S. Afr. J. Anim. Sci. 8, 111-113 (1978)

RESEARCH NOTE

OBSERVATIONS ON THE DEEP-FREEZING OF ANGORA GOAT SEMEN

Receipt of MS. 78.03.17

J.M. van der Westhuysen
Department of Human and Animal Physiology, University of Stellenbosch, Stellenbosch, 7600

(Key words: *Semen, goat, frozen)*
(Sleutelwoorde: *Semen, bok, gevries)*

Apart from gaining the many advantages of artificial insemination, the storing of deep-frozen semen also provides an alternative in cases of sexual quiescence in the male (Shelton, 1960; Marais, 1968; Van der Westhuysen, 1976). Since decreasing the average age of a flock has many economic advantages (Van der Westhuysen, 1977) attempts to increase the frequency of lambing in the Angora goat have been made. However, the absolute sexual quiescence in the male during female anoestrus hampers the out of season breeding to artificial stimulation (Pretorius & Van der Westhuysen, 1971; Marais, 1968; Van der Westhuysen 1976, 1977) and therefore necessitates fertilisation other than by natural service. Preliminary work on the indigenous Boer goat indicated that goat semen could be deep-frozen with success (Rossouw, 1974). Although other workers (Corteel, 1974; Fougner, 1974; Gonzalez Stagnaro, 1975) have also shown that deep-freezing and the post-thawing fertilizing ability of goat semen is satisfactory, no work has been done on the Angora goat. For this reason the effect of factors such as rate of cooling, equilibration time and removal of seminal plasma prior to deep-freezing of Angora goat semen were studied.

The semen was collected by artificial vagina from mature Angora goat rams and maintained at 32°C until diluted. The Tris-based diluent (Table 1), also at 32°C, was added to the semen in one step to achieve a dilution rate of 1:4. After cooling to 5°C, semen was aspirated into 0,5 mℓ straws and equilibrated before freezing in liquid nitrogen vapour.

In an experiment to study the effects of the rate of cooling from 32°C to 5°C and the duration of equilibration at 5°C on the success of freezing (Table 2), it was found that although neither the rate of cooling nor the duration of equilibration (0 -120 min.) affected the percentage motile sperm prior to freezing, the duration of the period of equilibration had a significant effect on the motility at thawing. An increase in the duration of equilibration was accompanied by an increase in the percentage motile sperm at thawing ($P < 0,01$).

In a further experiment, the effects of "washing" the semen with diluent to remove the seminal plasma prior to freezing and the effect of the duration of equilibration on the success of freezing, were studied. The semen was diluted 1:4 at 32°C and divided into equal volumes of which one was centrifuged (200 g) for 5 minutes, the supernatant aspirated and an equal volume of diluent added. The spermatozoa were then mixed with the diluent by gentle repeated inversion and/or "washing" by means of a pasteur pipette. Both washed and unwashed aliquots were then cooled to 5°C in 30 minutes and equilibrated for 0, 60, or 120 minutes. Prior to freezing in 0,5 mℓ straws the motility of each sample was determined.

This study (Table 3) again demonstrated that an equilibration period of at least 2 hours is required for the successful freezing of diluted semen. However, when semen was washed, freezing (even without any equilibration) yielded a significantly greater percentage of motile sperm at thawing than the unwashed semen. In addition, a trend for improved viability in the washed samples was also evident after an equilibration period of 2 hours. However, a degree of mechanical shock, deformities and poorer directional movement were observed and attributed to the centrifugation. For this reason the effects of centrifugal force, washing and equilibration time on the post-thawing viability were studied in a further experiment. The results confirmed that an increase in equilibration time from 2 to 4 hours improved the percentage motile sperm after thawing from 19,8% to 29,1% (N.S.). As before, washed semen proved to be superior to diluted semen as far as the percentage of motile sperm after thawing was concerned (Table 4). However, a degree of mechanical shock was again obvious, especially after fast centrifugation (Table 4). Since centrifuged semen tends to form a rather compact "pellet" at the bottom of the tube, mixing with fresh diluent is difficult and it is suspected that mixing by repeated "washing" with a pasteur pipette contributes to this mechanical shock. From the sperm concentration it was also obvious that many spermatozoa were lost when the diluent and seminal plasma were aspirated during the "washing".

From this study it can be concluded that Angora goat semen can be deep-frozen with a satisfactory recovery at thawing. It is obvious that the washing of semen and the consequent removal of the seminal plasma improved the viability of the spermatozoa over that of unwashed semen, but causes a degree of mechanical shock. Similarly, increasing the equilibration time to four hours is accompanied by increased sperm survival following deep-freezing. Although it is apparent that Angora goat semen can be deep-frozen successfully by prior cooling to 5°C in a period of 30 minutes and equilibration for 4 hours, the actual fertilizing ability of washed and unwashed semen can only be determined by large scale artificial insemination trials.

Table 1

Composition of the diluent used in the deep-freezing of Angora goat semen

Basic solution:		
Tris	2,8 g	
Fructose	1,4 g	in 100 mℓ/pH 7
Citric acid	1,35 g	
Working solution:		
Basic solution	66,5%	
Egg Yolk	22,0%	
Sorrenson's Phosphate Buffer	8,5%	
Glycerol	3,0%	

Acknowledgements

The author wishes to express his sincere gratitude to Messrs J. Greyling, J. Potgieter, Mrs E. Potgieter and the Insemina Cooperation, for assistance given during the course of this study.

Table 2

The effect of rate of cooling and duration of equilibration time on the mean (SD) percentage motile sperm at thawing of deep-frozen Angora goat semen

Cooling time (min) from 32°C to 5°C	30			60			120		
Equilibration time (min)	0	60	120	0	60	120	0	60	120
Number of samples	11	11	11	11	11	11	11	11	11
Motile sperm:									
Before freezing	64^a (8,0)	66^a (6,9)	68^a (8,3)	65^a (5,2)	64^a (7,9)	64^a (8,2)	64^a (8,4)	67^a (5,9)	64^a (7,4)
At thawing	2^a (2,9)	10^b (3,4)	18^b (4,0)	2^a (2,8)	18^b (6,4)	40^c (8,3)	1^a (1,5)	20^b (9,2)	31^c (7,8)

^{abc} Within each line of the table, means having the same superscript do not differ significantly from each other.

Table 3

The effects of washing of semen with diluent prior to deep-freezing and of equilibration time on the mean (SD) percentage motile sperm at thawing (Means ± (SD))

	Diluted Semen			Washed Semen		
Duration of equilibration (min)	0	60	120	0	60	120
Number of samples	6	6	6	6	6	6
Motile sperm (%)	$7,1^a$ (6,5)	$5,6^a$ (1,9)	$36,7^b$ (8,9)	$35,8^b$ (9,8)	$30,0^b$ (15,5)	$45,8^b$ (8,4)

Table 4

The effects of centrifigual force on the percentage motile sperm at thawing in semen following equilibration for 2 hours (Means ± SD)

	Semen only diluted	Semen washed and centrifuged for 5 min. at: 200 G	700 G
Motile sperm (%):			
Before freezing	73,75 ± 8,2	62,5 ± 23,8	67,5 ± 5,6
At thawing	15,9 ± 15,9	29,6 ± 16,09	23,85 ± 13,88

No significant differences

References

CORTEEL, J.M., 1974. Viabilité des spermatozoïdes de bouc conservés et congelés avenc ou sans leur plasma séminal: effect du glucose. *Annls. Biol. anim. Biochim. Biophys.* 14, 741.

FOUGNER, J.A., 1974. Intrauterin metode for inseminasjon med dypfrosen saed pa geit. *Norsk Veterinaertidsskrift* 86, 551.

GONZALEZ STAGNARO, G., 1975. Inseminacios artificial en cabras con semen congelado. *Zootechnia* 24, 151.

MARAIS, J.F.K., 1968. Aspekte van die vroeë embrioniese ontwikkeling by die Angorabok en die invloed van voeding daarop. M.Sc. Tesis, Universiteit van Stellenbosch, Stellenbosch.

PRETORIUS, P.S. & VAN DER WESTHUYSEN, J.M., 1971. Induction of breeding activity in anoestrous Angora goat does. *Agroanimalia* 3, 27.

ROSSOUW, A.F., 1974. 'n Voorlopige mededeling oor die bevriesing van Boerboksemen. *Tydskr. Veek.* 4, 165.

SHELTON, M., 1960. Influence of the presence of a male goat on the initiation of oestrous cycling and ovulation of Angora does. *J. Anim. Sci.* 19, 386.

VAN DER WESTHUYSEN, J.M., 1976. Induction of breeding activity in anoestrous angora goat does: Effect of progestagens, PMSG & teasing. *Agroanimalia* 8, 165.

VAN DER WESTHUYSEN, J.M., 1977. Some aspects of kid production in the Angora. *Angora goat and Mohair J.* 19, 37.