EFFECT OF DIFFERENT GLYCEROL CONCENTRATIONS ON FREEZING OF BUCK SEMEN

By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree

Master of Veterinary Science

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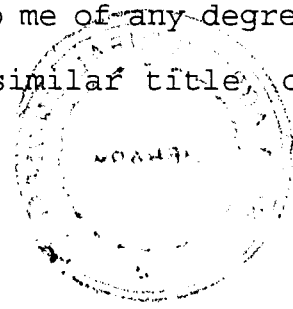
Mannuthy, Thrissur

1995

DECLARATION

I hereby declare that this thesis entitled "EFFECT OF DIFFERENT GLYCEROL CONCENTRATIONS ON FREEZING OF BUCK SEMEN" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.



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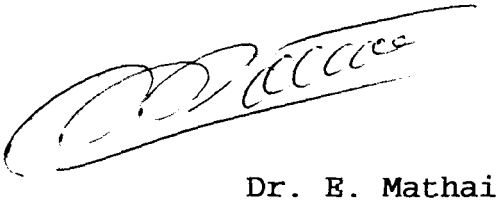
CERTIFICATE

Certified that this thesis entitled **"EFFECT OF DIFFERENT GLYCEROL CONCENTRATIONS ON FREEZING OF BUCK SEMEN"** is a record of research work done independently by **Sri. V. Prasanth**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.



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ACKNOWLEDGEMENTS

With profound gratitude I wish to place my indebtedness to Dr. E. Mathai, Professor, Department of Animal Reproduction and Chairman of Advisory Board for his valuable guidance, helpful suggestions, sustained encouragement and critical comments throughout the course of this work.

I am deeply indebted to Dr. C.P. Neelakanta Iyer, Professor and Head, Department of Animal Reproduction, and member of Advisory Committee for meticulous guidance, valuable suggestions and words of inspiration.

I express my deep sense of gratitude to Dr. Metilda Joseph, Assistant Professor, Department of Animal Reproduction and Dr. K.V. Raghunandanan, Associate Professor, Department of Animal Breeding and Genetics, for their valuable help and guidance at various stages of this work as members of the Advisory Committee.

I am thankful to Dr. A. Rajan, Dean, College of Veterinary and Animal Sciences for the facilities provided and timely help rendered during the study.

I would like to express my sincere thanks to Dr. K.C. George, Professor and Head, Department of Statistics and the staff for analysing the results.

My sincere thanks are due to Dr. K. Prabhakaran Nair, Professor, Dr. V. Sudarsanan, Professor, Dr. E. Madhavan, Professor, Dr. T. Sreekumaran, Associate Professor, Dr. K.N. Aravinda Ghosh, Associate Professor, Dr. K.V. Athman, Associate Professor, Dr. V. Vijayakumaran, Associate Professor Dr. S.P. Suresan Nair, Assistant Professor and Dr. K. Ramachandran, Assistant Professor of Department of Animal Reproduction for their advice and help rendered during various stages of the study.

I wish to thank Farm Assistants and the labourers of the Artificial Insemination Centre, Mannuthy, for helping me during the course of my work.

Special thanks are due to colleagues and friends for their whole-hearted help, co-operation and motivation.

The help rendered by the Librarian and Staff of College Library are appreciated with thanks.

I appreciate with thanks Mr. O.K. Ravindran, C/o Peagles, Mannuthy for his tireless efforts and skilled typing of this manuscript.

With immense pleasure, I place on record the unstinted encouragement and moral support extended by my parents in fulfilling this endeavour.

Last but not the least, I thank all the respondents of my study for their endurance and co-operation without which the study would not have been possible.

V. PRASANTH

Dedicated to my beloved parents

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Introduction

INTRODUCTION

Goat is one of the earliest domesticated animals. Small body size, high reproductive performance and efficient adaptability make it possible for goats to be reared in extreme environmental conditions. It can consume coarse fodder, wherein the actual nutrients are meagre and in crude form.

Goat is contributing to the national economy through the production of milk, meat, skin, pashmina and manure. Goat popularly known as the "Poor man's cow" play a significant role in the rural economy of our state.

Although India has the largest goat population in the world, our goats have lower productivity, when compared to their counterparts in advanced countries of the world. In order to rear goats under stallfed conditions and to increase their milk production capacity through scientific breeding and management, Indian Council of Agricultural Research, during IV five year plan launched the All India Co-ordinated Research Project on Goats for milk initially at two centres, Karnal in Haryana State and Mannuthy in Kerala State.

The recognised breed of goat native to Kerala is the Malabari (also called Tellichery). In the All India Co-ordinated Research Project on goats for milk at Mannuthy,

Malabari goats were crossed with Saanen and Alpine with an objective to evolve a milch breed adapted to Kerala.

Identification of superior sires and the maximum utilisation of their semen for breeding have been the well known method for promoting the livestock production all over the world. The large scale propagation of semen of proven bucks either through natural service or liquid semen in a population of goats spread over a wide area is not easy. Application of frozen semen technology in breeding of does is, therefore, a great promise for faster propagation of superior germplasm.

In Kerala, Artificial Insemination using frozen semen is well established in cows. But in goats, this programme is not implemented; as the technique of freezing of buck semen is still in its infancy. Once the technique of freezing of buck semen is brought on par with that in cattle, the facilities available for bull semen preservation and insemination can be well utilized for buck semen also without incurring much additional expenditure. A large number of goats with higher production potential can be produced by implementing scientific breeding with high quality buck semen. For this, effective methods of freezing of buck semen have to be practiced.

A cryoprotectant which protect spermatozoa from freezing injury should be added in the freezing medium. It is reported that glycerol is the most suitable cryoprotectant for buck

semen. Reports are available showing different levels of glycerol for buck semen extenders (Deka and Rao, 1986b; Chauhan and Anand, 1990; Deshpande and Mehta, 1991; Sinha et al., 1992b; Sinha et al., 1993). However lack of effective freezing technology for buck semen was found to be one of the major bottlenecks in implementing large scale Artificial Insemination in goats. Realising the importance of goats in our country in general and particularly in the state of Kerala, the present investigation on the freezing technology of goat semen was undertaken with the objective to study the effect of different concentrations of glycerol on post-thaw motility and fertility of frozen buck semen.

Review of Literature

REVIEW OF LITERATURE

Preservation of spermatozoa is a process in which not only the viability but also the fertilizing capacity is maintained during the period of storage. The cryoprotectant properties of glycerol made it very much advantageous to preserve semen at ultra low temperatures (Polge et al., 1949). The development of extenders using egg yolk provided the basis for most of the media used for preservation of spermatozoa (Salisbury et al., 1978).

Spermatozoa are damaged during freezing and thawing process mainly due to internal ice crystal formation causing structural alterations and increase in solute concentrations to toxic levels by withdrawal of pure water from both intracellular and suspending media of freezing (Salisbury et al., 1978).

2.1 Semen extenders

2.1.1 Citrate extenders

Sahni and Roy (1972) reported 50 to 60 per cent post-thaw motility for deep-frozen (-79°C) goat semen with egg yolk-citrate diluent. Deka and Rao (1985a) has done freezing of buck semen in egg yolk-citrate-fructose-glycerol extender.

Post- thaw motility and damaged acrosomes averaged 48.86 per cent and 18.85 per cent respectively.

2.1.2 Milk extenders

Sahni and Roy (1972) reported 50 to 60 per cent post-thaw motility for goat semen deep-frozen with heated cow milk. Corteel (1974) observed that addition of glucose in skim milk diluent for buck semen freezing increased post-thaw motility. Corteel et al. (1983) obtained 35 ± 2 and 27 ± 2 per cent post-thaw motility in goat semen frozen with 75 per cent milk plus 25 per cent saline and milk alone respectively. Deka and Rao (1985a) reported 65.86 per cent post-thaw motility for buck semen in skim milk-egg yolk–fructose-glycerol extender.

Melo and Nunes (1991) reported that cow's milk-glucose diluent was better than coconut milk diluent for freezing goat semen. Sinha et al. (1992a) reported that skim milk extender with six per cent glycerol resulted in better quality frozen buck semen.

2.1.3 Tris extenders

Haranath et al. (1982) reported 60 per cent post-thaw motility for buck semen frozen with a Tris-based diluent containing fructose, citric acid, egg yolk and glycerol. Buck spermatozoa tolerated a relatively wide range in concentration of Tris, but their survival depended on the type of sugar

included in the Tris diluent; glucose and fructose were more suitable components than lactose or raffinose; the best result was obtained with a four per cent (v/v) glycerol concentration in the diluted semen (Salamon and Ritar, 1982).

The percentage of live spermatozoa and spermatozoa with forward motility were significantly higher in buck semen frozen with Tris-egg yolk-citric acid-fructose-glycerol extender than in egg yolk-citrate-fructose-glycerol extender (Deka and Rao, 1985a). They obtained 68.43 per cent post-thaw motility in buck semen frozen with Tris-egg yolk-citric acid-fructose glycerol extender.

Memon et al., (1985) reported that there was no significant effect of extender on post-thaw motility of buck semen and the percentage of normal acrosomes was significantly higher in the Tris extender than in lactose-egg yolk-glycerol. Perez (1985) reported better post-thaw motility for buck semen in Tris-yolk-diluent than in Krebs-Ringer solution or in Krebs-Ringer-phosphate-glucose solution.

Deka and Rao (1986a) reported 68.35, 67.35 and 58.40 per cent post-thaw motility for buck semen frozen with Tris-egg yolk-citric acid-fructose-glycerol diluents containing 20, 10 and seven per cent egg yolk respectively. Semen from two Beetal goats were frozen in Tris-egg yolk-citric acid-fructose-glycerol

and the percentage post-thaw motility was64.00 ± 1.05 (Choudhury et al., 1987).

Chauhan and Anand (1990) reported egg yolk-Tris extender with seven per cent glycerol as the best extender for buck semen freezing.

Deshpande and Mehta (1991) reported Tris-egg yolk-citric acid-fructose with four per cent glycerol as the best dilutor for freezing of Surti buck semen. Sinha et al. (1991) reported that Tris extender was superior to skim milk and egg yolk citrate extenders for freezing of buck semen.

2.1.4 Other extenders

Liess and Ostrowski (1960) reported 40 to 60 per cent forward moving spermatozoa in goat semen frozen with a diluent containing spermasol, egg yolk and glycerol. Paggi (1971) tried five modifications of raffinose-egg yolk-glycerol diluent with different concentrations of raffinose for buck semen freezing; and reported 65 per cent post-thaw motility in a diluent having 89 parts 13 per cent raffinose, seven parts egg yolk and four parts glycerol with a pH between 6.7 and 7.4.

Survival of washed buck speratozoa was better when the resuspending-freezing medium contained 1.5 to 12 per cent (v/v) egg yolk than no egg yolk. Egg yolk concentration higher than

1.5 per cent (v/v) depressed the post-thawsurvival of non-washed spermatozoa (Ritar and Salamon, 1982).

Zheltobryukh and Ashurbegov (1984) reported 40 to 50 per cent post-thaw motility for buck semen processed in lactose-egg yolk.

Deka and Rao (1985b) reported that dimethyl sulphoxide (DMSO) was inferior to glycerol as a cryoprotectant for freezing of buck semen. The post-thaw buck sperm viability was 44 per cent in semen diluted with coconut milk diluent containing egg yolk, and 13 per cent in diluent containing no egg yolk (Arauja and Nunes, 1991).

2.2 Semen evaluation

2.2.1 Volume

Normal ejaculate volume was reported to be one ml in White German Improved bucks (Knoblauch, 1962), 0.4 to 1.2 ml (Kurian and Raja, 1965; Patil, 1970) and 0.55 $\pm$ 0.01 ml (Sarmah, 1983) in Malabari bucks and 0.72 $\pm$ 0.01 ml in Alpine x Malabari crossbred bucks (Sarmah, 1983).

Ejaculate volume of Pashmina goats averaged 0.62 $\pm$ 0.02 ml and there were significant difference in ejaculate volume between males and between collections (Mohan *et al.*, 1980).

Normal ejaculate volume was reported to be 0.77 ± 0.26 ml (Mann, 1980) and 0.73 ± 0.28 ml (Wuschko and Seifert, 1991) in African dwarf goats, 0.98 ml (Cetinkaya et al., 1980), 0.94 ± 0.11 ml (Dundar et al., 1983), 1.07 ± 0.10 ml (Sevinc et al., 1985) and 0.95 ± 0.03 ml (Bakshi et al., 1987) in Angora goats, 0.37 ± 0.03 ml (Saxena and Tripathi, 1980), 0.86 ± 0.09 ml (Singh et al., 1982) and 0.55 ml (Singh et al., 1985) in Jamnapari bucks, 0.45 ml (Sinha and Singh, 1982) and 0.46 ml (Singh et al., 1985) in Black Bengal bucks, 0.72 ml (Sinha and Singh, 1982) and 0.96 ml (Pandey et al., 1985) in Saanen bucks, 1.01 ± 0.04 ml (Singh et al., 1982) and 0.79 ml (Pandey et al., 1985) in Barbari bucks, 1.5 ml in Nubian goats (Ali and Mustafa, 1986), 0.80 ± 0.06 ml in Chegu goats (Mahmood et al., 1988), 0.84 ± 0.07 ml in Changthangi goats (Mahmood et al., 1988), and 0.94 ± 0.24 ml in Ganjam bucks (Pattnaik et al., 1991).

2.2.2 Colour

Knoblauch (1962) reported that White German Improved buck's semen was ivory in colour with creamy consistency. Patil (1970) noted milky yellow to thick creamy yellow colour for Malabari buck semen. Igboeli (1974) reported that semen colour of Zambian buck and Boer buck was ranging from creamy white to yellow, varied considerably between breeds, within breeds and between ejaculates of the same buck.

African dwarf goat's semen was ivory coloured and creamy in consistency (Mann, 1980), and Nubian goats semen colour was white to creamy white (Ali and Mustafa, 1986).

2.2.3 Density

White German Improved buck's semen density was 5/5 (Knoblauch, 1962). Normal density was reported to be 3.52 ± 0.03 (Sarmah, 1983) in Malabari bucks, 3.52 ± 0.03 (Sarmah, 1983) and 3.3611 ± 0.3438 (Joseph, 1983) in Alpine x Malabari crossbred bucks. There was significant difference in density between bucks and between ejaculates (Joseph, 1983).

2.2.4 Mass activity

Mass activity rate in '+' was reported to be in the range of two to four (Patil, 1970) and 3.52 ± 0.05 (Sarmah, 1983) in Malabari bucks, 3.80 ± 0.03 in Alpine x Malabari crossbred bucks (Sarmah, 1983), 4.19 ± 0.04 in Pashmina bucks (Mohan et al., 1980), 4.44 (Sinha and Singh, 1982) and 4.75 (singh et al., 1985) in Black Bengal bucks, 4.51 in Saanen bucks (Sinha and Singh, 1982), 3.15 ± 0.06 in Cheghu bucks (Mahmood et al., 1988) and 2.72 ± 0.11 in Changthangi bucks (Mahmood et al., 1988).

2.2.5 pH

Normal semen pH was reported to be 6.6 in White German Improved bucks (Kroblauch, 1962), 6.47 ± 0.16 (Patil, 1970) and 6.74 ± 0.02 (Sarmah, 1983) in Malabari bucks, 6.74 ± 0.01 in Alpine x Malabari crossbred bucks (Sarmah, 1983), 6.93 in African dwarf bucks (Mann, 1980), and 6.84 ± 0.02 in Pashmina bucks (Mohan et al., 1980).

There was significant difference in semen pH between bucks (Mohan et al., 1980). Normal semen pH was reported to be 6.79 in Black Bengal bucks (Sinha and Singh, 1982), 6.72 in Saanen bucks (Sinha and Singh, 1982), 6.685 ± 0.05 (Dundar et al., 1983) and 6.68 ± 0.17 (Sevinc et al., 1985) in Angora bucks. Sinha et al., (1983) reported that semen pH was significantly higher for Black Bengal than for Saanen bucks. Normal semen pH was reported to be 6.43 ± 0.06 in Cheghu bucks (Mahmood et al., 1988) and 6.52 ± 0.07 in Changthangi bucks (Mahmood et al., 1988).

2.2.6 Motility

Normal percentage sperm motility was reported to be 80 in White German Improved bucks (Knoblauch, 1962), 60 to 90 (Kurian and Raja, 1965), 40 to 85 with a mean of 66.14 ± 1.34 (Patil, 1970) and 79.18 ± 0.55 (Sarmah, 1983) in Malabari bucks,

82.10 ± 0.32 in Alpine x Malabari crossbred bucks (Sarmah, 1983)60.62 ± 0.04 in Pashmina bucks (Mohan et al., 1980), 86 (Cetinkaya et al., 1980), 83.49 ± 3.63 (Sevinc et al., 1985) and 86.69 ± 1.40 (Bakshi et al., 1987) in Angora bucks, 77.28 ± 7.75 (Mann, 1980) and 71 ± 10 (Wuschko and Seifert, 1991) in African dwarf bucks, 72.62 ± 1.06 (Saxena and Tripathi, 1980) and 74.0 ± 0.40 (Singh et al., 1982) in Jamnapari bucks and 78.30 ± 2.48 in Barbari bucks (Singh et al., 1982).

2.2.7 Sperm concentration

Normal sperm concentration per ml of semen was reported to be two to three billions (Kurian and Raja, 1965), 1.1 to 7.49 billions (Patil, 1970) and 3.49 ± 0.09 billions (Sarmah, 1983) in Malabari bucks, 3.28 ± 0.07 billions in Alpine x Malabari crossbred bucks (Sarmah, 1983), 3.674 (x 10^9) (Cetinkaya et al., 1980), 3.31 ± 0.24 (x 10^9) (Dundar et al., 1983), 3.11 ± 0.45 (x 10^9) (Sevinc et al., 1985) and 2.597 ± 86.34 (x 10^9) (Bakshi et al., 1987) in Angora bucks, 3.22 ± 1.22 (x 10^9) (Mann, 1980) and 3.22 ± 0.28 (x 10^9) (Wuschko and Seifert, 1991) in African dwarf bucks.

Sperm concentration of Pashmina bucks averaged 3.521 ± 1.18 (x 10^9)/ml and there were significant differences between males and between collections (Mohan et al., 1980).

Normal sperm concentration per ml of semen was reported to be 4.795 ± 292.97 (x 10^9) (Saxena and Tripathi, 1980), 2.293 ± 728 (x 10^9) (Singh et al., 1982) and 2.91033 (x 10^9) (Singh et al., 1985) in Jamnapari bucks, 2.44015 (x 10^9) (Sinha and Singh, 1982), and 2.61958 (x 10^9) (Singh et al., 1985) in Black Bengal bucks, 2780.30 millions in Saanen bucks (Sinha and Singh, 1982), 1920 ± 720 millions in Barbari bucks (Singh et al., 1982), 1.77 (x 10^9) in Nubian bucks (Ali and Mustafa, 1986), 3.25 ± 0.13 (x 10^9) in Cheghu bucks (Mahmood et al., 1988), 2.94 ± 0.16 (x 10^9) in Changthangi bucks (Mahmood et al., 1988) and 2.309 ± 0.0958 (x 10^9) in Ganjam bucks (Pattnaik et al., 1991).

2.2.8 Live sperm percentage

Normal live sperm percentage was reported to be 80 in White German Improved bucks (Knoblauch, 1962), 85 to 95 (Kurian and Raja, 1965), 20 to 86.6 (Patil, 1970) and 89.97 ± 0.58 (Sarmah, 1983) in Malabari bucks, 91.31 ± 0.35 in Alpine Malabari crossbred bucks (Sarmah, 1983) and 84.93 in African dwarf bucks (Mann, 1980).

Live sperm percentage of Pashmina bucks averaged 80.63 ± 0.29, and there was significant differences between bucks (Mohan et al., 1980). Normal live sperm percentage was reported to be 77.65 ± 1.04 (Saxena and Tripathi, 1980), 80.90 ± 2.32 (Singh et al., 1982) and 90.33 (Singh et al., 1985) in Jamnapari

bucks, 85.45 (Sinha and Singh, 1982) and 91.07 (Singh et al., 1985) in Black Bengal bucks, 85.21 (Singha and Singh, 1982) and 82.23 (Pandey et al., 1985) in Saanen bucks, 83.80 ± 0.26 (Singh et al., 1982) and 71.44 (Pandey et al., 1985) in Barbari bucks, 83.08 in Beetal bucks (Bordoloi and Sharma, 1983) and 77.45 in Assam bucks (Bordoloi and Sharma, 1983).

Misra and Mukherjee (1984) reported that percentage of live spermatozoa was less in older and heavier bucks than in younger and lighter bucks. Normal live sperm percentage was reported to be 76.3 ± 1.08 in Chegu bucks (Mahmood et al., 1988), 71.7 ± 2.09 in Changthangi bucks (Mahmood et al., 1988) and 83.83 ± 1.02 in Ganjam bucks (Pattnaik et al., 1991).

2.3 Incubation test

The forward velocity of spermatozoa was highest at 41 to 43°C, and the rate of glycolytic activity was maximum at 46.5°C (Beck and Salisbury, 1943). There are upper extremes of temperature which causes irreversible changes in the protoplasmic complex of living cells, resulting in their death (Salisbury and VanDemark, 1961).

The higher the temperature in the range of -196 to 50°C the faster the metabolism, and above 7°C the faster the motility of the cells and the shorter their life. Metabolic activity and

motility of spermatozoa increased outside the body as the temperature was raised above body temperature, and the total life expectancy of the cell was reduced. The motility and metabolic activity were higher, a few degrees above body temperature (Salisbury and VanDemark, 1961).

Joseph (1983) collected one ejaculate per day from Malabari crossbred bucks for incubation test and reported that the percentage of motile sperms at 0 hr, 10 minutes, 20 minutes and 30 minutes of incubation at 46.5°C were 84.3442 ± 4.3278, 60.885 ± 17.2038, 53.7049 ± 23.1771 and 36.8196 ± 22.9183 respectively.

2.4 Cold shock test

Ejaculated spermatozoa are highly sensitive to rapid decline in temperature, and this effect is generally known as cold shock. The rate of cooling is an important factor, influencing the intensity of cold shock, but spermatozoa from all species do not respond in the same manner to cold shock. In addition to species linked differences, there are marked variation in storage and freezability of sperms within species.

Salamon and Lightfoot (1967) opined that both undiluted and diluted ram semen showed greater susceptibility to cold shock with successive ejaculates. Cold shock test eliminate, all weak and infirm spermatozoa and hence this test is a better

criterion for evaluating the worth of a breeding bull than assessment based on either initial motility or live sperm concentration. This can be used as a basis for screening and grading of bulls for AI under field conditions (Tewari et al., 1969).

Susceptibility to cold shock may be related to the lipid composition of spermatozoa (Mann and Mann, 1981). The resistance to cold shock test provided a good means to study the thermal resistance of spermatozoa and could be a useful method in premonitoring freezability (Mohanty et al., 1985). The cold shock resisting ability of the spermatozoa was found to be positively correlated with the post-thaw recovery rate (Sharma et al., 1990).

Impairment of membrane integrity, demonstrated by the leakage of potassium, adenosine triphosphate, lipoproteins, enzymes, and other important intracellular constituents from gametes occur as a result of cold shock (Garner, 1991). For freezing buck semen egg yolk was necessary to avoid cold shock as the temperature was lowered during chilling (Dunner, 1993).

2.5 Methylene blue reduction test

Methylene blue receives the hydrogen ions from a hydrogen donator, a substrate, which has given up the ions by action of an intracellular enzyme, a dehydrogenase. The

temperature of 46.5°C was found suitable because the reduction of methylene blue proceeded at the fastest rate and the bacteria introduced at collection and in handling did not affect the tests at this temperature as many of the bacteria being killed during the incubation (Gunsalus et al., 1944).

Methylene Blue Reduction Test (MBRT) is used as an indicator of metabolic activity of spermatozoa due to the property of methylene blue to change the colour by the hydrogen ions liberated from anaerobic glycolysis of spermatozoa (Kumaran and Kumaran, 1968, Salisbury et al., 1978).

The mean time taken by Malabari buck semen for methylene blue reduction was 4.52 minutes (Patil, 1970). Mikus and Pilko (1975) reported that methylene blue reduction time averaged 43 seconds for ram semen collected once daily and 225 seconds for ram semen collected 11 times daily.

Methylene blue reduction time showed an increasing trend on increasing the ejaculation frequency; the mean MBR time in seconds were 133.0886 ± 61.4200, 299.7969 ± 116.0773 and 312.5348 ± 104.1285 for buck semen collected once daily, twice daily and thrice daily respectively (Joseph, 1983)

The crossbred bucks had higher values for MBRT than the indigenous bucks (Mittal, 1986). MBRT showed significant difference between the indigenous and crossbred bucks; the

values of MBRT were significantly higher in summer in crossbred bucks (Mittal, 1987). Kutty et al. (1995) reported that time taken for reduction of methylene blue by buck semen was 189.86 seconds.

2.6 Freezing of semen

2.6.1 Washing of spermatozoa

A thermolabile egg yolk coagulating factor was found in goat semen but not in bull, ram, boar and rabbit semen; the factor was present in the seminal plasma and was derived from the secretion of cowper's glands; spermatozoa washed once did not coagulate egg yolk and remained motile for 10-15 days in egg yolk-citrate at 4°C; twice washed spermatozoa were motile for one month (Iritani et al., 1961). The optimum conditions of temperature, yolk concentration, and pH for coagulation were investigated; the factor had properties similar to those of enzymes (Iritani and Nishikawa, 1961). Iritani and Nishikawa (1963) studied the release of some acids accompanied by the coagulating phenomena, and on the position of yolk constituents attacked by the coagulating enzymes; they tried the purification of the egg yolk-coagulating enzyme.

The percentage of motile spermatozoa was higher in the semen samples without plasma, both before and after freezing in skim milk diluent, with or without glucose (Corteel, 1974).

Loss in post-thaw motility of frozen goat semen after storage for three to 90 days loss was 16.6 per cent for unwashed spermatozoa and zero per cent for washed spermatozoa, and after storage for 91 to 180 days, the corresponding figures were 22.0 and 1.3 per cent (Corteel and Baril, 1975). Washing of buck semen before freezing in Tris-based diluent with egg yolk and glycerol, significantly increased post-thaw sperm motility (Westhuysen, J.M. van der, 1978).

Removal of seminal plasma by centrifugation of goat semen was beneficial for the survival of spermatozoa after thawing, but the effect depended on the intensity of washing; when the semen was diluted six to 11 fold, double washing was more effective than single washing (Ritar and Salamon, 1982).

Deka and Rao (1984a) reported better percentage of motile spermatozoa in goat semen without seminal plasma than in semen with seminal plasma.

Perez (1985) reported that ejaculates from goats washed twice had higher sperm motility and a higher percentage of acrosome defects than non-washed semen after freezing Memon et al., (1985) reported that significantly higher percentage of post-thaw motility and normal acrosomes in goat semen washed twice, 15 minutes each with Ringer's solution than in unwashed semen.

The percentage of live and of progressively motile spermatozoa upto 12 months of storage at -196°C did not differ between buck semen frozen with and without seminal plasma (Deka and Rao, 1987a). There was no significant difference in the conception rate in goats inseminated with frozen semen that had been washed in Tris or not washed before freezing (Deka and Rao, 1989). Haunhorst (1990) reported that buck sperm motility was not significantly affected by centrifugation. However Tuli and Holtz (1994) reported unfavourable effect of seminal plasma removal on post-thaw motility of buck semen.

2.6.2 Glycerolisation

Paggi (1971) reported that the diluted semen samples showing 65 per cent motility after thawing had 89 parts 13 per cent raffinose, seven parts egg yolk and four parts glycerol. Sahni and Roy (1972) has done freezing of buck semen using egg-yolk citrate and heated cow milk containing three, six and nine per cent glycerol as diluents; the results of post-thaw recovery with six per cent glycerol level were better than at the other two levels.

Salamon and Ritar (1982) reported that there was interaction between method of semen dilution, holding time at 5°C and glycerol concentration in freezing of buck semen. In buck semen frozen with Tris-egg yolk citric acid-fructose-glycerol extender, the post-thaw motility was higher for 6.4 per

cent glycerol than for four or nine per cent glycerol (Deka and Rao, 1986b). The best freezing regime for Korean native goat semen was 12°C per minute, using nine per cent glycerol (Park et al., 1989).

For buck semen, optimal results for motility, percentage of live spermatoza and acrosomal integrity before and after freezing were obtained with a 20 per cent dilution rate and seven per cent glycerol (Chauhan and Anand, 1990).

It was reported that egg yolk-citrate fructose and Tris-egg yolk-citric acid-fructose at four per cent glycerol level, were the best dilutors for freezing of Surti buck semen (Deshpande and Mehta, 1991). Skim milk extender with combination of six per cent glycerol and four hours equilibration resulted in better quality frozen buck semen (Sinha et al., 1992a).

It was reported that six per cent glycerol in Tris extender resulted in better quality frozen buck semen (Sinha et al., 1992b).

Based on progressive motility analysis of frozen buck semen, there was significant differences between level of glycerol, five per cent being significantly superior to three or four per cent level (Purohit et al., 1992).

Six per cent glycerol added to the initially extended semen with egg yolk citrate extender at 5°C yielded the highest

sperm motility and live sperm count in frozen buck semen (Sinha et al., 1993). Stepwise glycerolisation at 37°C gave higher progressive motility and percentage of live spermatozoa for Boer buck semen both before freezing and after thawing than one-step glycerolisation at 37°C or stepwise extension with glycerol after cooling to 5°C (Tuli and Holtz, 1994).

2.6.3 Equilibration

Post-thaw motility of buck semen frozen with Tris-egg yolk-citric acid-fructose-glycerol extender was higher for six hour equilibrated semen than for one or three hour equilibrated semen (Deka and Rao, 1986b). The findings of Tiwari and Bhattacharyya (1987) and Sinha et al., (1992a) revealed that four hours of equilibration period resulted in better quality frozen buck semen, in Tris extender. Purohit et al., (1992) reported that five hour equilibration period has shown significantly better post-thaw semen quality for frozen buck semen.

Skim milk extender with combination of six per cent glycerol and four hours equilibration resulted in better quality of frozen buck semen (Sinha et al., 1992b).

2.6.4 Freezing methods and effect of freezing

Sahni and Roy (1972) reported that, the mean percentage of abnormalities of head, mid-piece, coiled tail and free normal head in different stages of processing and freezing of buck semen did not differ significantly from that of neat semen in any extender (Deka and Rao, 1984b).

Deka and Rao (1986b) reported freezing of buck semen in 0.5 ml straws by suspending the straws 5 cm above liquid nitrogen for 10 minutes. Optimal freezing was attained by holding straws in vapour 4 cm above liquid nitrogen for $\geq$30 seconds, followed by plunging into liquid nitrogen. Initiation of ice crystallisation and the pattern of cooling of semen depended on size of straw and the type of rack used to hold the straws in the vapour during freezing, cooling curves differed greatly for semen frozen in straws in liquid nitrogen vapour and pellets on dry ice (Ritar et al., 1990a).

Addition of caffeine during deep preservation of goat semen in Tris medium was found to have a stimulatory effect on sperm motility (Sinha et al., 1994).

2.6.5 Thawing

In frozen bull semen thawed at 75°C for 12 seconds, the percentage of unstained spermatozoa after staining with

eosin-nigrosin was considerably higher than that after thawing at 35°C for 30 seconds (Aamdal and Andersen, 1968).

Significantly higher percentages of progressively motile and live sperms were recorded after rapid thawing (37°C for 12 to 15 seconds) than slow thawing (5°C for two minutes) of frozen goat semen; this may be due to short period of exposure of sperm to increased solute concentrations during rapid thawing (Deka and Rao, 1986c; Deka and Rao, 1987b). Lawrenz (1986) reported thawing of frozen goat semen at 70°C for 10 seconds immediately before insemination. Post-thaw motility of frozen buck semen was found to be 56.15, 54.58 and 47.85 per cent in Jamnapari, Barbari and Black Bengal respectively (Sinha et al., 1987).

Straws with deep frozen buck semen were thawed in a water bath at 40°C for 30 seconds (Torre et al., 1990) at 40°C for 10 seconds (Haunhorst, 1990) and at 37°C for 12 to 15 seconds (Sinha et al., 1993). The best temperature for thawing of frozen buck semen was found to be 37°C for 10 seconds in water bath (Das and Rajkonwar, 1994).

2.7 Insemination

Fougner (1974) reported that the conception rates of in portio, intra cervical and intra-uterine insemination averaged 22, 66 and 81 per cent respectively. Of 35 native goats

inseminated intra-cervically 68.6 per cent conceived to the first insemination (Stagnaro, 1975).

Kolk (1985) reported that post-thaw motility of buck semen frozen with modified Tris diluent was 55 to 65 per cent; of 40 females inseminated, six required a repeat insemination, to which four conceived. Lawrenz (1986) inseminated goats using frozen semen by a non-surgical intra-uterine technique.

Crossbred Saanen goats were inseminated intracervically using frozen semen (Torre et al., 1990). Using a vaginal speculum and forceps, an insemination cannula containing a semen pellet was introduced into the uterine horn of synchronized goats; Laproscopy confirmed the accurate placing of the semen in all goats (Mareco, 1993).

Garcia et al. (1994)reported that intra-uterine insemination in goat was facilitated with intravenous injection of 100 USP of oxytocin at insemination time. Eppleston et al. (1994) reported that there was a significant linear increase in fertility of frozen-thawed ram semen as the depth of insemination increased.

2.8 Fertility of frozen buck semen

Kidding percentage using frozen semen from German goats was only 13.33 per cent (Liess and Ostrowski, 1960). Bonfert

(1969) and Paggi (1971) reported a non-return rate of 65.9 per cent and 45 per cent respectively for inseminations using frozen buck semen.

Fougner (1974) reported 73.6 per cent conception rate for goats inseminated with frozen semen. The kidding percentage in does inseminated with 40 x 10^6, 80 x 10^6 and 120 x 10^6 motile frozen-thawed spermatozoa were 42.5, 54.0 and 62.5 respectively (Corteel et al., 1974).

Stagnaro (1975) reported that out of 35 goats inseminated with frozen-thawed semen 68.6 per cent of the females conceived to the first insemination, and the number of kids per kidding averaged 1.4.

The conception rate in goats due to insemination using chilled semen was 40.86 per cent based on kiddings or abortions; the Artificial Insemination Index which is the number of insemination required per kidding or abortion was 2.45 (Mathai et al., 1980). Conception rate for inseminations done with chilled semen of bucks stored upto 24 hours was 42.78 per cent and that in the case of inseminations with semen stored beyond 24 hours was only 29.26 per cent (Mathai and Nair, 1981).

Of 30 goats inseminated with frozen semen stored for 11 years, 66.66 per cent conceived on the first or second insemination; 70.37 per cent of goats inseminated with the same

batch of semen, shortly after freezing conceived; the effect of storage time on fertility was significant (Samouilidis et al., 1982). Valdes Leal (1984) reported that conception rates in inseminated using frozen semen and naturally mated nondescript female goats in Chile were 18.30 per cent and 89 to 91 per cent respectively.

The conception rates were 68.75, 50.00 and 58.33 per cent respectively for oestrous female goats inseminated with mixed chilled semen, Black Bengal semen and Saanen semen (Sinha and singh, 1984). The lambing percentage with frozen and fresh semen were 40 and 50.6 respectively in cross-bred ewes (Zanwar et al., 1984).

Of the 40 females inseminated using frozen semen of German Improved White goats, six required a repeat insemination, to which four conceived, the non return rate over two kidding season was 85 per cent (Kolk, 1985). When 72 and 47 female goats were inseminated with semen stored at 5°C for 48 hours in milk and egg yolk citrate, the kidding rates were 64.15 and 61.11 per cent respectively; the difference being non-significant (Bargohain et al., 1985). the non-return rate after insemination with frozen Saanen semen was 60.4 per cent (Zhang et al., 1985).

Insemination of oestrus induced goats with frozen semen which was centrifuged or not centrifuged before freezing,

resulted in kidding rates of 54.2 and 66.7 per cent respectively (Perez, 1985).

In the five non-surgical, intrauterine insemination trials using frozen buck semen, the 65-day non-return rate were 67.4, 77.4, 66.0, 64.7 and 65.0 per cent respectively; the kidding rate were 60.8 70.9, 62.3, 61.8 and 60.0 per cent respectively (Lawrenz, 1986). Pregnancy rates of 117 synchronized and 283 non-synchronized, inseminated does were 43.7 per cent and 71.6 per cent respectively (Summermatter, 1986).

The non-return rate was 78.95 per cent in goats inseminated with frozen semen in Tris diluent (Choudhury et al., 1987). Fertility rates were 45.16, 44.00 and 42.10 per cent for frozen-thawed semen of Jamnapari, Barbari and Black Bengal bucks, respectively (Sinha et al., 1987).

The conception rate in 130 goats inseminated with frozen semen that had been washed in Tris or not washed after collection were 66.10 and 69.35 per cent respectively (Deka and Rao, 1989). Moore et al., (1989) reported that laproscopic insemination of goats with frozen semen resulted in a higher kidding rate than cervical insemination. Of 12 females inseminated with frozen semen of Korean native goat, three produced live young (Park et al., 1989).

Of 26 female goats inseminated with semen diluted and frozen in egg yolk-Tris extender, 21 kidded (Chauhan and Anand, 1990). Pregnancy rate in goats inseminated laproscopically using frozen semen were more than that in goats inseminated cervically, the method of insemination and number of motile spermatozoa did not affect fecundity; with cervical insemination, fertility and fecundity improved with increasing depth of insemination into the reproductive tract (Ritar et al., 1990b).

Frozen semen was used for insemination in 67 crossbred Saanen goats; 42 does (63.7 per cent) conceived to the first insemination, six (8.8 per cent) to the second, and three (4.5 per cent) to the third; of the 51 does conceived, 50 kidded with an average litter size of 1.4 (Torre et al., 1990).

The use of frozen buck semen of crossbred containing 50 x 10^6 spermatozoa resulted in a 68 per cent conception rate compared with the mesopotamian semen, which resulted in a 41 per cent conception rate (Mylrea et al., 1991). Sixty goats were inseminated with fresh or frozen semen from five males; in two trials, conception rates were 73.3 and 80.0 per cent, and kidding rates were 60.0 and 76.0 per cent (Sungur et al., 1993).

Materials and Methods

MATERIALS AND METHODS

3.1 Semen collection

Normal semen ejaculates from five Malabari crossbred (Alpine x Malabari) bucks aged one to three years, maintained at Artificial Insemination Centre, under the Department of Animal Reproduction,College of Veterinary & Animal Sciences, Mannuthy, Thrissur, were utilized for this study. The bucks selected were normal healthy and maintained at standard feeding and management practices. Two ejaculates each were collected twice weekly from the bucks during the period from January, 1994 to April 1995 by artificial vagina method (Perry, 1969). Semen samples were kept in a water bath maintained at 37°C before dilution.

3.2 Semen extenders

Tris (hydroxy methyl) amino methane (1.21 g), citric acid (0.67 g) and fructose (0.5 g) were weighed accurately into six sterile 50 ml volumetric flasks numbered I to VI. Then 25 ml double glass distilled water was added in all the flasks and stirred well. Fresh egg yolk was collected aseptically and added 5 ml each to flasks numbered II to VI and mixed well using sterile glass rod. Four ml, five ml, six ml and seven ml each of

Fig.3.1 Collection of buck semen

Table 3.1 Composition of semen extenders

Ingredients	Tris buffer lated	Non-glycero-Tris extender	Glycerolated Tris extenders			
	I	II	III	IV	V	VI
[1] Tris (hydroxy methyl) amino methane (g)	1.21	1.21	1.21	1.21	1.21	1.21
[2] Citric acid (g)	0.67	0.67	0.67	0.67	0.67	0.67
[3] Fructose (g)	0.50	0.50	0.50	0.50	0.50	0.50
Egg yolk (ml)	-	5	5	5	5	5
[4] Glycerol (ml)	-	-	4	5	6	7
[5] Benzyl Penicillin(IU)	25000	25000	25000	25000	25000	25000
[6] Streptomycin (µg)	25000	25000	25000	25000	25000	25000
Aqua dest ad. (ml)	50	50	50	50	50	50

1. Loba-Chemie Indo austranal Co. Bombay
2. Qualigens Fine Chemicals, Bombay
3. D (-) Fructose Extra Pure; E. Merck (India) Ltd., Bombay
4. Qualigens Fine Chemicals, Bombay
5. Alembic Chemicals works Co. Ltd., Vadudara
6. Ambistryn-S (Inj.); Sarabhai Chemicals, Baroda

glycerol were added into the flasks numbered III, IV, V and VI respectively. Benzyl penicillin and streptomycin sulphate were added at the rate of 25000 IU and 25000 µg respectively in all flasks and stirred well. Then the volume of extenders were made upto 50 ml by adding double glass distilled water in all the six flasks. Extenders were placed in a water bath maintained at 37°C before dilution with semen.

3.3 Semen evaluation

For the study of normal characteristics of buck semen, all the ejaculates collected were subjected to preliminary evaluation observing colour, volume, density, mass activity and pH. Initial motility, sperm concentration and live sperm percentage were also estimated following the standard procedures (Roberts, 1971).

3.4 Incubation test

Part of ejaculates collected from April, 1994 to June, 1994 were used for incubation test. Samples with more than three plus mass activity were used for this test. From the ejaculates 0.25 ml each of semen were taken in two test tubes. First sample kept as such in the water bath at 37°C. In the other sample by adding Tris buffer (Flask No.I) made up the volume to 2.5 ml and centrifuged at 3000 rpm for five minutes. Discarded the supernatent fluid and again made up the volume to 2.5 ml by

adding Tris buffer. Again centrifuged for five minutes. Discarded the supernatant. Then by adding non-glycerolated Tris extender the volume made upto 2.5 ml. The semen in first test tube was diluted to 2.5 ml by adding non-glycerolated Tris extender. Recorded the motility of both the samples under high power of microscope. Both samples were immediately placed in a water bath at 46.5°C and recorded the motility percentage at 10 minutes interval upto 60 minutes period.

Repeated the test using six semen samples from each bucks.

3.5 Cold shock test

Ejaculates collected from the selected five bucks during July, 1994 to September, 1994 were used for cold shock test. Based on preliminary evaluation samples with more than three plus mass activity were used. From each ejaculate 0.25 ml each were taken in two test tubes. The first sample kept as such in the water bath at 37°C. The second sample was centrifuged for 10 minutes as in the incubation test. Final volume was retained as 0.25 ml with Tris buffer only. Sperms from each sample were stained using eosin-nigrosin stain (Campbell et al., 1956).

Immediately after preparing initial smears, both samples were placed in a water bath at 0°C. Smears were prepared using semen from both samples at five minutes interval till

30 minutes. Dried smears were examined under oil immersion objective of microscope and 300 sperms in each smear were counted. Calculated the percentage of live sperms in smears from both samples at different periods of time.

Repeated the test using six semen samples from each buck.

3.6 Methylene blue reduction test

Ejaculates collected twice weekly from selected bucks from October 1994 to December 1994 were used for this test. Semen samples with more than three plus mass activity, based on preliminary examination were used. From each ejaculate 0.2 ml sample was taken in two test tubes. First sample was kept in the water bath at 37°C. The second sample was centrifuged and washed as in the cold shock test and the final volume was retained to 0.2 ml.

0.8 ml egg yolk citrate extender (egg yolk 20 ml, 2.96 per cent sodium citrate 80 ml, Benzyl Penicillin 50000 IU and streptomycin 50000 µg) was added and mixed in both samples. Then 0.1 ml of 0.05 per cent methylene blue solution was added and mixed. Layered the contents in both test tubes for atleast one centimetre height with sterile liquid paraffin. Both test tubes were immediately placed in a water bath at 46.5°C and recorded

the time taken for complete disappearance of blue colour of methylene blue in test tubes.

Repeated this test using six semen samples from each buck.

3.7 Freezing of semen

3.7.1 Selection of samples

Buck semen collected from January 1995 to April, 1995 were used for freezing. Based on the preliminary evaluation samples with more than 70 per cent initial motility were used for freezing trials.

3.7.2 Washing of spermatozoa and initial extension

Semen sample was extended ten times with Tris buffer and centrifuged at 3000 rpm for five minutes. The supernatant fluid was pipetted out. Made up the extended volume by adding Tris buffer and repeated the centrifugation for five more minutes. The clear supernatant fluid was removed. The sediment after double washing was extended five times the original volume (1:5) with non-glycerolated Tris extender. The motility of the sample was assessed under high power objective of microscope. Semen with non-glycerolated Tris extender was divided into four equal parts in four sterilized test tubes. The mouth of the test tubes were closed with sterile aluminium foils.

Tris extenders in flasks numbered III, IV, V and VI were taken in separate test tubes. Volume of glycerolated extender taken in each test tubes was equal to the volume of non-glycerolated fraction. Test tubes containing glycerolated extender were also closed with aluminium foils.

3.7.3 Cooling of semen

Test tubes containing semen with non-glycerolated extender and test tubes containing glycerolated extender were kept in a 500 ml beaker containing water at 37°C. Beaker with test tubes containing semen and extender was placed in a cold handling chamber maintained at 5°C. straw clips, straw filling combs, sterile cotton, sterile towel, poly venyl alcohol (PVA) powder, air bubblers, bubbler dish, 0.5 ml straws of different colours and a tray containing water with drops of dettol were placed inside the cold handling chamber. Diluted semen and extenders were kept in the cold handling chamber for two hours or till the temperature of the water bath was lowered to 5°C.

3.7.4 Glycerolisation

After keeping two hours at 5°C, the glycerolatedfraction of the extender was mixed and added to the semen with non-glycerolated extender in three parts at 15 minute intervals. After glycerolisation, one drop each of the mixture from all test tubes were taken for assessing the motility.

3.7.5 Filling and sealing of straw

Different coloured straws with 0.5 ml capacity were used for filling semen with each combination of glycerol. Straws were arranged in straw clips. Polyvinyl alcohol (PVA) powder was spread about 5 mm thickness evenly in a glass dish with 9 cm diameter. Straws in clip were attached to comb by the factory seal end. The comb was connected to a vacuum pump by a small rubber tubing.

After gentle mixing semen was transferred into the bubbler dish in the cold handling cabinet and aspirated through the open end of the straws attached to the comb by the help of the vacuum pump. By applying the open ends of the straws into the bubbler comb, air spaces were created. Then the open end was dipped into polyvinyl alcohol powder to prepare laboratory seal. Removed the excess powder sticking to the straw, using a cotton pad. Released the straws from the clips and comb and immersed in water at 5°C in the cold handling cabinet. After 45 minutes, filled straws were taken out and wiped with dry towel and arranged on freezing racks.

3.7.6 Equilibration

Initially, three hour, four hour and five hour of equilibration periods were provided on trial basis for 10 straws

each, three times and subsequently on the basis of better results all straws were given four hour equilibration.

3.7.7 Freezing and storage

Wide mouthed liquid nitrogen refrigerator* was used for freezing. The freezing grill supporting the racks was placed at a depth of 30 cm below the brim of the refrigerator. The liquid nitrogen was poured into the refrigerator and kept the level touching the grill. After attaining the vapour pressure, freezing racks with straws arranged were transferred quickly from the cold handling cabinet to the freezing chamber. The straws in the rack were four centimetre above the liquid nitrogen level. Goblets were also placed over the grill. Closed the lid of refrigerator and allowed the straws to remain there for eight minutes. Then straws were collected by gloved cooled hand and placed into the precooled goblets and plunged into liquid nitrogen. After that the goblets along with straws were transferred into the storage container, **Cryocan BA-42. A thick paper label showing the date of collection, buck number and number of straws was kept on the handle of the canister for identification of doses. After 12 hours the post-thaw motility was recorded for each group of straws.

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* MVE Cryogenics, New Prague, Minn.

** IBP Co. Ltd., Nashik

3.7.8 Thawing of semen

After removing the neck tube plug of liquid nitrogen refrigerator, the tip of the forceps was cooled by holding it in the vapour through the neck for 30 seconds. Then lifted the canister upto the bottom level of the neck. With the pre-cooled forceps, straws were picked up within five seconds. By holding one end of the straw, shaked it twice to remove the drops of liquid nitrogen if any. Then placed the straws in water at 30°C for 30 seconds.

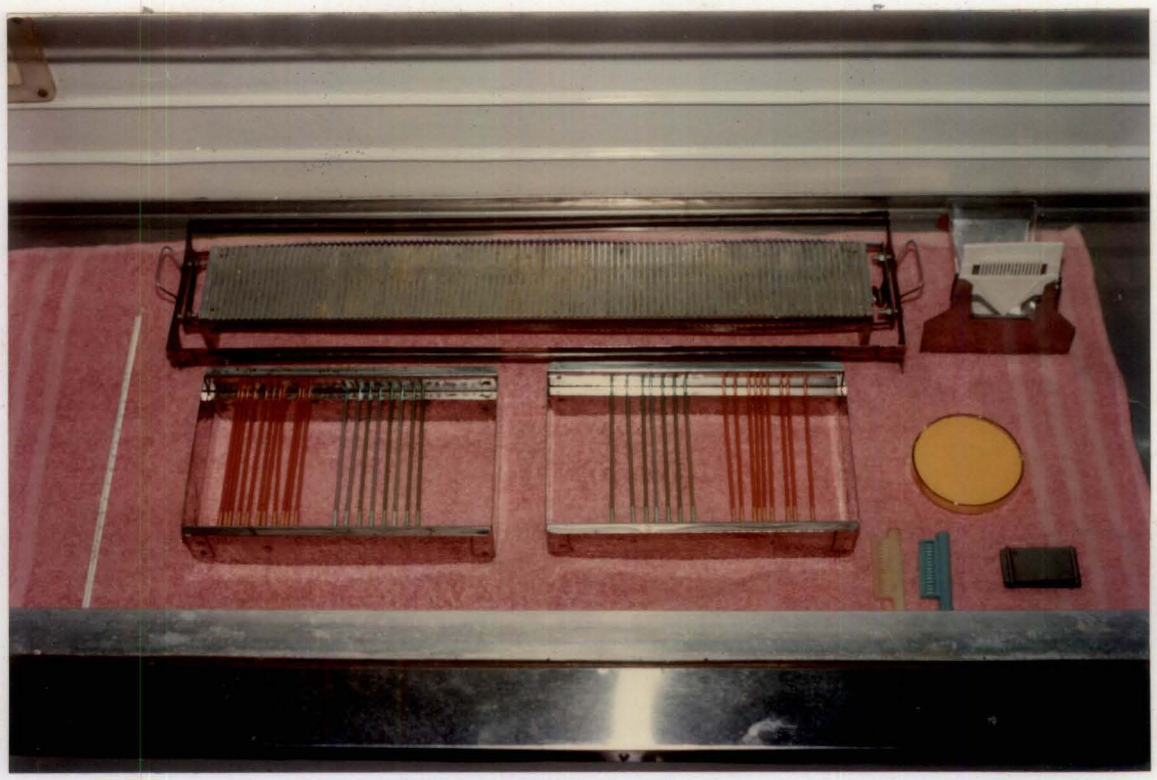
3.8 Insemination

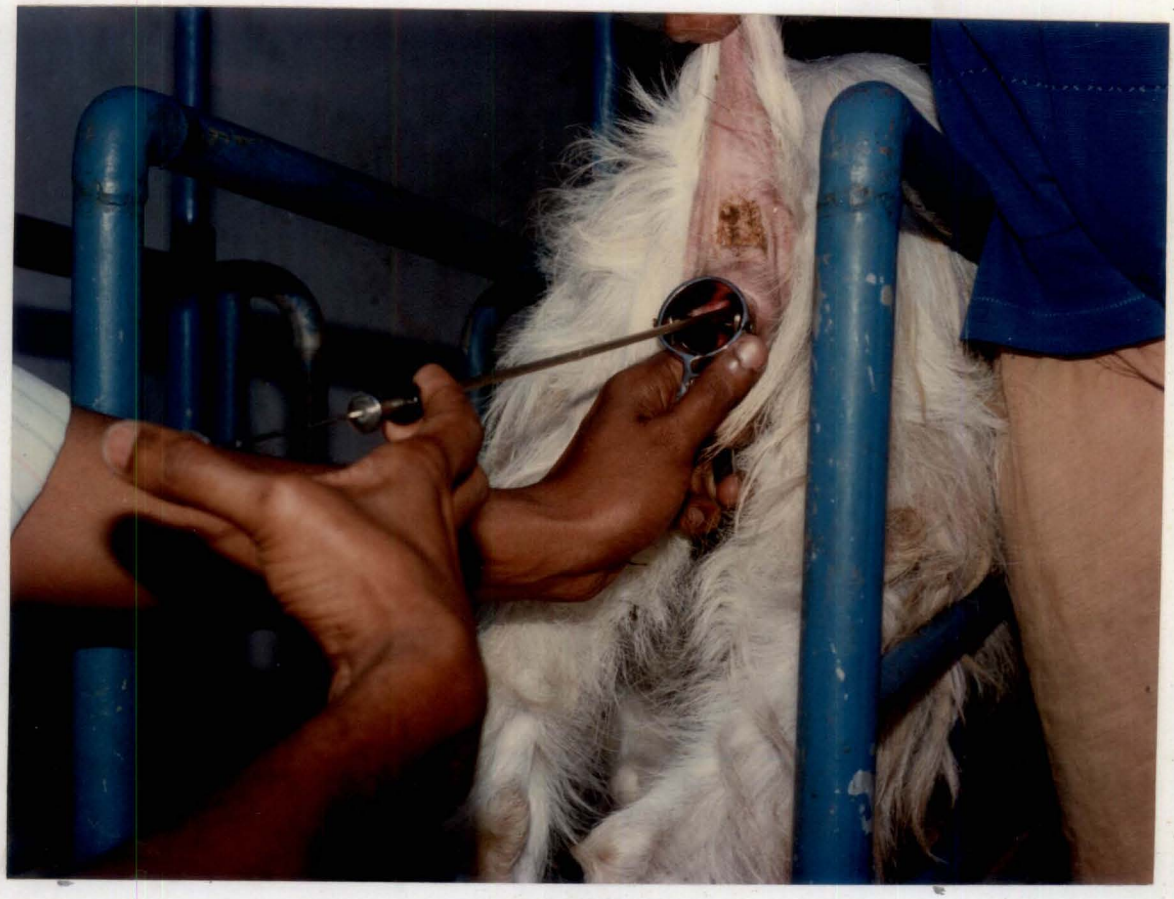
Insemination was carried out using frozen semen with a minimum of 35 per cent post-thaw motility in does brought for artificial insemination at the Artificial Insemination Centre attached to Department of Animal Reproduction. The doe was secured in the AI crate designed by Mathai (1983). Then lifted the hind quarters of the doe to a height of 50 cms using the lever of the crate. A sterile vaginal speculum lubricated with sterile jelly was used to dilate the vaginal cavity and to confirm whether animal is in heat. A torch was used to provide light in the dilated vaginal cavity whenever necessary.

Thawed straw was taken out of water and shaked by holding at the laboratory sealed end.

Fig.3.2 Freezing equipments

Fig.3.3 Artificial insemination in goats





Wiped the straw using sterile cotton. Then introduced into the insemination gun with laboratory sealed end outside.

Then the laboratory seal end was cut at right angle about one centimetre below the laboratory seal. Slipped one sterile sheath over the gun and secured it tightly using the ring of the gun.

The cervical opening of the doe in heat was located with sterilized vaginal speculum. Semen was deposited in cervical opening. After insemination the doe was kept in the lifted position in the crate for 10 minute.

3.9 Fertility study

Does that showed signs of heat after first insemination were again inseminated. Reply paid post card were sent to the owners of all does that were inseminated and not repeated for four months. On the basis of reply received actual kidding rate was calculated.

The results were statistically analysed as per standard procedures (Snedecor and Cochran, 1967).

Results

RESULTS

A study was carried out using semen samples from five Malabari crossbred bucks maintained at A.I. Centre, under the Department of Animal Reproduction, College of Veterinary and Animal Sciences, Mannuthy, Thrissur to find out the effect of different concentration of glycerol on post-thaw motility and fertility of frozen buck semen.

4.1 Semen evaluation

The results of the preliminary semen evaluation are presented in Table 4.1. The overall average volume of buck semen was 0.71 ± 0.02 ml. There was significant ($P<0.05$) difference in the volume of semen from different bucks.

The average colour index of all the ejaculates was found to be creamy, density was DDDD and the mass activity was ++++.

The overall average pH of buck semen was 6.85 ± 0.01. There was no significant difference in the pH of semen from different bucks.

The overall average percentage of sperm motility was 85.83 ± 1.05. There was significant difference ($P<0.05$) in sperm motility of different bucks.

The average sperm concentration of buck semen was 2842.33 ± 153.93 millions/ml. Between bucks there was highly significant difference in sperm concentration ($P<0.01$).

Overall average live sperm percentage of buck semen was 91.03 ± 0.56. Statistical analysis of the data revealed that there was highly significant difference between live sperm percentages of different bucks ($P<0.01$).

4.2 Incubation test

The percentage of motile sperms at zero, 10, 20, 30, 40, 50 and 60 minutes of incubation of spermatozoa with and without seminal plasma are given in Table 4.2. Statistical analysis revealed that there was no significant difference between motile sperm percentage in spermatozoa with and without seminal plasma at different time intervals upto 50 minutes of incubation (Table 4.3). However, at 60 minutes sperm motility percentage in spermatozoa with seminal plasma was significantly higher ($P<0.05$).

Initial percentage of sperm motility in spermatozoa sample with seminal plasma and without seminal plasma were 70.83 ± 1.31and 70.83 ± 1.40 respectively. Corresponding values at 10 minutes of incubation were 77.00 ± 1.52 and 74.50± 1.90 respectively. But from 10 minutes of incubation onwards motility was gradually decreasing (Fig.4.1).

Analysis (Table 4.3) revealed that there was significant difference in sperm motility of different bucks in spermatozoasamples incubated with and without seminal plasma at all time intervals ($P<0.01$).

4.3 Cold shock test

Effect of 0°C for varying periods of time on live sperm percentage of buck spermatozoa with and without seminal plasma are given in Table 4.4.

Analysis revealed that there was no significant difference in live sperm percentage of spermatozoa with and without seminal plasma at zero minutes (Table 4.5). But at all other time intervals live sperm percentage was significantly ($P<0.01$) higher in spermatozoa samples without seminal plasma than in spermatozoasamples with seminal plasma (Fig.4.2).

Initial live sperm percentage in spermatozoa sample with and without seminal plasma were 86.96 ± 1.09 and 87.37 ± 1.14respectively. Corresponding values at five minutes of cold shock were 7.75 ± 1.04 and 15.98 ± 2.24 respectively, showing a rapid reduction.

The live sperm percentages between bucks were highly significant ($P<0.01$) at zero minutes, and 20 minutes of cold

shock and significant ($P<0.05$) at five minutes, 15 minutes and 25 minutes. But not significant at 10 minutes and 30 minutes of cold shock.

4.4 Methylene blue reduction test

The overall average of time taken for reduction of methylene blue by spermatozoa with and without seminal plasma were 173.16 ± 8.77 seconds and 197.00 ± 9.97 seconds respectively (Table 4.6). On statistical analysis this time difference was not significant (Table 4.7). But statistical analysis revealed that there was significant difference in time taken for reduction of methylene blue by semen from different bucks ($P<0.05$).

4.5 Freezing of semen

Overall average percentage sperm motility of buck semen after washing in Tris buffer was 76.71 ± 0.79 (Table 4.13). Average percentage motility after glycerolisation at the level of four, five, six and seven per cent were 66.42 ± 1.15, 66.57 ± 1.15, 67.85 ± 1.39 and 64.85 ± 1.38 respectively (Table 4.13). The difference in average motility percentage (Table 4.14) in six per cent glycerolated sample and in seven per cent glycerolated sample was statistically significant ($P<0.05$). Maximum percentage motility after glycerolization was obtained in six per cent glycerolated semen.

There was highly significant ($P<0.01$) reduction in percentage of motility of washed spermatozoa after glycerolisation (Table 4.14).

Percentage sperm motility of individual bucks after freezing with four hour equilibration are given in Tables 4.8 to 4.12. Average post-thaw motility of buck semen frozen in four per cent, five per cent, six per cent and seven per cent glycerolated extender were 30.14 ± 1.78, 34.42 ± 1.87, 42.00 ± 1.84 and 34.71 ± 1.72 respectively (Table 4.13). There was highly significant reduction in percentage motility after glycerolisation to percentage motility after freezing ($P<0.01$) (Fig.4.3).

Maximum post-thaw motility was obtained for spermatozoa sample frozen in six per cent glycerolated extender. There was no significant difference in post-thaw motility percentage between semen frozen in five per cent glycerolated extender and seven per cent glycerolated extender.

4.6 Fertility study

Seventy five does in heat, brought to the AI Centre, Mannuthy were inseminated using buck semen frozen in six per cent glycerolated extender. Fifty seven were followed up. Out of that 27 does conceived, two aborted and 25 kidded. Thirty

does did not conceive. The conception percentage was 47.36. The kidding percentage calculated as 43.85.

Average gestation length was 149.85 $\pm$ 4.45 days. Three does (12 per cent does) gave birth to three kids, 12 does (48 per cent does) gave birth to two kids and 10 does (40 per cent does) gave birth to one kid. The number of kids per kidding averaged 1.7. Total kids born were 21 females and 22 males. Percentage of female and male kids out of total kids born were 48.83 and 51.16 respectively.

Table 4.1. Evaluation of buck semen (Average of six)

Buck No.	Volume (ml)	Colour	Density	Mass activity	pH	Motility percentage	Concentration (millions/ml)	Live sperm percentage
1	0.88±0.07	Creamy	DDDD	++++	6.86± 0.02	88.33± 1.66	3065.00±23.20	94.44±0.36
2	0.73±0.04	Creamy	DDDD	++++	6.83± 0.02	85.00± 2.23	3871.66±222.82	92.79±0.47
3	0.68±0.04	Creamy	DDDD	++++	6.83± 0.02	88.33± 1.66	3451.66±106.94	89.70±1.06
4	0.68±0.04	Creamy	DDDD	++++	6.86± 0.03	86.66± 2.10	1893.33±73.74	89.46±0.86
5	0.58±0.06	Creamy	DDDD	++++	6.85± 0.02	80.83± 3.27	1930.00±34.54	88.76±1.56
Average	0.71±0.02	Creamy	DDDD	++++	6.85± 0.01	85.83± 1.05	2842.33±153.93	91.03±0.56

Table 4.2. Incubation test using buck spermatozoa with and without seminal plasma (Average of six)

Buck No.	Volume (ml)	Mass activity	Percentage of motility at different periods of time after semen collection													
			0 min		10 min		20 min		30 min		40 min		50 min		60 min	
			With s.p.	Without s.p.	With s.p.	Without s.p.	With s.p.	Without s.p.	With s.p.	Without s.p.	With s.p.	Without s.p.	With s.p.	Without s.p.	With s.p.	Without s.p.
1	0.76	++++	73.33	74.16	79.16	75.00	71.66	65.00	64.16	58.33	62.50	55.00	58.33	47.50	48.33	32.50
2	0.75	++++	75.00	73.33	81.66	76.66	76.66	66.66	65.00	60.16	60.00	46.66	50.00	35.00	44.16	21.66
3	0.66	++++	71.66	72.50	80.00	76.66	68.33	63.33	50.00	47.50	29.16	25.83	20.83	17.50	8.33	6.66
4	0.78	++++	70.00	69.16	78.33	76.66	65.00	62.50	51.66	51.66	37.50	37.50	20.00	24.16	7.50	9.16
5	0.70	++++	64.16	65.00	65.83	67.50	55.83	57.50	44.16	44.16	27.50	23.33	20.00	15.83	5.00	4.16
Average	0.73	++++	70.83± 1.31	70.83± 1.40	77.00± 1.52	74.50± 1.90	67.50± 1.98	63.00± 1.97	55.00± 2.32	51.66± 2.30	43.33± 3.50	37.66± 3.26	33.83± 4.06	28.00± 3.33	22.66± 4.28	14.83± 2.58

s.p. - seminal plasma

Table 4.3. Analysis of variance - Incubation test

Source	Degrees of freedom	Mean square at different time intervals						
		0 min	10 min	20 min	30 min	40 min	50 min	60 min
Replication	5	76.667	233.750	148.750	100.667	173.000	166.417	80.750
Treatment	1	0.000	93.750	303.750	166.667	481.667	510.417	920.417 *
Buck	4	185.417 **	317.083 **	380.833 **	680.208 **	2709.792 **	3009.792 **	3317.708 **
Treatment x Buck	4	4.167	20.833	57.917	40.625	76.458	163.542	342.292
Error	45	45.926	59.491	95.972	131.778	176.519	234.194	149.269

** P<0.01

* P<0.05

Table 4. 4. Effect of zero degree celsius for varying periods of time on live sperm percentage of buck spermatozoa with and without seminal plasma (Average of six)

Buck No.	Volume (ml)	Mass activity	Percentage of livesperm at different periods of time after semen collection													
			0 min		5 min		10 min		15 min		20 min		25 min		30 min	
			With s.p.	Without s.p.	With s.p.	Without s.p.	With s.p.	Without s.p.	With s.p.	Without s.p.	With s.p.	Without s.p.	With s.p.	Without s.p.	With s.p.	Without s.p.
1	0.8	++++	92.1	89.64	5.22	22.26	3.53	15.61	2.62	13.78	1.98	8.08	1.72	6.61	0.86	5.27
2	0.75	++++	86.28	90.27	6.16	27.24	5.37	16.74	4.34	13.23	3.35	10.36	2.84	8.19	1.68	6.78
3	0.68	++++	87.17	89.63	5.90	10.1	4.56	7.59	2.95	4.64	2.38	4.27	1.92	3.55	1.30	2.69
4	0.76	++++	81.51	80.75	13.83	13.29	5.46	6.84	4.56	5.49	3.27	4.44	2.49	3.49	1.31	2.68
5	0.56	++++	87.74	86.55	7.66	7.00	5.07	5.69	4.12	5.05	3.48	4.13	2.80	3.40	2.17	2.58
Average	0.71	++++	86.96± 1.09	87.37± 1.14	7.75± 1.04	15.98± 2.24	4.80± 0.40	10.49± 1.52	3.72± 0.37	8.44± 1.24	2.89± 0.25	6.26± 0.73	2.35± 0.22	5.05± 0.67	1.46± 0.18	3.99± 0.61

s.p. - seminal plasma

Table 4.5. Analysis of variance - Cold shock test

Source	Degrees of freedom	Mean square at different time intervals						
		0 min	5 min	10 min	15 min	20 min	25 min	30 min
Replication	5	16.546	79.216	11.096	9.965	6.955	8.620	8.866
Treatment	1	2.485	1014.595**	486.268**	334.412**	169.781**	108.811**	96.064**
Buck	4	158.661**	195.817*	75.453	61.110*	24.318**	15.876*	10.744
Treatment x Buck	4	21.904	311.049	93.225	72.622	26.336	15.208	13.011
Error	45	30.415	64.719	32.141	19.490	6.286	6.027	4.879

** P<0.01

* P<0.05

Table 4.6. Methylene blue reduction test of buck spermatozoa with and without seminal plasma (Average of six)

Buck No.	Volume (ml)	Mass activity	Methylene blue reduction time (in seconds)	
			With s.p	Without s.p.
1	0.68	++++	177.5	184.1
2	0.75	++++	168.3	202.5
3	0.63	++++	202.5	247.5
4	0.83	++++	152.5	165.8
5	0.78	++++	160.0	175.0
Average	0.73	++++	173.16± 8.77	197.00± 9.97

Table 4.7 Analysis of variance - Methylene blue reduction test

Source	Degrees of freedom	Sum of squares	Mean square	F value
Replication	5	12722.083	2544.417	1.0622
Treatment	1	8520.417	8520.417	3.5571
Buck	4	30768.333	7692.083	3.2113*
Treatment x Buck	4	2223.333	555.833	0.2320
Error	45	107790.417	2395.343	

* $P<0.05$

Table 4.8. Freezing of semen - Buck No.1

Sl. No.	Volume (ml)	Mass activity	Motility percentage after washing in Tris buffer	Motility percentage after different level of glycerolisation				Motility percentage after freezing with different level of glycerol			
				4%	5%	6%	7%	4%	5%	6%	7%
1	1.0	++++	80	70	70	70	70	45	50	70	70
2	1.0	+++	70	60	70	60	60	30	35	40	40
3	1.0	+++(+)	80	70	70	70	70	40	40	45	45
4	0.5	++++	80	80	70	80	70	35	50	50	30
5	1.2	++++	80	70	70	80	60	30	35	40	35
6	0.4	+++(+)	80	60	60	70	60	25	30	45	45
7	1.2	++++	80	70	70	70	60	10	15	35	25
Average	0.9	++++	78.57	68.57	68.57	71.42	64.28	30.71	34.42	46.42	41.42

Table 4.9. Freezing of semen - Buck No.2

Sl. No.	Volume (ml)	Mass activity	Motility percentage after washing in Tris buffer	Motility percentage after different level of glycerolisation				Motility percentage after freezing with different level of glycerol			
				4%	5%	6%	7%	4%	5%	6%	7%
1	0.4	++++	80	70	70	70	70	20	30	50	30
2	0.8	++++	80	60	70	70	60	45	50	60	40
3	0.5	++++	80	80	80	80	80	25	35	50	45
4	0.8	++++	80	70	70	70	70	20	25	45	30
5	0.9	++++	80	70	70	75	60	25	45	45	40
6	0.8	++++	80	60	70	70	70	30	50	50	40
7	0.8	++++	80	70	60	70	70	30	40	40	30
Average	0.71	++++	80.00	68.57	70.00	72.14	68.57	27.85	39.28	48.57	36.42

Table 4.10. Freezing of semen - Buck No.3

Sl. No.	Volume (ml)	Mass activity	Motility percentage after washing in Tris buffer	Motility percentage after different level of glycerolisation				Motility percentage after freezing with different level of glycerol			
				4%	5%	6%	7%	4%	5%	6%	7%
1	0.3	++++	70	60	60	70	60	20	15	10	10
2	0.5	+++(+)	80	70	70	80	70	50	45	50	30
3	0.9	++++	80	80	70	70	80	20	35	40	40
4	1.0	++++	80	70	80	80	70	40	45	50	40
5	0.7	+++	70	60	50	60	60	15	20	30	35
6	0.7	+++(+)	70	50	50	50	50	25	20	30	30
7	0.6	++++	70	60	70	70	70	25	25	30	25
Average	0.67	++++	74.28	64.28	64.28	68.57	65.71	27.85	29.28	34.28	24.28

Table 4.11. Freezing of semen - Buck No.4

Sl. No.	Volume (ml)	Mass activity	Motility percentage after washing in Tris buffer	Motility percentage after different level of glycerolisation				Motility percentage after freezing with different level of glycerol			
				4%	5%	6%	7%	4%	5%	6%	7%
1	1.0	++++	75	70	70	70	70	20	20	35	30
2	1.1	+++	70	60	60	60	60	50	50	40	40
3	1.0	++++	80	60	60	50	50	20	30	20	20
4	0.7	+++	70	60	60	60	60	40	50	45	30
5	1.1	+++	80	70	70	70	70	40	45	55	40
6	0.8	+++(+)	70	60	60	50	60	30	35	40	20
7	1.2	++++	80	70	70	70	70	20	30	45	40
Average	0.98	+++(+)	75.00	64.28	64.28	61.42	62.85	31.42	37.14	40.00	31.42

Table 4.12. Freezing of semen - Buck No.5

Sl. No.	Volume (ml)	Mass activity	Motility percentage after washing in Tris buffer	Motility percentage after different level of glycerolisation				Motility percentage after freezing with different level of glycerol			
				4%	5%	6%	7%	4%	5%	6%	7%
1	1.0	+++(+)	70	70	70	70	70	50	40	30	40
2	1.0	++++	80	65	60	55	40	40	40	50	40
3	0.8	+++(+)	80	70	70	70	70	35	30	45	40
4	0.5	+++	80	70	70	70	70	30	35	40	20
5	1.0	++++	80	70	70	70	70	25	20	35	35
6	0.7	++++	70	60	60	60	60	30	20	45	30
7	0.6	++++	70	60	60	65	60	20	25	40	35
Average	0.8	++++	75.71	66.42	65.71	65.71	62.85	32.85	30.00	40.71	34.28

Table 4.13. Average spermatozoamotility percentage before and after freezing in extender containing different levels of glycerol (Average of seven)

Sl. No.	Volume (ml)	Mass activity	Motility percentage after washing in Tris buffer	Motility percentage after different level of glycerolisation				Motility percentage after freezing with different level of glycerol			
				4%	5%	6%	7%	4%	5%	6%	7%
1	0.90	++++	78.57	68.57	68.57	71.42	64.28	30.71	36.42	46.42	41.42
2	0.71	++++	80.00	68.57	70.00	72.14	68.57	27.85	39.28	48.57	36.42
3	0.67	++++	74.28	64.28	64.28	68.57	65.71	27.85	29.28	34.28	24.28
4	0.98	+++(+)	75.00	64.28	64.28	61.42	62.85	31.42	37.14	40.00	31.42
5	0.80	++++	75.71	66.42	65.71	65.71	62.85	32.85	30.00	40.71	34.28
Average	0.81	++++	76.71± 0.79	66.42± 1.15	66.57± 1.15	67.85± 1.39	64.85± 1.38	30.14± 1.78	34.42± 1.87	42.00± 1.84	34.71± 1.72

Table 4.14 Analysis of variance - Freezing of buck semen

Source	Degrees of freedom	Sum of squares	Mean square	F value
Replication	6	1747.460	291.243	4.0538
Treatment	8	91206.825	11400.853	158.6889**
Buck	4	1755.079	438.770	6.1073**
Treatment x Buck	32	1603.492	50.109	0.6975
Error	264	18966.825	71.844	

** P<0.01

FIG.4.1 INCUBATION TEST USING BUCK SPERMATOZOA WITH AND WITHOUT SEMINAL PLASMA

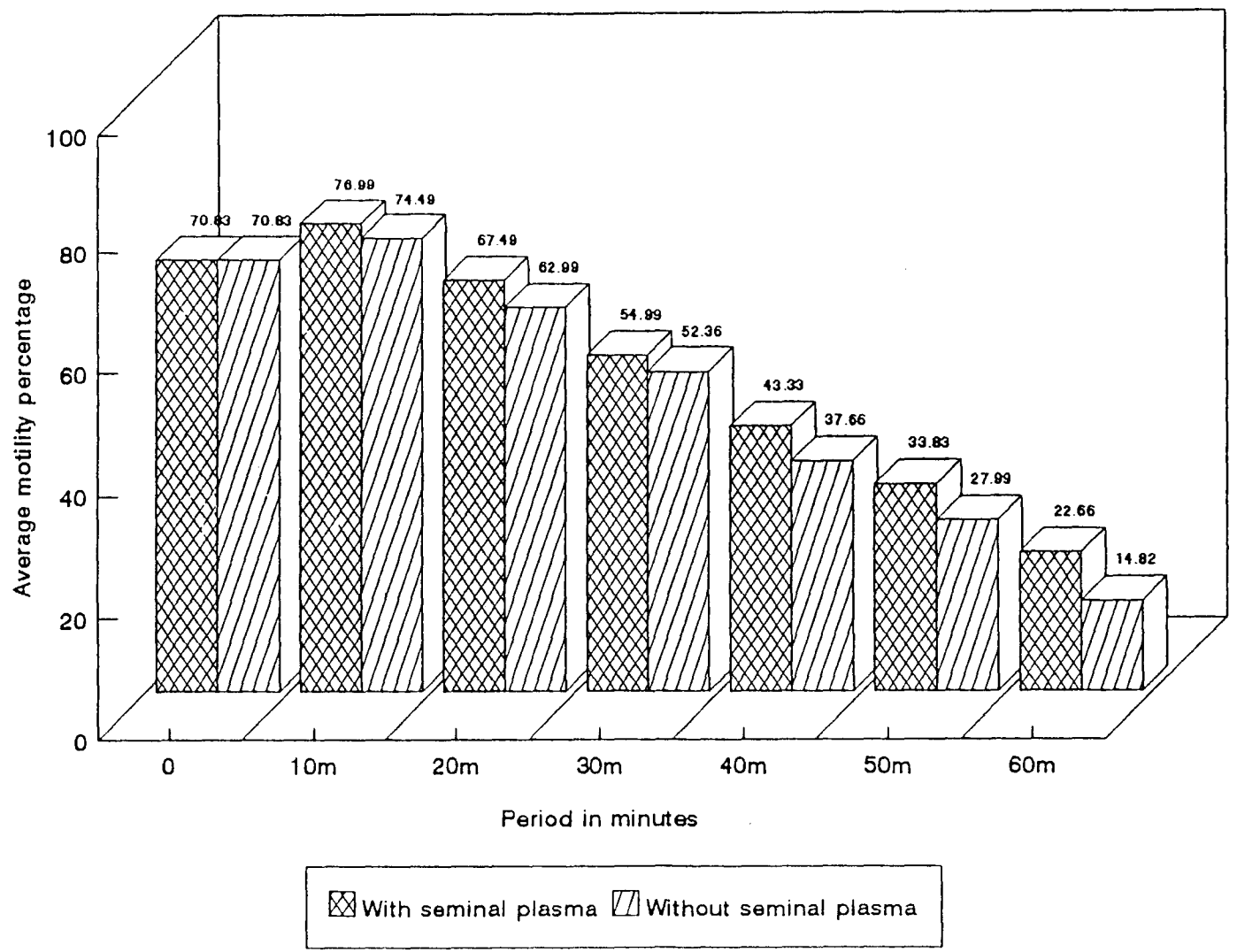


FIG.4.2 EFFECT OF ZERO DEGREE CELSIUS FOR VARYING PERIODS OF TIME ON LIVE SPERM PERCENTAGE OF BUCK SEMEN WITH AND WITHOUT SEMINAL PLASMA

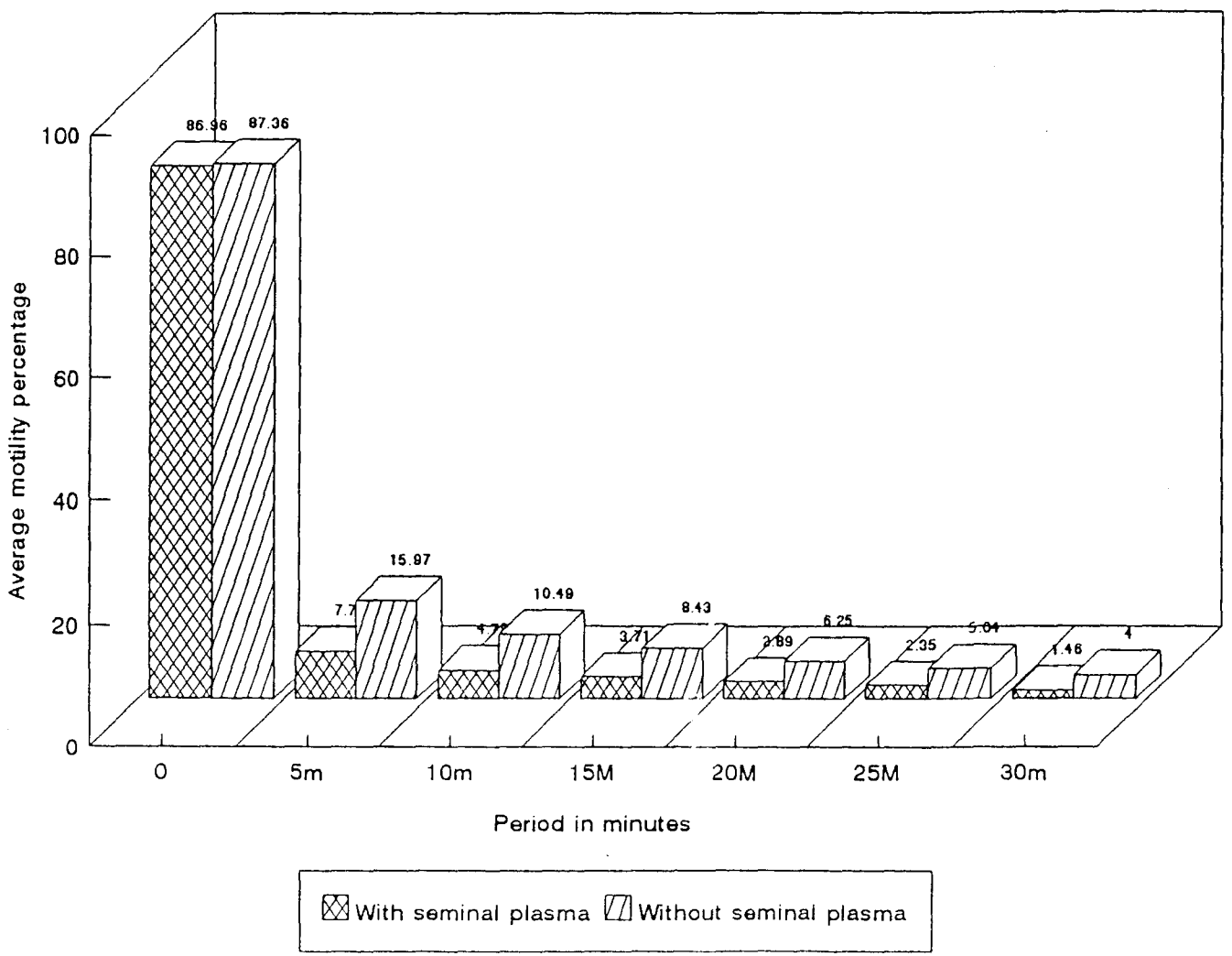
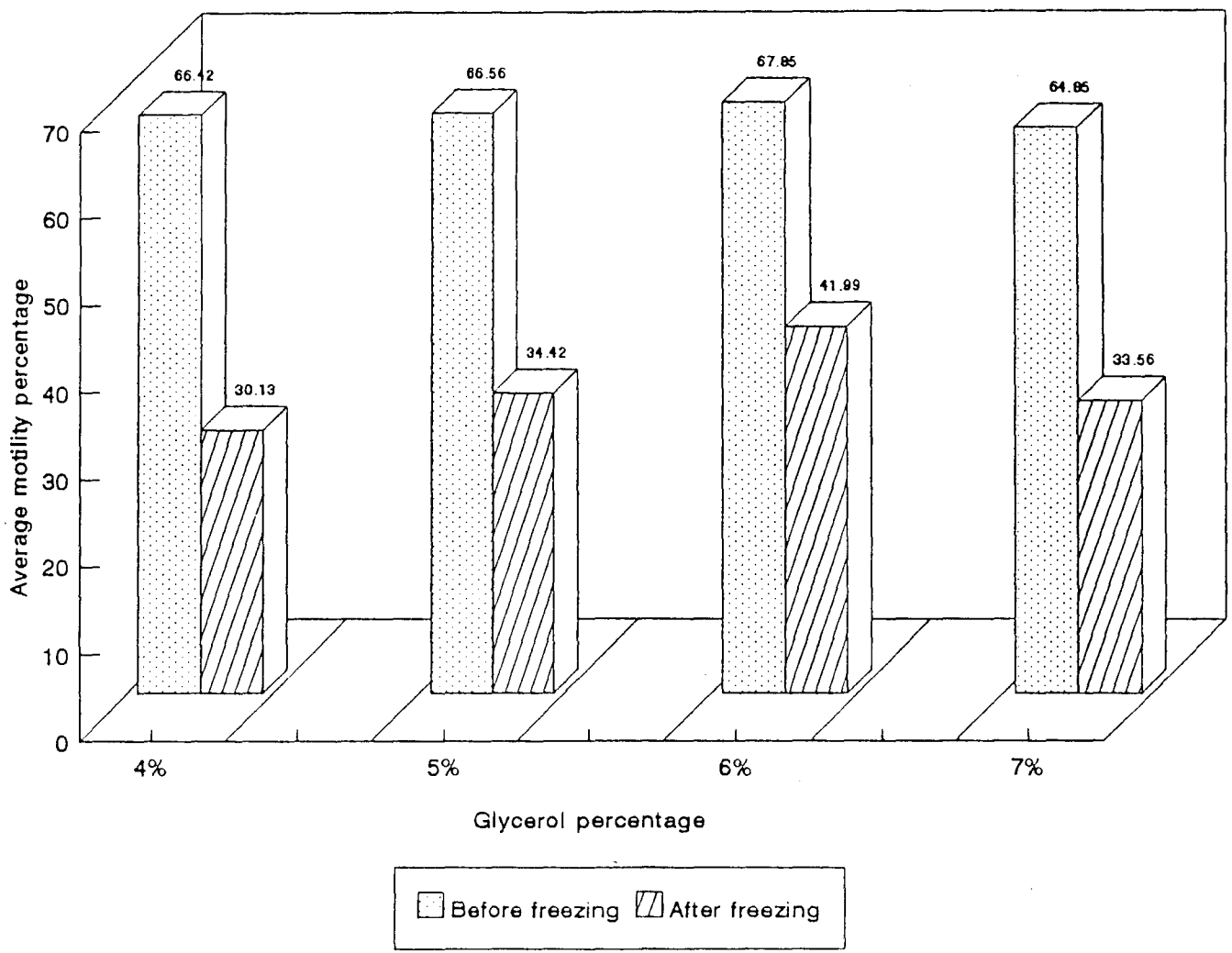


FIG.4.3 AVERAGE SPERM MOTILITY BEFORE AND AFTER FREEZING IN EXTENDER CONTAINING DIFFERENT LEVELS OF GLYCEROL



Discussion

DISCUSSION

5.1 Semen evaluation

Semen samples from five Malabari crossbred bucks were subjected to evaluation.

The mean semen volume was found to be 0.71 ± 0.02 (Table 4.1). This value is within the range of semen volume reported by Kurian and Raja (1965), Patil (1970), Mann (1980), Sinha and Singh (1982), Sarmah (1983), Pandey et al. (1985) and Wuschko and Seifort (1991). Higher values for semen volume have been reported by Knoblauch (1962); Cetinkaya et al. (1980), Singh et al. (1982); Dundar et al. (1983), Sevinc et al. (1985) and Bakshi et al. (1987). However semen volume reported by Saxena and Tripathi (1980) and Singh et al. (1985) were less than the volume obtained in this study. There was significant ($P<0.05$) difference in semen volume of different bucks. This is in accordance with the observation made by Mohan et al. (1980).

Colour of buck semen was found to be creamy (Table 4.1). This observation compares favourably with the reports of Knoblauch (1962), Patil (1970), Igboeli (1974), Mann (1980) and Ali and Mustafa (1986). The overall density of buck semen was DDDD (Table 4.1). This is in accordance with the observation made by Knoblauch (1962). The overall mass activity of buck

semen was ++++ (Table 4.1), which is within the range of mass activity reported by Patil (1970).

The overall average of buck semen pH was 6.85 ± 0.01 (Table 4.1). This value is well within the range of value reported by Patil (1970), Mohan et al. (1980), Sinha and Singh (1982) and Sarmah (1983). There was no significant difference between the pH of semen from different bucks. However Mohan et al. (1980) and Sarmah (1983) reported significant difference in pH of semen in different bucks.

The mean sperm motility percentage of buck semen was 85.83 ± 1.05 (Table 4.1) which is within the range reported by Kurian and Raja (1965), Patil (1970), Cetinkaya et al. (1980), Sarmah (1983), Sevinc et al. (1985) and Bakshi et al. (1987). There was significant difference between the percentages of sperm motility in different bucks ($P<0.05$).

The average sperm concentration of buck semen was 2842.33 ± 153.93 million/ml (Table 4.1). Sperm concentration reported by earlier workers were two to three billions (Kurian and Raja, 1965), 1.1 to 7.49 billions (Patil, 1970), 3.28 ± 0.07 billions (Sarmah, 1983) and 2780.30 millions (Sinha and Singh, 1982). There was highly significant difference in the sperm concentration between bucks ($P<0.01$). This is in accordance with the findings of earlier workers (Mohan et al., 1980; Sarmah, 1983).

The overall average live sperm percentage of buck semen was 91.03 ± 0.56 (Table 4.1). This value is within the range reported by Kurian and Raja (1965), Sarmah (1983) and Singh et al. (1985). There was highly significant difference between live sperm percentage of different bucks ($P<0.01$). This is in accordance with the findings of Mohan et al. (1980).

5.2 Incubation test

The percentage of motile sperms at different periods of incubation are given in Table 4.2.

Initial percentage sperm motility in sperm sample with seminal plasma and without seminal plasma were 70.83 ± 1.31 and 70.83 ± 1.40 respectively. Corresponding values at 10 minutes of incubation were 77.00 ± 1.52 and 74.50 ± 1.90 respectively. As per Salisbury and Van Demark (1961) the motility and metabolic activity of spermatozoa were higher at a temperature a few degrees above body temperature.

From 10 to 60 minutes of incubation, motility was gradually decreasing. This is in accordance with the findings of Joseph (1983). When the temperature was raised a few degrees above body temperature, the total life expectancy of the spermatozoa was reduced (Salisbury and Van Demark, 1961). This may be due to the increased rate of metabolic activities and exhaution of nutrients in the semen.

There was highly significant difference ($P<0.01$) in the percentage sperm motility of different bucks in sperm samples incubated with and without seminal plasma at all time intervals (Table 4.3). This shows the difference in the ability of spermatozoa from different bucks to withstand high temperature.

5.3 Cold shock test

Initial live sperm percentage in spermatozoa with and without seminal plasma were 86.96 ± 1.09 and 87.37 ± 1.14 respectively. On statistical analysis there was no significant difference in live sperm percentage in sperm with and without seminal plasma at zero minutes of cold shock (Table 4.5). But from zero minutes to 30 minutes of cold shock the live sperm percentage was higher in sperm samples without seminal plasma than in sperm samples with seminal plasma ($P<0.01$). This may be due to the superiority of Tris buffer over seminal plasma in protecting the spermatozoa during exposure to low temperaures.

Live sperm percentage in sperm sample with and without seminal plasma at five minutes of cold shock were 7.75 ± 1.04 and 15.98 ± 2.24 respectively, showing a rapid reduction. Spermatozoa are highly sensitive to rapid decline in temperature; the rate of cooling is an important factor, influencing the intensity of cold shock (Garner, 1991).

In the present study semen sample maintained at 37°C was placed in a water bath maintained at 0°C. That sudden reduction in temperature is the reason for rapid reduction in live sperm percentage after five minutes of cold shock. However spermatozoa without seminal plasma could withstand cold shock better than spermatozoa with seminal plasma.

The live sperm percentages of semen from different bucks were highly significant ($P<0.01$) at 0 minutes and 20 minutes and significant ($P<0.05$) at five minutes, 15 minutes and 25 minutes. But not significant at 10 minutes and 30 minutes (Table 4.5). There were marked difference in the response to cold shock by different animals in the same species;susceptibility to cold shock may be related to the lipid composition of spermatozoa (Mann and Mann, 1981).

5.4 Methylene blue reduction test

The overall average of time taken for reduction of methylene blue by spermatozoa with and without seminal plasma were 173.16 ± 8.77 seconds and 197.00 ± 9.97 seconds respectively (Table 4.6). This difference was not statistically significant (Table 4.7). Time taken for reduction of methylene blue reported by earlier workers were 271.2 seconds (Patil, 1970), 133.0886 ± 61.420 seconds (Joseph, 1983) and 189.86 seconds (Kutty et al., 1995).

Analysis revealed that there was significant difference ($P<0.05$) in time taken by different bucks (Table 4.7). This can be explained in terms of the variation in live sperm concentration in different bucks.

5.5 Freezing of semen

Overall average percentage sperm motility of buck semen after washing in Tris buffer was 76.71 ± 0.79 (Table 4.13). Corteel (1974), Corteel and Baril (1975), Westhuysen, J.M. van der (1978), Ritar and Salamon (1982), Perez (1985) and Memon et al. (1985) reported that percentage sperm motility was higher in sperm sample without seminal plasma than in sperm sample with seminal plasma. Deka and Rao (1987a) and Haunhorst (1990) opined that there was no significant reduction in sperm motility by centrifugation. However Tuli and Holtz (1994) reported unfavourable effect of removal of seminal plasma on post-thaw motility of buck semen.

Average percentage motility after glycerolisation at the level of four, five, six and seven per cent were 66.42 ± 1.15, 66.57 ± 1.15, 67.85 ± 1.39 and 64.85 ± 1.38 respectively (Table 4.13). Maximum percentage motility after glycerolisation was obtained in six per cent glycerolated semen. This is in accordance with the findings of earlier workers (Sahni and Roy, 1972; Deka and Rao, 1986b; Sinha et al., 1992b; Sinha et al., 1993). Chauhan and Anand (1990) reported maximum percentage

motility in seven per cent glycerolated semen. Purohit et al. (1992) reported maximum percentage motility in five per cent glycerolated semen. Paggi (1971) and Deshpande and Mehta (1991) reported maximum percentage motility in four per cent glycerolated semen. There was highly significant ($P<0.01$) reduction in percentage motility after centrifugation to percentage motility after glycerolisation (Table 4.14). This may be due to the interaction between spermatozoa and glycerol.

Average post-thaw motility percentage obtained in four, five, six and seven per cent glycerolated extender were 30.14± 1.78, 34.42 ± 1.87, 42.00 ± 1.84 and 34.71 ± 1.72respectively (Table 4.13). There was highly significant reduction in percentage motility after glycerolisation to percentage motility after freezing ($P<0.01$). This may be due to the death of weak spermatozoa due to thermal shock and internal ice formation. During the physical change in the media, the sperms are put under high stress and ice formation which causes mechanical injury in certain cases (Mathew, 1984). Even with the best freezing techniques there will be death of some viable cells during freezing (Hafez, 1993).

From this study it was clear that six per cent glycerolated extender was better than four per cent, five per cent, and seven per cent glycerolated extender for buck semen freezing. This is in accordance with the findings of earlier

workers (Sahni and Roy, 1972; Deka and Rao, 1986b; Sinha et al. 1992b; Sinha et al., 1993). Chauhan and Anand (1990) reported maximum post-thaw motility in seven per cent glycerolated semen, Purohit et al. (1992) in five per cent, Paggi (1971) and Deshpande and Mehta (1991) in four per cent glycerolated semen. The variation in regard to percentages of post-thaw motility in different investigations might be ascribed to differences in breeds, extenders, packages and freezing techniques.

5.6 Fertility study

Fifty seven does inseminated with frozen semen were followed up. Twenty seven does conceived. Conception percentage was 47.36. Percentage of conception reported by Valdes Leal (1984), Sinha et al. (1987) and Park et al. (1989) were lesser than the conception percentage obtained in this study. However Fougner (1974), Summermatter (1986), Deka and Rao (1989), Torre et al. (1990) and Mylrea et al. (1991) obtained conception percentage greater than the conception percentage obtained in this study.

Pregnancy rate in goats inseminated laproscopically using frozen semen were more than that in goats inseminated cervically; with cervical insemination, fertility and fecundity improved with increasing depth of insemination into the reproductive tract (Ritar et al., 1990b).

Out of 27 does conceived, 25 does kidded and two does aborted. The kidding percentage was 43.85. Kidding percentage reported by other workers were 13.33 (Liess and Ostrowski, 1960), 42.5 (Corteel et al., 1974), 60.8 (Lawrenz, 1986) and 25 (Park et al., 1989). Presumably, differences in managemental practices, methods of processing and freezing,fertility and skill of inseminators may account for variation in fertility rates in different investigations.

Average gestation length was 149.85 ± 4.45 days. Gestation length in goats, reported by earlier workers were 146.5 ± 1.2 days (Roy et al., 1962), 142 to 147 days (Sudarsanan and Raja, 1973), 148 to 156 days (Roberts, 1971), 148 to 156 days (Deshpande et al., 1982) and 145 to 151 days (Catchpole, 1991).

The incidence of single, twin and triplets obtained in this study were 40 per cent, 48 per cent and 12 per cent respectively. The incidence of single, twin and triplets in goats reported by earlier workers were 47 per cent, 35.29 per cent and 17.65 per cent respectively (Sudarsanan and Raja, 1973) and 61.3 per cent, 37 per cent and 1.64 per cent respectively (Nair and Mathai, 1979).

In this study, the number of kids per kidding averaged 1.7. According to Stagnaro (1975) it was 1.4. Total kids born

were 22 males and 21 females. The secondary sex ratio calculated was 51.16:48.83. This is in accordance with the secondary sex ratio reported by earlier workers (Sacker and Trail, 1966; Seth et al., 1968; Raja and Mukundan, 1973; Mittal, 1976; Nair and Mathai, 1979).

Summary

SUMMARY

A study was conducted to find out the effect of different concentrations of glycerol on post-thaw motility and fertility of frozen buck semen. The semen samples used for the study were collected from five Malabari crossbred bucks maintained at Artificial Insemination Centre, under the Department of Animal Reproduction, College of Veterinary and Animal Sciences, Mannuthy, Thrissur. The overall average volume of buck semen was 0.71 ± 0.02 ml. Significant ($P<0.05$) difference in semen volume was observed between bucks. The average colour index of buck semen was creamy, density was DDDD and the mass activity was ++++. The overall average pH of buck semen was 6.85 ± 0.01. There was no significant difference in the pH of semen between bucks. The overall average percentage of sperm motility was 85.83 ± 1.05. The motility percentage varied significantly ($P<0.05$) between bucks. The average sperm concentration was 2842.33 ± 153.93 millions/ml. There was highly significant ($P<0.01$) difference in sperm concentration between bucks. The overall average live sperm percentage was 91.03 ± 0.56. There was highly significant ($P<0.01$) difference in live sperm percentage between bucks.

Significantly higher ($P<0.05$) percentage sperm motility was noted in sperm with seminal plasma than in sperm without seminal plasma at 60 minutes of incubation at 46.5°C. Initial

sperm motility percentage in sperm sample with and without seminal plasma were 70.83 ± 1.31 and 70.83 ± 1.40 respectively. Corresponding values at 10 minutes of incubation were 77.00 ± 1.52 and 74.50 ± 1.90 respectively. From 10 minutes to 60 minutes of incubation motility was gradually decreasing. Highly significant difference ($P<0.01$) in sperm motility of different bucks was noted in sperm samples incubated with and without seminal plasma at all time periods.

Significantly higher ($P<0.01$) live sperm percentage was noted in sperm sample without seminal plasma than in sperm sample with seminal plasma at time periods upto 30 minutes of cold shock. Initial live sperm percentage in sperm sample with and without seminal plasma were 86.96 ± 1.09 and 87.37± 1.14 respectively. Corresponding values at five minutes of cold shock were 7.75 ± 1.04 and 15.98 ± 2.24respectively, showing a rapid reduction. The live sperm percentages between bucks were significant at different time periods except at 10 minutes and 30 minutes of cold shock.

The overall average of time taken for reduction of methylene blue by sperm with and without seminal plasma were 173.16 ± 8.77 seconds and 197.00 ± 9.97 seconds respectively, which was not significant. Between bucks there was significant difference ($P<0.05$) in methylene blue reduction time of spermatozoa.

Overall average percentage sperm motility after washing in Tris buffer was 76.71 ± 0.79. Average percentage motility after glycerolisation at four, five, six and seven percent level were 66.42 ± 1.15, 66.57 ± 1.15, 67.85 ± 1.39 and 64.85 ± 1.38 respectively. Maximum percentage motility after glycerolisation was obtained in six per cent glycerolated semen. Average post-thaw motility percentage obtained in four, five, six and seven percent glycerolated extenders were 30.14 ± 1.78, 34.42 ± 1.87, 42.00 ± 1.84 and 34.71 ± 1.72 respectively. Highly significant ($P<0.01$) reduction in motility percentage was noted after freezing. Maximum post-thaw motility was obtained in six per cent glycerolated extender. There was no significant difference between post-thaw sperm motility in five per cent and seven per cent glycerolated extender.

Using frozen semen 75 does were inseminated, and 57 were followed up. Out of 57 does, 27 conceived (47.36 per cent), 25 kidded (43.85 per cent) and two (3.50 per cent) aborted. Average gestation length was 149.85 ± 4.45 days. Twelve per cent kiddings were triplets, 48 per cent were twins and 40 per cent were singles. The number of kids per kidding averaged 1.7. Percentage of male and female kids born were 51.16 and 48.83 respectively.

From this study it could be inferred that Tris extender with six per cent glycerol was superior to Tris extender with four per cent, five per cent or seven per cent glycerol for better post-thaw motility and fertility of frozen buck semen.

References

REFERENCES

*Aamdal, J and Andersen, K. (1968). Fast thawing of semen frozen in straws. VI Congr. Reprod. Insem. Artif., Paris., 1968. Cited in Anim. Breed. Abstr. (1969) 37 (1): Abstr. 337.

Ali, B.H. and Mustafa, A.I. (1986). Semen characteristics of Nubian goats in the Sudan. Anim. Reprod. Sci. 12 (1): 63-68.

*Arauja, A.A.DE. and Nunes, J.F. (1991). Use of coconut milk with added egg yolk as a cryoprotectant for goat semen. In Anais, IX Congresso Brasileiro de Reproducao Animal, Belo Horizonte, Brazil, 22 a 26 de Juho de 1991. Vol.II Belo Horizonte, Brazil, Colegio Brasileiro de Reproducao Animal: 434. Cited in Anim. Breed. Abstr. 60 (6): Abstr. 3742.

Bakshi, S.A., Patil, V.K., Srivastava, A.K., Jagtap, D.Z and More, B.K. (1987). Studies on semen evaluation and fertility rate of Angora and 7/8 Angora bucks. Livest. Adviser 12 (10): 13-18.

Bargohain, A.C., Deka, B.C. and Rajkonwar, C.K. (1985). Preservation of buck semen. Indian Vet. J. 62(1): 81-82.

Beck, G.H. and Salisbury, G.W. (1943). Rapid methods for estimating the quality of bull semen. J. Dairy Sci. 26: 483-494.

*Bonfert, A. (1969). Is goat A.I. with frozen semen ready for practical use? Tierzuchter **21**: 264. Cited in Anim. Breed. Abstr. **37**(4): Abstr. 3779.

Bordoloi, R.K. and Sharma, P.K. (1983). Variation in the percentage of abnormalities and percentage of live spermatozoa in different breeds of goat. Indian Vet J. **60**(6): 455-456.

*Campbell, R.C., Dott, H.M. and Glover, T.D. (1956). Nigrosin-eosin as a stain for differentiating live and dead spermatozoa. J. Agric. Sci. **48**(1): 1-8. Cited in Anim. Breed. Abstr. (1957) **25**(2): Abstr. 545.

Catchpole, H.R. (1991). Hormonal mechanisms in pregnancy and parturition. In: Cupps, P.T. Reproduction in Domestic Animals. 4th Edn. Academic Press, Inc. Harcourt Brace Jovanovich, Publishers, San Diego, p. 362.

*Cetinkaya, R., Cekgul, E. and Ekici, A. (1980). Characteristics of Angora goat semen and fertility of frozen semen. Lalahan Zootekni Arastirma Enstitusu Dergisi **20**(3-4): 67-88. Cited in Anim. Breed. Abstr. (1983) **51**(7): Abstr. 4386.

Chauhan, M.S. and Anand, S.R. (1990). Effect of egg yolk lipids on the freezing of goat semen. Theriogenology **34**(5): 1003-1013.

Choudhury, A.H., Deka, B.C. and Rajkonwar, C.K. (1987). Effect of diluters on motility and fertility of frozen goat semen. Cheiron **16**(2): 94-96.

*Corteel, J.M. (1974). Viability of goat spermatozoa deep frozen with or without seminal plasma: effect of glucose. Annales de Biologie Animale, Biochemie, Biophysique **14**(4B): 741-745. Cited in Anim. Breed. Abstr. (1975) **43**(11): Abstr. 5293.

*Corteel, J.M. and Baril, G. (1975). Effect of "Washing" on the preservation of goat semen at low temperatures. Elevage et Insemination. (146): 26-30. Cited in Anim. Breed. Abstr. (1976) **44** (2): Abstr. 701.

*Corteel, J.M., Courot, M. and Ortavant, R (1974). Fertility of synchronized multiparous goats inseminated with liquid or deep frozen semen. In Proceedings of the International Symposium on physio-pathology of Reproduction and Artificial Insemination in Small Ruminants, Thessaloniki, 16-19 May, 1974. Cited in Anim. Breed. Abstr. (1975). **43** (10): Abstr. 4624.

*Corteel, J.M., Nunes, J.F., Baril, B. and Leboeuf, B. (1983). Effect of modification of diluent on survival of washed goat spermatozoa. Paper presented at the 9th meeting of the Asociacion Latino americana reproducion Animal in 1983, Nouzilly, France. Cited in Anim. Breed. Abstr. (1987). **55** (6): Abstr. 3657.

Das, K.K. and Rajkonwar, C.K. (1994). Effects of thawing temperature on the quality of frozen buck semen. Indian Vet. Med. J. **18** (3): 167-170.

Deka, B.C. and Rao, A.R. (1984a). Effect of separation of seminal plasma on quality of frozen buck semen. Indian J. Anim. Reprod 5 (2): 56-59.

Deka, B.C. and Rao, A.R. (1984b). Changes in the morphology of buck spermatozoa before and after freezing. Indian J. Anim. Reprod. 5 (1): 124-126.

Deka, B.C. and Rao, A.R. (1985a). Effect of extenders on sperm motility and acrosomal integrity of frozen buck semen. Indian Vet. J. 62 (5): 414-417.

Deka, B.C. and Rao, AR. (1985b). Effect of glycerol and DMSO on sperm motility in frozen buck semen. Indian J. Anim. Reprod. 6 (2): 95-96.

Deka, B.C. and Rao, A.R. (1986a). Effect of egg yolk levels on quality of frozen buck semen. Indian Vet. J. 63 (11): 909-912.

Deka, B.C. and Rao, A.R. (1986b). Effect of glycerol level in Tris-based extender and equilibration period on quality of frozen goat semen. Theriogenology 26 (2): 231-238.

Deka, B.C. and Rao, A.R. (1986c). Effect of thawing method on quality of frozen goat semen. Indian J. Anim. Reprod. 7 (2): 68-69.

Deka, B.C. and Rao, A.R. (1987a). Effect of storage at -196°C on quality of goat semen with and without seminal plasma in Tris-based extender. Cheiron 16 (2): 65-69.

Deka, B.C. and Rao, A.R. (1987b). Effect of extenders and thawing methods on post-thawing preservation of goat semen. Indian Vet. J. **64** (7): 591-594.

Deka, B.C. and Rao, A.R. (1989) Effect of washing on the fertility of frozen goat semen. Indian Vet J. **66** (3): 268-269.

Deshpande, S.B. and Mehta, V.M. (1991). Effect of dilutors and different glycerol levels on pre-freeze and post-freeze sperm motility and live sperm count in Surti buck semen. Indian J. Anim. Sci. **61** (10): 1093-1095.

Deshpande, B.R., Kaikini, A.S. and Pargaonkar, D.R. (1982). Ovulation, fertilization, implantation, gestation, placenta and placentation. In : Sane, C.R.; Luktuke, S.N.; Deshpande, B.R.; Kaikini, A.S.; Velhankar, D.P.; Hukeri, V.B. and Kodagali, S.B. A text book reproduction in farm animals (Theriogenology). Varghese publishing house, Bombay. p.247.

*Dundar, Y., Tekin, N. and Altintas, A. (1983). Semen fructose and fructolysis and some chemical constituents in seminal plasma from Angora goats. Lalahan Zootekni Arastirma Enstitusu Dergisi 23 (3-4): 100-113. Cited in Anim. Breed. Abstr (1985) **53** (1): Abstr. 264.

Dunner, S. (1993). Freezing buck semen diluted in amine organic buffers. Anim. Prod. **56** (3): 387-391.

Eppleston, J., Salamon, S., Moore, N.W. and Evans, G. (1994). The depth of cervical insemination and site of intrauterine insemination and their relationship to the fertility of frozen - thawed ram semen. Anim. Reprod. Sci. **36** (3-4): 211-225.

*Fougner, J.A. (1974). Intra-Uterine method of inseminating goats with frozen semen. Norsk Veterinaertidsskrift **86** (11): 551-553. Cited in Anim. Breed. Abstr. (1975) **43** (11): Abstr. 5294.

Garcia, C.J., Padilla, R.G., De-Leon, T.M. and Martinez, C.A. (1994). Intrauterine artificial insemination through cervix in goats with synchronized oestrus. J. Reprod. Fertil. Abstr. Series (13): 49.

Garner, D.L. (1991). Artificial insemination. In: Cupps, P.T. (Editor), Reproduction in domestic animals. 4th Edn. Academic press, Inc, San Diego, California. p.263.

Gunsalus, I.C., Campbell, J.J.R., Beck, G.H. and Salisbury, G.W. (1944). The bacteriology of semen. II. The effect of bacteria upon rapid tests for semen quality. J. Dairy Sci. **27**: 357-364.

Hafez, E.S.E. (1993). Preservation and cryopreservation of gametes and embryos. In: Reproduction in farm animals. 6th Edn. Lea & Febiger, Philadelphia, p.517.

Haranath, G.B., Dutt, K.L. and Rao, Y.V.R. (1982). Freezing technique of buck and ram semen and study of its post-thaw survival rate at Indo-Swiss Project, Visakhapatnam. Livest. Adviser **7** (8): 49-53.

*Haunhorst, E. (1990). Deep freezing of goat semen, with reference to morphology of the head cap of spermatozoa and a computerized Videomicrography system for evaluating spermatozoal motility. Inaugural Dissertation, Fachbereich Veterinarmedizin, Fraie Universitat, Berlin, Germany (1990), p.121. Cited in Anim. Breed. Abstr. (1992) **60** (6): Abstr. 3752.

*Igboeli, G. (1974). A comparative study of the semen and seminal characteristics of two breeds of goat. East African Agric. Forest. J. **40** (2): 132-137. Cited in Anim. Breed. Abstr. (1976) **44** (3): Abstr. 1237.

*Iritani, A. and Nishikawa, Y. (1961). Studies on the egg yolk coagulating factor in goat semen. I. Properties of the coagulating factor and influential conditions for coagulation. In the proceedings for the Silver Jubilee, the Laboratory of Animal Husbandry, College of Agriculture, Kyoto University, March, 1961: 97-104. Cited in Anim. Breed. Abstr. (1964) **32** (3): Abstr. 2144.

*Iritani, A. and Nishikawa, Y. (1963). Studies on the egg yolk-coagulating factor in goat semen. III. Release of some acids accompanied by the coagulating phenomena. J. Anim. Reprod. 8: 109-112. Cited in Anim. Breed. Abstr. (1964) 32 (3): Abstr. 2145.

*Iritani, A., Nishikawa, Y. and Fukuhara, R. (1961). Studies on the egg yolk-coagulating factor in goat semen. I. Localisation of the coagulating factor and decline of pH following coagulation. In the proceedings for the Silver Jubilee: the Laboratory of Animal Husbandry, College of Agriculture, Kyoto University, March, 1961: 89-96. Cited in Anim. Breed. Abstr. (1964) 32 (3): Abstr, 2143.

Joseph, M. (1983). Effect of frequency of ejaculation on semen characteristics and libido in cross-bred bucks. M.V.Sc. Thesis. Kerala Agricultural University, Thrissur, Kerala.

*Knoblauch, H. (1962). Investigations on goat ejaculates. Zuchthyg. Fort Pftstor. Besam. Haustiere 6: 9-22. Cited in Anim. Breed. Abstr. (1963) 31 (2): Abstr. 1309.

*Kolk, J. (1985). Artificial insemination in goats - no problem with deep freezing and insemination under practical conditions. Praktische Tierarzt 66 (6): 490-492. Cited in Anim. Breed. Abstr. (1986) 54 (5): Abstr. 3053.

Kumaran, J.D.S. and Kumaran, J.S. (1968). Artificial insemination and animal reproduction. 2nd Edn. U.P. Agricultural University Press, Pantnagar, Nainital, India.

Kurian, N.I. and Raja, C.K.S.V. (1965). Studies on the semen characteristics of Malabari bucks. Kerala Vet. **4**: 131-133.

Kutty, C.I., Mathai, E. and Iyer, C.P.N. (1995). Metabolic rate of ejaculated buck spermatozoa. J. Vet. Anim. Sci. (under publication).

*Lawrenz, R. (1986). Artificial insemination of Angora and Boer goats with deep frozen semen. J. South African Vet. Ass. **57** (2): 109-111. Cited in Anim. Breed. Abstr. (1987) **55** (6): Abstr. 3666.

*Liess, J. and Ostrowski, J.E.B. (1960). Artificial insemination in goats using deep-frozen semen (-79°C). Dtsch. tierarztl. Wschr. **67**: 127-129. Cited in Anim. Breed. Abstr. **28** (4): Abstr. 2062.

Mahmood, S., Biswas, J.C. and Koul, G.L. (1988). A note on the semen quality of Cheghu and Changthangi bucks. Indian Vet. Med. J. **12** (3): 179-181.

*Mann, J (1980). Spermatological studies on West African dwarf bucks (Capra hircus L.) in the German Federal Republic. Thesis, Justus - Liebig - Universitat Giessen, German Federal Republic. pp.112. Cited in Anim. Breed. Abstr. (1981) **49** (5): Abstr. 2709.

Mann, T. and Mann, C.L. (1981). Biochemistry of spermatozoa: Chemical and Functional correlations in ejaculated semen, andrological aspects. In: Male reproductive function and semen. Springer - Verlag Berlin Heidelberg, New York. p.211.

Mareco, G. (1993). Transcervical intrauterine insemination in goats. Veterinaria Argentina **10** (97): 455-457.

Mathai, E. (1983). Artificial insemination crate for goats. Kalpadhenu **10**(5): 37.

Mathai, E. and Nair, B.R.K. (1981). Effect of age of semen on conception rate in goats. Kerala J. Vet. Sci. **12** (2): 220-223.

Mathai, E., Nair, B.R.K. and Iyer, C.P.N. (1980). A.I. in goats with special reference to the effects of season and phase of the moon on the (Oestrus) cycle. Kerala J. Vet. Sci **11** (1): 33-41.

Mathew, A. (1984). Principles and practice deep freezing bull semen. 2nd Edn. Indo-Swiss Project, Kerala, India.

*Melo, A.C.M. and Nunes, J.F. (1991). Use of coconut milk and milk-glucose as diluents for frozen goat semen. In Anais, IX Congresso Brasileiro de Reproducao Animal, Belo Horizonte, Brazil, 22 a 26 de Junho de 1991 Vol. II Belo Horizonte, Brazil; Colegio Brasileiro de Reproducao Animal : 435. Cited in Anim. Breed. Abstr. (1992) **60** (6): Abstr. 3757.

*Memon, M.A., Bretzlaff, K.N. and Ott, R.S. (1985). Effect of washing on motility and acrosome morphology of frozen-thawed goat spermatozoa. American J. Vet. Res. **46** (2): 473-475. Cited in Anim. Breed. Abstr. (1987) **55** (1): Abstr. 301.

*Mikus, M. and Pilko, P. (1975). Biology of reproduction in sheep. Vyskumny Ustav Ovciarsky V. Trencine : 57-65. Cited in Anim. Breed. Abstr. (1976) **44** (3): Abstr. 1217.

Misra, B.S. and Mukherjee, D.P. (1984). Breed differences in the characteristics of goat spermatozoa. Indian Vet. J. **61** (4): 315-322.

Mittal, J.P. (1976). A study on mortality of kids. Indian Vet. J. **53** (9): 681-684.

Mittal, J.P. (1986). Reproductive characteristics of indigenous and crossbred bucks maintained under desert conditions. Indian J. Anim. Sci. **56** (6): 688-692.

Mittal, J.P. (1987). Male reproductive characteristics of indigenous and crossbred goats under Indian arid zone. Indian J. Anim. Sci **57** (2): 158-161.

Mohan, G., Mazumder, N.K. and Goswami, K.K. (1980). Note on semen characteristics of Pashmina goats. Indian J. Anim. Sci. **50** (10): 898-900.

Mohanty, D.N., Biswas, R.K. and Dugwekar, Y.G. (1985). Cold shock resistance test as an indicator of spermatozoan freezability in Holstein-Friesian bulls. Indian J. Anim. Sci. **55** (2): 107-109.

*Moore, R.W., Dow, B.W. and Staples, L.D. (1989). Artificial insemination of farmed feral goats with frozen - thawed semen. Proceedings of the New Zealand Society of Animal Production **49**: 171-173. Cited in Anim. Breed. Abstr. (1990) **58** (12): Abstr. 7990.

*Mylrea, G.E., Evans, G. and English, A.W. (1991). Conception rates in European fallow does (Dama dama dama) following intrauterine insemination with frozen - thawed semen from mesopotamian fallow (Dama dama mesopotamica) and crossbred (Dama dama dama x Dama dama mesopotamica) bucks. Australian Vet. J. **68** (9): 294-295. Cited in Anim. Breed. Abstr. **60** (3): Abstr. 1780.

Nair, B.R.K. and Mathai, E. (1979). Effects of certain genetic and non-genetic factors on the secondary sex ratio in goats. Kerala J. Vet. Sci. **10**: 1-8.

*Paggi (1971). Artificial insemination in goats. XIV. Experiments on the deep freezing of buck semen. Vet. Med. Dissertation, Tierarztl. Hochsch, Hanover: 51. Cited in Anim. Breed. Abstr. **39**: Abstr. 4828.

Pandey, R.P., Sinha, S.N., Singh, B. and Akhtar, M.H. (1985). Characteristics of semen and fertility rate in Saanen and Barbari bucks. Indian J. Anim. Sci. **55** (9): 773-774.

*Park, C.S., Yang, M.H., Hwang, D.S., Lee, K.S. and Seo, K.W. (1989). Study on fresh and deep-frozen storage of Korean native goat spermatozoa Korean J. Anim. Sci. **31** (7): 412-419. Cited in Anim. Breed. Abstr. **57** (12): Abstr. 7890.

Patil, R.V. (1970). Studies on goat semen. M.Sc. Thesis, University of Calicut, Kerala.

Pattnaik, U.C., Mohanty, B.N., Ray, S.K.H., Mohanty, D.N. and Panda, G.M. (1991). A note on the semen characteristics of Ganjam bucks. Indian J. Anim. Reprod. **12** (2): 151.

*Perez, J.V. (1985). Importance of various semen treatments used in the freezing of goat semen. Thesis, Tierarztliche Hochschule, Hannover: 108. Cited in Anim. Breed. Abstr. (1988) **56** (3): Abstr. 1491.

Perry, E.J. (1969). The artificial insemination of farm animals. 4th Edn. Oxford and IBH Publishing Company, Calcutta. pp.114-116, 218-219.

Polge, C., Smith, A.U. and Parkes, A.S. (1949). Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature 164: 666. In: Salisbury, G.W. and Van Demark, N.L. (1961). Physiology of reproduction and artificial insemination of cattle. W.H. Freeman & Company, San Francisco. p.27.

Purohit, J.R., Bhorekar, M.R. and Mangurkar, B.R. (1992). Studies on deep freezing of buck semen. V International Conference on Goats, New Delhi, 2-8 March, 1992. Pre-Conference Proceedings Abstractsp.290.

Raja, C.A.R. and Mukundan, G. (1973). Age at first kidding, kidding rate and kidding interval in Malabari and Jamnapari-Malabari crossbreds. Kerala J. Vet. Sci. **4** (2): 165-169.

*Ritar, A.J. and Salamon, S. (1982). Effects of seminal plasma and of its removal and of egg yolk in the diluent on the survival of fresh and frozen-thawed spermatozoa of the Angora goats. Australian J. Biol. Sci. **35** (3): 305-312. Cited in Anim. Breed. Abstr. (1983) **51** (2): Abstr. 968.

Ritar, A.J., Ball, P.D. and O'May, P.J. (1990a). Examination of methods for the deep freezing of goat semen. Reprod. Fertil. Develop. **2** (1): 27-34.

*Ritar, A.J., Ball, P.D. and O'May, P.J. (1990b). Artificial insemination of Cashmere goats; effect on fertility and fecundity of intravaginal treatment, method and time of insemination, semen freezing process, number of motile spermatozoa and age of females. Reprod. Fertil. Develop. **2** (4): 377-384. Cited in Anim. Breed. Abstr. **58** (11): Abstr. 7369.

Roberts, S.J. (1971). Veterinary obstetrics and genital diseases. 2nd Edn. Scientific Book Agency, Calcutta. pp.83-84, 709-711.

Roy, A., Datta, I.C., Sahani, K.L., Singh, B. and Sen Gupta, B.P. (1962). Studies on certain aspects of sheep and goat husbandry. Artificial breeding and reproductive phenomena in sheep and goats. Indian J. Vet. Sci. 32: 269-275.

*Sacker, G.D. and Trail, J.C.M. (1966). Production characteristics of a herd of East African Mubende goats. Trop. Agric. Trin. 43: 43-51. Cited in Anim. Breed. Abstr. 34 (2): Abstr. 1338.

Sahni, K.L. and Roy, A. (1972). A study on the effect of deep-freezing (-79°C) on post thawing revival of sheep and goat spermatozoa. Indian J. Anim. Sci. 42 (2). 102-105.

Salamon, S. and Lightfoot, R.J. (1967). Effect of cold shock, liquid storage and pellet freezing on successive ramejaculates. Australian J. Agric. Res. 18: 959-972. In: Joseph, M. (1983). "Effect of frequency of ejaculation on semen characteristics and libido in cross-bred bucks". M.V.Sc. Thesis. Kerala Agricultural University, Thrissur.

*Salamon, S. and Ritar, A.J. (1982). Deep freezing of Angora goat semen: effects of diluent composition and method and rate of dilution on survival of spermatozoa. Australian J. Biol. Sci. 35 (3): 295-303. Cited in Anim. Breed. Abstr. (1983) 51 (2): Abstr. 969.

Salisbury, G.W. and Van Demark, N.L. (1961). Principles and techniques of spermatozoan preservation. In: Physiology of reproduction and artificial insemination of cattle. W.H Freeman & Company, San Francisco, pp.383-384.

Salisbury, G.W., Van Demark, N.L. and Lodge, J.R. (1978). Physiology of reproduction and artificial insemination. 2nd Edn. W.H. Freeman and Company, San Francisco.

*Samouilidis, S., Foukos, A. and Tsakalof, P. (1982). The fertility of buck semen after an 11-year storage in liquid nitrogen (-196°C). Bulletin Hellenic Vet. Med. Society. **33** (1): 53-55. Cited in Anim. Breed. Abstr. (1983) **51** (7): Abstr. 4397.

Sarmah, A.K. (1983). Preliminary trials on preservation of buck semen in glycerol containing diluents. M.V.Sc. Thesis. Kerala Agricultural University, Thrissur, Kerala.

Saxena, V.B. and Tripathi, S.S (1980). Note on the physico-chemical and morphological attributes of semen of Jamnapari bucks. Indian J. Anim. Sci. **50** (9): 775-777.

Seth, O.N., Saraswath, K.C., Chorey, P.A., Nathani, S.P. and Roy, A. (1968). The effect of alteration in management practices on kidding percentages and lactational performance of Barbari goats and the survival of new born kids. Indian J. vet. Sci. **38** (1): 93-100.

*Sevinc, A., Tekin, N., Yurdaydin, N. and Kale, N (1985). Sperm characteristics of male Angora goats reared at Cifteler State Farm. Veterinerlik Ve Hayvancilik **9** (3): 264-273. Cited in Anim. Breed. Abstr. (1986) **54** (1): Abstr. 334.

Sharma, M.L., Mohan, G.; Benjamin, B.R. and Sahni, K.L. (1990). The cold shock resistance test as an indicator of post thaw recovery rates among Friesians and Triple crossbred bulls. Indian Vet. med. J. **14** (3): 170-172.

Singh, M.P., Sinha, S.N. and Singh, B. (1982). Semen characteristics of Jamunapari and Barbari bucks. Indian Vet. med. J. **6** (1): 41-43.

Singh, D.K., Sinha, M.P., Singh, R.A., Singh, C.S.P. and Singh, K.K. (1985). Comparative studies on seminal quality of pure and crossbred bucks. Indian Vet. Med. J. **9** (1): 56-58.

Sinha, M.P. and Singh, B.K. (1982). Studies on the semen characteristics of Black Bengal and Saanen bucks. Indian Vet. Med. J. **6** (4): 253-257.

Sinha, M.P. and Singh, B.K. (1984). Keeping quality of heterospermic buck semen and its influence on conception rate. Indian J. Anim. Sci **54** (3): 278-280.

Sinha, M.P., Singh, B. and Singh, B.K. (1983). Effect of heterospermy on preservation of semen and conception rate in goats. Indian Vet. Med. J. **7** (3): 168-170.

Sinha, S.N., Singh, B.K. and Sinha, A.K. (1987). Post-thaw motility and fertility of frozen sperms of bucks of different breeds. Indian J. Anim. Reprod. 8 (1): 28-31.

Sinha, S., Deka, B.C. and Tamuli, M.K. (1991). Effect of extenders on quality of frozen goat semen. Indian J. Anim. Reprod. 12 (2): 146-148.

Sinha, S., Deka, B.C, Borgohain, B.N. and Tamuli, M.K. (1992a). Study on freezing of goat semen in skim milk extenders with different glycerol levels and equilibration periods. Indian J. Anim. Reprod. 13 (1): 38-41.

Sinha, S., Deka, B.C., Tamuli, M.K. and Borgohain, B.N. (1992b). Effect of equilibration period and glycerol level in Tris extender on quality of frozen goat semen. Indian Vet. J. 69 (12): 1107-1110.

Sinha, S., Deka, B.C., Borgohain, B.N and Bhuyan, D. (1993). Study of freezing of goat semen in egg yolk citrate extender with three glycerol levels and equilibration periods. Indian Vet Med. J. 17 (3-4): 150-153.

Sinha, M.P., Sinha, A.K. and Singh, B.K. (1994). Effect of caffeine on motility and fertility of frozen goat semen. Indian J. Anim. Reprod. 15 (1): 53-56.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. 6th Edn. Oxford and IBH publishing company, Calcutta.

*Stagnaro, G.G. (1975). Artificial insemination of goats with frozen semen. Zootechnia **24** (3-4): 151-163. Cited in Anim. Breed. Abstr. **43** (11): Abstr. 5295.

Sudarsanan, V. and Raja, C.K.S.V. (1973). Observation on conception, gestation period, multiple birth and infertility in Malabari goats. Kerala J. Vet. Sci. **4**: 96-98.

*Summermatter, P. (1986). Position of goat insemination Kleinviehzuchter **34** (4): 113-116. Cited in Anim. Breed. Abstr. (1988) **56** (3): Abstr. 1494.

*Sungur, H., Goncagul, T. and Ozsar, S. (1993). Artificial insemination of Angora goats with frozen and fresh semen and control of fertility. Lalahan Hayvancilik Arastirma Enstitusu Dergisi **33** (1-2): 59-64. Cited in Anim. Breed. Abstr. **62** (2): Abstr. 953.

Tewari, R.B.L., Abbhi, H.L., Sengupta, B.P. and Roy, A. (1969). A note on the possibility of using 'Cold shock resistance' test as a dependable index for preliminary screening and grading of A.I. bulls, under field conditions. Indian Vet. J. **46** (5): 424-429.

Tiwari, S.B. and Bhattacharyya, N.K. (1987). A successful protocol on deep freezing of buck semen. Central Institute for research on goats, Makhdoom, Mathura. p.5

*Torre, M., Herak, M., Sukalic, M., Mihajlovic, R., Premzl, B., Simic, H., Selanec, S. and Dejanovic, D. (1990). Artificial insemination of goats in season using deep frozen semen. Veterinarska Stanica 21 (4): 257-262. Cited in Anim. Breed. Abstr. (1993) **61** (6): Abstr. 3164.

Tuli, R.K. and Holtz, W. (1994). Effect of glycerolisation procedure and removal of seminal plasma on post-thaw survival and GOT-release from Boer goat spermatozoa. Theriogenology **42** (3): 547-555.

*Valdes Leal, J.F. (1984). Some reproductive characters in goats and efficiency of artificial insemination using frozen semen Advances en Production Animal **9** (1-2): 206-207. Cited in Anim. Breed. Abstr. (1986) **54** (7): Abstr. 4530.

*Westhuysen, J.M., van der (1978). Observations on the deep-freezing of Angora goat semen. South African J. Anim. Sci. **8** (2): 111-113. Cited in Anim. Breed. Abstr. (1979) **47** (10): Abstr. 5548.

*Wuschko, S.and Seifert, H. (1991). Investigations on semen quality and semen freezing in West African goats. Reihe Agrarwissenschaften **40** (1): 113-115. Cited in Anim. Breed. Abstr. (1992) **60** (8): Abstr. 5144.

Zanwar, S.G., Agarwal, S.K. and Totey, S.M. (1984). Fertility of cross-bred ewes inseminated with frozen semen. Indian Vet. J. **61** (9): 786-788.

*Zhang, J.C., Liu, J.R., Lu, Y.Z. and Yang, H.L. (1985). An experiment on the use of frozen semen in goats. Chinese J. Anim. Sci. (5): 14-16. Cited in Anim. Breed. Abstr. (1986) **54** (8): Abstr. 5304.

*Zheltobryukh, N.A. and Ashurbegov, K.K. (1984). Freezing of goat semen in sugar-egg yolk media. Referativnyi Zhurnal **58** (5): 462. Cited in Anim. Breed. Abstr. **52** (8): Abstr. 4749.

*Original not consulted

EFFECT OF DIFFERENT GLYCEROL CONCENTRATIONS ON FREEZING OF BUCK SEMEN

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the
requirement for the degree

Master of Veterinary Science

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Mannuthy, Thrissur

1995

ABSTRACT

With the object of studying the effect of different concentrations of glycerol on post-thaw motility and fertility of frozen buck semen, five Malabari crossbred (Alpine x Malabari) bucks maintained at Artificial Insemination Centre and 75 does in heat, brought to the A.I. centre, College of Veterinary and Animal Sciences, Mannuthy, Thrissur were used.

The average volume of semen was 0.71 ± 0.02 ml. Semen volume varied significantly between bucks. The average colour index was creamy, density was DDDD and the mass activity was ++++. The average pH of semen was 6.85 ± 0.01. The average sperm motility percentage was 85.83 ± 1.05. Motility percentage varied significantly between bucks. The average sperm concentration was 2842.33 ± 153.93 millions/ml. Highly significant difference in sperm concentration was noted between bucks. The average live sperm percentage was 91.03 ± 0.56. There was highly significant difference in live sperm percentage between bucks.

Significantly higher percentage of sperm motility was noted in spermatozoa with seminal plasma than in spermatozoa without seminal plasma at 60 minutes of incubation. Slight increase in motility percentage was noted for spermatozoa with and without seminal plasma at 10 minutes of incubation. From 10 minutes to 60 minutes motility was gradually decreasing.

Between bucks there was highly significant difference in sperm motility of incubated spermatozoa.

Live sperm percentage was significantly higher for spermatozoa without seminal plasma at all time periods of cold shock. There was a rapid reduction in live sperm percentage at five minutes of cold shock. The live sperm percentages between bucks were significant at different time periods except at 10 minutes and 30 minutes of cold shock.

The average time taken for reduction of methylene blue by spermatozoa with and without seminal plasma were 173.16 ± 8.77seconds and 197.00 ± 9.97 seconds respectively. Significant difference was noted in methylene blue reduction time between bucks.

Average percentage sperm motility after washing was 76.71 ± 0.79. Maximum percentage motility after glycerolisation was obtained in six per cent glycerolated semen (67.85 ± 1.39). There was highly significant reduction in motility percentage after glycerolisation. Maximum post-thaw motility was obtained in six per cent glycerolated extender (42.00 ± 1.84). Highly significant reduction in motility percentage was noted after freezing. The conception percentage and kidding percentage were 47.36 and 43.85 respectively. Average gestation length was 149.85 ± 4.45 days. The number of kids per kidding averaged 1.7. Percentage of male and female kidsborn were 51.16 and 48.83 respectively.

From this study it could be inferred that Tris extender with six per cent glycerol was superior to Tris extender with four per cent, five per cent or seven per cent glycerol for better post-thaw motility and fertility of frozen buck semen.