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THE RELATIONSHIP BETWEEN NUMBERS OF SPERMATOZOA INSEMINATED AND FERTILIZATION RATE OF OVA IN EWES TREATED WITH FLUORO-PROGESTAGEN INTRAVAGINAL SPONGES IN SUMMER AND AUTUMN

T. D. QUINLIVAN*

Department of Animal Husbandry, University of Sydney, Sydney, N.S.W., 2006

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Summary. Two experiments were conducted, during December and March, designed primarily to determine the oestrous response and fertilization rate following the insemination of varying numbers of spermatozoa in ewes treated with intravaginal sponges impregnated with Cronolone, a synthetic progestagen. The main results were:

1. Incidence of oestrus: December; (a) treated ewes, 45·7%; (b) untreated teased ewes, 47·5%; (c) untreated non-teased ewes, 30·0%; March; (d) treated ewes, 94·8% (a versus b, NS; a versus c, $P < 0{\cdot}01$; a versus d, $P < 0{\cdot}001$).

2. Peak incidence of oestrus after withdrawal of sponges: December, 72 hr; March, 48 hr ($P < 0{\cdot}001$).

3. Overall percentage of ewes which yielded fertilized ova: December, 64·3; March, 83·2 ($P < 0{\cdot}001$).

4. Numbers of spermatozoa and fertilization in treated ewes: December, linear increase in percentage of ewes fertilized, with increasing sperm numbers, from 44·0% (300×10^6 spermatozoa) to 76·0% (1500×10^6 spermatozoa); March, no effect of numbers of spermatozoa, with 83·2% ova fertilized (100 to 1500×10^6 spermatozoa).

5. Number of spermatozoa and fertilization in untreated versus treated ewes: December (300×10^6 spermatozoa); untreated, 43·3%, treated 44·0% (NS); March (100 to 1500×10^6 spermatozoa); untreated 78·8%, treated 83·2% (NS).

6. Effect of breed of ram on percentage of treated ewes fertilized in December: Merino, 75·6%, Border Leicester, 47·5% ($P < 0{\cdot}001$). Similar differences were observed in untreated ewes.

7. In both December and March, 21·4% of fertilized ova showed unequal cleavage or anuclear fragmentation.

* Present address: N.Z. Romney Survey, Box 231, Feilding, New Zealand.

8. The estimated mean numbers of spermatozoa recovered from the Fallopian tubes of progestagen-treated and untreated ewes 24 hr after the insemination of 300×10^6 spermatozoa were 807 and 5019 ($P < 0{\cdot}001$).

INTRODUCTION

Quinlivan & Robinson (1967, 1969) have shown an alteration in the pattern of transport and survival of spermatozoa throughout the genital tract of the ewe at the first synchronized oestrus following withdrawal of intravaginal sponges impregnated with the synthetic progestagen 17α-acetoxy-9α-fluoro-11β-hydroxypregn-4-ene-3,20-dione (Cronolone, Searle; Robinson, 1965; Shelton, 1965). This work also demonstrated that the insemination of numbers of spermatozoa within the range 80 to 500 ($\times 10^6$) had no discernible effect on the numbers recovered from the Fallopian tubes 4, 12, 24 and 36 hr after insemination, or on the fertilization rate of ova.

However, on the basis of extensive field work using semen of differing qualities and dilutions, Robinson (1967) considered that an increase in the number of spermatozoa inseminated, far in excess of normal requirements, may lead to a subsequent improvement in conception rate. Two experiments were designed to test this hypothesis, the first during the early summer, in ewes which had all lambed 3 months previously, and the second 3 months later during the breeding season.

The experiments were conducted at the McCaughey Memorial Institute, Jerilderie, N.S.W., in December 1966 and March 1967.

MATERIALS AND METHODS

Experimental animals

In Exp. 1, 1200 Merino ewes, 5 years old, plus eleven Merino and fourteen Border Leicester rams and three vasectomized rams were used. All ewes had lambed 3 to 4 months previously.

In Exp. 2, 350 Merino ewes, 4 years old, eleven Merino rams and twenty vasectomized rams were used.

Experimental design

In Exp. 1, the 1200 ewes were randomized into two flocks, Flock 1 of 960 ewes and Flock 2 of 240. All ewes of Flock 1 were treated with impregnated sponges, while those of Flock 2 were untreated. One half of Flock 2 was exposed to vasectomized rams 6 weeks before the experiment (120 'teased ewes'). The remaining ewes were not so exposed (120 'unteased').

The two flocks were used in the following four tests. Tests 1 and 2 measured the incidence of oestrus while 3 and 4 measured the percentage of fertilized eggs recovered.

Test 1. *Flock* 1 *only*. Effect of duration of insertion of sponges on incidence of oestrus in Merino ewes in early summer: 480 ewes were treated for 12 days and 480 for 16 days. The numbers of ewes served were recorded.

Test 2. *Flocks* 1 *and* 2. Effect of teasing and of intravaginal sponges on the incidence of oestrus in Merino ewes in early summer: 960 treated ewes were

compared with 120 untreated 'teased', and 120 untreated 'unteased' ewes. The numbers of ewes served over a 20-day period were recorded, commencing on the day of sponge withdrawal.

Test 3. *Flock* 1 *only*. Effect of duration of insertion of sponges, breed of ram, and numbers of spermatozoa on percentage of ewes fertilized: 360 ewes, 180 treated for 12 days and 180 for 16 days, were incorporated into a factorial test:

Duration of insertion — 12 versus 16 days
Breed of ram — Merino versus Border Leicester
Number of spermatozoa ($\times 10^6$) — 300, 600, 900, 1200, 1500, > 1500*

* Hand service (see p. 91)

$2 \times 2 \times 6$; n = 15; N = 360.

The numbers of eggs recovered, fertilized and with spermatozoa attached to the zona pellucida, were recorded.

Test 4. *Flocks* 1 *and* 2. Effect of intravaginal sponges, compared with no treatment, and of breed of ram on percentage of ewes fertilized: the sixty treated ewes from Flock 1 inseminated with 300×10^6 spermatozoa were incorporated into a factorial test with sixty untreated ewes from Flock 2 inseminated with the same number of spermatozoa from the same rams (2×2; n = 30; N = 120). Data recorded were as for Test 3.

Failure of the Border Leicester rams to produce sufficient semen necessitated a departure from strict orthogonality in Tests 3 and 4 (see appropriate tables of results).

In Exp. 2, the 350 ewes were randomized into two flocks, Flock 3 of 250 ewes and Flock 4 of 100. All ewes of Flock 3 were treated for 16 days with progestagen-impregnated sponges, while those of Flock 4 were untreated.

The two flocks were used in the following three tests.

Test 1. *Flock* 3 *only*. Effect of numbers of spermatozoa on the percentage of ewes fertilized following treatment with intravaginal sponges: 210 oestrous ewes of Flock 3 were inseminated and subsequently ova were recovered at laparotomy. The seven treatments (n = 30) were: 100, 300, 600, 900, 1200, 1500, > 1500 spermatozoa.

Test 2. *Flocks* 3 *and* 4. Effect of intravaginal sponges compared with no treatment on percentage of ewes fertilized: the thirty treated ewes from Flock 3 inseminated with 300×10^6 spermatozoa were compared with thirty untreated ewes from Flock 4 inseminated with the same number of spermatozoa (n = 30, N = 60).

Data recorded were as for Exp. 1, Tests 3 and 4.

Test 3. *Flocks* 3 *and* 4. Comparison of numbers of spermatozoa recovered from the Fallopian tubes of treated and untreated ewes 24 hr after insemination: twenty oestrous ewes of Flock 3 and twenty of Flock 4 were inseminated with 300×10^6 spermatozoa; 24 hr later, the Fallopian tubes were flushed for the recovery of spermatozoa (n = 20, N = 40).

Management

In Exp. 1, the ewes were randomized into two flocks on 27th October 1966, when six vasectomized rams fitted with harnesses and crayons were joined with

the 120 untreated 'teased' ewes. Thereafter, the time schedule shown in Table 1 applied.

In Exp. 2, the ewes were randomized into two flocks on 5th March 1967, when insertion of impregnated sponges commenced at the rate of fifty every 2nd day. Thereafter, the time schedule shown in Table 2 applied.

TABLE 1

TIME SCHEDULE—EXPERIMENT 1

Operation	*Date* 1966	*Flock* 1	*Flock* 2	
		Treated	*Untreated*	
			Teased	*Unteased*
Randomization	Oct. 27	960 ewes	240 ewes	240 ewes
Vasectomized rams	Oct. 27 to Nov. 10	—	6 rams	—
Vasectomized rams	Nov. 11 to Nov. 23	—	—	—
Vasectomized rams	Nov. 24 to Dec. 8	—	6 rams	—
Vasectomized rams—new raddle	Dec. 8 to Dec. 28	—	12 rams	
Insertion of sponges in batches	Nov. 30 to Dec. 12	960 ewes	—	—
Removal of sponges and introduction of twenty-five vasectomized rams to Flock 1	Dec. 16	192 ewes	—	—
	18	192 ewes	—	—
	20	192 ewes	—	—
	22	192 ewes	—	—
	24	192 ewes	—	—
Artificial insemination and hand service (on detection of oestrus)	Dec. 18 to 26	360 ewes	60 ewes	

TABLE 2

TIME SCHEDULE—EXPERIMENT 2

Operation	*Date* 1967	*Flock* 3 *Treated*	*Flock* 4 *Untreated*
Randomization	March 5	250 ewes	100 ewes
Vasectomized rams	March 22 to 31	—	5 rams
Insertion of sponges in batches	March 5 to 13	250 ewes	—
Removal of sponges and introduction of fifteen vasectomized rams	March 21	50 ewes	—
	23	50 ewes	—
	25	50 ewes	—
	27	50 ewes	—
	29	50 ewes	—
Artificial insemination and hand service (on detection of oestrus)	March 23 to 31	210 ewes	50 ewes

In both experiments, ewes were inspected twice daily for oestrus, at 07.00 and 17.00 hours.

Artificial insemination

All ewes were inseminated once with undiluted semen, as soon as possible after first detection of oestrus. Semen was collected by artificial vagina from

several animals and pooled. Because of the low volume produced by the Border Leicesters in Exp. 1, collection was from ten rams per pool compared with four for the Merinos.

Each ejaculate was examined for density and motility and only semen of high density and motility was used. The ejaculates were pooled, the number of spermatozoa estimated by haemocytometer counts, and the volumes determined to provide the required numbers of spermatozoa. Counts for the pooled samples were $2{\cdot}75$ to $3{\cdot}25 \times 10^9$ spermatozoa/ml for the Border Leicester rams (Exp. 1) and $3{\cdot}70$ to $5{\cdot}60 \times 10^9$ spermatozoa/ml for the Merinos (Exps 1 and 2). Estimates of motility and percentage 'normal' and 'abnormal' were comparable for the two breeds, but the volumes differed: Border Leicester, 0·1 to 0·2 ml; Merino, 0·6 to 0·9 ml.

Hand service

Each ewe allocated for hand service was served twice—once by each of two rams on which semen checks had been made. By analogy with the sperm counts made on semen samples collected for artificial insemination, the approximate numbers of spermatozoa received per ewe served twice by rams of the two breeds were: Border Leicester, 500 to 1100×10^6; Merino, 4500 to $10{,}100 \times 10^6$.

Recovery of ova

Ova were recovered *in vivo* by the method described by Hunter, Adams & Rowson (1955). Each ovum was mounted and examined under phase contrast microscopy at × 200 to 400. Cleavage was accepted as the criterion for fertilization. Any ovum that appeared abnormal was cleared in 25% glacial acetic acid in alcohol and subsequently stained with 1% natural orcein in 45% glacial acetic acid.

Hormone treatment

The polyurethane sponges were 3·5 cm in diameter and impregnated in the laboratory with 30 mg Cronolone dissolved in 5 ml ethanol. They were suspended from racks, the progestagen in alcohol was injected with a multi-dose automatic syringe, and the sponges were then air dried for 24 hr.

Estimates of residual Cronolone were made on eighteen sponges from Exp. 1, and twenty-four from Exp. 2, by the method described by Morgan, Lack & Robinson (1967), and are shown in Table 3.

TABLE 3

ESTIMATES OF RESIDUAL CRONOLONE IN INTRAVAGINAL SPONGES INSERTED FOR VARYING LENGTHS OF TIME

Experiment	*No. of sponges assayed*	*Duration of insertion (days)*	*Mean residual Cronolone (mg)**	*Estimated mean absorbed (mg)*
1	6	0	$27{\cdot}9 \pm 2{\cdot}8$	—
	6	12	$11{\cdot}9 \pm 7{\cdot}3$	16·0
	6	16	$12{\cdot}2 \pm 4{\cdot}7$	15·7
2	12	0	$27{\cdot}5 \pm 4{\cdot}2$	—
	12	16	$14{\cdot}6 \pm 4{\cdot}7$	12·9

* Within 95% confidence limits.

Recovery and counting of spermatozoa

The methods of flushing, counting and statistical analysis were as described by Quinlivan & Robinson (1967).

RESULTS

EXPERIMENT 1—EARLY SUMMER

Tests 1 *and* 2

Number of ewes which exhibited oestrus. Eight (0·8%) of the 960 treated ewes lost sponges. Of the remainder, 439 (46·1%) exhibited oestrus within 120 hr of withdrawal (Table 4). There was no significant effect of duration of treatment on either the total number in oestrus or the time of onset after removal of the sponges.

Over the 21-day period of observations, fifty-seven (47·5%) of the 120 untreated 'teased' ewes and thirty-six (30·0%) of the 120 'unteased' ewes exhibited oestrus ($P < 0·01$).

The oestrous response of the progestagen-treated ewes differed significantly from that of the untreated 'unteased' ewes (45·7 versus 30·0%, $P < 0·01$) but did not differ from that of the 'teased' ewes (45·7 versus 47·5%, NS).

Test 3

Number of progestagen-treated ewes with fertilized ova. The original design of the test called for 360 ewes, 180 inseminated to each of the two breeds. Due to the failure of the Border Leicester rams to produce sufficient semen, the actual numbers used were:

Breed of ram	*Inseminated*	*Flushed for ova*	*Yielded ova*
Merino	216	208	180
Border Leicester	144	136	120
Total	360	344	300

Sufficient ewes of those inseminated were flushed for ova to provide twenty Border Leicester-bred and thirty Merino-bred ewes from which ova were recovered for comparison for each number of spermatozoa used (Table 5, Text-fig. 1). Overall, fifty-seven of 120 ewes (47·5%) inseminated with Border Leicester semen were fertilized compared with 136 of 180 (75·6%) inseminated with Merino semen ($P < 0·001$). There was a significant linear effect ($P < 0·01$) of number of spermatozoa (300 to 1500×10^6), ranging from twenty-two of fifty (44·0%) to thirty-eight of fifty (76·0%). There was no interaction between breed and number of spermatozoa, and no effect of duration of treatment with progestagen.

Only seven of twenty ewes (35·0%) hand mated to Border Leicester rams produced fertilized ova compared with twenty-six of thirty (86·7%) mated to Merinos ($P < 0·001$).

Number of ova with spermatozoa attached to the zona pellucida. One hundred and forty-two (40·7%) of the 349 ova recovered from the 300 treated ewes had spermatozoa attached to the zona pellucida (Table 6).

There was a highly significant effect of breed of ram ($P < 0·001$) and a linear effect of numbers of spermatozoa which approached overall significance

TABLE 4

EXP. 1, TESTS 1 AND 2: INCIDENCE OF OESTRUS FOLLOWING WITHDRAWAL OF CRONOLONE-IMPREGNATED INTRAVAGINAL SPONGES IN EARLY SUMMER; DECEMBER 1966

Duration of insertion of sponges	*No. of ewes*			*No. of ewes first detected in oestrus at intervals in hr after sponge withdrawal*									*Total*	
	Total	*Lost sponges*	*Available*	24	36	48	60	72	84	96	108	120	*No.*	%
A. *Treated ewes*														
12 days	480	2	478	—	—	44	42	82	34	11	2	1	216	45·0
16 days	480	6	474	1	—	37	31	99	30	22	3	—	223	46·5
Total	960	8	952	1	—	81	73	181	64	33	5	1	439	45·7
B. *Untreated ewes*														
0—Teased	120	—	—	3-week period of observations									57	47·5
0—Unteased	120	—	—										36	30·0
Total	240												93	38·8

χ^2 tests for significance

1. Distribution of times of detection of oestrus—12 versus 16 days insertion: $\chi^2_{(8)} = 9{\cdot}89$; NS.
2. Total ewes in oestrus: 12 versus 16 days insertion: NS.
   Treated versus untreated—total: $\chi^2_{(1)} = 3{\cdot}88$; $P < 0{\cdot}05$.
   Treated versus untreated—teased: NS.
   Treated versus untreated—unteased: $\chi^2_{(1)} = 10{\cdot}75$; $P < 0{\cdot}01$.
   Untreated—teased versus unteased: $\chi^2_{(1)} = 7{\cdot}74$; $P < 0{\cdot}01$.

TABLE 5

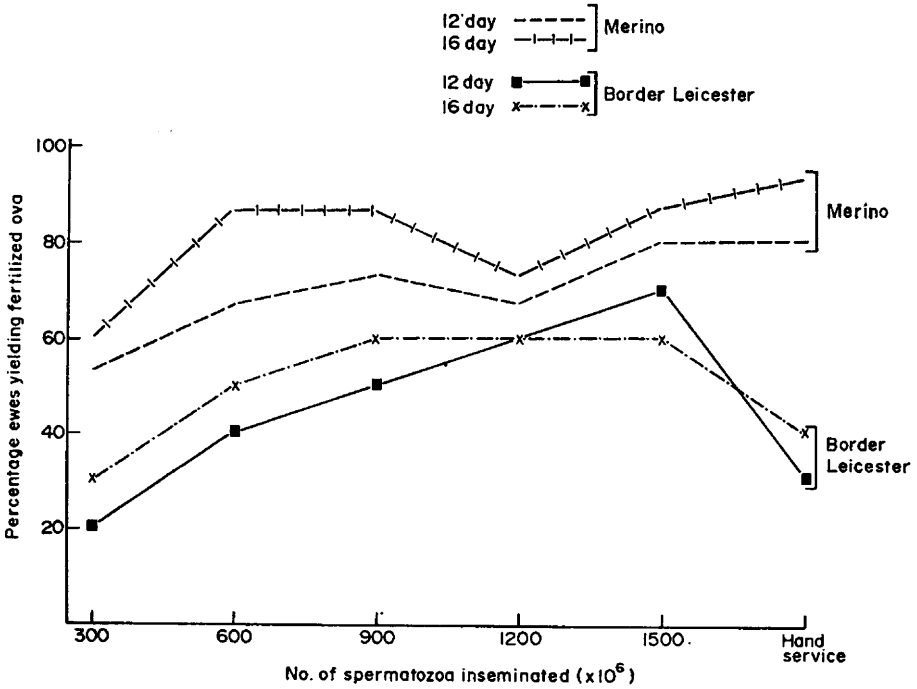
EXP. 1, TEST 3: NUMBER OF TREATED EWES WHICH YIELDED FERTILIZED OVA IN EARLY SUMMER; DECEMBER 1966

Breed of ram	*Duration of insertion of sponges (days)*	*No. of ewes fertilized following*						*Total ewes fertilized*	
		Artificial insemination with no. of spermatozoa ($\times 10^6$)					*Hand service*		
		300	600	900	1200	1500		*No.*	%
Border Leicester	12	2	4	5	6	7	3	27	45·0
(n = 10)	16	3	5	6	6	6	4	30	50·0
Total (n = 20)		5	9	11	12	13	7	57	—
Per cent		25·0	45·0	55·0	60·0	65·0	35·0	—	47·5
Merino (n = 15)	12	8	10	11	10	12	12	63	70·0
	16	9	13	13	11	13	14	73	81·1
Total (n = 30)		17	23	24	21	25	26	136	—
Per cent		56·7	76·7	80·0	70·0	83·3	86·7	—	75·6
Grand total (n = 50)		22	32	35	33	38	33	193	—
Per cent		44·0	64·0	70·0	66·0	76·0	66·0	—	64·3

A total of 344 ewes, of which 300 yielded ova at laparotomy.

Significant main effects (inseminated ewes only): No. of spermatozoa (linear) $P < 0{\cdot}01$.
Breed of ram $P < 0{\cdot}01$.

Significant interaction: Nil.



TEXT-FIG. 1. Percentage of progestagen-treated ewes which yielded fertilized ova relative to the number of spermatozoa inseminated in early summer—December 1966.

($0{\cdot}05 < P < 0{\cdot}10$) and which interacted significantly with breed of ram ($P \fallingdotseq 0{\cdot}05$). There was no significant effect of duration of treatment.

Only one of the twenty-five ova recovered from ewes hand served by the

TABLE 6

EXP. 1, TEST 3: NUMBER OF OVA WITH SPERMATOZOA ON THE ZONA PELLUCIDA, FROM EWES TREATED IN EARLY SUMMER; DECEMBER 1966

Breed of ram	*Ova recovered*	*No. of spermatozoa inseminated* ($\times 10^6$)					*Hand service*	*Total*
		300	600	900	1200	1500		
Border Leicester	Total	21	24	22	22	21	25	135
	With sperm.	5	7	7	5	6	1	31
	%	23·8	29·2	31·8	22·7	28·6	4·0	23·0
Merino	Total	37	35	32	37	34	39	214
	With sperm.	10	16	19	21	21	24	111
	%	27·0	45·7	59·4	56·8	61·8	61·5	51·9
	Grand total	58	59	54	59	55	64	349
	With sperm.	15	23	26	26	27	25	142
	%	25·9	39·0	48·2	44·1	49·1	39·1	40·7

The 349 ova were recovered from 300 ewes.
Significant main effects (inseminated ewes only):
No. of spermatozoa (linear) $0{\cdot}05 < P < 0{\cdot}10$.
Breed of ram $P < 0{\cdot}001$.
Significant interaction: Number of spermatozoa × breed $P \fallingdotseq 0{\cdot}05$.

Border Leicester rams had spermatozoa attached, compared with twenty-four of thirty-nine of those from Merino matings ($P < 0{\cdot}001$).

Test 4

Number of ewes with fertilized ova. Table 7 shows the numbers and percentages of untreated and treated ewes that yielded fertilized ova following insemination with 300×10^6 spermatozoa. There was no effect of treatment but a significant effect of breed of ram ($P < 0{\cdot}01$).

TABLE 7

EXP. 1, TEST 4: NUMBER OF PROGESTAGEN-TREATED AND UNTREATED EWES WHICH YIELDED FERTILIZED OVA FOLLOWING INSEMINATION WITH 300×10^6 SPERMATOZOA FROM TWO BREEDS OF RAM IN EARLY SUMMER; DECEMBER 1966

Treatment	*Border Leicester Rams*			*Merino rams*		
	No. of ewes		*% ewes fertilized*	*No. of ewes*		*% ewes fertilized*
	Yielded ova	*Fertilized*		*Yielded ova*	*Fertilized*	
Progestagen-treated	20	5	25·0	30	17	56·7
Untreated	15	4	26·7	15	9	60·0
Total	35	9	25·7	45	26	57·8

The fifty progestagen-treated ewes yielded fifty-eight ova; the thirty untreated ewes yielded thirty-one ova (NS).
Significant main effect: Breed of ram $P < 0{\cdot}01$.

Seven of the thirty-one ova recovered from the untreated ewes had spermatozoa attached to the zona pellucida as compared with fifteen of the fifty-eight from the fifty treated ewes (22·6 and 25·8%; NS).

EXPERIMENT 2—AUTUMN

Test 1

Number of ewes which exhibited oestrus. Three of the 250 progestagen-treated ewes (1·2%) lost sponges. Of the remainder, ten (4·0%) failed to exhibit oestrus. Thus, 237 (94·8%) of the 250 treated ewes exhibited oestrus within 96 hr of withdrawal of sponges (Table 8). Peak onset was 48 hr after removal of the sponges, and 228 (96·2% of oestrous ewes) were served between 36 and 72 hr.

TABLE 8

EXP. 2, TEST 1: INCIDENCE OF OESTRUS FOLLOWING WITHDRAWAL OF CRONOLONE-IMPREGNATED INTRAVAGINAL SPONGES IN AUTUMN; MARCH 1967

Total ewes	*Lost sponges*	*No. of ewes first detected in oestrus at intervals in hr after sponge withdrawal*							*Total*	
		24	36	48	60	72	84	96	*No.*	%
250	3	2	15	116	73	24	5	2	237	94·8

Number of ewes with fertilized ova. Ova were recovered from 190 treated ewes of which 158 (83·2%) yielded fertilized eggs (Table 9). There were no significant differences between numbers of spermatozoa or hand service.

TABLE 9

EXP. 2, TEST 1: NUMBER OF TREATED EWES WHICH YIELDED FERTILIZED OVA IN AUTUMN; MARCH 1967

No. of ewes which yielded:	*No. of spermatozoa inseminated* ($\times 10^6$)						*Hand service*	*Total*
	100	300	600	900	1200	1500		
Ova	27	27	28	25	27	28	28	190
Fertilized ova	23	24	25	18	22	24	22	158
% ewes fertilized	85·2	88·9	89·3	72·0	81·5	85·7	78·6	83·2

A total of 210 ewes, of which 190 yielded ova at laparotomy.

Number of ova with spermatozoa attached to the zona pellucida. One hundred and forty-five (64·2%) of the 226 ova recovered from the 190 treated ewes had spermatozoa attached to the zona pellucida (Table 10).

TABLE 10

EXP. 2, TEST 1: NUMBER OF OVA WITH SPERMATOZOA ON THE ZONA PELLUCIDA, FROM EWES TREATED IN AUTUMN; MARCH 1967

Ova recovered	*No. of spermatozoa inseminated* ($\times 10^6$)						*Hand service*	*Total*
	100	300	600	900	1200	1500		
Total	32	33	29	34	32	32	34	226
With sperm.	18	20	20	20	21	26	20	145
%	56·3	60·6	68·9	58·8	65·6	81·3	58·8	64·2

The 226 ova were recovered from 190 ewes.
Effect of number of spermatozoa (linear)—$0{\cdot}05 < P < 0{\cdot}10$.

There was a linear relationship between percentage of ova with attached spermatozoa and numbers of spermatozoa inseminated ($0{\cdot}05 < P < 0{\cdot}10$).

Test 2

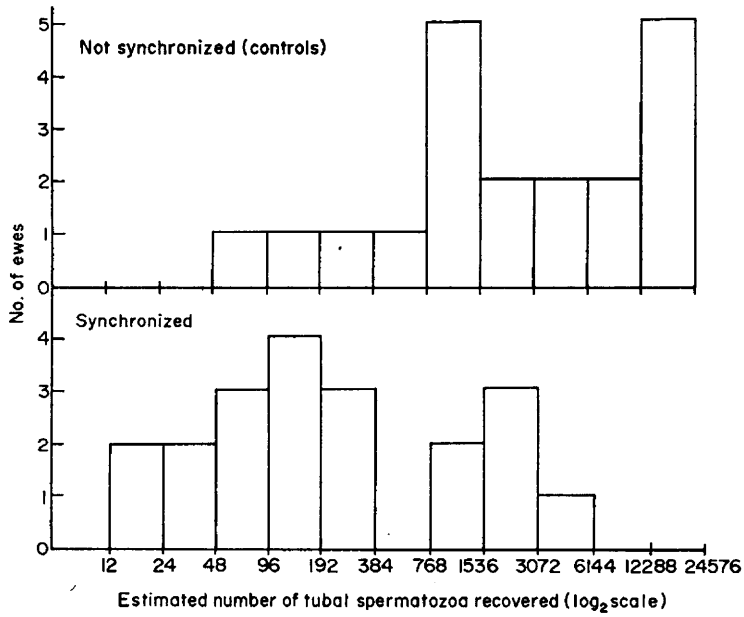
Number of ewes with fertilized ova. Twenty-four of the thirty untreated ewes yielded fertilized ova as compared with twenty-three of twenty-seven treated animals (80·0 versus 85·2%; NS).

Nineteen of the thirty-three ova recovered from untreated animals had spermatozoa attached as compared with eighteen of thirty-two in the treated ewes (57·6 versus 56·3%; NS).

Test 3

Number of spermatozoa recovered. The estimated mean numbers of spermatozoa recovered from the Fallopian tubes of treated and untreated ewes 24 hr after the insemination of 300×10^6 spermatozoa were 807 and 5019 ($P < 0{\cdot}001$).

Text-fig. 2 shows the distribution of numbers of spermatozoa. There was evidence of a bimodal distribution in both groups of ewes. In the untreated ewes, one was centred about 768 to 1536 spermatozoa and the other about



TEXT-FIG. 2. Distribution curves of number of ewes which yielded tubal spermatozoa 24 hr after artificial insemination.

12,288 to 24,576 spermatozoa. In the progestagen-treated animals, one mode was centred about 96 to 192 spermatozoa and the other about 1536 to 3072 spermatozoa. The difference in these distributions was significant ($P < 0{\cdot}01$).

EXPERIMENTS 1 AND 2—COMPARISON OF DATA

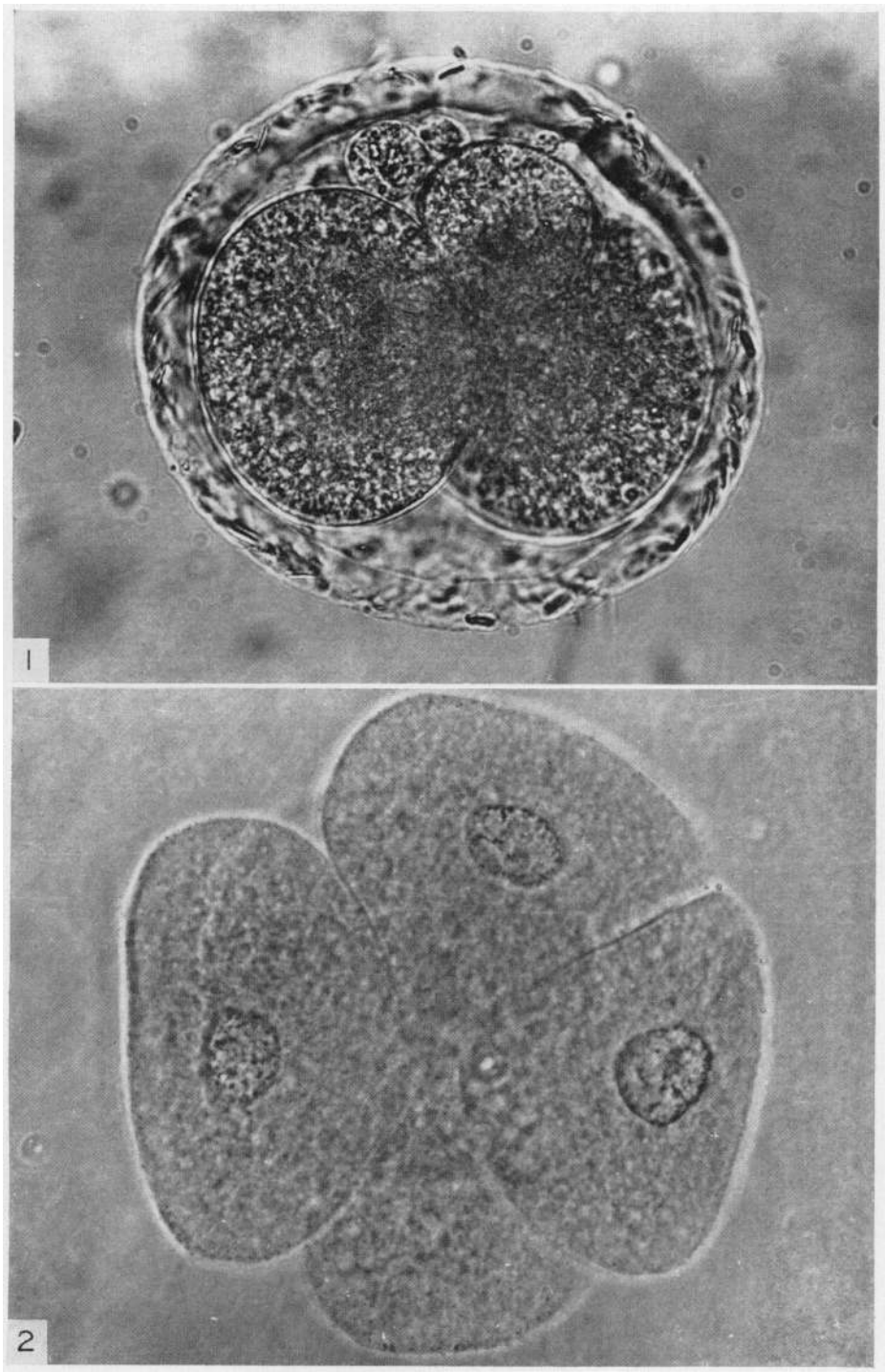
Number of morphologically 'atypical' fertilized ova

Table 11 shows the distribution of ova classed into four groups. Overall, of sixty-five ova recovered from untreated ewes, thirty-eight were classed as cleaved and all appeared 'normal'. Of 575 recovered from treated ewes, 392 were classed as cleaved of which eighty-four (21·4% of cleaved ova) were 'atypical' as illustrated in Plate 1. The incidence of such ova was similar in summer and autumn.

Number of ewes which exhibited oestrus

During the early summer, 45·7% of 5-year-old Merino ewes exhibited oestrus, compared with 94·8% of 4-year-olds in the autumn ($P < 0{\cdot}001$).

The peak onset of oestrus following removal of the sponges during the early summer was at 72 hr, and during the autumn at 48 hr. The distribution of time of onset relative to removal of the sponges was significantly different ($P < 0{\cdot}001$).



Apparently abnormal ('atypical') fertilized ova recovered from progestagen-treated ewes.

FIG. 1. A two-cell egg with anucleate fragments. × 360.

FIG. 2. A cleared and stained, unequally cleaved, five-cell egg (one blastomere obscured), with two anucleate cells. × 504.

(*Facing p.* 98)

Number of ewes yielding fertilized ova

Overall, 64·3% of the ewes mated in the summer and 83·2% of the ewes mated in the autumn yielded fertilized ova ($P < 0{\cdot}001$).

Of the ewes inseminated with from 300 to 1500 ($\times 10^6$) Merino spermatozoa, 73·3% were fertilized in the summer as compared with 83·7% in the autumn

TABLE 11

EXPS 1 AND 2: THE CLASSIFICATION OF OVA RECOVERED FROM UNTREATED AND TREATED EWES

Classification of ova	*No. of ova recovered*							
	Experiment 1—Summer				*Experiment 2—Autumn*			
	Untreated ewes		*Treated ewes*		*Untreated ewes*		*Treated ewes*	
	No.	%	*No.*	%	*No.*	%	*No.*	%
Cleaved—'normal'	13	40·6	169	48·4	25	75·8	139	61·5
'atypical'*	0	0·0	46	13·2	0	0·0	38	16·8
Fractured	0	0·0	4	1·1	1	3·0	8	3·5
Non-fertilized	19	59·4	130	37·3	7	21·2	41	18·2
Total	32	100·0	349	100·0	33	100·0	226	100·0

All untreated ewes were artificially inseminated. Data for AI and natural mating pooled for progestagen-treated ewes.

* 'Atypical' ova showed unequal cleavage or apparent fragmentation.

($P < 0{\cdot}05$). There was no significant effect of number of spermatozoa and no interaction. There was no significant difference between ewes hand mated to the Merino rams in the two seasons (86·7% summer versus 78·6% autumn).

Number of ova with spermatozoa on the zona pellucida

Overall, 40·7% of the ewes mated in the summer and 64·2% of the ewes mated in the autumn yielded ova with spermatozoa attached ($P < 0{\cdot}001$).

Of the ewes inseminated with from 300 to 1500 ($\times 10^6$) Merino spermatozoa, 49·7% yielded ova with spermatozoa attached in the summer, as compared with 66·9% in the autumn ($P < 0{\cdot}01$). The increase with increasing number of spermatozoa inseminated occurred in both seasons, so there was no interaction. There was no seasonal difference between ewes hand-mated to Merino rams.

DISCUSSION

These experiments show that the stage of the breeding season has an important influence on the response of ewes to intravaginal treatment with progestagen. This is manifested by effects upon the proportion of ewes in oestrus, the time of onset of oestrus, the proportion of ova fertilized and, particularly where British breed rams are used, the number of spermatozoa required for optimum chances of fertilization.

It is important to recognize the confounding effects resulting from the introduction of rams and the cessation of progestagen treatment on ovulation

and oestrus early in the breeding season. Thus, the percentage of progestagen-treated ewes detected in oestrus within 5 days when mated on cessation of treatment was 45·7. The percentages of untreated ewes were 47·5 for ewes previously exposed to rams ('teased') and 30·0 for ewes not exposed. This latter difference is significant and confirms the observation of Cunningham, Deas & Fitzsimmons (1967) concerning the necessity for adequate control animals in any evaluation of methods available for the advancement of the breeding season.

By contrast with the 45·7% of treated ewes observed in oestrus in the summer, 94·8% were in oestrus in the autumn. They also came into oestrus earlier in the autumn which, by analogy with the observations of Scaramuzzi (1968) and Fletcher (1969) on spayed ewes, suggests a greater production of oestrogen resulting in earlier onset and a longer duration of oestrus; that is, oestrus is of greater intensity than in the summer.

These effects on the oestrous response are carried further into the subsequent process of fertilization. It is well established (Quinlivan & Robinson, 1967, 1969) that the ewe treated with intravaginal progestagen has a pattern of transport and survival of spermatozoa which differs from that in the normal ewe, resulting in lower numbers of spermatozoa in the Fallopian tubes and susceptibility to failure of fertilization. This susceptibility is greater in the summer than in the autumn, and is further aggravated if British breed rams (Border Leicester) are used in the summer. Semen of the latter, as compared with that of Merino rams, resulted in markedly fewer fertilized ova, fewer with spermatozoa attached, and a much greater dependence on large numbers of spermatozoa for fertilization. The deficiency of the Border Leicester semen was not confined to the treated ewe—it applied also to the untreated—and it is not a function of artificial insemination; hand served ewes were also infertile.

In view of the linear increase in fertilization rates in summer with increase in numbers of spermatozoa, and also the breed difference, it is concluded that varied numbers of spermatozoa are required for maximum fertility at different seasons of the year and with different breeds. In this experiment in the summer, 1500×10^6 Border Leicester spermatozoa were required to provide 65% fertilization, while 600×10^6 Merino spermatozoa provided 77%, and there was no significant increase thereafter with increasing numbers. In the autumn, there was no advantage in using more than 100×10^6 Merino spermatozoa (mean 83% fertilization). While these data refer specifically to progestagen-treated ewes, they need to be considered in evaluating the numbers of spermatozoa required for maximum chances of conception using different breeds of rams at different times of the year, factors not usually considered in estimates of the minimum numbers of spermatozoa required for artificial insemination (Emmens & Robinson, 1962; Salamon, 1962).

The problem of poor fertilization in treated ewes, associated with an abnormal pattern of transport and survival of spermatozoa (Quinlivan & Robinson, 1967, 1969) appears much more serious very early in the breeding season (December—summer) than in the middle of the season (March—autumn). In the autumn, the problem is marginal; 83·2% of recovered ova were fertilized, only 7 to 12% below the figure commonly quoted for normal ewes. In the

summer, the figure is somewhat lower (75·6% for Merino rams, 47·5% for Border Leicesters). In the latter case, the problem is partly alleviated by massive sperm numbers but even with numbers as high as 1500×10^6, only 65% of ova were fertilized with Border Leicester semen and 83% with Merino.

Evidence for the existence of a problem of transport and survival of spermatozoa, which can only partly be overcome by the use of excessively large numbers, is provided by the data for number of ova with attached spermatozoa. Very few ova (23%) from ewes inseminated in summer with Border Leicester semen had spermatozoa attached, while those inseminated with Merino semen showed a linear increase from 27 to 62% of such ova with increasing numbers of spermatozoa inseminated. In the autumn, the mean number of such ova was much higher (64%) and again there was a positive association with numbers of spermatozoa inseminated. These observations confirm the conclusion of Quinlivan & Robinson (1967) that the presence of ova with attached spermatozoa serves as a useful guide to the effectiveness of transport and survival of spermatozoa.

It was not possible to assess the potential capacity for development of the 'atypical' ova recovered from the treated ewes. Recently, Killeen (1969) has shown that such eggs transferred to donor ewes may develop normally. There is, however, the distinct possibility that such ova are the result of late fertilization. Counts of the numbers of spermatozoa in the Fallopian tubes 24 hr after insemination in the autumn (Exp. 2) showed differences between progestagen-treated and untreated ewes. Earlier, Quinlivan & Robinson (1967) had shown that maximum accumulation of tubal spermatozoa in normal ewes is 24 hr after insemination and of treated ewes, 36 hr. Hence, there may be some difference between treated and untreated ewes in the time, relative to ovulation, at which penetration by a spermatozoon occurs.

Robinson (1970) has shown a pattern of seasonal variation in response to intravaginal progestagen treatment, similar to that reported here. In the summer, the incidence of ovulation without oestrus ('silent' heats) is high, the onset of oestrus relative to cessation of treatment is late, and the pregnancy rate following two inseminations 8 to 10 hr apart is low (52%). In the autumn, most ewes exhibit oestrus with ovulation, the onset of oestrus is early and the pregnancy rate is high (80%). Data presented in this paper conform to this pattern for each parameter and show that seasonal differences in fertility can be accounted for by differences in fertilization rates. These may be affected by breed of ram and to some extent by the numbers of spermatozoa inseminated at the particular season. The contribution to failure of pregnancy of early mortality of the 'atypical' ova is an open question.

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