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Fertility of Fresh and Frozen-Thawed Semen of the Angora Goat

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Abstract

In six experiments, involving a total of 954 does, fresh and frozen–thawed semen processed in a Tris-based diluent was used for artificial insemination.

There was an improvement in fertility with increasing depth of insemination into the genital tract (up to 1·0 cm, 1·5–3·0 cm into the cervix, into uterus), but the effect was more pronounced for frozen–thawed than for fresh-diluted semen. The two types of semen yielded indistinguishable kidding results after intra-uterine insemination. When the insemination was made at the second oestrus after synchronization with intravaginal sponges, the fertility for the categories of depth of insemination was not improved by double insemination and deposition of twice the total number of fresh motile spermatozoa ($120\times10^6+120\times10^6$) than by single insemination, or by the increase of fresh or frozen–thawed motile sperm numbers (60×10^6 *v.* 120×10^6) in the single inseminate.

When at the synchronized oestrus the depth of insemination was not recorded and equal total numbers of frozen–thawed motile spermatozoa (60×10^6 *v.* 120×10^6) were deposited by both single and double inseminations, the latter method of insemination was of no advantage within either sperm dose. Further, the rates of kidding were not different for single insemination of frozen–thawed semen 48 or 59 h after removal of intravaginal sponges, for washed (seminal plasma removed) or non-washed spermatozoa, and for does injected with 75 or 300 i.u. pregnant mare serum gonadotrophin at sponge removal.

Introduction

Artificial insemination of dairy goats is practised in several countries, mainly in Europe, and the fertility of semen frozen–thawed in a variety of diluents has also been examined by a number of investigators (reviewed by Corteel 1973, 1977; Bonfert 1974; Fougner 1979). There is limited information on the fertility of fresh and frozen–thawed Angora semen (Moore and Eppleston 1979; Van der Westhuysen *et al.* 1980).

In the present study fresh or frozen–thawed semen of the Angora buck was used at the first and second oestrus after synchronization for single or double inseminations with different numbers of motile spermatozoa. In addition, the fertility in relation to the depth of insemination into the genital tract was examined.

A brief report on part of the results has been presented elsewhere (Ritar and Salamon 1981).

Materials and Methods

Semen was collected from mature Angora bucks by use of an artificial vagina and only ejaculates which showed good initial motility (80–85%) and sperm concentration (not less than $3\cdot0\times10^9$/ml) were processed. Altogether, from four to seven ejaculates from individual bucks were processed for

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frozen storage or for fresh use. In each experiment different bucks were used and their semen was not tested previously for coagulation with egg yolk.

For freezing, the semen was diluted 1 : 2 (semen : diluent) at 30°C with a diluent containing 375 mM Tris, 41·625 mM glucose, 124 mM citric acid, 9% (v/v) egg yolk, and 6% (v/v) glycerol (Salamon and Ritar 1982). The diluted semen was cooled to 5°C in 1·5 h then frozen in pellet form (0·10–0·14 ml) on dry ice and transferred into liquid nitrogen. Only semen batches showing not less than 40% motile spermatozoa (after thawing two to three pellets) were laid down in the semen bank.

In experiment 6 the pooled semen from ejaculates of four bucks was split into two equal portions. One part was diluted 1 : 2 (semen : diluent, non-washed). The other part was subjected to washing, and for this the semen was diluted 1 : 1 (semen : diluent) with the above medium containing no egg yolk or glycerol, and then centrifuged for 10 min at 900 *g*. After the removal of the supernatant and resuspension of spermatozoa in the same amount of washing medium used for the first centrifugation the procedure was repeated. The twice-washed spermatozoa were resuspended to the same concentration as the non-washed semen with the Tris-based diluent containing 6% (v/v) glycerol and 9% (v/v) egg yolk, then further processed and frozen as described above.

The frozen pellets were stored in liquid nitrogen for 1–4 months before using for insemination. The pellets were thawed in dry test tubes (three pellets per tube) held in a water bath at 37°C. Immediately after thawing or collection of semen for fresh use, the percentage of progressively motile spermatozoa was assessed under a coverslip on a warm stage (37 °C) and the cell concentration was determined by haemocytometer counts. The concentration of motile spermatozoa was adjusted to 400×10^6/ml in the thawed semen and to 600×10^6/ml in the fresh semen by extending both with the above Tris–glucose diluent containing no egg yolk or glycerol. The semen was used for insemination within 25 min after thawing or collection.

The experiments were conducted during the breeding period (February–May) and when frozen–thawed semen was involved freezing was done in January–early February. The animals used were Angora does (expts 1, 2, 5), and feral does (expts 3, 4, 6). For synchronization of oestrus, the animals were treated for 16–18 days with intravaginal sponges containing 45 mg fluorogestone acetate (FGA, Chrono-gest, Intervet (Australia) Pty. Ltd., Sydney). In experiment 3, sponges containing 60 mg 6α-methyl-17α-acetoxyprogesterone (MAP, Repromap, Upjohn Pty. Ltd., Sydney) were inserted for 18 days.

In experiments 1, 2 and 3, the does were inseminated at the second oestrus after synchronization with sponges. The does were run with goat-wethers, injected with testosterone (200 mg Durateston, Intervet (Australia) Pty. Ltd., Sydney) and the oestrous animals were removed from the herd twice daily at 0800 and 1800 h. The inseminations were carried out 10–14 h (single insemination), or 10–14 and 24 h (double insemination) after detection. In experiments 4, 5 and 6, the inseminations were performed 48 or 59 h (single insemination), or 48 and 59 h (double insemination) after sponge removal and intramuscular injection of pregnant mare serum gonadotrophin (PMSG). The dose of PMSG was 300 i.u. (expt. 4); 200 i.u. (expt. 5), and varied in experiment 6. The animals were allocated randomly into treatment groups. For insemination, the does were restrained by raising their hindquarters over a rail, and then left undisturbed for several hours in pens in the shed. The depth of semen deposition up to 1 cm or 1·5–3·0 cm into the cervix, or into the uterus, was recorded for each animal in experiments 1, 2, 3 and 6.

In experiment 1, blood samples were collected by jugular venepuncture on day 20 after insemination, and radioimmunoassays for plasma progesterone were done as described by Thorneycroft and Stone (1972) and modified by R. J. Scaramuzzi (personal communication). Plasma samples were assayed in duplicate and the minimum detectable level of progesterone was 0·3 ng/ml. The intra- and interassay coefficients of variation were $< 6\%$ and $< 10\%$ respectively. The does were diagnosed pregnant when plasma progesterone concentrations were greater than 1·5 ng/ml (Corteel 1976). In experiment 2, the returns to service (days 15–60 after insemination) were recorded.

The statistical significance of treatment comparisons was determined by χ^2 analysis.

Experimental Details and Results

Experiment 1

In this experiment the does detected in oestrus in the morning (0800 h) or in the evening (1800 h) at the second oestrus after synchronization (Chrono-gest)

received single or double insemination with fresh-diluted and pooled semen from two bucks. The dose of each inseminate was 0·2 ml and contained 120×10^6 motile spermatozoa.

Table 1. Rates of pregnancy and of kidding in relation to the time of oestrus detection and number and depth of insemination with fresh-diluted semen (expt 1)

Insemination was at the second oestrus after synchronization with intravaginal sponges. Single insemination was carried out 10 and 12 h after detection of oestrus in the morning (0800 h) and evening (1800 h) respectively. Double inseminations were done 10 and 24 h after morning detection and 14 and 24 h after evening detection. Each inseminate (0·2 ml) contained 120×10^6 motile spermatozoa

Time of oestrus detection and depth of insemination	No. of does inseminated	Percentage of does Pregnant	Kidded	Ratio of kidded to pregnant (%)
Single insemination				
Morning detection	39	84·6	74·4	87·9
Evening detection	25	84·0	76·0	90·5
Depth of insemination				
Up to 1 cm into cervix	13	76·9	53·8	70·0
1·5–3·0 cm into cervix	42	85·7	78·9	92·0
Into the uterus	9	88·9	88·9	100·0
Mean	64	84·4	75·0	88·9
Double insemination				
Morning detection	40	72·5	47·5	65·6
Evening detection	22	59·1	54·5	92·3
Depth of insemination				
Up to 1 cm into cervix	18	77·7	50·0	64·4
1·5–3·0 cm into cervix	38	60·5	44·7	74·0
Into the uterus	6	83·3	83·3	100·0
Mean	62	67·7	50·0	73·8
Overall mean: single and double inseminations				
Morning detection	79	78·5	60·8	77·4
Evening detection	47	72·3	66·0	91·2
Depth of insemination				
Up to 1 cm into cervix	31	77·4	51·6	66·7
1·5–3·0 cm into cervix	80	73·8	62·5	84·7
Into the uterus	15	86·7	86·7	100·0

In all, 95% of does were detected in oestrus 20–24 days after sponge removal. The fertility results are presented in Table 1. The mean rates of pregnancy and of kidding were higher ($P < 0·05$ and $P < 0·01$) for single than double insemination, but the mean rates of embryonic survival (estimated by the ratio of number of does kidded to the number pregnant) were not significantly different for the two methods of insemination. Fertility for the animals detected in oestrus at 0800 h (morning) or at 1800 h (evening) was indistinguishable. There was no interaction between numbers of inseminations and time of oestrus detection, or between number of inseminations and depth of insemination for any of the parameters examined. The fertility for the two times of oestrus detection was similar within category of depth of insemination

when single or double inseminations were performed. Therefore the data for the times of oestrus detection were pooled within the categories of depth of insemination. Fertility for the animals involved in the three categories of depth of insemination were not markedly different within either single or double insemination. However, when the data for number of inseminations were pooled and the means for the depth of insemination compared, the improvement in the rates of kidding and embryonic survival with the increasing depth of semen deposition was statistically evident ($P < 0{\cdot}05$). The mean rates of pregnancy for depth of insemination were not markedly different. Of the total of 96 does considered pregnant by the progesterone assay, 79 (82·3%) kidded.

Experiment 2

In this experiment the fertility of fresh-diluted and frozen–thawed semen after single insemination with 60×10^6 or 120×10^6 motile spermatozoa was examined.

Table 2. Fertility after single insemination with two sperm doses of fresh-diluted and frozen–thawed semen (expt 2)

Insemination was at the second oestrus after synchronization with intravaginal sponges

Sperm dose and depth of insemination	No. of does inseminated	Percentage of does Non-return	Kidded
Fresh-diluted semen			
Sperm dose			
60×10^6 motile sperm (0·10 ml)	48	68·8	60·4
120×10^6 motile sperm (0·20 ml)	48	62·5	58·3
Depth of insemination			
Up to 1 cm into cervix	12	50·0	41·7
1·5–3·0 cm into cervix	32	62·5	53·1
Into the uterus	52	69·2	67·3
Frozen–thawed semen			
Sperm dose			
60×10^6 motile sperm (0·15 ml)	45	46·7	46·7
120×10^6 motile sperm (0·30 ml)	45	46·7	51·1
Depth of insemination			
Up to 1 cm into cervix	21	14·3	14·3
1·5–3·0 cm into cervix	23	39·1	43·5
Into the uterus	46	65·2	67·4

Semen for fresh use and for freezing was collected from the same two individual bucks. Oestrus was detected in 90% of does 20–24 days after sponge (Chrono-gest) removal, and the oestrous does received the insemination 10–14 h after detection. There was no difference between the two bucks within type of semen and dose of inseminate. The results, pooled for bucks, are presented in Table 2. The mean rate of non-return to service was higher for fresh-diluted than for frozen–thawed semen (65·6% *v.* 46·7%, $P < 0{\cdot}01$), but this was not reflected at kidding (59·4% *v.* 48·9%, $0{\cdot}10 < P < 0{\cdot}25$). The rates of kidding after insemination of 60×10^6 and 120×10^6 motile spermatozoa were similar (50 out of 93, 53·8%; 51 out of 93, 54·8%). There was no interaction between the type of semen and number of spermatozoa inseminated. The fertility for the two doses of fresh or of frozen–thawed spermatozoa within depth

of insemination was also indistinguishable. Examination of data pooled for sperm doses (Table 2) revealed a marked improvement in fertility with increasing depth of insemination for frozen–thawed but not for fresh-diluted semen (type of semen × depth of insemination: percentage non-return, $P < 0{\cdot}05$; percentage kidded, $P < 0{\cdot}01$). The fertility results for the two types of semen were similar after intra-uterine insemination. The number of animals for treatments within the category of depth of insemination was comparable.

Experiment 3

In this experiment fresh-diluted and frozen–thawed semen (each obtained from four ejaculates of one buck) was used for single and double insemination at the second oestrus after synchronization with Repromap sponges. Each inseminate of 0·10 ml fresh-diluted or 0·15 ml frozen–thawed semen contained 60×10^6 motile spermatozoa.

Table 3. Number of does kidding after single and double insemination with fresh-diluted and frozen–thawed semen (expt 3)

Insemination was at the second oestrus after synchronization with intravaginal sponges and was carried out 10–14 h (single insemination) and 10–14 h and 24 h (double insemination) after detection of oestrus

Number and depth of insemination	No. of does inseminated	Percentage of does that kidded
Fresh-diluted semen		
Single insemination (60×10^6 motile sperm)	35	20·0
Double insemination (120×10^6 total motile sperm)	39	59·0
Depth of insemination		
Up to 1 cm into cervix	45	33·3
1·5–3·0 cm into cervix	15	46·7
Into the uterus	14	51·1
Frozen–thawed semen		
Single insemination (60×10^6 motile sperm)	30	43·3
Double insemination (120×10^6 total motile sperm)	32	40·6
Depth of insemination		
Up to 1 cm into cervix	14	21·4
1·5–3·0 cm into cervix	30	26·7
Into the uterus	18	83·3

Of the does run with teasers, 80% were detected in oestrus 20–32 days after sponge removal. The results are presented in Table 3. There was an interaction between the type of semen and number of inseminations ($P < 0{\cdot}05$) which was due to the low rate of kidding after single insemination with fresh-diluted semen. In the latter case, the fertility was depressed in each category of depth of insemination, but the differences with regard to double insemination were not significant. When frozen–thawed semen was used, the rates of kidding were similar for single and double insemination within the depth of semen depostion. The analysis of the data pooled for the number of inseminations (Table 3) revealed that the type of semen and depth of insemination

interacted ($P < 0{\cdot}01$). As in the previous experiment, the improvement in fertility with increasing depth of insemination was more pronounced for the frozen–thawed than for fresh-diluted semen. However, in the interaction there was an implication of the difference between the numbers of animals for the three categories of depth of insemination for the two types of semen.

Experiment 4

In this experiment the frozen–thawed semen was used for single insemination at 48 or 59 h, and for double insemination at 48 and 59 h after sponge (Chrono-gest) removal. A group of goats received double insemination with fresh-diluted semen. At each insemination 60×10^6 motile spermatozoa were deposited in 0·15 ml frozen–thawed or in 0·10 ml fresh-diluted semen volumes. The semen for fresh use and for freezing was collected from the same two bucks and in both cases the ejaculates were pooled before dilution and processing.

Table 4. Number of does kidding in relation to number and time of insemination (expt 4)

At each insemination 60×10^6 motile sperm were deposited in 0·15 ml frozen–thawed or in 0·10 ml fresh-diluted semen volumes

No. of insem-inations	Time of insem-ination (h)[A]	No. of does kidded/ No. inseminated (%)
	Frozen–thawed semen	
1	48	14/35 (40·0)
1	59	11/37 (29·7)
2	48 and 59	24/45 (53·3)
	Fresh-diluted semen	
2	48 and 59	50/73 (68·5)

[A] Time after sponge removal.

The results are presented in Table 4. The rates of kidding for single inseminations with frozen–thawed semen at 48 or 59 h after sponge removal were indistinguishable. The mean rate of kidding for single insemination was lower than for double insemination with frozen–thawed semen (34·7% *v.* 53·3%, $P < 0{\cdot}05$). There was no difference in fertility of frozen–thawed and fresh-diluted semen when double insemination was performed with both types of semen.

Experiment 5

In this experiment a total of 60×10^6 or 120×10^6 motile frozen–thawed spermatozoa were deposited by single insemination 48 or 59 h, and by double insemination 48 and 59 h after sponge (Chrono-gest) removal. Semen was collected from three bucks and the ejaculates were pooled before processing for freezing. For single insemination, the inseminate volumes were 0·15 and 0·30 ml for 60×10^6 and 120×10^6 motile spermatozoa respectively. When double inseminations were performed, half of the semen dose was deposited at the first insemination and the other half at the second insemination (i.e. 0·075+0·075 ml and 0·15+0·15 ml).

The results are presented in Table 5. The mean rates of kidding for the three insemination treatments were similar. The difference in the fertility after insemination of 60×10^6 and 120×10^6 total motile spermatozoa did not attain significance ($0{\cdot}05 < P < 0{\cdot}10$). There was no interaction.

Table 5. Number of does kidding after insemination of equal numbers of frozen–thawed motile spermatozoa by single and double inseminations (expt 5)

No. of inseminations	Time of insemination (h)[A]	No. of does kidded/ No. inseminated (%)		Mean percentage of does that kidded
		60×10^6 total motile sperm	120×10^6 total motile sperm	
1	48	15/36 (41·6)	20/39 (51·3)	46·7
1	59	15/36 (41·6)	19/38 (50·0)	45·9
2	48 and 59	12/35 (34·3)	17/34 (50·0)	42·0
Totals and means		42/107 (39·3)	56/111 (50·5)	

[A] Time after sponge removal.

Table 6. Number of does kidding after double insemination with non-washed and twice-washed and subsequently frozen–thawed spermatozoa (expt 6)

Time of insemination 48 and 59 h after sponge removal and each time 0·15 ml of semen containing 60×10^6 motile sperm was deposited. PMSG was injected (i.m.) at sponge removal

Dose of PMSG and depth of insemination	No. of does inseminated	Percentage of does that kidded
Non-washed spermatozoa		
PMSG		
75 i.u.	26	53·8
300 i.u.	24	70·8
Depth of insemination		
Up to 1 cm into cervix	13	46·2
1·5–3·0 cm into cervix	14	71·4
Into the uterus	23	65·2
Mean	50	62·0
Twice-washed spermatozoa		
PMSG		
75 i.u.	24	54·2
300 i.u.	24	50·0
Depth of insemination		
Up to 1 cm into cervix	15	33·3
1·5–3·0 cm into cervix	18	61·1
Into the uterus	15	60·0
Mean	48	52·1

Experiment 6

In this experiment the non-washed or twice-washed and subsequently frozen–thawed spermatozoa were used for double insemination of does which received 75 or 300 i.u.

PMSG at the removal of sponges (Chrono-gest). The inseminations were carried out 48 and 59 h after sponge removal, each time with a dose of 0·15 ml containing 60×10^6 motile spermatozoa.

The results are presented in Table 6. The differences between the rates of kidding for treatment of spermatozoa before freezing or for dose of PMSG, and for depth of insemination were not significant. There were no interactions.

Discussion

During insemination in the goat, unlike the sheep, the semen can be deposited into the uterus in a relatively high proportion of animals for which the fertility is better than for the does inseminated into the cervix (Corteel 1976; Fougner 1976). An improvement in the fertility in both the goat (Peskovatskov *et al.* 1975) and sheep (Saidullin 1977; Milovanov *et al.* 1978; Stoyanov 1980; Varnavskij and Varnavskaja 1980; Grigorjan and Nazarjan 1981), can also be achieved by deposition of semen deep (up to 3 cm) into the cervix. The depth of insemination into the genital tract depends on the anatomical structure of the cervix of individual animals and the skill of the inseminator. In this study, all inseminations were performed by one operator, therefore the variability in the distribution of animals in the three categories of depth of insemination (between expts 1, 2, 3 and 6) was a reflection of differences between herds. For example, the proportions of does in which intra-uterine insemination could be performed were 12, 53, 24 and 39% in experiments 1, 2, 3 and 6 respectively. It was clear that, in experiments 1, 2, 3 and 6, the proportion of animals within and between categories of depth of insemination had an influence on the mean fertility for the treatments. Thus, the 'factor' of depth of insemination was included in the analysis of the data.

In this study, as in the work by other investigators (mentioned above), the depth of deposition of semen into the genital tract had an influence on fertility. However, the improvement in fertility with the increasing depth of insemination was more pronounced for frozen–thawed than for fresh-diluted semen (Tables 2 and 3). This points to an altered pattern of transport of the frozen–thawed spermatozoa in the cervix, a finding previously noted in the ewe (Lightfoot and Salamon 1970).

When the semen was used at the second oestrus after synchronization, the fertility was not influenced by treatments such as double insemination with deposition of twice the number of fresh motile spermatozoa than by single insemination (Table 1), or the increase of fresh or frozen–thawed motile sperm numbers in the single inseminate (Table 2). Both former treatments aimed at an increase in the sperm population in the segments of the reproductive tract. No explanation can be offered for the poor rate of kidding (20%) after single insemination with fresh-diluted semen in experiment 3 (Table 3). In this case, the fertility was also depressed in each category of depth of insemination.

When the frozen–thawed semen was used at the synchronized oestrus and the depth of insemination was not recorded, double insemination had a beneficial effect on fertility (Table 4). The benefit of the method was, however, most likely accounted for on the basis of increased total number of motile spermatozoa deposited, as indicated by the results of experiment 5, in which equal numbers of spermatozoa were deposited by both single and double inseminations. In the latter case, double insemi-

nation was of no advantage, but the increased total sperm dose (60×10^6 *v.* 120×10^6) yielded some improvement in the fertility (Table 5). Corteel (1976) found that in dairy goats the benefit of double insemination was not attributable to the increased total number of spermatozoa deposited and subsequently Corteel *et al.* (1980) recommended two inseminations with frozen–thawed semen, each time with not less than 60×10^6–75×10^6 motile spermatozoa.

In this study, the inseminations during the second oestrus after synchronization were carried out 10–24 h after detection of oestrus. The times of insemination were most likely within the appropriate range as judged from the work of Peskovatskov *et al.* (1974) and Fougner (1976). There are no observations in Angora does on the time of ovulation during the natural oestrus which in the Norwegian goat was found to occur 24–36 h (Fougner 1976) and in the Black Bengal goat 24–32 h from the onset of oestrus (Rao and Bhattacharyya 1980). Ovulation at the synchronized oestrus has been found to occur 56–66 h after PMSG injection at sponge removal (Ritar *et al.* 1981), and the data presented here (Tables 4 and 5) indicate that single or double inseminations can be carried out 48–59 h after the removal of sponges. The results of Moore and Eppleston (1979) with fresh Angora semen support the performance of inseminations within the above time ranges after the cessation of intravaginal sponge treatment for 16–18 days. Corteel *et al.* (1972) found that in dairy does the fertility was not influenced when two inseminations at the synchronized oestrus were performed at intervals of 12, 14 or 24 h within a period of 24–60 h after removal of intravaginal sponges. The authors, however, did not compare in their study single insemination at different times with double insemination.

Removal of seminal plasma by washing the spermatozoa before freezing has been found beneficial for post-thawing *in vitro* survival of the sperm cells (Corteel 1974, 1975*b*; Ritar and Salamon 1982). Although in the comparative fertility test in this study (Table 6) and of Corteel (1975*a*) the rates of kidding were not different for twice-washed and non-washed spermatozoa, the question regarding the need for removal of seminal plasma before freezing the spermatozoa can be considered still open.

In the final experiment in which the animals were injected with 75 or 300 i.u. PMSG there was only a marginal benefit in fertility at the synchronized oestrus after treatment with the higher dose of gonadotrophin. It is pertinent that Ritar *et al.* (1981) found that during the non-breeding period (November–December) injection of 400 i.u. PMSG was necessary (preferably 48 h before sponge removal) to achieve satisfactory synchrony of ovulation(s) with regard to insemination at fixed time after removal of intravaginal sponges.

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