# FERTILITY OF PROGESTAGEN-TREATED EWES IN RELATION TO THE NUMBERS AND CONCENTRATION OF SPERMATOZOA IN THE INSEMINATE

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*Abstract*

Eight hundred Merino ewes in which oestrus was synchronized with progestagen-impregnated sponges were incorporated into an experiment of parallelogram design ($4 \times 5$; $n = 40$) in which four doses (numbers) of spermatozoa were inseminated in five concentrations (numbers/ml), which involved a range in volume of inseminate from 0·012 ml to 1·6 ml. The purpose was to determine the importance on fertility of the number of spermatozoa relative to the degree of dilution, i.e. their concentration and the consequent volume in which a given number was inseminated. All ewes were inseminated 48 hr after withdrawal of sponges.

Fertility within the 20 treatment combinations of the experiment ranged from 5·3 to 47·4%. There was a linear effect of number of spermatozoa in the inseminate over the whole range of numbers used: 50–400 ($\times 10^6$). There was a linear and quadratic effect of concentration: increasing concentration up to $200 \times 10^7$ spermatozoa per millilitre was associated with increasing fertility, with no further increase to $400 \times 10^7$.

Insemination of 0·05 or 0·10 ml of undiluted semen was more effective than was the use of larger volumes of diluted semen containing the same numbers of spermatozoa. Wherever possible undiluted semen should be used for the artificial insemination of the progestagen-treated ewe, and the maximum dilution must not exceed 1 : 1 ($\doteqdot 200 \times 10^7$ spermatozoa/ml).

## I. Introduction

Fertility following artificial insemination of ewes in which oestrus has been synchronized generally has been lower than that observed in untreated ewes, particularly when diluted semen has been used. The initial use of intravaginal sponges (Robinson 1965) resulted in apparently normal fertility following the insemination of 0·2 ml of undiluted semen or natural mating with a high percentage of rams. Three of four studies where fertilization rates have been measured in synchronized ewes inseminated with undiluted semen have indicated fertilization rates within the normal range (Moore *et al.* 1967; Quinlivan 1967; Quinlivan and Robinson 1967; Allison and Robinson 1970). On the other hand, Moore *et al.* (1967) and Robinson and Moore (1967) have shown that the use of diluted semen has resulted in fertilization and lambing rates lower than normal and conclude that the accepted criteria for sperm numbers and dilution rates applicable to "normal" ewes (Emmens and Robinson 1962) may require modification for ewes in which oestrus is controlled.

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The numbers of spermatozoa inseminated may be varied by altering either the volume (concentration of spermatozoa remaining constant) or the concentration (volume remaining constant) of the inseminate, or a combination of both. Although there is a vast literature concerning artificial insemination, and some authors have obtained a correlation between numbers of spermatozoa and fertility (Panyševa 1940; Semkov and Kolev 1966), the importance of concentration of spermatozoa in the inseminate and the consequent volume required to provide a given number of spermatozoa has not been determined. Sinclair (1957) concluded that increasing the volume of undiluted semen above 0·10 ml was of little value while Jones, Martin, and Lapwood (1969) found no difference in fertility within the range of 0·02–0·08 ml. Lightfoot and Salamon (1970), working with frozen semen, reported that increasing the concentration of the inseminate increased the numbers of spermatozoa recovered from the cervix 30 min after insemination. Finally, Quinlivan and Robinson (1967) found that increasing the numbers of spermatozoa from 80 to 1500 ($\times 10^6$) by increasing the volume of undiluted semen had little effect on the numbers of spermatozoa in the Fallopian tubes 24 hr after insemination.

The population of spermatozoa in the ovine cervix is probably maximal within 15 min of deposition of semen (Mattner 1963) and motility of the spermatozoa appears essential for the establishment of this population (R. J. Lightfoot and B. J. Restall, personal communication). It is reasonable to conclude that a high concentration of motile spermatozoa at the semen–mucus interface immediately proximal to the external cervical os would be conducive to initial rapid entry into the cervix. Assuming that dilution of semen (with a resultant decrease in the concentration of spermatozoa) does reduce the chances of a large number of spermatozoa entering the cervix, this could be expected to be of particular importance in the progestagen-treated ewe in which there exists an intrinsic problem of sperm transport and survival (Quinlivan and Robinson 1967, 1969). Consequently the following experiment was conducted in an effort to determine the relative importance on fertility of (1) number of spermatozoa in the inseminate, and (2) their concentration, and to define the volume/concentration combinations most likely to provide maximum chance of conception in such ewes.

## II. Materials and Methods

### (a) *Experimental Design*

Eight hundred 5-yr-old Merino ewes were incorporated into a $5 \times 4$ parallelogram design ($n = 40$) which incorporated five concentrations of spermatozoa in the inseminate and four numbers of spermatozoa (see Table 2). Associated with these combinations were volumes of inseminate ranging from 0·012 to 1·60 ml. The loss of 40 ewes from the experiment due to loss of sponges, death, and other causes reduced the number to 760, with a harmonic mean of 38 in each of the 20 cells.

### (b) *Hormone Administration and Detection of Oestrus*

All ewes were treated for 16 days with intravaginal sponges prepared in the laboratory and containing 30 mg Cronolone. Such sponges release an average of 20–25 mg of steriod over a 16-day period. Insertion was over a period of 4 days, starting on January 30. Withdrawals were made from 100 ewes at 0800 and 1800 hr on each of 4 successive days and ewes were immediately placed with 15 raddled vasectomized rams. Oestrus was recorded at 12-hourly intervals but all animals were inseminated 47–49 hr (mean 48) after sponge withdrawal, irrespective of the occurrence of oestrus. After insemination, ewes which had already exhibited oestrus were transferred to a nearby paddock while the remainder were returned to the teaser rams.

### (c) *Artificial Insemination*

Semen was collected by artificial vagina from 8 to 10 rams at each of the eight periods of insemination. The ejaculates were assessed visually and those of high quality were pooled. The concentration of spermatozoa per millilitre of pooled ejaculate ranged from 410 to 450 ($\times 10^7$), as assessed in an EEL photocolorimeter calibrated against haemocytometer counts. The proportions of heat-treated milk required to provide concentrations of 200, 100, 50, and $25 \times 10^7$ spermatozoa per millilitre were calculated and the appropriate dilutions made. Undiluted semen was used to provide the highest concentration ($400 \times 10^7$/ml) and, as the volume used was pre-determined, the actual numbers used at this concentration were from 2·5 to 12·5% higher than specified. No corrections were made due to the minor nature of this error.

Four or five ewes from each of the 20 treatment combination groups were inseminated at each of the eight insemination periods, a check being maintained to ensure an even distribution of oestrous and non-oestrous ewes. All were inseminated within $1\frac{1}{4}$ hr of collection of semen. Small volumes of inseminate (0·012 and 0·025 ml) were measured in a micropipette attached to a tuberculin syringe.

### (d) *Observations on Lambing Performance*

One month before lambing the identification number of each ewe was branded on the flank with Siromark sheep-branding fluid. During lambing the flock was examined daily; newly born lambs were caught and marked and the identification numbers of dams recorded.

### (e) *Statistical Analysis*

The data for fertility were subjected to an analysis of variance to single degrees of freedom, following transformation of percentages to angles (Claringbold, Biggers, and Emmens 1953). The regression equations were derived from this analysis.

## III. Results

### (a) *Oestrus*

Oestrus was recorded in 680 of the 760 ewes for which complete data are available (89·5%), with the time distribution shown in Table 1.

Table 1

DISTRIBUTION OF TIME OF ONSET OF OESTRUS RELATED TO LAMBING PERFORMANCE

| | Time of Onset of Oestrus (hours after sponge withdrawal) | | | | | Non-oestrous Ewes | Total |
|---|---|---|---|---|---|---|---|
| | 36 | 48* | 60 | 72 | 84 | | |
| Ewes inseminated | 63 | 345 | 186 | 78 | 8 | 80 | 760 |
| Ewes lambed | 20 | 117 | 40 | 17 | 1 | 17 | 212 |
| Percentage lambed | 31·7 | 33·9 | 21·5 | 21·8 | 12·5 | 21·3 | 27·9 |

| | |
|---|---|
| Significance of interaction between percentage ewes lambed and: | |
| Time of onset of oestrus | $\chi^2_4 = 12{\cdot}38$; $P < 0{\cdot}02$ |
| Oestrous or non-oestrous ewes | $\chi^2_1 = 1{\cdot}96$; n.s. |
| Oestrus detected before (36, 48 hr) or after insemination (60, 72, 84 hr) | $\chi^2_1 = 11{\cdot}98$; $P = 0{\cdot}001$ |

* Time of artificial insemination.

TABLE 2

PERCENTAGE OF EWES WHICH LAMBED FOLLOWING INSEMINATION WITH DIFFERENT VOLUMES OF SEMEN AND CONCENTRATIONS OF SPERMATOZOA

All ewes were teased to determine the time of onset of oestrus but insemination was carried out on a time basis, 48 hr after withdrawal of sponges. Each percentage is based on 37–40 ewes inseminated

| Volume of the Inseminate (ml) | Concentrations of Spermatozoa: $400\times10^7$/ml* | | $200\times10^7$/ml | | $100\times10^7$/ml | | $50\times10^7$/ml | | $25\times10^7$/ml | | Mean Lambed (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. of Sperm (millions) | Ewes Lambed (%) | No. of Sperm (millions) | Ewes Lambed (%) | No. of Sperm (millions) | Ewes Lambed (%) | No. of Sperm (millions) | Ewes Lambed (%) | No. of Sperm (millions) | Ewes Lambed (%) | |
| 0·012 | 50 | 26·3 | | | | | | | | | |
| 0·025 | 100 | 21·1 | 50 | 28·9 | | | | | | | |
| 0·050 | 200 | 42·1 | 100 | 31·6 | 50 | 23·7 | | | | | |
| 0·100 | 400 | 47·3 | 200 | 36·8 | 100 | 34·2 | 50 | 26·3 | | | |
| 0·200 | | | 400 | 47·4 | 200 | 23·7 | 100 | 23·7 | 50 | 5·3 | 22·1 |
| 0·400 | | | | | 400 | 44·7 | 200 | 21·1 | 100 | 15·8 | 25·3 |
| 0·800 | | | | | | | 400 | 23·7 | 200 | 10·5 | 26·8 |
| 1·600 | | | | | | | | | 400 | 23·7 | 37·4 |
| Mean lambed (%): | 34·2 | | 36·2 | | 31·6 | | 24·2 | | 13·8 | | 27·9 |

* Undiluted semen (see text).

### (*b*) *Lambing*

Table 2 presents the lambing data and Table 3 the analysis of variance. Overall, 212 (27·9%) ewes lambed yielding 260 lambs. There were no differences between treatments in the proportion of multiple births.

There was a highly significant ($P < 0{\cdot}001$) linear increase in fertility with increasing numbers of spermatozoa in the inseminate over the whole range from 50 to 400 ($\times 10^6$). This accounted for 21·6% of the total variance and had the following form, shown in Figure 1,

$$y = 8{\cdot}67 + 10{\cdot}52x,$$

where $\sin^2 y$ = proportion of ewes lambed and $x = \log_{10}$ number of spermatozoa ($\times 10^6$).

TABLE 3

ANALYSIS OF VARIANCE OF LAMBING DATA SHOWING THE PERCENTAGE CONTRIBUTION OF THE SOURCES OF VARIATION

| Source of Variation | Degrees of Freedom | Mean Square | Percentage of Variation |
|---|---|---|---|
| Number of spermatozoa | (3) | | |
| Linear | 1 | 250·91*** | 21·6 |
| Quadratic | 1 | 19·21 | 1·7 |
| Cubic | 1 | 17·47 | 1·5 |
| Concentration of spermatozoa | (4) | | |
| Linear | 1 | 524·90*** | 45·3 |
| Quadratic | 1 | 119·98* | 10·3 |
| Cubic | 1 | 0·46 | 0·0 |
| Quartic | 1 | 0·77 | 0·1 |
| Interaction | 12 | 18·87 | 19·5 |
| Total sum of squares | 19 | 1159·96 | 100·0 |
| Theoretical variance [$n(k_0) = 38$] | $\infty$ | 22·20 | |

* $P < 0{\cdot}05$. *** $P < 0{\cdot}001$.

There was a highly significant ($P < 0{\cdot}001$) linear increase in fertility with increasing concentration of spermatozoa in the inseminate and a significant ($P < 0{\cdot}05$) quadratic component. Together these accounted for 55·6% of the total variance and had the following form, shown in Figure 2,

$$y = -54{\cdot}34 + 76{\cdot}46x - 16{\cdot}14x^2,$$

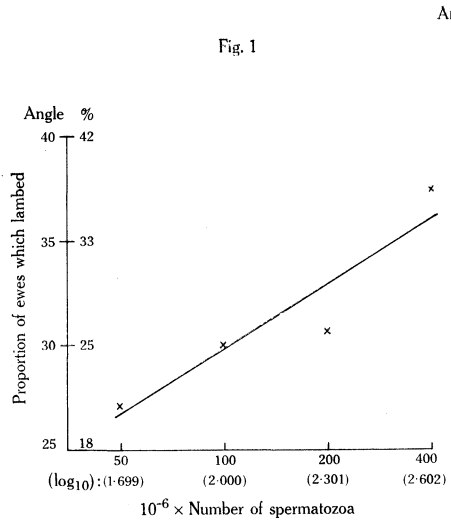
where $\sin^2 y$ = proportion of ewes lambed and $x = \log_{10}$ concentration of spermatozoa per millilitre ($\times 10^7$) in the inseminate.

There were no significant interactions.

The time to onset of oestrus relative to that of insemination was important. Of those ewes recorded in oestrus before insemination, 33·6% lambed compared with 21·3% of those which either came into oestrus after insemination or did not exhibit oestrus (Table 1).

## IV. Discussion

These data show that at higher dilutions (i.e. above 4 diluent : 1 semen) the concentration of spermatozoa in the inseminate has a substantially greater effect on fertility than has the number of spermatozoa used. Figure 2 shows that halving the concentration by four successive dilutions from 400 to 200, 100, 50, and 25 ($\times 10^7$) spermatozoa per millilitre is associated with expected reductions in fertility of 0, 4, 8, and 10%, despite the use of the same numbers of spermatozoa but in increasing volumes of inseminate.



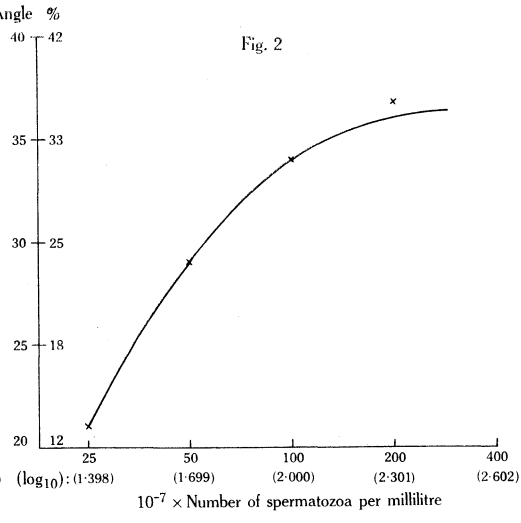




Fig. 1.—Linear regression of proportion of ewes which lambed on the number of spermatozoa in the inseminate. For regression equation, see text.

Fig. 2.—Curvilinear regression of proportion of ewes which lambed on the concentration of spermatozoa in the inseminate. For regression equation, see text.

Each doubling of the numbers of spermatozoa per inseminate from 50 to 100, 200, and 400 million, by three successive increases in volume, is associated with an expected 5% increase in fertility (Fig. 1) and there is no evidence that maximum potential fertility is reached at the highest number. It could be argued that this is due to failure to obtain optimum fertility with the large volumes of more diluted semen, but inspection of Table 2 does not support this argument. Fertility is still increasing in a linear fashion with each successive increase in volume of undiluted semen, and it appears that full potential fertility may not have been attained even with 0·1 ml of such semen containing 400 million spermatozoa. It can be concluded that for the insemination of progestagen-treated ewes at first oestrus after treatment it is advisable to use 0·1–0·2 ml of undiluted semen. If semen must be diluted, the rate must not exceed 1 : 1.

These requirements appear to be considerably more rigid than those applicable to ewes experiencing a normal oestrus. It is generally accepted that "normal" fertility in such ewes can be attained using semen diluted at rates up to 4 : 1 and containing 50–150 million spermatozoa in the inseminate (Emmens and Robinson 1962;

Salamon and Robinson 1962) and there are reports of satisfactory conception rates following much greater dilution (Dauzier, Thibault, and Wintenberger 1954; Gančev 1961; Lopyrin and Manuĭlov 1967). For practical purposes one can manipulate either volume of the inseminate or its concentration of spermatozoa in order to provide a given number of spermatozoa. The data presented here show the importance of volume/concentration relationships as they apply to the progestagen-treated ewe. The same principles probably apply to the sheep in "normal" oestrus but with rather different volume/concentration relationships.

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