozoa herwin was groter in geval van groter getalle geïnsemineer. Servikale slymmonsters is verder van dié ooie wat op 'n tydsbasis geïnsemineer is geneem en die slym het dikker en gevolglik meer onderdringbaar vir spermselle geword namate die siklus gevorder het. In geval van hierdie ooie het die manipulasie wat met die monsterneming gepaard gegaan het skynbaar konsepsie (40%) nadelig beïnvloed.

SUMMARY:

Six groups of 45 Karakul ewes were treated with 40 mg MAP sponges inserted for 15 days. Five of these groups also received 400 IU PMSG on the 13th day. All the ewes were inseminated 12 h after the onset of oestrus and again 9 to 10 h later, either artificially with 80 to 2000 x 10^6 spermatozoa in volumes of 0,2 or 0,5 ml or by natural service. One of the groups was inseminated at a fixed time 48 h subsequent to sponge withdrawal. The conception rates were 57,8% after AI and 68,6% following mating. The number of spermatozoa inseminated did not affect the conception rate but this was lower when the spermatozoa were in the higher volume of fluid. Spermatozoa were flushed from the tubes of 5 ewes in each group 24h after the first insemination and the numbers of sperm recovered was greater when large numbers had been inseminated. Cervical mucus were taken from one group and it was evident that the mucus became thicker as oestrus proceeded. The manipulation of the cervix to collect mucus appeared to reduce the conception rate in this group (40%).

Despite its widespread use in Russia and the central European countries, artificial insemination is not common practice in other major sheep producing countries. In South Africa some of the factors preventing its use are the poorer conception rates obtained with stored semen, the past inability to store semen for any length of time as well as the unpredictability of oestrus on any given day. However, following the development of convenient and effective methods of oestrus synchronisation (Robinson, 1965) and improved techniques of preserving semen (Visser, 1974), renewed interest in exploiting AI was apparent.

Experimental control of oestrus in sheep has resulted in variable fertility and it has subsequently been found that low sperm numbers in the tubes, after AI with diluted semen, was a major factor responsible (Quinlivan & Robinson 1967; Schindler & Amir, 1973).

The experiments described in this paper were designed to determine the relationship between the number of spermatozoa inseminated, the volume of the inseminate, the number of spermatozoa in the Fallopian tubes and the conception rate of Karakul ewes inseminated at a fixed time without reference to the behaviour of the ewe.

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Procedure

Two hundred and seventy parous Karakul ewes were randomly allotted to 6 groups of 45 and treated as shown in Table 1. One month prior to commencement of the trial, semen from 10 breeding rams was collected twice weekly by artificial vagina and examined for density, motility and percentage live sperm cells. Ewes were mated or inseminated at the controlled cycle only, 12 h after first oestrus and again 9 to 10 h later. Random samples of ejaculates were examined every day for the estimation of sperm numbers to ensure that the total number of live spermatozoa per ejaculate was in excess of 2000 x 10^6. Semen for insemination was collected from all rams and the ejaculates of four to five rams pooled after examination. Any ejaculate which failed to attain a 4,4 grading for density and motility (maximum 5,5) was discarded. Random samples of pooled ejaculates were examined for percentage of abnormal spermatozoa and sperm numbers determined by haemocytometer counts. Satisfactory samples were pooled and used either undiluted or diluted 1 : 4 with sterilized skimmed milk (30°C). Diluted samples were subsequently examined microscopically to ensure sperm were motile and to decide on the correct volume for insemination. Pooled semen was used for insemination of approximately equal numbers of ewes in each treatment group.

Table 1

Treatment schedule according to method of mating and numbers of spermatozoa introduced

Group	Hormone treatment	Method of mating and approximate numbers of spermatozoa introduced
1	40 mg MAP sponges for 15 days plus 400 I.U. PMSG on Day 13	Handmating 12 h after first oestrus (> 2 000 x 10^6 spermatozoa)
2	–do–	Artificial insemination with 0,2 ml undiluted semen (> 400 x 10^6 spermatozoa)
3	–do–	Artificial insemination with 0,5 ml diluted semen (1 : 4 warmed cowes milk, > 200 x 10^6 spermatozoa)
4	–do–	Artificial insemination with 0,2 ml diluted semen (> 80 x 10^6 spermatozoa)
5	–do–	As for Group 4 but AI, without teasing, on a time basis 48 h after sponge withdrawal
6	60 mg MAP sponges for 15 days	AI as for Group 4

Table 2

Numerical scores for visual assessment of cervical mucus of Karakul ewes inseminated on a time basis

Mucus trait	Score			
	0	1	2	3
Consistency	–	Thick and caseous	Cloudy and copious	Clear and copious
Cellular content	–	Dry clumps of cornified cells	Scattered cells	Few cells
Ferning	Absent	Few faint patterns	Scattered patterns	Well defined patterns

Cervical mucus was taken from all the ewes in Group 5 immediately after sponge withdrawal and subsequently every 12 h until insemination at 48 h. The mucus was collected as described by Le Roux & Nel (1968) and subjective gradings were made according to a numerical scale given in Table 2.

Five ewes were randomly selected from each group and laparotomised 24 h subsequent to first insemination. Following midline laparotomy under pentobarbitone (Sagatal-Maybaker) anaesthesia, the fallopian tubes were flushed with saline (Quinlivan & Robinson, 1969). The flushings from both tubes were pooled and collected in a glass bottle and frozen. Following thawing the pooled sample from every ewe was made up to 5 ml with normal saline. After thorough mixing and the adding of one drop congo red-nigrosin, aliquots were placed by pasteur pipette into leucocyte counting chambers of a haemocytometer. After a counting chamber had been filled the residue in the pipette was returned to the holder. The contents were then remixed and a second sample taken. At least 15 minutes were allowed for spermatozoa to settle after which intact sperm and loose sperm heads were counted (X 200). The number of spermatozoa in each chamber was counted using the standard technique for white blood cells. The mean for 5 chambers was determined for each flushing. After counting 5 blocks in each chamber this number multiplied by 10 000 gave an estimate of the total number of spermatozoa recovered from each ewe.

In every subgroup 40 ewes were allowed to go to term and a complete record of service, oestrus and lambing data was kept so as to facilitate comparison between treatments. Oestrus and lambing data were analysed by Chi-square and sperm numbers by analysis of variance of corrected log means (Snedecor & Cochran, 1967).

Results and Discussion

Synchronisation of oestrus and lambing results

The various treatment effects on oestrous response and conception are shown in Table 3. A total of 3 (1,3%) sponges were lost and these ewes were not included in

Table 3

Conception and lambing results subsequent to AI with different dilution rates of MAP and MAP-PMSG-treated Karakul ewes during the early breeding season (N = 40)

Factors	Treatment groups according to method of mating and numbers of spermatozoa introduced						Total and mean (%)
	1	2	3	4	5	6	
Lost sponges	1	–	–	–	2	–	3 (1,3)
Synchronised oestrus within 5 days (%)	89,7	100,0	95,5	92,5	Inseminated on a time basis	97,5	95,0
Lambing % of ewes mated	68,6	65,0	57,9	64,9	40,0	59,0	54,9
% Viable lambs of ewes mated	102,9	90,0	74,0	94,6	45,0	61,5	78,0
% Multiple births of ewes lambed	45,8	34,6	27,3	50,0	18,8	8,7	31,9

the analysis. A highly satisfactory overall response of 95% oestrous ewes within 5 days of sponge withdrawal was observed. On the whole oestrus was accellerated by a mean of 27,4 h ($P < 0,01$) where PMSG was given in conjunction with MAP sponges compared with MAP alone. The lambing results indicated only slight differences except for Group 5 which were inseminated without teasing with subsequent poorer fertility (40% ; $P < 0,05$).

Under the extensive grazing and flock management conditions prevailing in Australia and even in those countries where AI of sheep has been most highly developed viz. the Soviet Union, Central Europe and South America, lambing rates of 55 to 65% usually follow AI with undiluted semen (Jones, Martin & Lapwood, 1969). If these figures are a reliable criterion, then the mean conception rate of 57,8 (range 40–65%) following AI in the present study, could be regarded as satisfactory. Futhermore these results were obtained after a double insemination at the synchronised cycle only and might have been better in the event of returns being rebred at the second cycle.

The effect of initial numbers of spermatozoa inseminated on subsequent conception and the incidence of multiple births, as shown in Table 3, was negligible. However it would appear that greater inseminate volumes (0,5 ml; Group 3) had a depressing effect on both these parameters. This could have been as a result of a physical inability to get the greater volume of fluid into the cervix.

Table 4

Estimated mean number of spermatozoa recovered from the tubes of Karakul ewes 24 h after handmating or AI

Main effects	Mean number of spermatozoa recovered		
	Arithmetic ± SE	Log 10	Reconstituted corrected mean
Sperm numbers introduced			
Group 1. > 400 x 10^6	37120 ± 1600	4,5443 a	35010
Group 2. > 800 x 10^6	17480 ± 1060	4,1522 b	14200
Group 3. > 400 x 10^6	3200 ± 327	3,4512 bc	2826
Group 4. > 160 x 10^6	2640 ± 282	3,3792 bc	2395
PMSG application			
Group 6. – 0	4800 ± 383	3,6461 a	4428
Group 4. – 400	2640 ± 282	3,3792 a	2395
Premating treatment			
Group 4. Teased	2640 ± 282	3,3792 a	2395
Group 5. Not teased	2020 ± 293	3,1680 a	1472

abc = Within each set of observations numbers having the same superscript are not significantly different from each other.

Numbers of spermatozoa recovered

Table 4 shows the estimated mean number of spermatozoa recovered from tubal flushings. There were highly significant ($P < 0,01$) differences in numbers of spermatozoa recovered attributable to numbers introduced.

These results agree with the findings of Kennedy & Kennedy (1972) and Allison (1972), but Quinlivan & Robinson (1967) reported in contrast that numbers inseminated did not influence numbers recovered. In addition, numbers of spermatozoa actually recovered in the present study, particularly from handmated ewes, greatly exceeded the numbers previously reported to be recovered from ewes inseminated during the controlled cycle (Quinlivan & Robinson, 1967; 1969; Kennedy & Kennedy, 1972). However the ewes in the present study were mated or inseminated at least 12 h after oestrus had been established, whereas previous workers inseminated at first signs of oestrus. Presumably the inhibiting effect of progestagen on sperm transport (Quinlivan & Robinson, 1967) as well as the effect on sperm loss though breakage and fagocytosis (Hawk, 1972), had been alleviated by delaying insemination.

Significantly more spermatozoa were recovered from handmated compared with artificially inseminated ewes (35010 cf 6473; $P < 0,01$). This is in agreement with the findings of Lightfoot & Restall (1971). Contrary to the hypothesis of Quinlivan & Robinson (1967) it thus would appear that sperm transport to the tubes might well be influenced by the introduction of large numbers of spermatozoa either through natural service or by concentrating in small inseminate volumes.

Although greater numbers of spermatozoa inseminated resulted in greater recovery rates this did not result in a substantial increase in fertility. However smaller volumes inseminated did prove beneficial possibly through the physical effect of the inseminator being able to deposit the smaller fluid volume into the cervix. It thus would appear that good conception rates can be obtained provided that small volumes are used and sperm density is $\geq 80 \times 10^6$.

Insemination on a time basis

The advantages of AI on a fixed time basis without prior teasing are obvious. However, the conception rate of 40% recorded for Group 5 (Table 3) was significantly lower than for Group 4 (64,9%). There are three possible causes for this:

(a) the ewes were not teased
(b) the cervix was repeatedly manipulated to obtain mucus, and
(c) the insemination was not related to observed oestrus.

The first two of these might well have resulted in changes in the rate of transport of the gametes. However, the evidence from sperm recovery in tubal flushings (Table 4) suggests that sperm transport at least was unaffected and the same is probably true for the ova.

Table 5

Mean mucus scores of successful and dry Karakul ewes inseminated on a time basis 48 h after sponge withdrawal

Factors	Numerical mean scores			
	Consistency (1 to 3)		Ferning (0 to 3)	
Parity	Lambed	Dry	Lambed	Dry
Number	16	24	16	24
Mean score ±SE	2,29 ± 0,11	1,63 ± 0,13	2,56 ± 0,13	1,38 ± 0,19

It is possible to monitor the course of oestrus by observing the consistency and ferning pattern of the cervical mucus (Dun & Restall, 1961; Le Roux & Nel, 1967) and when the results of insemination of ewes in Group 5 is compared with the mucus score at the time of insemination (Table 5), there is a suggestion that those ewes which did not lamb had a lower score than those which did lamb. If this is substantiated by further work it should be possible to obtain reasonable conception rates by inseminating ewes without using a teaser ram. Furthermore, insemination on a time or any other basis should have as a prerequisite the absolute minimum of any form of manipulation or handling of the ewes concerned.

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