SEMEN FREEZING AND SUBSEQUENT INSEMINATION RESULTS IN SOUTH AFRICAN ANGORA GOATS

M.A. Snyman[1#] & F. Jooste[2]

[1] Grootfontein Agricultural Development Institute, Private Bag X529, Middelburg (EC), 5900

[2]GeneCo, Small Stock Reproduction Centre, P.O. Box 37, Philipstown, 8795

E-mail: [#]GrethaSn@daff.gov.za

INTRODUCTION

Genetic improvement in a breed is dependent on the rate of genetic change achieved in stud animals and the rate and efficiency of distribution of this superior genetics to the lower levels of the breed structure. The Angora Ram Breeders wanted to expedite this process by making superior genetics available to the entire industry through frozen semen. However, the low historic conception rates achieved with laparoscopic insemination with frozen semen in Angora goats were unacceptable and would inhibit the widespread use of this technology in the industry. Therefore, various trials were conducted over a 10-year period with the aim to develop a frozen semen protocol for South African Angora goats.

After five years of investigations, one problem area identified was the variability and poor semen quality of some Angora rams, which meant that a low percentage of semen ejaculates collected could successfully be frozen. Another problem was the poor post-thaw survivability and durability of the semen, which lead to a low percentage of the frozen semen doses being suitable for long term freezing. Subsequent results on a project involving the preparation of rams for a frozen semen protocol indicated that with proper preparation of rams before the semen collection process, semen quality of the rams would not pose a problem (Baca et al., 2014). Since 2013, a protocol for freezing semen in straws has been implemented, which yielded acceptable post-thaw motility and durability. Various synchronisation protocols for the ewes, as well as insemination methods have subsequently been evaluated. The most important results of these different trials will be summarised in this paper, as well as recommendations made regarding the preparation of rams before the collection of semen and ewes before artificial insemination.

PREPARATION OF RAMS FOR SEMEN COLLECTION

Preparation of rams for semen collection for freezing should start at least eight weeks beforehand to ensure optimum spermatozoa production during collection. The quality and quantity of semen produced are influenced by the nutritional level of the ram (Martin et al., 1994a; Martin et al., 1994b; Thwaites, 1995; Almeida et al., 2007). The results presented by Braden et al. (1974), Oldham et al. (1978), Alkass et al. (1982), Fernandez et al. (2004) and Kheradmand et al. (2006) confirm that spermatozoa production, as well as total number of spermatozoa per ejaculate, can be affected by energy and protein content of the diet. Zinc, Selenium, Vitamin E and Vitamin A are essential elements during the spermatozoa production processes and evidence exists that supplementation with these vitamins and trace minerals improves semen quality (Abdulkareem et al., 2005; Cheah & Yang, 2011). Rams should thus be administered vitamin and mineral supplements during the pre-collection period.

An example of a high energy-high protein diet is given in Table 1. It is important that the rams should be adapted to the diet over a two-week period. Rams should be fed this diet at 2-3% of their body weight. Lucerne hay should also be provided at 500 g/ram/day. Half of the daily feed portion should be provided in the morning and half in the afternoon. Clean drinking water should be available on an *ad libitum* basis.

Table 1. Example of a high energy-high protein diet

Ingredient	**Percentage**
Lucerne hay	31
Maize stover	5
Maize	45
Fishmeal	2.5
Cottonseed oil cake meal	15
Feed lime	1
Ammonium chloride	0.5
Nutrient	**Percentage**
Crude protein	16.1
Energy (TDN)	68.5
Crude fibre	13.6
Ca	0.94
P	0.39
Urea	0

Rams should be exercised daily by letting them walk at a fast pace for at least an hour. The aim is to have rams in an excellent body condition (body condition (BCS) score between 3 and 4 out of 5), but not overfat (BCS over 4 out of 5). The rams could be exposed to mature synchronised ewes for four weeks before semen collection. Photoperiod plays a role in the quality and quantity of spermatozoa produced. Therefore, the normal autumn breeding season for Angora goats is preferable for the collection and freezing of ejaculates (Ritar, 1991).

PREPARATION OF EWES BEFORE ARTIFICIAL INSEMINATION

There are several factors that affect the results of artificial insemination in ewes. Among these are various environmental elements, year, season, farm or production systems, management program of the ewe before breeding, health and physiological status of the ewes, age and parity of the ewe, synchronisation treatment, sire, artificial insemination technique and technician, semen deposition site, insemination time and spermatozoa dose rate (Shackell et al., 1990; Hill et al., 1998; Paulenz et al., 2002; Anel et al., 2005; Rahman et al., 2008; Beilby et al., 2009; Arrebola et al., 2012; Kulaksiz & Daşkin, 2012).

Ewes should have a body condition score (BCS) of at least 2.5 on a scale out of 5, six weeks before artificial insemination. From six weeks before the date of artificial insemination, ewes need to be on a rising plane of nutrition and the BCS should increase. On natural pasture of the Karoo and Eastern Cape, ewes should receive 200 to 500 grams of a production lick daily depending on the BCS at the start of the program. Good quality roughage must be available at all times.

For an artificial insemination program, it is preferable to use ewes that have kidded at least once and to avoid using ewes older than 7 years of age, as they could have an increased risk of reproductive disorders and decreased rates of ovulation with quality ovulated oocytes compared to younger ewes (Kulaksiz & Daşkin, 2012). According to Rahman et al. (2008), the 9 to 11 day progesterone treatment, followed by a luteolytic dose of prostaglandin administered in the period 48 hours prior to the removal of the CIDR (Controlled internal drug release device), is widely used in insemination programs in goats.

Recommended 17-day (traditional protocol) and 9-day (short protocol) synchronisation programs for ewes for trans-cervical insemination with fresh semen and laparoscopic insemination with frozen-thawed semen are summarised in Tables 2 to 5. These programs were used for the insemination trials during this study.

Table 2. Synchronisation program for ewes for trans-cervical insemination using fresh semen on a 17-day synchronisation period

Day	Time	Procedure
0	Any time	Insert CIDRs
17	13:00	Remove CIDRs
		Inject 200 IU PMSG*
18	16:00	Overnight no food or water Add teaser rams
19	09:00	AI (44 to 48 hours after CIDR removal)

* Pregnant mare serum gonadotropin

Table 3. Synchronisation program for ewes for laparoscopic insemination using frozen semen on a 17-day synchronisation period

Day	Time	Procedure
0	Any time	Insert CIDRs
17	07:00	Remove CIDRs
		Inject 200 IU PMSG*
18	16:00	Overnight no food or water Add teaser rams
19	09:00	AI (50 to 56 hours after CIDR removal)

* Pregnant mare serum gonadotropin

Table 4. Synchronisation program for ewes for trans-cervical insemination using fresh semen on a 9-day synchronisation period

Day	Time	Procedure
0	Any time	Insert CIDRs
9	14:00	Remove CIDRs
		Inject 200 IU PMSG*
		Inject 1 mL Lutalyse
10	16:00	Overnight no food or water Add teaser rams
11	11:00	AI (44 to 48 hours after CIDR removal)

* Pregnant mare serum gonadotropin

Table 5. Synchronisation program for ewes for laparoscopic insemination using frozen semen on a 9-day synchronisation period

Day	Time	Procedure
0	Any time	Insert CIDRs
9	09:00	Remove CIDRs
		Inject 200 IU PMSG*
		Inject 1 mL Lutalyse
10	16:00	Overnight no food or water Add teaser rams
11	11:00	AI (50 to 56 hours after CIDR removal)

* Pregnant mare serum gonadotropin

SEMEN FREEZING PROTOCOLS

Semen was frozen according to various protocols in either pellets or straws during the study. Ejaculates of acceptable quality were collected throughout the study period. Fresh semen quality was therefore not a constraining factor during the semen freezing protocols. The percentage post-thaw motile spermatozoa obtained with various protocols over the duration of the study is summarised in Table 6. It can be concluded that post-thaw motility rates above 50% can be achieved with the straw-freezing protocols followed since 2013.

Table 6. Summary of percentage post-thaw motile spermatozoa of semen frozen during the study

Protocol	Year	Treatment of rams	% Motile spermatozoa after thawing
Pellets	2008	High quality ration	40 to 60%
Pellets	2010	High quality ration	24%
Pellets	2012	High energy-high protein diet	Low post-thaw % live spermatozoa
Straws	2013	High energy-high protein diet	35 to 50%
Pellets	2013	High energy-high protein diet	30 to 40%
Straws	2013	High energy-high protein diet	40 to 50%
Straws	2014	High energy-high protein diet	65%
Straws	2014	High energy-high protein diet	60%
Straws	2016	High energy-high protein diet	38 to 65%

Post-thaw survival rates of 42% to 58% were reported by Loubser & Van Niekerk (1983) for Angora goat semen frozen from February to June. It is recommended that at least 20 million progressively motile spermatozoa be placed in the uterus by laparoscopic insemination, or 100 million progressively motile spermatozoa by trans-cervical insemination (Shipley et al., 2007). The lower the percentage live spermatozoa of a frozen semen sample, the higher the dose rate should be to ensure successful conception.

There was much variation in freezability, subsequent post-thaw quality and durability of semen among the different sires in all the trials conducted over all the collection periods. It is well known that there is large variation in semen quality traits and freezability among individuals within breeds, as well as within flocks (Thurston et al., 2002).

INSEMINATION TRIALS

Since 2015, several insemination trials were done on the farms of two producers to evaluate the semen frozen in straws in terms of conception rate (number of ewes scanned pregnant per number of ewes that were inseminated) and / or kidding percentage (number of ewes that kidded per number of ewes that were inseminated). The trans-cervical and laparoscopic insemination techniques were used in the evaluation process. During the 2015 trials, trans-cervical and laparoscopic insemination with either fresh or frozen semen were compared. In 2016, the 9-day and 17-day synchronisation periods, as well as insemination with fresh or frozen semen were compared. One vs. two laparoscopic inseminations was compared during 2017.

Two hundred and twenty mature ewes of the Grootfontein Student Angora Stud (GSAS) and two hundred and ninety mature ewes of Mr Ray Hobson were used in the trials from 2015 until 2017. Sires from the respective producers were taken to the small stock reproduction centre of GeneCo in December of that year or January of the following year, where semen was collected and frozen in straws. The ewes at the two farms were prepared according to the management and treatment programs indicated in Tables 2 to 5. The results of the various insemination trials are summarised in Table 7.

Table 7. Results of the various insemination trials

Trial-Year	Procedure*	Semen	Conception percentage	Kidding percentage
GSAS - 2015	Lapro-AI	Frozen - straws	33.0	29.0
Mr Ray Hobson - 2015	Lapro-AI	Frozen - straws	52.2	41.2
GSAS - 2016	Lapro-AI CIDR-17 days	Frozen - straws	15.0	22.0
GSAS - 2016	Lapro-AI CIDR-9 days	Frozen - straws	56.0	61.0
GSAS - 2017	Lapro-AI 1 time AI	Frozen - straws	-	30.5
GSAS - 2017	Lapro-AI 2 times AI	Frozen - straws	-	31.6
Average	**Lapro-AI**	**Frozen - straws**	**39.1**	**35.9**
GSAS - 2015	Lapro-AI	Fresh (AV)	25.0	22.0
Mr Ray Hobson - 2015	Lapro-AI	Fresh (AV)	10.0	0.0
Average	**Lapro-AI**	**Fresh (AV)**	**17.5**	**11.0**
GSAS - 2014	TC-AI	Frozen - straws	47.7	17.7
GSAS - 2015	TC-AI	Frozen - straws	25.0	22.0
Mr Ray Hobson - 2015	TC-AI	Frozen - straws	20.0	16.6
Average	**TC-AI**	**Frozen - straws**	**30.9**	**18.8**
Mr Ray Hobson - 2015	TC-AI	Fresh (AV)	25.0	15.6
GSAS - 2016	TC-AI CIDR-17 days	Fresh	20.0	27.0
GSAS - 2016	TC-AI CIDR-9 days	Fresh	41.0	39.0
Average	**TC-AI**	**Fresh**	**28.7**	**27.2**
Mr Ray Hobson - 2015	**Traditional**	**Fresh (AV)**	**73.3**	**63.3**

***** Lapro-AI = Laparoscopic insemination; TC-AI = Trans-cervical insemination; AV = Artificial vagina

Variable results were obtained for conception rates and kidding percentages with trans-cervical and laparoscopic insemination with frozen-thawed semen in the different trials done over the years. In general trans-cervical insemination with either fresh or frozen semen did not yield acceptable results. Although laparoscopic insemination with frozen-thawed semen produced better results than with fresh semen, variable results were obtained in the different years. As far as specific aspects are concerned, from the results of the 2016-trial at Grootfontein it is clear that the shorter 9-day synchronisation cycle yielded better conception results when using CIDRs in Angora goats than the longer 17-day cycle. According to Rahman et al. (2008), the shorter 9- to 11-day progesterone treatment is widely used in goats. No difference in scanning and kidding results was obtained between one or two inseminations at GSAS. In contrast, Yotov et al. (2016) reported 5 to 7% higher conception rates with two vs. one

inseminations in Bulgarian White milk goats with different oestrus synchronisation methods.

During 2017, laparoscopic insemination of Angora ewes with semen frozen in straws at GeneCo was done at the farms of four producers. The synchronisation program as indicated in Table 5 was followed. The ultrasound scanning results are summarised in Table 8.

Table 8. Conception results of the producer trials conducted during 2017

Producer	Conception percentage	District	Remarks
Producer A	Sire 1 = 75% Sire 2 = 50%	Fraserburg	Very dry conditions before mating. Flushing: 500 g lucerne hay, 300 g maize, 250 g lick.
Producer B	Sire 1 = 53% Sire 2 = 70% Sire 3 = 33% Sire 4 = 56% Sire 5 = 86%	Aberdeen	
Producer C	Sire 1 = 5% Sire 2 = 20%	Willowmore	Flushing: 300 g Ram, lamb and ewe pellets.
Producer D	Sire 1 = 30%	Willowmore	
Average	**47.8%**		

***** Lapro-AI = Laparoscopic insemination; Kidding percentages not available

Variable results were obtained among the farms and the sires. These results indicate that it is possible to obtain acceptable conception rates with laparoscopic insemination with frozen-thawed semen when following the prescribed protocols.

Conception rates and kidding / lambing percentages obtained with trans-cervical and laparoscopic insemination with frozen-thawed semen in goats and sheep reported in literature are summarised in Table 9. The results obtained with the current study are slightly lower than those reported in literature for goats and sheep summarised in Table 9.

Table 9. Conception rates and kidding / lambing percentages obtained with trans-cervical and laparoscopic insemination with frozen-thawed semen in goats and sheep reported in literature

Reference	Breed	Procedure*	Conception percentage	Kidding / Lambing percentage
Goats				
Loubser et al. (1983)	Angora	Cervical -AI	34.8	
Ritar & Salamon (1983)	Angora	Cervical-AI	14.3 to 39.1	14.6 to 43.5
	Angora	TC-AI	65.2	67.4
Ritar et al. (1990)	Cashmere	Cervical-AI	39.1	
	Cashmere	Lapro-AI	52.1 to 63.6	34.0 to 63.6
Ritar & Ball (1991)	Cashmere	Lapro-AI		50.0 to 56.8
Goonewardene et al. (1997)	Alpine and Saanen	Lapro-AI		41.0
Salvador et al. (2005)	Murciano-Granadina	TC-AI	48.2	
Apu et al. (2012)	Black Bengal	Cervical-AI		43.9
Kulaksiz & Daşkin, 2012	Saanen	Lapro-AI	59.5	
Ramukhithi et al. (2012)	Boer goat	Lapro-AI	30.8 to 36.4	
	Indigenous veld goat		33.3 to 50.0	
Kharche et al. (2013)	Jamunapari	Cervical-AI		34.4
Yotov et al. (2016)	Bulgarian White milk goats	Cervical-AI	37.0 to 60.0	
Sheep				
Olesen (1993)	Various	Cervical-AI	58.0	51.5
Donovan et al. (2004)	Norwegian and Irish	Cervical-AI	36.0 to 45.0	
Anel et al. (2005)	Churra	Lapro-AI		44.9
Avendano-Reyes et al. (2007)	Pelibeuy	Lapro-AI		20.0 to 65.0
Fukui et al. (2007)	Suffolk	Lapro-AI	65.5 to 66.7	62.1 to 66.7

***** Lapro-AI = Laparoscopic insemination; TC-AI = Trans-cervical insemination

Furthermore, Kulaksiz & Daşkin (2012) quoted Dickson et al. (2001) and Lowinger et al. (2001) who respectively reported 59.5% (Alpine and Saanen goats) and 0.0% to 40.0% (Argentinean goat flocks) conception rates with laparoscopic insemination with frozen-thawed semen.

CONCLUSIONS

Quality of fresh semen of Angora rams should not pose a problem during the freezing process of semen. However, differences among rams do exist, as is the case in other breeds and species. A workable protocol for the freezing of semen in straws is available that can yield acceptable conception rates with laparoscopic insemination. It should be kept in mind that the preparation and management of the ewes play an important role in the success of this protocol.

Wide variation in conception rates was achieved with frozen-thawed semen during these trials. Results varied from excellent to poor. Further research and trials should focus on eliminating this variation. Timing of ovulation, failure of fertilisation or incorrect insemination dose could be factors contributing to the variation observed and should be investigated.

ACKNOWLEDGEMENTS

The following people / institutions are acknowledged for their contribution to this project:

- Mohair South Africa for funding of the project
- Mr Ray Hobson and participating breeders for their inputs
- Dr Fanie Steyn and Ramsem for their contribution to the execution of the project
- Dr Frans Jooste for his contribution to the execution of the project and subsidising of the 2017-trials
- Mr Hans Greeff and the GSAS for participating and funding of the 2015 and 2017 GSAS projects.

REFERENCES

Abdulkareem, T.A., Al-Haboby, A.H., Al-Mjamei, S.M. & Hobi, A.A., 2005. Sperm abnormalities associated with Vitamin A deficiency in rams. Small Rumin. Res. 57, 67-71.

Alkass, J.E., Bryant, M.J. & Walton, J.S., 1982. Some effects of level of feeding and body condition upon sperm production and gonadotropin concentrations in the ram. Anim. Prod. 34, 265-277.

Almeida, A.M., Schwalbach, L.M.J., Cardoso, L.A. & Greyling, J.P.C., 2007. Scrotal, testicular and semen characteristics of young Boer bucks fed winter veld hay: The effect of nutritional supplementation. Small Rumin. Res. 73, 216-220.

Anel, L., Kaabi, M., Abroug, B., Alvarez, M., Anel, E., Boixo, J.C., De la Fuente, L.F. & De Paz, P., 2005. Factors influencing the success of vaginal and laparoscopic artificial insemination in Churra ewes: a field assay. Theriogen. 63, 1235-1247.

Apu, A.S., Yahia Khandoker, M.A.M., Husain, S.S., Fakruzzaman, M. & Notter. D.R., 2012. A Comparative Study of Fresh and Frozen-Thawed Semen Quality in Relation to Fertility of

Black Bengal Goats. Iranian J. Appl. Anim. Sci. 2(2), 157-161.

Arrebola, F.A., Pardo, B., Sanchez, M., Lopez, M.D. & Perez-Marin, C.C., 2012. Factors influencing the success of an artificial insemination program in Florida goats. Spanish J. Agric. Res. 10(2), 338-344.

Avendano-Reyes, L., Alvarez-Valenzuela, F.D., Molina-Ramirez, L., Rangel-Santos, R., Correa-Calderon, A., Rodriguez-Garcia, J., Cruz-Villegas, M., Robinson, P.H. & Famula, T.R., 2007. Reproduction performance of Pelibuey ewes in response to estrus synchronization and artificial insemination in Northwestern Mexico. J. Anim. Vet. Adv. 6(6), 807-812.

Baca, A., Snyman, M.A. & Kilian, E., 2014. Effect of frequency of mineral and vitamin supplementation on semen quality of Angora goat sires. Grootfontein Agric. 14(1), 11-24.

Beilby, K.H., Grupen, C.G., Thomson, P.C., Maxwell, W.M.C. & Evans, G., 2009. The effect of insemination time and sperm dose on pregnancy rate using sex-sorted ram sperm. Theriogen. 71, 829-835.

Braden, A.W.H., Turnball, K.E., Mattner, P.E. & Moule G.R., 1974. Effect of protein and energy content of the diet on the rate of sperm production in rams. Aust. J. Biol. Sci. 27, 67-73.

Cheah, Y. & Yang, W., 2011. Functions of essential nutrition for high quality spermatogenesis. Adv. Biosci. Biotechnol. 2, 182-197.

Donovan, A., Hanrahan, J.P., Kummen, I.E., Duffy, P. & Boland, M.P., 2004. Fertility in the ewe following cervical insemination with fresh or frozen thawed semen at a natural or synchronized oestrus. Anim. Rep. Sci. 84, 359-368.

Fernández, M., Giráldez, F.J., Frutos, P., Lavín, P. & Mantecón, A.R., 2004. Effect of undegradable protein supply on testicular size, spermiogram parameters and sexual behaviour of mature Assaf rams. Theriogen. 62(1-2), 299-310.

Fukui, Y., Kohno, H., Togari, T. & Hiwasa, M., 2007. Fertility of ewes inseminated intrauterinally with frozen semen using extender containing bovine serum albumin. J. Reprod. Dev. 53, 95-962.

Goonewardene, L., Whitmore, W., Jaeger, S. & Emond, S., 1997. Effect of prebreeding maintenance diet on artificial insemination in Alpine and Saanen goats. Theriogen. 48, 151-159.

Hill, J.R., Thompson, J.A. & Perkins, N.R., 1998. Factors affecting pregnancy rates following laparoscopic insemination of 28447 Merino ewes under commercial conditions: a survey. Theriogen. 49, 697-709.

Kharche, S.D., Jindal, S.K., Priyadharsini, R., Kumar, S., Goel, A.K., Ramachandran, N. & Rout, P.K., 2013. Fertility following frozen semen Artificial Insemination in Jamunapari goats. Indian J. Anim. Sci. 83(10), 1071-1073.

Kheradmand, A., Babaei, H. & Ali Batavani, R., 2006. Effect of improved diet on semen quality and scrotal circumference in the ram. Vet. Arhiv. 76(4), 333-341.

Kulaksiz, R. & Daşkin. A., 2012. Reproductive performance of primiparous and multiparous Saanen goats after laparoscopic intrauterine insemination: a field study. Turk. J. Vet. Anim. Sci. 36(2), 201-204.

Loubser, P.G., Greyling, J.P.C. & Viljoen, K.S., 1983. Artificial insemination of Angora goat does with pelleted deep-frozen semen. S. Afr. J. Anim. Sci. 13(2), 134-135.

Loubser, P.G. & Van Niekerk, C.H., 1983. Seasonal changes in sexual activity and semen quality in the Angora ram. 2. Semen volume, quality and freezability. S. Afr. J. Anim. Sci. 13(3), 161-163.

Martin, G.B., Fisher, J.S., Blackberry, M.A., Boukhliq, R., Hotzel, M.J., Shepherd, K. & Walkden-Brown, S.W., 1994a. Nutritional and photoperiodic control of testicular size in Suffolk and Merino rams. Proc. Aust. Soc. Anim. Prod. 20, 427.

Martin, G.B., Tjondronegoro, S. & Blackberry, M.A., 1994b. Effect of nutrition in testicular size and the concentrations of gonadotrophins, testosterone and inhibin in plasma of mature male sheep. J. Reprod. Fertil. 101, 121-128.

Oldham, C.M., Adams, N.R., Ghendi, D.R. & Mackintosh, J.B., 1978. The influence of level of feed intake on sperm producing capacity of testicular weight tissue in rams. Aust. J. Agric. Res. 29, 173-179.

Olesen, I., 1993. Effects of cervical insemination with frozen semen on fertility and litter size of Norwegian sheep. Livest. Prod. Sci. 37, 169-184.

Paulenz, H., Adnoy, T., Fossen, O.H., Soderquist, L. & Berg, K.A., 2002. Effect of deposition site and sperm number on the fertility of sheep inseminated with liquid semen. Vet. Rec. 150, 299-302.

Rahman, A.N.M.A., Abdullah, R.B. & Wan-Khadijah, W.E., 2008. Estrus synchronization and superovulation in goats: A review. J. Biol. Sci. 8(7), 1129-1137.

Ramukhithi, F.V., Nedambale, T.S., Sutherland, B., Greyling, J.P.C. & Lehloenya, K.C., 2012. Oestrous synchronisation and pregnancy rate following artificial insemination (AI) in South African indigenous goats. J. Appl. Anim. Res. 40(4), 292-296.

Ritar, A.J., 1991. Seasonal changes in LH, Androgens and testes in the male Angora goat. Theriogen. 36(6), 959-972.

Ritar, A.J. & Ball, P.D., 1991. Fertility of young Cashmere goats after laparoscopic insemination. J. Agric. Sci. 117(2), 271-273.

Ritar, A.J., Ball, P.D. & O'May, P.J., 1990. Artificial insemination of Cashmere goats: effects on fertility and fecundity of intravaginal treatment, method and time of insemination, semen freezing process, number of motile spermatozoa and age of females. Reprod. Fert. Dev. 2(4), 377-384.

Ritar, A.J. & Salamon, S., 1983. Fertility of Fresh and Frozen-Thawed Semen of the Angora Goat. Aust. J. BioI. Sci. 36, 49-59.

Salvador, I., Viudes-de-Castro, M.P., Bernacer, J., Gómez, E.A. & Silvestre, M.A., 2005. Factors Affecting Pregnancy Rate in Artificial Insemination with Frozen Semen During Non-Breeding Season in Murciano-Granadina Goats: a Field Assay. Reprod. Dom. Anim. 40(6), 526-529.

Shipley, C.F.B., Buckrell, B.C., Mylne, M.J.A., Pollard, J. & Hunton, J.R., 2007. Artificial insemination and embryo transfer in sheep. In: Current therapy in large animal theriogenology. Eds. R.S. Youngquist & W.R. Threlfall. Sounders Elsevier, St. Louis, MO, USA. pp. 629-641.

Shackell, G.H., Kyle, B. & Littlejohn, R.P., 1990. Factors influencing the success of a large scale artificial insemination programme in sheep. Proc. New Zeal. Soc. Anim. Prod. 50, 427-30.

Thurston, L.M., Watson, P.F. & Holt, W.V., 2002. Semen cryopreservation: a genetic explanation for species and individual variation? CryoLetters 23, 255-262.

Thwaites, C.J., 1995. Effect of undernutrition on the size and tone of the ram's testes. Small Rum. Res. 16, 283-286.

Yotov, S.A., Velislavova, D.V. & Dimova, L.R., 2016. Pregnancy rate in Bulgarian White milk goats with natural and synchronized estrus after artificial insemination by frozen semen during breeding season. Asian Pacific J. Reprod. 5(2), 144-147.