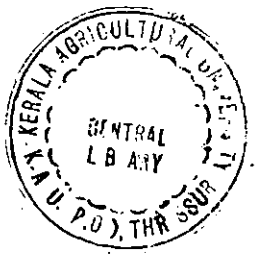
FACTORS AFFECTING CONCEPTION RATE ON ARTIFICIAL INSEMINATION IN GOATS



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Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
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DECLARATION

I hereby declare that the thesis entitled **"FACTORS AFFECTING CONCEPTION RATE ON ARTIFICIAL INSEMINATION IN GOATS"** 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, associate ship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled **"FACTORS AFFECTING CONCEPTION RATE ON ARTIFICIAL INSEMINATION IN GOATS"** is a record of research work done independently by **Ramya Rajan V.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associate ship to her.

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We, the undersigned members of the Advisory Committee of Ramya Rajan V., a candidate for the degree of Master of Veterinary Science in Animal Reproduction, agree that the thesis entitled **"FACTORS AFFECTING CONCEPTION RATE ON ARTIFICIAL INSEMINATION IN GOATS"** may be submitted by **Ramya Rajan V.**, in partial fulfillment of the requirement for the degree.

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Ramya Rajan V.

Dedicated to

my beloved Parents

and

Husband

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Introduction

1. INTRODUCTION

India ranks second among the countries of the world in goat population with 20 distinct breeds of goat. According to 2003 census goat population in India and Kerala are 12.51 million and 12.13 lakhs respectively. Kerala has its own native breeds like Malabari and Attapadi black. Recently cross breeding with Jamunapari, Saanen, Boer and Alpine are also becoming popular among farmers due to increased demand for meat and milk.

Goat popularly known as the "Poor man's cow" plays a significant role in the rural economy of Kerala State. Since goats can be easily managed by women in the households, backyard goat husbandry is popular in Kerala. Along with this, on account of their short generation interval and higher rates of prolificacy goat rearing is becoming a major source of income among village people.

One of the major drawbacks in improving the local stock of goats is the lack of effective methods for large scale scientific breeding with sires of improved genetic potential. Semen from superior bucks can be preserved for short term by chilling and long term by freezing. The large scale propagation of germplasm from proven bucks using liquid semen is not easy, especially in a population of goats spread over a wide area. The effective remedy for this problem is to standardise the various aspects of frozen semen technology in goats and adoption of this technique in an extensive way.

Artificial insemination is the most widely used assisted reproductive technology and the one that has made the most significant contribution to genetic improvement of livestock world wide. Combined with an appropriate system for sire evaluation, AI offers a relatively simple and low cost method for dissemination of valuable genes. (Baldassarre and Karatzas, 2004).

The success of an AI programme depends on the proper management of semen collection, storage and use. There is detrimental interaction between buck seminal plasma and the preservation media due to the presence of certain components in

bulbourethral gland secretion. Efficient processing methods and diluents have to be elaborated for storage of goat semen (Leboeuf *et al.*, 2000).

The conception rate of inseminated goats is highly variable under field and farm conditions due to various factors. Mathai and Nair (1981) reported that conception rate decreases when semen was stored beyond 24 hours. According to Prasanth and Mathai (1996), there was significant reduction in motility percentage after freezing due to the death of weak spermatozoa from thermal shock and internal ice formation which resulted in reduced conception rate. Ritar and Salamon (1983) opined that there was improvement in fertility rates with increasing depth of insemination. In order to achieve satisfactory fertility, it is necessary to inseminate close to the time of ovulation (Leboeuf *et al.* 2000). No systematic study has been undertaken on conception rate on AI in goats under field conditions. Hence the present study is undertaken with the following objectives.

1. To assess the effect of duration of storage of liquid semen on conception rate.

2. To compare the fertility of frozen semen with that of liquid semen.

3. To assess the effect of intensity of heat signs on conception rate.

4. To assess the effect of depth of penetration of the artificial insemination gun into the cervix during AI on conception rate.

Review of Literature

2. REVIEW OF LITERATURE

2.1 SEMEN EVALUATION

2.1.1 Volume

Knoblauch (1962) recorded an average ejaculate volume of 1.0 ml in White German improved bucks. The volume of semen per ejaculate was found to vary from 0.2 ml to 1.2 ml with a mean of 0.5 ± 0.13 ml in Malabari bucks (Patil and Raja, 1978). Cetinkaya *et al.* (1980) reported an average ejaculate volume of 0.98 ml in Angora goats.

The average ejaculate volume was reported to be 0.94 ± 0.11 in Angora goats (Dundar *et al.*, 1983). The average ejaculate volume was found to be 0.955 ± 0.049 ml in Sannen and 0.788 ± 0.047 ml in Barbari bucks (Pandey *et al.*, 1985). Sevinc *et al.* (1985) recorded an average ejaculate volume of 1.07 ± 0.10 ml in Angora bucks. The average ejaculate volume was reported to be 0.70 ± 0.022, 0.72 ± 0.032 and 0.69 ± 0.024 ml in Sannen, Barbari and Sannen × Barbari bucks respectively (Prasad *et al.*, 1986).

Pattnaik *et al.* (1991) recorded an average ejaculate volume of 0.94 ± 0.24 ml in Ganjam bucks. Wuschko and Seifert (1991) reported an average ejaculate volume of 0.73 ± 0.28 ml for adult and 0.42 ± 0.22 ml for young West African bucks. Tuli and Holtz (1992) studied the semen characteristics of Boer bucks in different seasons of the year and found that ejaculate volume did not differ much between summer (1.79 ± 0.15 ml) and winter (1.77 ± 0.14 ml) months.

The average ejaculate volume was 0.66, 0.57 and 0.58 ml for Alpine, Anglo – Nubian and Caninde bucks respectively (Filho *et al.*, 1993). Prasanth (1995) recorded an average ejaculate volume of 0.71 ± 0.02 ml in Malabari crossbred bucks. Singh and Nasir (1995) reported an average ejaculate volume of 1.38 ± 0.05 ml in Sirohi bucks. The mean ejaculate volume for local and crossbred rams raised in the United Arab Emirates was 0.59 and 0.90 ml, respectively (Saleh , 1997).

The average volume of semen was reported to be 1.33 ± 0.07 ml for Malabari crossbred bucks (Ranjini, 1998).

Kutty and Mathew (2000) reported an average ejaculate volume of 0.75 ml both in Malabari and Alpine × Malabari bucks. Srinivas *et al.* (2002) studied the effect of season on semen volume of native bucks of Andhra Pradesh and reported the lowest volume (0.49 ± 0.03 ml) of semen in summer season and highest volume (0.76 ± 0.03 ml) in winter season. Afsal (2003) recorded an average ejaculate volume of 1.27 ± 0.06 ml in Boer bucks. The average ejaculate volume was reported to be 1.20 ± 0.04 ml in Florida bucks (Dorado *et al.*, 2009).

2.1.2 Colour

Knoblauch (1962) recorded ivory coloured ejaculate with creamy consistency for White German improved bucks. Colour of the seminal ejaculates of Saanen bucks was creamy, whereas those of Barbari bucks varied from yellowish to creamy (Pandey *et al.*, 1985).

Colour of ejaculate of Malabari crossbred buck was found to be creamy (Prasanth, 1995; Ranjini, 1998 and Afsal, 2003). Kutty and Mathew (2000) reported that the colour of ejaculate of Malabari and it's Alpine cross varied from yellowish white to deep yellow. The intensity of yellow colour was more during winter season and lesser during summer season.

2.1.3 Density

A semen density of 3.3611 ± 0.3438 was reported in Alpine Malabari crossbred bucks (Joseph, 1983). Density of semen from Malabari crossbred bucks was reported as DDDD (Prasanth, 1995; Ranjini, 1998 and Afsal, 2003).

Kutty and Mathew (2000) reported that density of semen from Malabari and Alpine – Malabari crossbred bucks was less during winter and more during summer (3.39 Vs 3.61). It was further recorded that density was more during long day period than short day (3.53 Vs 3.40).

2.1.4 Mass activity

The average mass activity of semen of Saanen, Barbari and Saanen × Barbari bucks was reported to be 4.16 ± 0.075, 4.35 ± 0.06 and 4.18 ± 0.068 respectively (Prasad *et al.*, 1986). Pattnaik *et al.* (1991) reported that the mass activity of semen from Ganjam bucks was 4.12 ± 0.05. The mass activity of semen from Malabari crossbred bucks was reported to be ++++ (Prasanth, 1995 and Ranjini, 1998). The average mass activity of semen of Malabari and Alpine × Malabari bucks was reported to be 3.31 (Kutty and Mathew, 2000). The average mass activity was found to be 4.46 ± 0.06 and 4.36 ± 0.08 in winter and summer seasons respectively (Srinivas *et al.*, 2002).

2.1.5 Hydrogen ion concentration

Normal semen pH was reported to be 6.6 for White German improved bucks (Knoblauch, 1962). The average pH value of Malabari buck semen was found to be 6.47 ± 0.16 (Patil and Raja, 1978). Dundar *et al.* (1983) recorded an average pH of 6.685 ± 0.05 in Angora goats. Sevinc *et al.* (1985) reported that the semen pH averaged 6.68 ± 0.17 in Angora goats. Prasad *et al.* (1986) recorded an average pH of 6.60 ± 0.002 in Saanen, 6.78 ± 0.002 in Barbari and 6.68 ± 0.002 in Saanen × Barbari bucks.

The average semen pH was reported to be 6.47 ± 0.02 for Sirohi bucks (Singh and Nasir, 1995). The average semen pH was reported to be 6.85 ± 0.01 (Prasanth, 1995) and 7.275 ± 0.040 (Ranjini, 1998) in Malabari crossbred bucks. Kutty and Mathew (2000) reported that the semen pH of Alpine × Malabari crossbred buck was lowest during spring season and highest during monsoon season. The pH obtained was 6.16 during spring and 6.39 during monsoon. Afsal (2003) reported that the average pH value of Boer goat semen was 6.98 ± 0.03.

2.1.6 Sperm concentration

Patil and Raja (1978) studied the characteristics of Malabari goat semen and found that the sperm concentration was in the range of 1100 to 7490 millions per ml

with an average of 3534 ± 176.1 millions per ml. The sperm concentration of Angora goat semen was in a range of 1660 to 5200 millions per ml with an average of 3674 millions per ml (Cetinkaya *et al.*, 1980). The average sperm concentration of semen was 4503.256 ± 114.75 millions per ml in Saanen goats and 2792.445 ± 80.52 millions per ml in Barbari goats (Pandey *et al.*,1985).

Sevinc *et al.* (1985) reported an average sperm concentration of 3110 millions per ml in Angora goats. Prasad *et al.* (1986) recorded the spermatozoan concentration of Saanen, Barbari and their crossbreds to be 2820.96 ± 7.43, 2117.65 ± 32.45 and 2375.47 ± 7.10 millions per ml respectively. Sperm concentration was reported to be 2309.17 ± 95.89 millions per ml in Ganjam bucks (Pattnaik *et al.*, 1991).

The average sperm concentration of Malabari crossbred buck semen was 2842.33 ± 153.93 millions per ml (Prasanth, 1995). The normal sperm concentration of Sirohi bucks was reported to be 2348.69 ± 47.10 millions per ml (Singh and Nasir, 1995). Ranjini (1998) reported an average sperm concentration of 2972 ± 293 millions per ml in Malabari crossbred bucks.

Kutty and Mathew (2000) studied the effect of season on sperm quality and found that the sperm concentration did not vary significantly between different seasons of the year. These authors recorded maximum concentration in summer (3133 millions per ml) and minimum in post monsoon season (2105 millions per ml). Semen concentration of native goats of Andhra Pradesh was reported to be 2450 ± 0.31 millions per ml in summer and 2350 ± 0.02 millions per ml in winter (Srinivas *et al.*, 2002). Afsal (2003) reported an average sperm concentration of 2956.67 ± 81.74 millions per ml in Boer bucks. The sperm concentration of Florida buck semen was reported to be 3690 ± 0.08 millions per ml (Dorado *et al.*, 2009).

2.1.7 Initial motility

Normal percentage of sperm motility was reported to be 80 in White German improved bucks (Knoblauch, 1962). The initial motility percentage of spermatozoa in Malabari goats was found to be in the range of 40 and 85 with a mean of 66.14 ± 1.34

(Patil and Raja, 1978). An average sperm motility of 86 per cent was reported in Angora goats (Cetinkaya *et al.,* 1980).

Sevinc *et al.* (1985) reported an average sperm motility of 83.49 ± 3.63 per cent in Angora goats. The initial motility was found to be 75 to 80 per cent in Ganjam bucks (Pattnaik *et al.,* 1991) and 71± 10 per cent in West African dwarf goats (Wuschko and Seifert, 1991).

Filho *et al.* (1993) reported an average sperm motility of 50.7, 56.8 and 63.2 per cent in Alpine, Anglo – Nubian and Caninde bucks respectively. The overall average percentage of sperm motility in Malabari crossbred bucks was 85.83 ± 1.05 (Prasanth, 1995). Singh and Nasir (1995) recorded an average initial motility of 72.66 ± 1.72 per cent in Sirohi bucks.

The average percentage of individual sperm motility was observed to be 81.78 ± 0.53 and 78.06 ± 0.68 in winter and summer seasons respectively (Srinivas *et al.,* 2002). Zamfirescu and Nadolu (2007) reported that normal percentage of progressively motile sperms was 86.81 ± 0.88 and 85.0 ± 2.24 per cent in Alpine and Saanen goats respectively.

2.1.8 Sperm viability

The normal live sperm percentage was reported to be 80 in White German improved goats (Knoblauch, 1962). Patil and Raja (1978) reported a live sperm percentage of 63.38 ± 2.58 in Malabari goats with a range of 22 to 88 per cent. Normal live spermatozoan count was reported to be 83.227 per cent in Saanen and 71.443 per cent in Barbari breeds of goats (Pandey *et al.*, 1985).

The percentage of live sperm was reported to be 91.13 ± 0.986 for the semen of Saanen breed of goat, 92.53 ± 0.291 for Barbari goats and 91.07 ± 0.544 for Saanen × Barbari crossbred animals (Prasad *et al.*, 1986). Pattnaik *et al.* (1991) reported a live sperm percentage of 84.83 ±1.02 in Ganjam bucks. The percentage of live spermatozoa of Boer buck semen was found to be 75.08 ± 2.53 in summer and 73.05 ± 3.10 in winter.

The average live sperm percentage in fresh semen of Malabari crossbred buck was reported to be 91.03 ± 0.56 (Prasanth, 1995). Normal live sperm percentage of Sirohi buck semen was reported to be 84.80 ± 1.05 (Singh and Nasir, 1995). An average live sperm percentage of 90.025 ± 0.801 (Ranjini, 1998) and 94.69 ± 0.67 (Simon, 1999) was reported in Malabari crossbred bucks.

Live spermatozoa percentage in native goats of Andhra Pradesh was reported to be 87.05 ± 0.62 in winter and 81.11 ± 0.51 in summer season (Srinivas *et al.*, 2002). Afsal (2003) reported an average live sperm percentage of 88.16 ± 0.50 in Boer bucks.

2.1.9 Sperm abnormality

The average abnormal count of semen was reported to be 4.34 ± 0.48 per cent in Malabari bucks (Patil and Raja, 1978) and 2.3 per cent in Angora bucks (Cetinkaya *et al.*, 1980). According to Sevinc *et al.* (1985) average abnormal count of spermatozoa in Angora goats was 2.06 ± 0.42 per cent. The percentage of abnormal spermatozoa in fresh semen was recorded as 7.54 ± 0.449 in Saanen, 5.94 ± 0.252 in Barbari and 7.63 ± 0.442 in Saanen × Barbari crossbred bucks (Prasad *et al.*, 1986).

Pattnaik *et al.* (1991) reported that the fresh semen of Ganjam bucks showed 5.42 ± 0.31 per cent abnormalities. According to Filho *et al.* (1993) the incidence of sperm abnormalities was 15.14 and 10.95 per cent for Anglo – Nubian and Caninde goats respectively. The average abnormal count of semen was reported to be 3.050 ± 0.245 per cent (Ranjini, 1998) and 1.31 ± 0.67 (Simon, 1999) in Malabari crossbred bucks. The percentage of abnormal spermatozoa in native goats of Andhra Pradesh was 8.98 ± 0.23 in summer and 9.28 ± 0.20 in winter (Srinivas *et al.*,2002). The mean percentage of abnormal spermatozoa was reported to be 3.20 ± 0.27 in Boer goats (Afsal, 2003).

2.1.10 Acrosome morphology

2.1.10.1 Acrosome staining

Acrosomal structure of ram spermatozoa was prominently stained when air dried smears of diluted semen was fixed for 15 minutes in buffered formol saline and stained for 90 minutes in six per cent buffered solution of Giemsa stain (Watson, 1975). The stained acrosomes were further classified as intact, damaged and entirely lost ones.

Benjamin (1985) compared various staining methods for acrosome and classified acrosomal abnormalities in to four categories viz., knobbed, incomplete, ruffled and abnormal nuclear cap.

Tamuli and Watson (1994) combined the two common staining techniques, Nigrosin-Eosin and Giemsa to distinguish acrosomal change in live sperm sub population and reported that this novel staining technique was capable of determining four categories of spermatozoa, live-acrosome intact, live-acrosome damaged, dead acrosome intact and dead acrosome damaged.

Kutty *et al.* (1996) reported a more simplified Nigrosin-Eosin-Giemsa staining technique to distinguish acrosome damaged live and dead sperms. The staining technique required very short time and no fixative was needed.

2.1.10.2 Acrosome integrity

The average percentage of intact acrosomes in the fresh semen of Malabari crossbred bucks was reported to be 91.175 ± 0.636. It was also reported that the percentage of swollen, ruffled and entirely lost acrosome in fresh semen were 3.925, 3.300 and 1.600 respectively (Ranjini, 1998). The percentage of acrosome abnormality was found to be 0.70 ± 0.15 in fresh Malabari crossbred buck semen (Simon, 1999).

Srinivas *et al.* (2002) recorded that in native goats of Andhra Pradesh in summer 72.68 ± 0.97 per cent of spermatozoa and in winter 77.26 ± 0.76 per cent of spermatozoa had intact acrosome. Mean percentage of spermatozoa with abnormal acrosome in the fresh semen of Boer bucks was 1.00 ± 0.13 (Afsal, 2003).

2.2 PRESERVATION OF SEMEN

2.2.1 Refrigeration storage of semen

2.2.1.1 Diluents

Deka and Rao (1980) observed Tris egg yolk glucose and milk extenders to be superior to egg yolk citrate extender in maintaining sperm motility on preservation up to 72 hours.

Mathew *et al.* (1984) observed that Tris egg yolk diluent containing 20 and 25 per cent eggyolk with a molality of 0.2 and pH of 7 were superior for preservation of spermatozoa at 3 - 5°C and Tris diluent with 5 per cent egg yolk with pH 7.0 was superior at 6 to 8°C.

Roca *et al.* (1997) reported that unwashed Murciano-Granadina spermatozoa could be usefully preserved at 5°C after dilution in Tris-based extender with 2 per cent egg yolk.

2.2.1.2 Dilution rate

Although greater extension of ram or buck semen was possible, rates of 1:1 to 1:10 were most practical to avoid a severe decline in fertility due to a rapid loss in motility (Roberts, 1971).

Paulenz *et al.* (2005) proposed a dilution rate in such a way that each insemination dose contained 200 $\times 10^6$ motile spermatozoa after dilution.

Dilution rate was fixed in such a way that minimum safe numbers of motile spermatozoa (in million) for AI in goats was 100 and 150 respectively for fresh and liquid stored semen by cervical insemination (Nuti, 2007).

2.2.1.3 Packaging of semen

Plastic vials of various sizes with plastic caps were used for storage of extended semen (Roberts, 1971). Plastic straws with a volume of 0.25 or 0.5 ml were used for packaging of semen (Paulenz *et al.*, 2005).

2.2.1.4 Motility

Balakrishnan (1979) reported an average motility of 75.39, 62.97, 49.90, 37.42 and 19.30 per cent after 24, 48, 72, 96 and 120 hours of storage respectively at 5°C. Singh *et al.* (1982) reported that the percentage of motility were 76.60 ± 3.48, 53.60 ± 2.81, 41.90 ± 0.96, 29.50 ± 1.01 and 13.30 ± 0.78 in Tris yolk glycerol diluent at 0, 24, 48, 72 and 96 hours of preservation respectively.

According to Sarmah (1983) the motility of buck semen preserved under refrigeration temperature were 79.63, 73.75 and 65.50 per cent respectively at 0, 24 and 72 hours of preservation with Tris yolk diluent. Puranik *et al.* (1994) suggested that Tris-fructose-citric acid was a good diluent with 52 per cent sperm motility at 72 hours of preservation.

Simon (1999) reported that the mean sperm motility of Malabari crossbred buck semen at 0, 24 and 48 h of preservation were 73.47 ± 4.53, 70.55 ± 0.17 and 62.50 ± 1.27 per cent respectively. The mean sperm motility of Marwari buck semen recorded at 0, 24, 48 and 72 h of preservation in 7.5 per cent egg yolk level were 85.00 ± 1.29, 63.33 ± 1.05, 50.00 ± 1.82 and 44.17 ± 1.54 per cent respectively. The corresponding values in 10 per cent egg yolk were 85.00 ± 1.29, 69.17 ± 1.54, 61.67 ± 1.67 and 55.00 ± 2.24 per cent respectively. (Ranjan *et al.*, 2009).

2.2.1.5 Sperm viability

The percentage of live sperms at 0,24,48,72 and 96 hours of preservation in Tris yolk glycerol was found to be 86.00 ± 2.38, 63.10 ± 2.01, 49.30 ± 2.67, 36.60 ± 1.81 and 21.00 ± 1.69 respectively (Sarmah, 1983).

Simon (1999) reported that the live sperm percentage of Malabari crossbred buck semen at 0, 24 and 48 h of preservation was 88.24 ± 0.56, 80.82 ± 0.53 and 72.72 ± 1.70 respectively.

The live sperm count of Marwari buck semen were 89.00 ± 1.53, 86.17 ± 2.75, 84.67 ± 1.87 and 83.17 ± 1.01 per cent respectively at 0, 24, 48 and 72 h of preservation in diluent with 10 per cent egg yolk level at refrigeration temperature (Ranjan *et al.*, 2009).

2.2.1.6 Sperm abnormality

According to Sarmah (1983) the total sperm abnormalities in buck semen were 5.42, 6.57 and 6.76 per cent when preserved in Tris diluent at 0, 24 and 72 hours respectively.

The percentage of sperm abnormalities of Malabari crossbred buck semen at 0, 24 and 48 h of preservation were 2.97 ± 0.37, 3.68 ± 0.51 and 4.74 ± 0.48 respectively (Simon, 1999).

2.2.1.7 Acrosome integrity

Sarmah (1983) reported that the acrosome defects of buck semen preserved in Tris diluent were 0.87, 2.76 and 3.62 per cent at 0, 24 and 72 hours of preservation respectively.

The percentage of acrosome abnormalities were 7.2 ± 0.58, 8.58 ± 0.60 and 9.31 ± 0.66 at 0, 24 and 48 h of preservation respectively (Simon, 1999).

2.2.2 Cryopreservation

2.2.2.1 Diluents

Corteel *et al.* (1974) observed that addition of glucose in skim milk diluent for freezing of buck semen increased the post thaw motility. Cetinkaya *et al.* (1980) reported that egg yolk sodium citrate (EYC) diluent was best for freezing of buck semen. According to Salmon and Ritar (1982) best result was obtained with Tris diluent containing four per cent glycerol.

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). Deka and Rao (1987b) reported that the semen frozen in Tris egg yolk citric acid fructose glycerol extender maintained the highest sperm motility and lowest acrosomal damage.

According to Chauhan and Anand (1990) egg yolk-Tris extender with seven per cent glycerol was the best diluent for freezing of buck semen.

The mean sperm motility and live sperm count were significantly higher in Egg yolk citrate fructose glycerol extender and Tris egg yolk citric acid fructose glycerol extender than goat milk glycerol diluent at different stages of buck semen freezing (Deshpande and Mehta, 1991)

The combinations of 6 per cent glycerol and 4 hours of equilibration in Tris and skim milk extenders and 6 per cent glycerol and 6 hours of equilibration in EYC extender were found to be better for freezing of buck semen (Sinha *et al.*, 1991). It was also evident from the study that Tris extender was superior to skim milk and EYC extenders for freezing of buck semen.

Sinha *et al.* (1992a) reported that skim milk extender with six per cent glycerol resulted in better quality frozen buck semen.

The lowest percentage of swollen, separating and entirely lost acrosome was reported after freezing of buck semen in Fructose egg yolk glycerol extender with three hours equilibration period and seven per cent glycerol (Das and Rajkonwar, 1994d). According to Das and Rajkonwar (1995) the highest percentage of motile buck spermatozoa after freezing in Maltose egg yolk glycerol extender and lactose egg yolk glycerol diluent was with three hours of equilibration and seven per cent glycerol.

Dorado *et al.* (2007) reported that semen doses cryopreserved in milk extender provided greater pregnancy rates after intra-cervical insemination than in Tris extender.

2.2.2.2 Steps in freezing

2.2.2.2.1 Removal of seminal plasma

A specific problem in the preservation of goat semen was the detrimental effect of seminal plasma on the viability of the spermatozoa in diluents containing egg yolk or in milk-based media. The problem regarding egg yolk diluents was due to an enzyme originating from the bulbourethral gland secretion in seminal plasma, named egg yolk coagulating enzyme (EYCE). Spermatozoa washed once did not coagulate egg yolk and remained motile for 10 – 15 days in egg yolk – citrate at 4°C; spermatozoa washed twice were motile for one month (Iritani *et al.*, 1961). The EYCE was identified as phospholipase A and exhibited a lipase activity on egg yolk lecithin contained in the semen extenders resulting in fatty acid and lysolecithin production (Iritani and Nishikawa, 1961 and 1963).

Similarly, a protein from the caprine bulbourethral gland (SBUIII), identified as 55-60 kDa glycoprotein lipase (BUSgp60), was found to be responsible for hydrolysis of plasma membrane triglycerides and triglycerides in the skimmed milk resulting in fatty acid production (Pellicer-Rubio *et al.*, 1997).

The conventional method of overcoming the harmful interactions of seminal plasma and eggyolk or milk proteins was to dilute the goat semen sample in a

buffered diluent and then separate the seminal plasma from the sperm by centrifugation. Cells were washed either once or twice, each for 10 – 15 min at 550-950 x *g* (Ritar and Salamon, 1982; Memon *et al.*, 1985).

Corteel and Baril (1975) reported that the post-thaw motility of goat semen one to two days after freezing averaged 44.4 and 43.7 per cent in unwashed and washed spermatozoa respectively, but after storage for 3 to 90 days, the additional loss in motility 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.

Removal of seminal plasma by centrifugation of goat semen was beneficial for maximizing post-thaw motility and acrosomal integrity of goat semen, but the effect depended on the intensity of washing (Ritar and Salamon, 1982). These authors further suggested that when the semen was diluted 6 to 11 fold, double washing was more effective than single washing.

Memon *et al.* (1985) reported that percentage of post-thaw motility and normal acrosomes were significantly higher in goat semen washed twice with Ringer's solution than in unwashed semen. Removal of seminal plasma from goat semen was beneficial in preserving the integrity of the spermatozoa after freezing, regardless of the extender used.

Chauhan and Anand (1990) showed that in Jamunapari bucks unwashed spermatozoa could be usefully preserved at low temperature after dilution in Tris-based extender with a high concentration of egg yolk.

Azeredo *et al.* (2001) opined that removal of seminal plasma decreased motility and vigor rates in frozen samples.

2.2.2.2.2 Dilution

Dilution rates of 1:1-1:23 were used successfully (Evans and Maxwell, 1987). Ritar and Ball (1993) suggested dilution rates of 1:0.5, 1:1 or 1:2 when Tris-based

diluent was used. According to Nuti (2007) dilution rate was fixed in such a way that minimum safe numbers of motile spermatozoa (in million) for AI in goats was 180 for frozen semen by cervical insemination.

2.2.2.2.3 Glycerolisation

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). Chauhan and Anand (1990) reported that for buck semen, optimal results for motility, percentage of live spermatozoa and acrosomal integrity before and after freezing were obtained with seven per cent glycerol.

Deshpande and Mehta (1991) concluded that four per cent glycerol gave best post thaw motility when buck semen was frozen in Tris buffer. 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 of frozen buck semen (Sinha *et al.,* 1992b). According to Purohit *et al.* (1992) five per cent glycerol was superior to three or four per cent for freezing of buck semen.

Tuli and Holtz (1994) suggested that stepwise addition of glycerol to semen at 37°C was better than one step addition with regard to progressive motility and percentage of live spermatozoa. Prasanth (1995) opined that better post thaw motility was obtained when six per cent glycerol was used as cryoprotectant than five or seven per cent levels for freezing Malabari buck semen.

Farshad and Akhondzadeh (2008) reported that 7 per cent glycerol was the best cryoprotective agent for cryopreservation of goat semen. Batista *et al.* (2009) recommended 4 per cent glycerol and 12 per cent egg yolk as cryoprotective agents for cryopreservation of Majorera goat semen.

2.2.2.2.4 Packaging of semen

The glass ampules with round bottoms were used to prevent breaking during freezing (Roberts, 1971). The semen samples were filled in 0.25 ml French straws and frozen in liquid nitrogen vapour (Memon *et al.,* 1985). Deka and Rao (1986c), Prasanth (1995), Ranjini (1998), Simon (1999) and Afsal (2003) packed semen in 0.5 ml French straws for manual freezing of buck semen.

Diluted buck semen was frozen either in pellets or in straws (Evans and Maxwell, 1987; Tuli *et al.*, 1991; Leboeuf *et al.*, 2000). Ritar *et al.* (1990a, b) reported that sperm frozen in pellets yielded superior post thaw motility compared to sperm frozen in straws. However, cell motility was similar for sperm frozen in 0.25 and 0.5 ml straws (Purdy, 2006)

According to Leboeuf *et al.* (2000) the velocity of cooling of straws was regulated by the distance of the straws from the level of liquid nitrogen and the size of the straw; fine (0.25 ml) or medium (0.50 ml). In pellet freezing the velocity of cooling was regulated by the volume of the pellet.

2.2.2.2.5 Equilibration

Memon *et al.* (1985) suggested an equilibration period of 2 hours at 5°C. Deka and Rao (1986b) concluded that post-thaw motility of buck semen frozen with Tris-egg yolk-citric acid-fructose-glycerol extender was higher when equilibrated for six hours than equilibrated for one or three hours.

Sinha *et al.* (1992a) reported that four hours of equilibration period in Tris extender resulted in better quality frozen buck semen. According to Purohit *et al.* (1992) five hour equilibration period resulted in 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).

According to Das and Rajkonwar (1996) semen equilibrated for 3 h in 7 per cent glycerol gave better results compared to 1 or 6 h of equilibration. Sivaselvam *et al.* (2000) reported that four hours equilibration period resulted in good quality frozen buck semen in Tris extender.

2.2.2.2.6 Freezing methods

Deka and Rao (1986b) did 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 four cm above liquid nitrogen for 30 seconds, followed by plunging into liquid nitrogen (Ritar *et al.,* 1990a).

According to Evans and Maxwell (1987) freezing was done by placing the rack containing the samples into the liquid nitrogen vapour at a height of 3-4 cm above the liquid for 7-8 min and then plunged into liquid nitrogen for storage.

To maximize the post-thaw recovery of motile and viable buck sperm frozen with the Tris-egg yolk diluent the semen straws should be placed 4 cm above liquid nitrogen for 4.5 min and then plunged into the liquid nitrogen for storage (Ritar *et al.*, 1990b).

Purdy (2006) suggested that 0.5 ml straws should be frozen 4 cm above liquid nitrogen for 5 min, and then plunged into the liquid nitrogen, while 0.25 ml straws should be placed 16 cm above the liquid nitrogen for 2 min, lowered to 4 cm for 3 min, and plunged into the liquid nitrogen for storage.

Dorado *et al.* (2007) proposed a freezing technique in which 0.5 ml straws were put into straw holders and frozen in liquid nitrogen vapour, 5 cm above the liquid nitrogen level for 20 min, before being plunged into the liquid nitrogen for storage.

2.2.2.2.7 Thawing

Memon *et al.* (1985) proposed a thawing temperature of 35°C for 2 minutes.

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 (Deka and Rao, 1986c; Deka and Rao, 1987b).

Tuli *et al.* (1991), using the semen of Boer goat, found that thawing of straws at 70°C for 7 seconds was superior to either at 37°C for 2 min, or at 40°C for 20 seconds. The best post thaw motility of spermatozoa was recorded at a thawing temperature of 37°C for 10 seconds (Das and Rajkonwar, 1994).

Purdy (2006) reported that increasing the thawing temperature to 70°C and thawing the semen straw for only 7 seconds resulted in significantly higher progressive motility and plasma membrane integrity compared to thawing straws at 37°C for 2 min or 40°C for 20 seconds . Dorado *et al.* (2007) proposed a thawing temperature of 39°C for 30 seconds with regard to freezing of buck semen.

2.2.2.3 Sperm motility

Corteel and Baril (1975) recorded a post thaw motility of 44.4 and 43.7 per cent for unwashed and washed spermatozoa respectively. Cetinkaya *et al.* (1980) reported a post thaw motility of 40 – 55 per cent in Angora bucks. Percentage of progressively motile sperm after equilibration and after freezing in extender containing glycerol 6.4 per cent as cryoprotectant was 79.80 ± 0.72 and 68.50 ± 0.78 respectively (Deka and Rao, 1985).

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). The mean sperm motility of Beetal goat semen after freezing in Tris, skim milk and EYC extenders was found to be 65.20 ± 0.80, 56.02 ± 1.87 and 47.99 ± 2.00 per cent respectively(Sinha *et al.*,1991). Das and Rajkonwar (1995) reported a post thaw motility of 48.79 ± 0.66 per cent in Beetal bucks after 3 hours of equilibration period at 7 per cent glycerol level.

For semen frozen in 0.5 ml straws and thawed at 37°C for 15 seconds or at 55°C for 8 seconds, the average forward sperm motility was 62.7 and 58.7 per cent

respectively vs. 59.0 and 59.9 per cent for similarly treated semen frozen in 0.25 ml straws (Mendez *et al.*, 1995).

Average percentage sperm motility of Malabari crossbred buck semen after washing, glycerolisation and freezing was 76.71 ± 0.79, 67.85 ± 1.39 and 42.0 ± 1.84 respectivelyin six percent glycerol (Prasanth and Mathai, 1996). The percentage of sperm motility after washing of semen and initial extension, after cooling to 5°C, after glycerolisation and equilibration and after freezing and thawing was 82.00 ± 0.61, 75.88 ± 1.31, 70.50 ± 0.96 and 44.00 ± 0.79 respectively (Ranjini, 1998).

The sperm motility percentage was 61.80 ± 0.95 and 55.83 ± 1.12 after glycerolisation and equilibration respectively. The percentage of sperm motility were 35.42 ± 0.71, 34.45 ± 0.74 and 33.17 ± 1.14 at zero, fifteen and thirty days of freezing respectively (Simon, 1999).

Sivaselvam *et al.* (2000) obtained a post thaw motility of 54.50 ± 0.01 per cent for Tellicherry buck semen frozen in Tris based diluent containing 20 per cent egg yolk and seven percent glycerol. Azeredo *et al.* (2001) recorded a post thaw motility of 28.32 per cent in Saanen bucks. Tambing *et al.* (2002) reported that the mean post-thaw motility was 52.60, 44.31 and 45.28 when buck semen was frozen in 6, 5 and 7 per cent glycerol respectively.

Average percentage of sperm motility of Boer buck semen after washing in Tris buffer and after freezing in Tris extender was 67.00 ± 1.47 and 43.91 ± 1.86 respectively (Afsal, 2003).

Baruah *et al.* (2003) reported that in Beetal × Assam local crossbred goat the mean post thaw sperm motility was 54.58 ± 1.30 , 48.75 ± 1.39 and 51.67 ± 0.71 per cent at 5, 3 and 7 h of equilibration periods respectively.

Purdy (2006) reported that cell motility was similar for sperm frozen in 0.25 (33 per cent post-thaw motility) and 0.5 ml straws (34 per cent post-thaw motility). Alpine and Saanen goat semen showed a post thaw motility of 46.34 ± 2.66 and 56.12 ± 4.02 per cent respectively (Zamfirescu and Nadolu, 2007). The mean sperm motility

of Black Bengal goat semen after freezing in Triladyl based diluents was found to be ranging from 42.86 ± 3.25 to 52.33 ± 1.45 per cent (Apu *et al.*, 2009).

2.2.2.4 Sperm viability

Live sperm percentage of frozen goat semen was reported to be 65.63 ± 0.98 and 55.47 ± 1.11 after rapid thawing (37°C for 12- 15 sec.) and slow thawing (5°C for 2 min) respectively (Deka and Rao, 1986b).

Deshpande and Mehta (1991) reported that the percentage of live sperms immediately after dilution , five hours after dilution, immediately after freezing and 24 hours freezing were 82.00 ± 2.14, 76.83 ± 2.23,45.33 ± 4.52 and 44.50 ± 2.98 respectively at six per cent glycerol level.

The percentage of sperm viability after washing of semen and initial extension, after cooling to 5°C, after glycerolisation and equilibration and after freezing and thawing was 86.75 ± 0.79, 83.00 ± 0.72, 74.33 ± 0.84 and 54.25 ± 0.59 respectively (Ranjini, 1998).

Simon (1999) reported that live sperm percentage were 78.79 ± 1.43 and 70.82 ± 2.01 after glycerolisation and equilibration respectively. It was also reported that live sperm percentage of frozen goat semen were 59.70 ± 1.03, 57.69 ± 1.30 and 57.83 ± 0.90 at zero, fifteen and thirty days of freezing respectively.

Tambing *et al.* (2000) reported that after freezing, the mean percentage of live sperm were 65.03 , 52.00 and 52.10 in diluent containing 6, 5 and 7 per cent glycerol respectively. Mean percentage of live spermatozoa in freeze thawed Boer buck semen was 52.61 ± 1.56 (Afsal, 2003).

2.2.2.5 Sperm abnormality

Deka and Rao (1984b) reported that the mean abnormality percentage in fresh semen, after initial dilution and cooling, after equilibration and after freezing and thawing were 0.20 ± 0.07, 0.78 ± 0.14, 1.05 ± 0.20 and 1.50 ± 0.19 respectively in

Egg yolk citrate diluent, 0.20 ± 0.07, 0.85 ± 0.15, 1.23 ± 0.19 and 1.26 ± 0.17 respectively in Tris diluent, 0.20 ± 0.07, 0.85 ± 0.15, 1.05 ± 0.17 and 1.18 ± 0.16 respectively in Skim milk and 0.20 ± 0.07, 1.28 ± 0.24, 1.53 ± 0.30 and 1.54 ± 0.27 respectively in Raffinose containing diluent.

The sperm abnormality percentage after washing of semen and initial extension, after cooling to 5°C, after glycerolisation and equilibration and after freezing and thawing was 3.75 ± 0.57, 4.70 ± 0.30, 5.50 ± 0.43 and 7.13 ± 0.71 respectively (Ranjini, 1998).

The percentage of sperm abnormalities were reported to be 5.32 ± 0.39 and 5.44 ± 0.42 after glycerolisation and equilibration respectively (Simon, 1999). It was also reported that the percentage of sperm abnormalities were 6.79 ± 0.41, 6.72 ± 0.55 and 7.42 ± 0.45 at zero, fifteen and thirty days of freezing respectively.

2.2.2.6 Acrosome integrity

Deka and Rao (1985) reported that the percentage of total damaged acrosomes were 1.50 ± 0.3, 2.10 ± 0.40, 5.73 ± 0.91 and 18.60 ± 2.74 respectively in fresh semen, in cooled diluted semen at 5°C, after equilibration and after freezing and thawing in Egg yolk citrate fructose glycerol diluent. It was also reported that the percentage of total damaged acrosomes were 2.50 ± 0.23, 5.07 ± 0.52 and 12.37 ± 1.84 respectively in cooled diluted semen, after equilibration and after freezing and thawing in Tris egg yolk citric acid fructose diluent.

The mean percentage of intact acrosomes after freezing in Tris hydroxymethyl aminomethane-citric acid-egg yolk-glycerol diluent were 69.29 ± 4.61 and 66.07 ± 5.06 for washed and unwashed semen respectively (Memon *et al.*, 1985).

The percentage of intact acrosomes in Beetal buck semen after freezing in Tris, skim milk and EYC extenders was found to be 79.01 ± 0.44, 55.80 ± 0.38 and 51.90 ± 0.54 per cent respectively (Sinha *et al.*, 1991).

Das and Rajkonwar (1996) reported that after freezing the percentage of swollen, separating and detached acrosomes were 14.41, 0.81 and 0.39 respectively for semen equilibrated for 3 h in 7 per cent glycerol.

Singh and Purbey (1996) reported that the percentage of intact acrosomes in buck semen diluted in Tris extender and frozen in liquid nitrogen declined from 88.76 ± 0.43 in pre freeze to 68.90 ± 0.82 in post thaw semen. The percentage of swollen, ruffled, fractured, separating and entirely lost acrosomes were 14.68 ± 0.21, 7.94 ± 0.35, 13.12 ± 0.06 and 3.98 ± 0.20 in Tris extender.

The average percentage of intact acrosomes after washing of semen and initial extension, after cooling to 5°C, after glycerolisation and equilibration and after freezing and thawing was 86.05 ±1.25, 81.83 ±2.18, 79.58 ± 1.70 and 76.43 ± 2.04 respectively (Ranjini, 1998).

Simon (1999) reported that the percentage of acrosomal abnormalities were 9.62 ± 0.75 and 10.81 ± 0.71 after glycerolisation and equilibration respectively. It was also reported that the percentage of acrosomal abnormalities were 14.50 ± 0.95, 14.76 ± 0.96 and 14.76 ± 0.77 at zero, fifteen and thirty days of freezing respectively.

Tambing *et al.* (2000) reported that after freezing the mean percentage of intact acrosome were 47.54, 37.00 and 37.14 in 6, 5 and 7 per cent glycerol respectively. Baruah *et al.* (2003) reported that in Beetal × Assam local crossbred goats the mean percentage of intact acrosome after freezing was 81.33± 0.17 at 5, 80.58 ± 0.25 at 3 and 79.21 ± 0.31 at 7 h of equilibration periods.

2.3 ARTIFICIAL INSEMINATION

2.3.1 HEAT DETECTION

2.3.1.1 Behavioral signs

Mehta *et al.* (1991) reported that smelling, mounting, bleating and switching of tail were the prominant signs of oestrus in goats. According to Goel and

Agrawal (1994) the salient signs of oestrus in goats were frequent bleating, wagging of tail, frequent micturition, teasing of other goats which are either in oestrum or non-oestrum, homosexual behaviour and tendency to seek the buck. A rapid side to side or up and down tail flagging was found to be a good sign of oestrus (Jainudeen and Hafez, 2000).

Kumar and Yadav (2000) recorded signs of oestrus as frequent bleating, switching of tail, restlessness in seeking the buck, arching and stretching of the body, mounting and allowing mounting by other does and allowing mating. The does in oestrus exhibited reduced apetite and produced less milk.

Joseph (2003) reported that wagging of tail was the most prominent behavioural sign exhibited by superovulated Malabari goats. Bleating, frequent urination, reduction in feed and water intake were also noticed.

Afsal (2003) reported that behavioural signs noticed in the oestrus does were wagging of tail (65.33 per cent), frequent bleating (56.67 per cent), standing to be mounted (34 per cent), circling with the buck (26 per cent), and mounting on other animals (14 per cent).

Nuti (2007) opined that behavioural signs of estrus included increased frequency of bleating, frequent urination, obvious restlessness, tail flagging, increased curiosity and attentiveness to the herd handler, noticeable change in milk volume in lactating animals, exhibition of male behaviour by does and receptivity to mounting.

According to Ramya *et al.* (2008) predominant heat signs were urination (57.5 per cent), wagging of tail (45 per cent) and bleating (32.5 per cent).

2.3.1.2 Clinical signs

Goel and Agrawal (1994) reported that the vulvar discharge was thin and watery during early estrum, mucinous during mid-oestrus and thick and cheesy in late oestrum.

According to Afsal (2003) major clinical signs of estrus in goats included vulval oedema (43.33 per cent), vulval redness (21.33 per cent) and discharge from genitalia (14 per cent).

Joseph (2003) reported that major clinical signs of estrus in superovulated Malabari goats were congestion of vaginal mucosa, vulval oedema and mucus discharge from vulva.

Clinical signs of oestrus included swelling of the vulva, discharge of vaginal mucus and hyperemia of the vulva (Nuti, 2007).

2.3.1.4 Heat detection aids

Mathai *et al.* (1980) used a vasectomised teaser buck for heat detection. Different practical methods of oestrus detection include entire males provided with an apron, vasectomised males, androgenised females fitted with a harness and marking crayon (Leboeuf *et al.*, 2000). A "buck rag" which is a cloth that has been wiped over the scent glands of a male in rut, stored in a closed jar was used for heat detection (Nuti, 2007).

Ramya *et al.* (2008) used buck jar technique to detect oestrus in goats and reported urination as the prominent heat sign.

2.3.1.5 Intensity of heat

Joseph (2003) used scores to express intensity of oestrum in oestrus synchronised goats as one, two, three and four based on behavioural signs on approach of the buck and by observing physical changes in the external genitalia. An average score of 3.44 ± 0.15 and 3.18 ± 0.21 was reported in peripubertal and adult goats respectively.

2.3.2 TECHNIQUES OF INSEMINATION

Lawrenz (1986) inseminated goats using frozen semen by a non-surgical intra-

uterine technique. Moore *et al.* (1989) compared conception rates after cervical and laparoscopic inseminations in oestrus synchronised goats and observed that laparoscopic AI resulted in a higher kidding rate than cervical AI.

Eppleston *et al.* (1994) reported that one of the important factors affecting the fertility of frozen – thawed ram semen inseminated into the cervix of sheep was the depth of insemination. It was also reported that the fertility was increased by 7-12 per cent for each cm increase in depth of insemination.

Salamon and Maxwell (1995b) studied various methods to improve fertility in ewes and suggested that the most effective method is to increase the depth of deposition of frozen thawed semen into the cervical canal. However the best method was found to be intra uterine insemination by laparoscopy.

Naqvi *et al.* (1998) recorded a mean lambing rate of 22.7 per cent in Malpura ewes inseminated using transcervical insemination technique. According to Ax *et al.* (2000) there were four methods for artificial insemination in goats; vaginal, cervical, transcervical and laparoscopic or intrauterine methods.

Baldassarre and Karatzas (2004) opined that vaginal insemination was successful for fresh semen whereas intra-cervical insemination for refrigerated and frozen semen. However, in order to achieve high pregnancy rates with frozen semen, intrauterine deposition of semen was required.

Paulenz *et al.* (2005) reported that in Norwegian Dairy goats (using liquid semen stored at room temperature) cervical insemination using 200×10^6 motile spermatozoa per dose resulted in 25-day non-return and kidding rates of 87.0 and 78.0 per cent, while vaginal insemination gave 85.5 and 74.3 per cent respectively.

Nuti (2007) opined that if the cervix had been penetrated at least 13 mm, the sperm could negotiate the remainder of the cervical canal. In no instance should penetration be greater than 38 mm to avoid perforation of the uterine wall or entry into one of the uterine horns. If a depth of at least 32 mm had been achieved, the semen should be deposited.

2.4 PREGNANCY DIAGNOSIS

2.4.1 Ultrasonography

Ultrasonic techniques based on the Doppler principle could diagnose multiple pregnancies with an acceptable accuracy, but could not readily distinguish between females carrying two, three or more than three fetuses (Ishwar, 1995).

Ishwar (1995) reported that with real time B- mode scan ultrasound, pregnancy could be detected as early as 25 days of gestation. It was also reported that the ideal time for transabdominal scanning was between 40 to 70 days of gestation when the pregnant uterus was lying against the right body wall.

Karen *et al.* (2001) opined that A-mode ultrasound could not predict the foetal number and the viability of the foetus.

Singh *et al.* (2004) reported that ultrasonography by real time B mode with 5MHz transrectal transducer was reliable, safe and accurate and practical in diagnosing early pregnancy as early as 25 days post breeding.

Suguna *et al.* (2008) reported that the embryonic vesicle was detected on day 21 and day 28 and the embryo proper on day 28 and day 35 using the transrectal and transabdominal methods, respectively. Heart beat was observed as early as day 21 of gestation and it was recordable by day 28 using the transrectal approach. However, via transabdominal scanning the detection and recording of the heart beat was possible only on day 35 of gestation. Singles and twins were differentiated on day 35 and day 42 by the transrectal and the transabdominal approaches, respectively. Skeletal structures such as the skull, rib cage and vertebral column were first viewed on day 56 in both approaches.

Aly *et al.* (2009) reported that the embryonic vesicle was first detected by transabdominal ultrasonography at about 27.87 ± 3.48 days of pregnancy. It was also

reported that the embryo proper with a beating heart was first observed at an average of 30.36 ± 4.75 days of pregnancy.

According to Sreejith (2009) the embryonic vesicle was detected earliest on day 19 of gestation by transrectal scanning and on day 26 by transabdominal scanning.

Hussein (2010) reported that B- mode real-time ultrasonography could be used as a reliable mean for early detection of gestation as early as 19-27 days after mating. With the transabdominal probe, fluid-filled vesicles were reliably located from day 24.7 ± 0.4 onwards, whereas the foetal heart beat first seen as late as day 27.0 ± 0.6.

2.4.2 Abdominal palpation

. Pratt and Hopkins (1975) reported that abdominal palpation technique could be used with an accuracy of 80 to 90 per cent in ewes at 90 - 130 days of pregnancy. Smith (1980) reported that the foetus could not be detected by abdominal palpation in goats before 110 days of pregnancy.

According to Goel and Agrawal (1990) pregnancy diagnosis could be done by abdominal palpation in goats that were in natural standing position by pressing the abdominal wall on both sides to feel the fetal mass. It was also reported that diagnosis by abdominal palpation was not possible at 51 to 60 days of pregnancy but the percentage of accuracy increased to 70 at 61-70 days, 90.3 at 71-80 days and 95.4 at 80 days and later.

Rajasekaran *et al.* (1992) used abdominal palpation as a method for pregnancy diagnosis and the percentage of animals diagnosed as pregnant, non-pregnant and doubtful were 27.5, 45 and 27.5 respectively. Out of the doubtful cases 71.4 per cent were diagnosed as pregnant and 28.6 per cent as non-pregnant by the use of X-ray.

Ewes and does in the late stages of pregnancy could be diagnosed by abdominal palpation technique (Ishwar, 1995). Gordon (1997) opined that abdominal palpation could be used for pregnancy diagnosis in late pregnancy in slab-sided, thin, relaxed does.

Kutty (1999) developed bimanual palpation technique and suggested that it could be used for diagnosing pregnancy in small ruminants. This method could be successfully for diagnosing pregnancy from 28 to 30 days.

According to Matsas (2007) pregnancy diagnosis in goats could be done during the last days of pregnancy by either palpating the gravid uterus or by ballottement of the fetus low in the right flank through the abdominal wall.

2.5 CONCEPTION RATE

2.5.1 Chilled semen

Balakrishnan (1979) obtained 44 per cent pregnancy rate in Malabari × Saanen crossbred goats after insemination with chilled semen diluted in Tris extender. The conception rate in goats after insemination using chilled semen was 40.86 per cent (Mathai *et* al., 1980).

Mathai and Nair (1981) reported that conception rate for inseminations done with chilled semen of bucks stored up to 24 hours was 42.78 per cent and for semen stored beyond 24 hours it was only 29.26 per cent.

Bargohain *et al.* (1985) reported that when buck semen stored at 5°C for 48 hours in milk and egg yolk citrate were used for insemination the kidding rates were 64.15 and 61.11 per cent respectively.

Senthilkumar (2002) observed a conception rate of 45.45 per cent after insemination with chilled semen in Malabari crossbred goats. Molina *et al.* (2007) reported a conception rate of 62.4 per cent using fresh cooled semen from Payoya goats.

2.5.2 Frozen semen

Cetinkaya *et al.* (1980) reported a conception rate of 37.5 per cent using frozen semen from Angora bucks. According to Sinha *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.

Chauhan and Anand (1990) recorded a fertility rate of 81 per cent for Jamunapari buck semen. Fertility results of frozen-thawed buck semen after cervical insemination was 33.3 per cent and after laparoscopic insemination was 56.8 per cent (Ritar and Ball, 1993). Prasanth (1995) recorded a conception rate of 47.36 per cent in Malabari × Alpine crossbred goats after insemination using frozen semen.

According to Naqvi *et al.* (1998) the overall lambing rate achieved from deposition of frozen-thawed semen into the cervix or uterus using the transcervical technique was 22.7 per cent even though the mean post-thaw motility of semen was 78.7 per cent.

Afsal (2003) recorded a conception rate of 21.68 per cent in oestrus synchronized crossbred Malabari does inseminated using frozen semen. The fecundity after AI of cryopreserved Saanen and Alpine semen were 28.2 and 46.6 per cent respectively (Zamfirescu and Nadolu, 2007).

Molina *et al.* (2007) reported a conception rate of 44.6 per cent using frozen semen in Payoya goats. Dorado *et al.* (2007) reported that fertility rates obtained by cervical insemination were 47.62 per cent in Florida goats, 51 per cent in Angora goats, 39.1 per cent in Cashmere goat, 50.53 per cent in Beetal and Bengal goats and 57 per cent in the Murciano-Granadina goat. Fertility rates ranged from 36.4 to 47.4 per cent in Black Bengal goats (Apu *et al.*, 2009).

Materials and Methods

3. MATERIALS AND METHODS

3.1. SEMEN COLLECTION

Normal ejaculates from an adult Malabari crossbred buck maintained at the Artificial Insemination Centre, under the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur were utilized for the study. Ejaculates were collected thrice weekly at an interval of two to three days from the buck using Danish type artificial vagina maintained at 42 to 45°C under adequate pressure . Adult male bucks were used as teasers. Two false mounts were given for stewing the buck before taking collection. Immediately after collection the semen samples were transferred to a water bath maintained at 37°C.

3.2 SEMEN EVALUATION

3.2.1 Volume

The volume of semen was noted from the graduations on the collection vial.

3.2.2 Colour

The colour of semen was recorded by visual observation and graded based on the intensity of cream colour. Semen samples with a colour of thick creamy were utilized for study.

3.2.3 Density

The density of semen was expressed from D to DDDD based on visual examination of opacity of a drop of semen taken on a glass slide. Semen samples with a minimum of DDD only were utilized for study.

3.2.4 Mass activity

A small drop of semen was taken on a clean glass slide and examined under low power (100x) of microscope for waves and swirls. Mass activity was expressed from + to + + + +. Samples with a minimum of + + + mass activity were selected for study.

3.2.5 Initial motility

Good quality semen samples were diluted (1:1 to 1:3) using 0.2M Tris – yolk buffer with pH 7 and maintained at 37°C.

Composition of Tris – yolk buffer

Tris buffer – 3.03g
Citric acid – 1.63g
Fructose - 1.25 g
Distilled water – to 100ml
Benzyl penicillin – 1000 IU per ml
Streptomycin sulphate – 1000 µg per ml
Egg yolk – 7.4 per cent

A small drop of diluted semen was placed on a clean glass slide and a cover slip was placed over the drop. Motility was observed under high power of microscope (400x) and percentage of progressively motile sperms were assessed. Ejaculates having a minimum of 80 per cent initial motility were chosen for further processing.

3.2.6 Hydrogen ion concentration

A small piece of pH indicator paper was immersed in a drop of fresh semen placed on a glass slide. The change in colour obtained on the pH paper was compared with that of the standard.

3.2.7 Sperm concentration

The sperm concentration was determined using hemacytometer. Number of sperms in eighty small squares in Neubauer counting chamber was multiplied by 10^7 to obtain the number of sperms per ml of semen.

3.2.8 Sperm viability

One drop of two per cent Eosin and four drops of ten per cent Nigrosine were mixed on a clean glass slide. One small drop of diluted semen sample was mixed with the stain mixture gently and uniformly. From this mixture a moderately thick smear was made on a clean glass slide, air dried and examined under oil immersion objective (100 ×10x) of the microscope. A minimum of 100 sperms were counted from different microscopic fields keeping a note of dead and live sperms from the staining characteristics and percentage of each was worked out.

3.2.9 Sperm abnormality

Percentage of abnormal sperms was estimated using Eosin – Nigrosine staining technique as described for sperm viability. From different microscopic fields a minimum of 100 sperms were counted keeping a note of specific abnormalities.

3.2.10 Acrosome integrity

3.2.10.1 Giemsa staining

Acrosome integrity of diluted semen sample was assessed at 0 h of preservation using Giemsa staining technique (Plate-1). Smears were prepared from diluted semen on grease free glass slides, air dried and fixed with methanol for 15 minutes. These were washed in tap water, air dried and immersed in Giemsa working solution for three hours.

The stained slides were then washed in tap water, air dried and observed under oil immersion objective of microscope (1000x).

Giemsa stock solution

Giemsa stain powder – 1g
Methanol – 66ml
Glycerol – 60 ml

Giemsa working solution

Giemsa stock solution – 6ml
Sorenson's 0.1 M phosphate buffer – 4ml
Deionised water – 90ml

Sorenson's 0.1M phosphate buffer (pH 7.0)

A. 0.1M Potassium dihydrogen phosphate solution
Potassium dihydrogen phosphate (Anhydrous) – 13.609g
Double glass distilled water – 1000ml

B. 0.1M Disodium hydrogen orthophosphate solution
Disodium hydrogen phosphate – 14.198g
Double glass distilled water – 1000ml

Sorenson's 0.1 M phosphate buffer was prepared by mixing 17 ml solution 'A' and 33 ml of solution 'B' and the pH was adjusted to7.0.

3.3. PRESERVATION OF SEMEN

3.3.1 Refrigeration storage of semen

A total of 16 ejaculates having a minimum of 80 per cent initial motility were used for chilling.

3.3.1.1 Semen extender

Tris egg yolk extender used for evaluation of initial motility was used for refrigeration preservation of semen.

3.3.1.2 Dilution

Dilution was done in such a way that each dose (.25 ml) of semen contained 200 million motile sperms. Dilution rate varied from 1:1 to 1:3.

3.3.1.3 Packaging of semen

Tris diluted semen was filled manually in 0.25 ml sterile French straws. These straws were sealed using polyvinyl alcohol powder. After cleaning the adhering powder, straws were immersed in water at 37°C in a tray and were subjected to refrigeration (3-5°C).

3.3.1.4 Storage of semen

Straws were kept under refrigeration for a maximum period of 72 h.

3.3.1.5 Semen evaluation during refrigeration

Sperm motility, viability, abnormality and acrosome integrity were assessed at 0

h, 24 h, 48 h and 72 h of preservation.

3.3.2 Cryopreservation of semen

A total of six ejaculates, which showed a minimum of 80 per cent initial motility, were used for study.

3.3.2.1 Preparation of semen extenders

Tris hydroxy methyl amino methane (1.21 g), citric acid (0.67 g) and fructose (0.5 g) were weighed accurately into three sterile 50 ml volumetric flasks numbered I to III. Then 25 ml double glass distilled water was added in all the flasks and stirred well. Fresh egg yolk (chicken egg) was collected aseptically and added 5 ml each to flasks numbered II and III and mixed well using sterile glass rod. Six ml of glycerol was added into the flask numbered III. Benzyl penicillin and streptomycin sulphate were added at the rate of 1000 IU per ml and 1000 µg per ml respectively in all flasks and stirred well. Then the volume of extenders was made up to 50 ml by adding double glass distilled water in all the three flasks. Extenders were placed in a water bath maintained at 37°C. The solution in the flask I was used for washing of fresh semen, that in flask II was the non glycerolated portion of the extender for initial dilution and that in flask III was the glycerolated fraction of the extender used in freezing of semen.

3.3.2.2 Removal of seminal plasma and initial extension (Stage I)

3.3.2.2.1 Removal of seminal plasma

Semen sample was extended 10 times with Tris buffer and centrifuged at 3000 rpm for five minutes. The supernatant fluid was pipetted out. The pellet was extended again by 10 fold in Tris buffer and repeated the centrifugation for five more minutes at 3000 rpm and the clear supernatant fluid was removed.

3.3.2.2.2 Initial extension

The pellet formed after washing was extended (1:1) with the non glycerolated Tris extender. The semen diluted with non glycerolated Tris extender and the glycerolated Tris extender were taken in separate vials and closed with aluminium foils. Volume of glycerolated extender taken in the vial was equal to the volume of non-glycerolated fraction.

3.3.2.3 Cooling of semen to 5°C (Stage II)

The vial containing semen with non glycerolated extender and vial containing glycerolated extender were kept in a 250 ml beaker containing water at 37°C. This beaker was kept under refrigeration for one and a half to two hours or till the temperature of the water bath was lowered to 5°C.

3.3.2.4 Glycerolisation and equilibration (Stage III)

3.3.2.4.1 Glycerolisation

The glycerolated fraction of the extender was mixed and added to the semen with non-glycerolated extender in three parts at 15 minute intervals so that the final concentration of glycerol was six per cent. The dilution rate ranged form 1:1 to 1:3, so that each dose (.25 ml) of semen contained 200 million motile sperms.

3.3.2.4.2 Filling and sealing of straws

After glycerolisation semen was filled in precooled 0.25 ml sterile French straws manually. An air space of 1 cm was created at the open end. Then the open end was dipped into polyvinyl alcohol powder to prepare laboratory seal. The excess powder sticking to the straws were removed using a cotton pad. The straws were immersed in

water at 5°C. After 45 minutes the straws loaded with semen were taken out and wiped with dry towel and arranged on freezing racks.

3.3.2.4.3 Equilibration

The straws were equilibrated at 5°C for four hours before freezing.

3.3.2.5 Freezing and thawing of semen (Stage IV)

3.3.2.5.1 Freezing and storage

At the end of equilibration manual freezing of straws were carried out in a styroform box. The liquid nitrogen was poured into the styroform box to such a level that the straws in the rack would be four centimeter above the liquid nitrogen level. This was closed and kept for a few minutes to get cooled. Freezing racks with straws were transferred quickly from the refrigerator to the styroform box. Closed the styroform box and allowed the straws to remain there for 4.5 minutes. Then the straws were collected by cooled gloved hand and placed into the precooled goblet and plunged into liquid nitrogen. After that the goblets along with straws were transferred into the storage container, cryocan. A thick paper label showing the date of collection and number of straws was tied on the handle of the canister for identification of semen straws.

3.3.2.5.2 Thawing of semen

After 24 hours of cryopreservation the neck tube plug of liquid nitrogen container was removed and the tip of the forceps was cooled by holding it in the vapour through the neck for 30 seconds. Then the canister was lifted up to 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 shook it twice to remove the drops of liquid nitrogen if any. Then the straws were placed in water at 37°C for 10 seconds.

3.3.2.6 Evaluation of semen during processing and after freezing

The percentage of sperm motility, sperm viability, sperm abnormality and acrosome integrity were estimated at the end of initial extension (stage I), after cooling to 5°C (stage II), after glycerolisation and equilibration (stage III) and after freezing and thawing (stage IV).

Semen samples having a minimum of 35 per cent post-thaw motility were used for insemination.

3.4 ARTIFICIAL INSEMINATION

3.4.1 Heat detection and selection of does

Does brought to Artificial Insemination Centre, under the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, were selected for fertility studies.

Heat detection was done using buck jar technique. By rubbing a piece of cotton on the caudomedial aspect of horn, pheromone containing secretion was collected from the buck and kept in an air tight jar (Plate-2). When the buck jar was shown to doe in heat they elicited various heat signs like frequent urination, bleating, wagging of tail and flehmen reaction (Plate-3). Confirmation of oestrus was done by speculum examination. Changes in the vulva, vaginal mucus membrane (oedema/moistness/hyperaemia) and cervical os (mucus/hyperaemia/opening) were also recorded. The intensity of heat was assessed based on heat signs and examination of genitalia as shown below.

Table 1. Intensity of oestrum in goats

Intensity of heat	Score	Description
Very good	4	Behavioral signs obviously very intense and changes in the external genitalia very prominent
Good	3	Behavioral signs intense and changes in external genitalia less prominent
Fair	2	Behavioral signs mild, changes in external genitalia evident
Poor	1	Absence of behavioral signs, changes in external genitalia evident

Selected does were at random allotted to four groups with eleven animals in each group. Does in first three groups were inseminated using chilled semen having motility over 35 per cent at 0-24 h, 24-48 h and 48-72 h of preservation. Animals of fourth group were inseminated using frozen semen having a minimum of 35 per cent post thaw motility.

3.4.2 Insemination technique

Insemination was done by controlling does in an AI crate and lifting the hind limbs of the does before introduction of speculum. Straw was loaded in appropriate gun and transcervical insemination was done. Owners were advised to present the animals for AI if heat signs were noticed 24 hours after the first insemination.

3.4.3 Assessment of depth of penetration of AI gun

Depth of penetration of AI gun was measured using another sheath as yardstick

(Plate-4) (Nuti, 2007). Depth of penetration of AI gun into the cervix was assessed and classified into four groups as 0-10 mm, 10-20 mm, 20-30 mm and 30-40 mm.

3.5 ASSESSMENT OF CONCEPTION RATE

Pregnancy diagnosis of inseminated does was done after 2 months using ultrasonography or after 3 months by abdominal palpation. Out of the 44 animals, pregnancy diagnosis by ultrasonography was done in 20 animals (chilled semen-9 and frozen semen-11) and by abdominal palpation in 24 animals (chilled semen-24 and frozen semen-0).

3.5.1 Ultrasonography

Ultrasound equipment (DC-6 VET DIAGNOSTIC ULTRASOUND SYSTEM MINDRAY), which produces two dimensional gray scale real time images, was used for the ultrasound scanning. A linear array transducer (5 to 8 MHz) and a sector transducer (3 to 5 MHz) were used.

3.5.1.1 Transabdominal scanning

To perform transabdominal ultrasonography, the hair on the right inguinal region and abdomen cranial to the pelvic brim was removed. Ultrasound gel-couplant was applied to the shaved area and to the sector transducer to assure good acoustic transmission. Goats were placed in left lateral recumbancy and ultrasonographic examination was done. Ultrasonography was done from the right side, as on left side the filled rumen could impede proper observation of the uterus (Plate-5). Does were considered positive when there was the detection of gestational sac, foetus, foetal heartbeat, placentomes or foetal skeleton (Plate-6).

3.5.1.2 Transrectal scanning

Ultrasonographic examination was performed using a real time B-mode scanner equipped with linear array transducer. The does were restrained in standing position. Ultrasound gel-couplant was applied to the transducer to assure good acoustic transmission. After clearing the faeces from rectum the transducer was introduced gently into the rectum in a downward fashion until the bladder appeared on the screen. The uterine horns were observed cranial to the bladder and then the probe was rotated laterally at 90° clockwise and 180° anticlockwise to visualize typical echoic/anechoic structures viz. foetal fluid, foetus, foetal heart flickering and placentome (Plate-7). Does were diagnosed pregnant from the observation of gestational sac, foetus, foetal heartbeat, placentomes or foetal skeleton (Plate-8).

3.5.2 Abdominal palpation

Abdominal palpation was done by standing behind the animal and lifting the ventral abdomen just in front of the udder with both hands (Plate-9). Abdomen was swung with a slight lifting. On swinging, in positive cases the enlarged uterus with foetus could be palpated with the palm.

3.6 STATISTICAL ANALYSIS OF DATA

The data were analysed statistically as per the method of Snedecor and Cochran (1994).

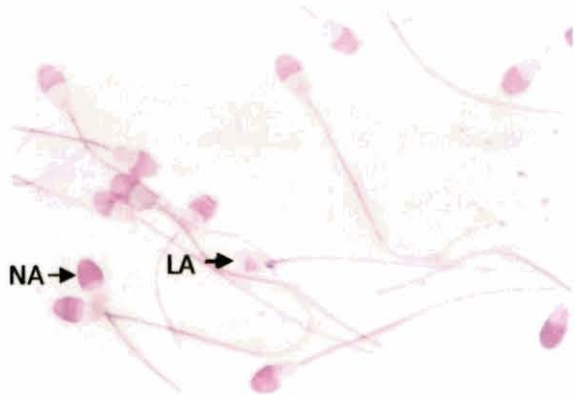

Plate – 1 Buck spermatozoa with normal acrosome (NA) and lost acrosome (LA) – Giemsa staining

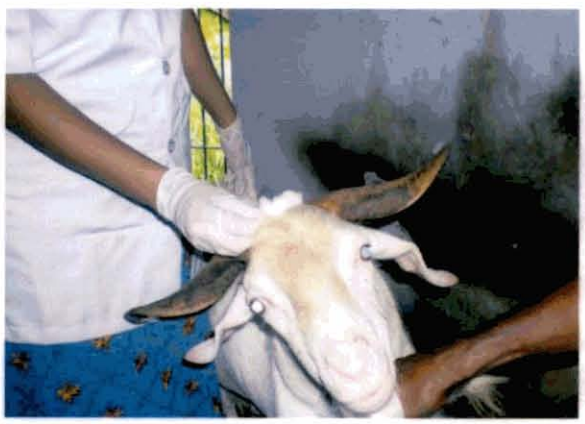

Plate – 2 Collection of buck pheromone

Plate – 3 Detection of heat using Buck Jar

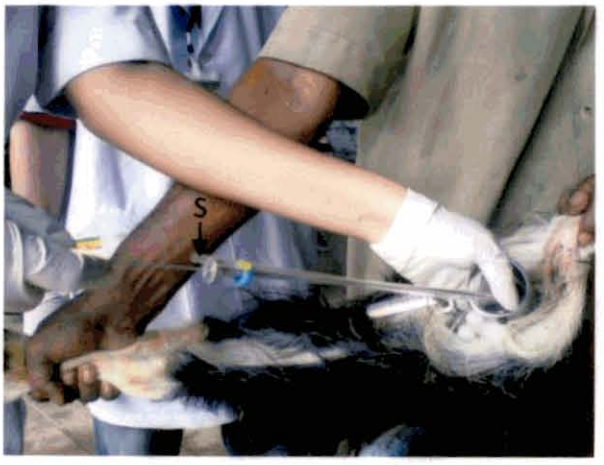

Plate – 4 Measurement of depth of penetration of AI gun using sheath (S) as yard stick

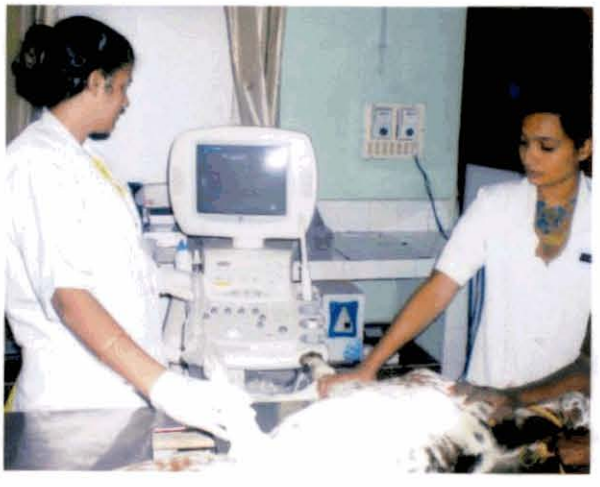

Plate – 5 Transabdominal ultrasound scanning

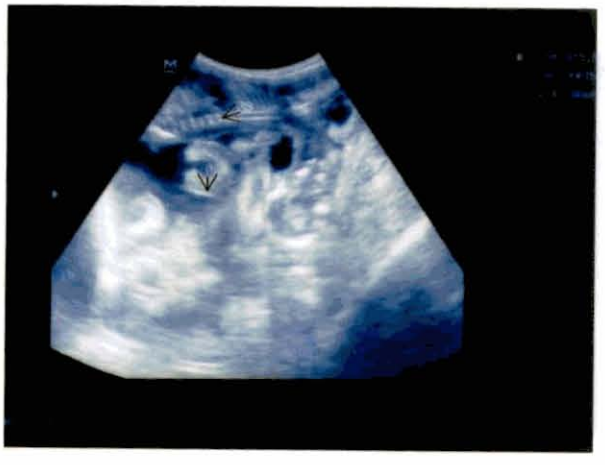

Plate – 6 Transabdominal ultrasonogram of uterus of a doe on day 60 of pregnancy. Echogenic foetal skeleton (FS) and placentomes (P) can be seen

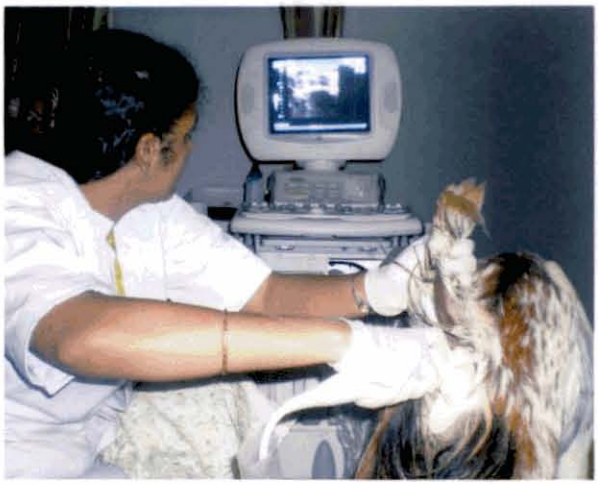

Plate – 7 Transrectal ultrasound scanning

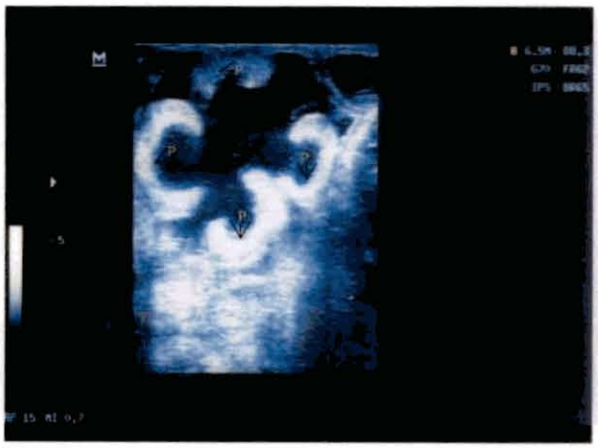

Plate – 8 Transrectal ultrasonogram of uterus of a doe on day 60 of pregnancy. Placentomes (P) can be seen

Plate – 9 Abdominal palpation

Results

4. RESULTS

The study was carried out to find out the factors affecting conception rate on artificial insemination in goats using 22 normal ejaculates from an adult Malabari crossbred buck maintained at Artificial Insemination Centre, under the department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy. A total of 44 adult healthy does brought to the AI Centre for insemination were selected for the study.

4.1 SEMEN CHARACTERISTICS OF FRESH EJACULATES OF MALABARI CROSSBRED BUCK

The volume of buck semen ranged from 0.7-2 ml with an average of 1.25 ± 0.97 ml. The colour of all the ejaculates was found to be creamy with yellowish tinge. The mean density and mass activity were DDDD and ++++ respectively.

The overall average pH was 6.89 ± 0.21 ranging from 6.8-7. The average sperm concentration was 2781.82 ± 51.69 millions per ml ranging from 2180-3110 millions per ml.

The percentage of initial motility was in a range of 80-85 with a mean of 82.77 ± 0.33. Mean live sperm percentage of fresh semen was 90.14 ± 0.53.

Average percentage of abnormal spermatozoa in the fresh semen as observed in the Nigrosin-Eosin stained smear was 2.28 ± 0.12. The percentage of intact acrosomes in the fresh semen averaged 92.27 ± 0.21.

4.2 SEMEN QUALITY DURING PRESERVATION BY CHILLING

A total of 16 ejaculates having a minimum of 80 per cent initial motility were used for chilling.

4.2.1 At 24 hours of preservation

The mean percentage of sperm motility, live sperms, sperm abnormality and intact acrosomes were 70.25 ± 0.60, 83.42 ± 1.27, 2.97 ± 0.13 and 90.27 ± 0.18 respectively.

4.2.2 At 48 hours of preservation

The mean percentage of sperm motility, live sperms, sperm abnormality and intact acrosomes were 61.13 ± 0.72, 78.84 ± 1.47, 4.12 ± 0.15 and 87.71 ± 0.37 respectively.

4.2.3 At 72 hours of preservation

The mean percentage of sperm motility, live sperms, sperm abnormality and intact acrosomes were 42.81 ± 0.95, 74.57 ± 1.53, 5.34 ± 0.11 and 85.09 ± 0.44 respectively.

4.2.4 Effect of duration of chilling on semen quality

There was significant difference in sperm motility, viability, abnormality and acrosome integrity between various storage periods ($p<0.01$). The data is presented in Table 2.

4.3 SEMEN QUALITY DURING AND AFTER CRYOPRESERVATION

A total of six ejaculates, which showed a minimum of 80 per cent initial motility, were used for study.

4.3.1 Initial extension (Stage I)

The mean percentage of sperm motility, live sperms, sperm abnormality and intact acrosomes at the end of initial extension were 78.50 ± 0.50, 84.67 ± 0.91, 3.82 ± 0.12 and 85.68 ± 0.72 respectively.

4.3.2 Cooling to 5°C (Stage II)

The mean percentage of sperm motility, live sperms, sperm abnormality and intact acrosomes on cooling to 5°C were 73.67 ± 0.21, 79.19 ± 0.48, 4.75 ± 0.15 and 80.84 ± 1.73 respectively.

4.3.3 Glycerolisation and equilibration (Stage III)

The mean percentage of sperm motility, live sperms, sperm abnormality and intact acrosomes on completion of glycerolisation and equilibration were 63.00 ± 0.63, 69.25 ± 1.48, 5.58 ± 0.51 and 77.61 ± 1.09 respectively.

4.3.4 Freezing and thawing (Stage IV)

The mean percentage of sperm motility, live sperms, sperm abnormality and acrosome integrity after freezing and thawing were 37.67 ± 0.49, 60.36 ± 0.77, 6.34 ± 0.22 and 76.06 ± 1.41 respectively.

4.3.5 Effect of processing and freezing on semen quality

There was significant difference between sperm motility, viability, abnormality and acrosome integrity at various stages of processing and freezing ($p<0.01$) except acrosome integrity of stage III and stage IV. The data is presented in Table 3.

4.4 EFFECT OF METHODS OF PRESERVATION ON SEMEN QUALITY

There was significant difference ($p<0.01$) between quality of frozen semen and chilled semen at various storage periods. The data is presented in Table 4.

4.5 ARTIFICIAL INSEMINATION

A total of 44 healthy does in oestrus were selected for fertility trials. None of

the animals were presented for insemination 24 hours after first insemination.

4.5.1 Heat signs

Predominant behavioural signs observed using "Buck jar" technique were bleating (79.55 per cent), wagging of tail (65.91 per cent), frequent urination (45.45 per cent) and flehmen reaction (13.64 per cent). The major clinical signs were vulval oedema, moistness and hyperaemia of vagina, mucus discharge and opening of cervical os.

4.5.2 Intensity of heat signs

Out of the 44 does, percentage of animals which showed intensity of heat score of two, three and four were 25, 59.09 and 15.91 respectively. None of the animals showed heat intensity score of one. Average score of heat intensity was 2.91 ± 0.10.

4.5.3 Depth of penetration of AI gun into cervix

The depth of penetration of AI gun into the cervical lumen ranged from 6 - 36 mm with an average of 20.07 ± 1.35 mm. Out of the total 44 does inseminated, in 45.45 per cent (20/44), 20.45 per cent (9/44) and 18.18 per cent (8/44) the depth of penetration of AI gun was in the range of 10-20 mm, 20-30 mm and 30-40 mm respectively. But in 15.91 percentage of animals (7/44) the depth of penetration of AI gun was in the range of 0-10 mm.

4.5.4 Correlation between intensity of heat and depth of penetration of AI gun

No significant correlation was observed between intensity of heat and depth of penetration of AI gun ($p>0.05$).

4.6 CONCEPTION RATE

4.6.1 Overall conception rate (both chilled and frozen)

Out of 44 does inseminated, 27 were diagnosed as pregnant with an overall conception rate of 61.36 per cent.

4.6.2 AI using chilled semen.

Out of 33 does inseminated, 24 were diagnosed as pregnant with an overall conception rate of 72.73 per cent.

4.6.2.1 Group I (0-24 h)

Among the eleven animals of this group, nine were confirmed as pregnant and two as non-pregnant based on abdominal palpation at three months (eight does) and ultrasonography at two months (three does). The conception rate was 81.82 per cent.

4.6.2.2 Group II (24-48 h)

Of the eleven animals of this group seven were confirmed as pregnant and four as non-pregnant based on abdominal palpation (nine does) and ultrasonography (two does). The conception rate was 63.64 per cent.

4.6.2.3 Group III (48-72 h)

Among the eleven animals of this group eight were confirmed as pregnant and three as non-pregnant based on abdominal palpation (seven does) and ultrasonography (four does). The conception rate was 72.73 per cent.

4.6.2.4 Effect of duration of chilling on conception rate

The data regarding effect of duration of chilling on conception rate is presented in Table 5. Chi- square test showed that there was no significant difference

($p > 0.05$) between storage periods on conception rate.

4.6.3 AI using frozen semen (Group IV)

Of the eleven animals of group IV three were confirmed as pregnant and eight as non-pregnant based on ultrasonography. The conception rate was 27.27 per cent.

4.6.4 Effect of methods of preservation on conception rate

The conception rate of does inseminated using chilled semen (at various storage periods) and frozen semen are plotted in Fig. 1. Overall conception rate of does inseminated using chilled and frozen semen are shown in Fig. 2. Chi-square test showed that there was significant difference ($p<0.01$) in conception rate between chilled and frozen semen.

4.7 EFFECT OF INTENSITY OF HEAT ON PREGNANCY STATUS

The details regarding intensity of heat score and overall conception rate are furnished in Table 6 and Fig.3.

No significant correlation was observed between intensity of heat and pregnancy status of does inseminated using chilled semen at various storage periods. Significant correlation ($p<0.05$) was observed between intensity of heat and pregnancy status when AI was performed using frozen semen. No significant correlation ($p>0.05$) was observed between intensity of heat and overall pregnancy status (Table 7).

4.8 EFFECT OF DEPTH OF PENETRATION OF AI GUN ON PREGNANCY STATUS

The details regarding depth of penetration of AI gun and overall conception rate are furnished in Table 8 and Fig.4.

No significant correlation was observed between depth of penetration of AI gun and pregnancy status at various storage periods of chilled semen. But significant correlation ($p<0.05$) was noticed between depth of penetration of AI gun and pregnancy status of animals inseminated using frozen semen. There was no significant correlation ($p>0.05$) between depth of penetration of AI gun and overall pregnancy status (Table 9).

Table 2. Effect of duration of chilling on semen quality

Serial No.	Parameter	Period of storage	Mean ± S.E.	t value
1	Motility	0 h 24 h	83.06 ± 0.36 70.25 ± 0.60	21.12**
		24 h 48 h	70.25 ± 0.60 61.13 ± 0.72	23.64**
		48 h 72 h	61.13 ± 0.72 42.81 ± 0.95	31.06**
2	Sperm viability	0 h 24 h	89.96 ± 0.70 83.42 ± 1.27	6.84**
		24 h 48 h	83.42 ± 1.27 78.84 ± 1.47	8.61**
		48 h 72 h	78.84 ± 1.47 74.57 ± 1.53	10.68**
3	Sperm abnormality	0 h 24 h	2.02 ± 0.09 2.97 ± 0.13	10.43**
		24 h 48 h	2.97 ± 0.13 4.12 ± 0.15	18.09**
		48 h 72 h	4.12 ± 0.15 5.34 ± 0.11	13.21**
4	Acrosome integrity	0 h 24 h	92.50 ± 0.21 90.27 ± 0.18	18.67**
		24 h 48 h	90.27 ± 0.18 87.71 ± 0.37	8.44**
		48 h 72 h	87.71 ± 0.37 85.09 ± 0.44	17.74**

** denotes significant at 1 per cent level

Table 3. Effect of processing and freezing on semen quality

Serial No.	Parameters	Stage of freezing	Mean ± S.E.	t value
1	Motility	Stage I II	78.50 ± 0.50 73.67 ± 0.21	12.04**
		Stage II III	73.67 ± 0.21 63.00 ± 0.63	13.75**
		Stage III IV	63.00 ± 0.63 37.67 ± 0.49	50.80**
2	Sperm viability	Stage I II	84.67 ± 0.91 79.19 ± 0.48	10.10**
		Stage II III	79.19 ± 0.48 69.25 ± 1.48	8.12**
		Stage III IV	69.25 ± 1.48 60.36 ± 0.77	8.20**
3	Sperm abnormality	Stage I II	3.82 ± 0.12 4.75 ± 0.15	8.45**
		Stage II III	4.75 ± 0.15 5.58 ± 0.15	7.41**
		Stage III IV	5.58 ± 0.15 6.34 ± 0.22	4.94**
4	Acrosome integrity	Stage I II	85.68 ± 0.72 80.84 ± 1.73	6.30**
		Stage II III	80.84 ± 1.73 77.61 ± 1.09	2.96**
		Stage III IV	77.61 ± 1.09 76.06 ± 1.41	1.499^{NS}

** denotes significant at 1 per cent level

^{NS} denotes non significant

Table 4. Effect of methods of preservation on semen quality

Serial No.	Parameter	Preservation methods	Mean ± S.E.	t value
1	Motility	Freezing Chilling(0 h)	37.67 ± 0.49 83.06 ± 0.36	68.55**
		Freezing Chilling(24 h)	37.67 ± 0.49 70.25 ± 0.60	31.34**
		Freezing Chilling(48h)	37.67 ± 0.49 61.13 ± 0.72	19.14**
		Freezing Chilling(72h)	37.67 ± 0.49 42.81 ± 0.95	3.20**
2	Sperm viability	Freezing Chilling(0 h)	60.36 ± 0.77 89.96 ± 0.70	23.70**
		Freezing Chilling(24 h)	60.36 ± 0.77 83.42 ± 1.27	10.70**
		Freezing Chilling(48h)	60.36 ± 0.77 78.84 ± 1.47	7.44**
		Freezing Chilling(72h)	60.36 ± 0.77 74.57 ± 1.53	5.53**
3	Sperm abnormality	Freezing Chilling(0 h)	6.34 ± 0.22 2.02 ± 0.09	21.70**
		Freezing Chilling(24 h)	6.34 ± 0.22 2.97 ± 0.13	13.76**
		Freezing Chilling(48h)	6.34 ± 0.22 4.12 ± 0.15	8.11**
		Freezing Chilling(72h)	6.34 ± 0.22 5.34 ± 0.11	4.50**
4	Acrosome integrity	Freezing Chilling(0 h)	76.06 ± 1.41 92.50 ± 0.21	18.30**
		Freezing Chilling(24 h)	76.06 ± 1.41 90.27 ± 0.17	16.19**
		Freezing Chilling(48h)	76.06 ± 1.41 87.71 ± 0.37	11.28**
		Freezing Chilling(72h)	76.06 ± 1.41 85.09 ± 0.44	8.16**

** denotes significant at 1 per cent level

Table 5. Effect of duration of chilling on conception rate

Duration of storage of chilled semen	Number of does inseminated	Number of does conceived	Conception rate (%)	Chi-square value
0-24 h	11	9	81.82	0.92^{NS}
24-48 h	11	7	63.64	
48-72 h	11	8	72.73	

^{NS} denotes non significant

Table 6. Intensity of heat signs and overall conception rate

Intensity of heat score	Number of does inseminated	Number of does conceived	Conception rate (%)
1	-	-	-
2	11	7	63.64
3	26	15	57.69
4	7	5	71.43
Overall	44	27	61.36

Table 7. Effect of Intensity of heat on pregnancy status in goats inseminated using chilled and frozen semen

Intensity of heat score	Chilled semen									Frozen semen			Overall
	Group I (0-24 h storage)			Group II (24-48 h storage)			Group III (48-72 h storage)			Overall	Group IV			
	Number of does inseminated	Number of does conceived (%)	r	Number of does inseminated	Number of does conceived (%)	r	Number of does inseminated	Number of does conceived (%)	r	r	Number of does inseminated	Number of does conceived (%)	r	r
1	0	0		0	0		0	0			0	0		
2	3	3(100)	-0.082	4	2(50)	-0.129	2	2(100)	-0.124	-0.113	2	0	0.677*	0.029
3	6	4(66.67)		5	5(100)		8	5(62.5)			7	1(14.29)		
4	2	2(100)		2	0		1	1(100)			2	2(100)		

r = Spearman's rank Correlation Coefficient

*Correlation is significant at the .05 level

Table 8. Depth of penetration of AI gun and overall conception rate

Depth of penetration of AI gun (mm)	Number of does inseminated	Number of does conceived	Conception rate (%)
0-10	7	4	57.14
10-20	20	12	60
20-30	9	4	44.4
30-40	8	7	87.5
Overall	44	27	61.36

Table 9. Effect of depth of penetration of AI gun on pregnancy status in goats inseminated using chilled and frozen semen

Depth of penetration of AI gun (mm)	Chilled semen										Frozen semen			Overall
	Group I (0-24 h storage)			Group II (24-48 h storage)			Group III (48-72 h storage)			Overall	Group IV			
	Number of does inseminated	Number of does conceived (%)	r	Number of does inseminated	Number of does conceived (%)	r	Number of does inseminated	Number of does conceived (%)	r	r	Number of does inseminated	Number of does conceived (%)	r	r
0-10	2	2(100)	0.113	3	2(66.67)	-0.090	1	0	0.323	0.108	1	0	0.679*	0.109
10-20	7	5(71.43)		5	3(60)		5	4(60)			3	0		
20-30	0	0		2	1(50)		3	2(66.67)			4	1(25)		
30-40	2	2(100)		1	1(100)		2	2(100)			3	2(66.67)		

r = Spearman's rank Correlation Coefficient

*Correlation is significant at the .05 level

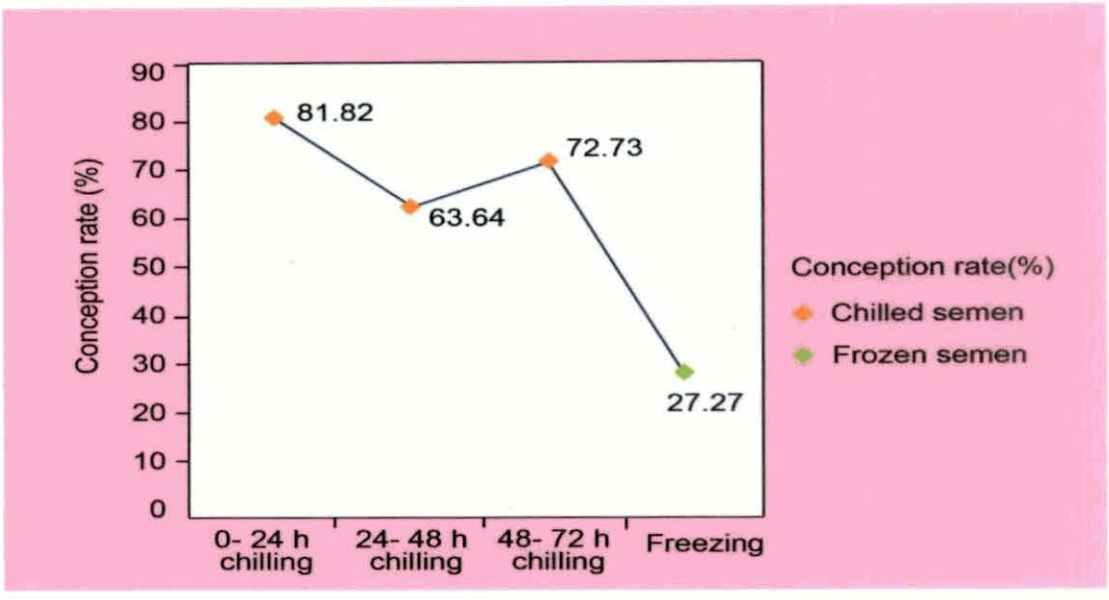

Fig. 1. Conception rate of does inseminated using chilled semen (various storage periods) and frozen semen

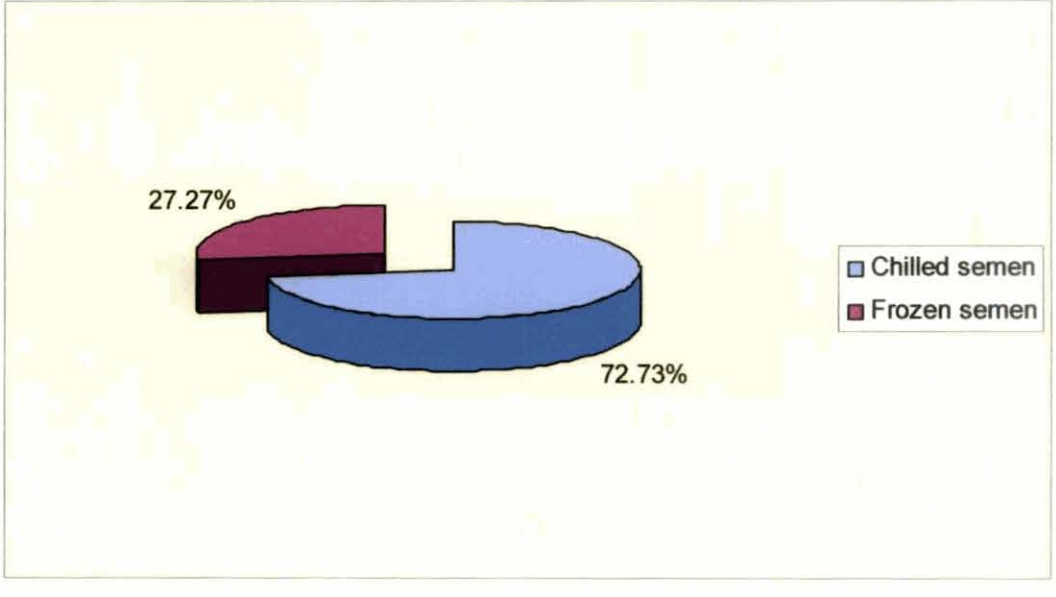

Fig. 2. Overall conception rate of does inseminated using chilled semen and frozen semen

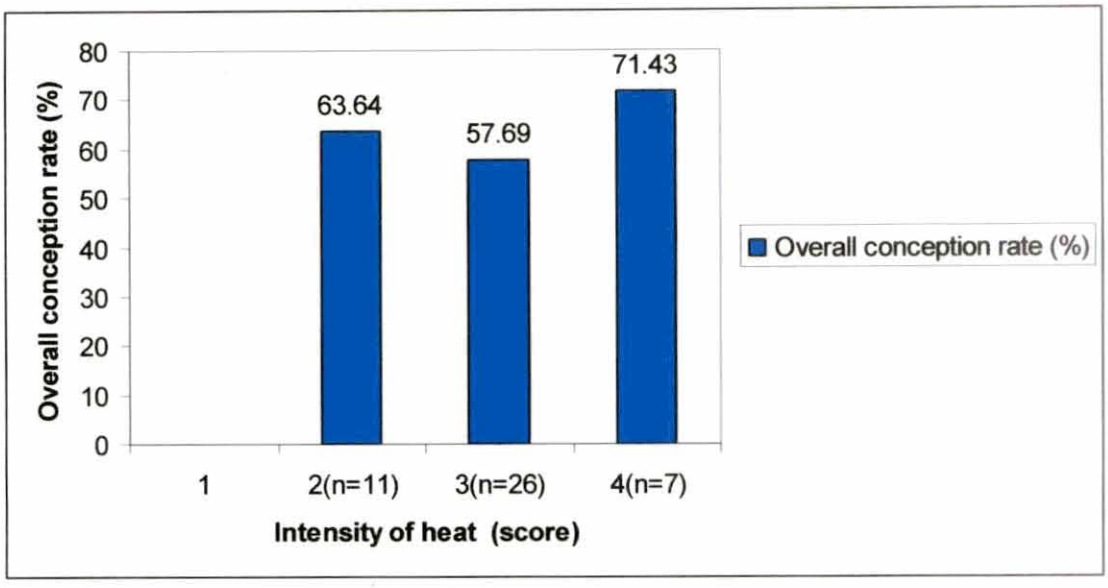

Fig. 3. Effect of intensity of heat on overall conception rate

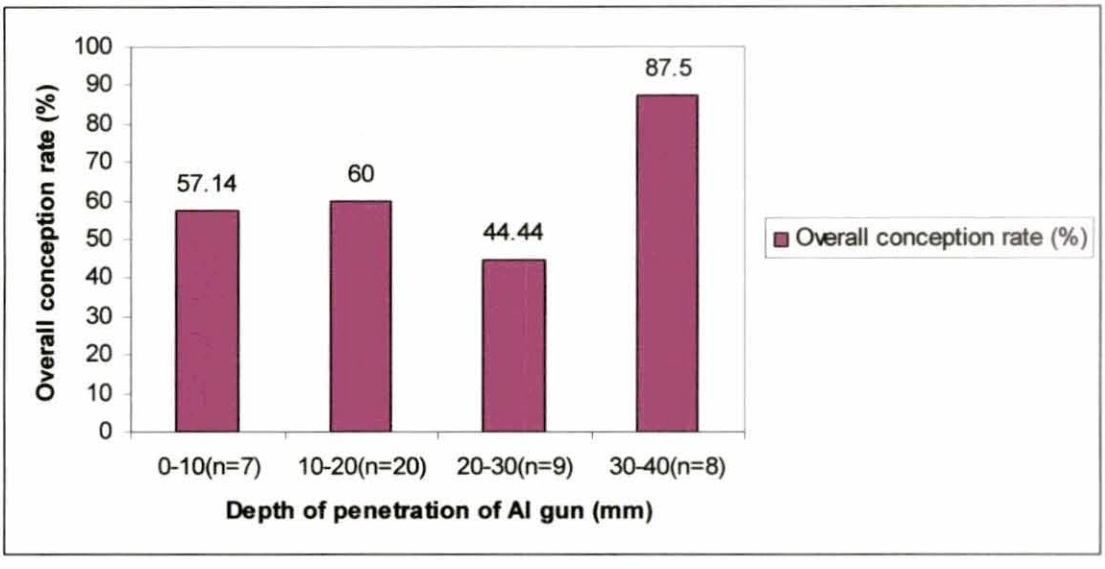

Fig. 4. Effect of depth of penetration of AI gun into the cervix on overall conception rate

Discussion

5. DISCUSSION

A total of 22 ejaculates from an adult Malabari crossbred buck were utilized for assessing factors affecting conception rate on artificial insemination in goats. Semen was collected thrice weekly at an interval of two to three days using artificial vagina. Forty four adult healthy does brought to the AI Centre for insemination were selected for the study.

5.1 Semen characteristics of fresh ejaculates of Malabari cross bred buck

The mean semen volume was found to be 1.25 ± 0.97 ml. The value is in agreement with the results recorded by Ranjini (1998) in Malabari cross bred bucks, Afsal (2003) in Boer bucks and Dorado *et al.* (2009) in Florida bucks. Lower values have been reported by Patil and Raja (1978) in Malabari bucks, Prasanth (1995) and Kutty and Mathew (2000) in Malabari crossbred bucks. Srinivas *et al.* (2002) studied the effect of season on semen volume of native bucks of Andhra Pradesh and reported the lowest volume (0.49 ± 0.03 ml) of semen was in summer season and highest (0.76 ± 0.03 ml) in winter season. However, higher values have been reported by Tuli and Holtz (1992) in Boer bucks. The conflicting results obtained for semen volume by different workers might be attributed to the fact that the volume of the ejaculate and the number of spermatozoa per ejaculate are inversely proportional to the number of ejaculates collected per day and the number of days the males has been used for semen collection (Lindsay, 1991). It can also be affected by breed, age of bucks and the method of semen collection. Teasing of bucks before service can improve the semen volume (Roberts, 1971).

The colour of all the ejaculates was creamy with yellowish tinge. Pandey *et al.* (1985) reported that colour of the seminal ejaculates of Saanen bucks was creamy, whereas those of Barbari bucks varied from yellowish to creamy. Prasanth (1995) and Ranjini (1998) in Malabari cross bred bucks and Afsal (2003) in Boer bucks recorded similar observations.

The overall density of buck semen was DDDD. This is in full agreement with

the earlier reported values (Prasanth, 1995; Ranjini, 1998 and Afsal, 2003).

The mean mass activity of buck semen was ++++, which is in agreement with the observations of Prasanth (1995) and Ranjini (1998) in Malabari cross bred bucks.

The average pH was found to be 6.89 ± 0.21. This is comparable to the values observed by Prasad *et al.* (1986) in Saanen and Barbari bucks and Prasanth (1995) in Malabari cross bred bucks. Afsal (2003) obtained an average pH of 6.98 ± 0.03 in Boer bucks. Lower values have been reported by Patil and Raja (1978) in Malabari and Kutty and Mathew (2000) in Malabari cross bred bucks, while Ranjini (1998) recorded a pH of 7.28 ± 0.04 in Malabari cross bred bucks.

The mean sperm concentration of buck semen was 2781.82 ± 51.69 millions per ml, which is comparable to the values recorded by Pandey *et al.* (1985) in Barbari bucks and Prasad *et al.* (1986) in Saanen bucks. Prasanth (1995) in Malabari cross bred bucks obtained a sperm concentration of 2842.33 ± 153.93 millions per ml. Higher values have been observed by Patil and Raja (1978) in Malabari bucks, Cetinkaya *et al.* (1980) in Angora bucks and Pandey *et al.* (1985) in Saanen bucks. The sperm concentration of Florida buck semen was reported to be 3690 ± 0.08 millions per ml (Dorado *et al.*, 2009). However, Prasad *et al.* (1986) in Barbari bucks, Pattnaik *et al.* (1991) in Ganjam bucks and Srinivas *et al.* (2002) in native bucks of Andhra Pradesh obtained lower sperm concentration.

The mean sperm motility percentage of buck semen was 82.77 ± 0.33 which is comparable with the values reported by Patil and Raja (1978) in Malabari goats and Sevinc *et al.* (1985) in Angora goats. The average percentage of individual sperm motility was observed to be 81.78 ± 0.53 and 78.06 ± 0.68 in winter and summer seasons respectively in indigenous bucks of Andhra Pradesh (Srinivas *et al.*, 2002). Lower values were reported by Pattnaik *et al.* (1991) in Ganjam bucks, Wuschko and Seifert (1991) in West African dwarf goats and Singh and Nasir (1995) in Sirohi bucks. Filho *et al.* (1993) reported an average sperm motility of 50.7, 56.8 and 63.2 per cent in Alpine, Anglo – Nubian and Caninde bucks respectively. However, Cetinkaya *et al.* (1980) in Angora goats, Prasanth (1995) in Malabari cross bred bucks

and Zamfirescu and Nadolu (2007) in Alpine and Saanen bucks obtained higher motility percentage.

Mean percentage of live sperm was 90.14 ± 0.53 which is within the range of values reported by Prasad *et al.* (1986) in Saanen, Barbari and Saanen × Barbari bucks, Prasanth (1995) and Ranjini (1998) in Malabari cross bred bucks. Simon (1999) recorded an average live sperm percentage of 94.69 ± 0.67 in Malabari crossbred bucks. Lower values have been recorded by Patil and Raja (1978), Pandey *et al.* (1985) and Tuli and Holtz (1992).

Average percentage of abnormal spermatozoa in the fresh semen was 2.28 ± 0.12 which is comparable to the values observed by Cetinkaya *et al.* (1980) in Angora bucks. According to Sevinc *et al.* (1985) average abnormal count of spermatozoa in Angora goats was 2.06 ± 0.42 per cent. Many workers reported higher values (Patil and Raja, 1978; Prasad *et al.,* 1986; Pattnaik *et al.*, 1991; Filho *et al.*, 1993 and Srinivas *et al.*, 2002).

The percentage of intact acrosomes averaged 92.27 ± 0.21. Comparable result was reported by Ranjini (1998). Higher values have been reported by Simon (1999). Mean percentage of spermatozoa with abnormal acrosome in the fresh semen of Boer bucks was 1.00 ± 0.13 (Afsal, 2003). However, Srinivas *et al.* (2002) recorded lower values in native goats of Andhra Pradesh. Sane *et al.* (1991) suggested that reduction in the number of spermatozoa with intact acrosomes in semen was due to epididymal ageing of spermatozoa or improper handling of semen.

Joseph and Nair (1991) opined that age, size, breed, season, nutrition and frequency of ejaculation are the major factors that lead to variable semen characteristics of buck semen noticed by different workers.

5.2 SEMEN QUALITY DURING AND AFTER PRESERVATION BY CHILLING

5.2.1 At 24 hours of preservation

5.2.1.1 Sperm motility

The mean percentage of motile sperm was 70.25 ± 0.60. According to Simon (1999) the mean sperm motility of Malabari crossbred buck was 70.55 ± 0.17 per cent at 24 h of preservation. Balakrishnan (1979) and Sarmah (1983) observed slightly higher values. However Singh *et al.* (1982) and Ranjan *et al.* (2009) reported lower values.

5.2.1.2 Sperm viability

The mean percentage of live sperm was 83.42 ± 1.27. The mean live sperm count of Marwari buck semen was 86.17 ± 2.75 per cent at 24 h of preservation in 10 per cent egg yolk level at refrigeration temperature (Ranjan *et al.*, 2009). But much lower values were recorded by Sarmah (1983) and Simon (1999) in Malabari cross bred bucks.

5.2.1.3 Sperm abnormality

The mean sperm abnormality was 2.97 ± 0.13 per cent. However, higher values were recorded by Sarmah (1983) and Simon (1999).

5.2.1.4 Acrosome integrity

The mean percentage of sperms with intact acrosome was 90.27 ± 0.18 which is almost similar to the observation made by Simon (1999) in Malabari crossbred bucks. Sarmah (1983) reported that the acrosome defects of buck semen were 2.76 per cent at 24 hours of preservation in Tris diluent.

5.2.2 At 48 hours of preservation

5.2.2.1 Sperm motility

The mean percentage of sperm motility was 61.13 ± 0.72 which is comparable with the observation made by Balakrishnan (1979). Simon (1999) reported that the mean sperm motility of Malabari crossbred buck semen at 48 h of preservation was 62.50 ± 1.27 per cent. However, lower values were recorded by Singh *et al.* (1982) and Ranjan *et al.* (2009).

5.2.2.2 Sperm viability

The mean percentage of live sperm was 78.84 ± 1.47. Sarmah (1983) and Simon (1999) obtained much lower values. The live sperm count of Marwari buck semen was 84.67 ± 1.87 per cent at 48 h of preservation in Tris diluent with 10 per cent egg yolk at refrigeration temperature (Ranjan *et al.*, 2009).

5.2.2.3 Sperm abnormality

The mean sperm abnormality percentage was 4.12 ± 0.15. The percentage of sperm abnormalities of Malabari crossbred buck semen at 48 h of preservation was 4.74 ± 0.48 per cent (Simon, 1999).

5.2.2.4 Acrosome integrity

The mean percentage of sperms with intact acrosome was 87.71 ± 0.37 per cent which is lower than the value reported by Simon (1999).

5.2.3 At 72 hours of preservation

5.2.3.1 Sperm motility

The mean percentage of motile sperms was 42.81 ± 0.95 which is comparable to the value obtained by Ranjan *et al.* (2009). Much higher values are recorded by

Balakrishnan (1979), Sarmah (1983) and Puranik (1994). However, Singh *et al.* (1982) recorded a motility percentage of 29.50 ± 1.01 in Tris yolk glycerol diluent at 72 hours of preservation.

5.2.3.2 Sperm viability

The mean percentage of live sperms was 74.57 ± 1.58. Ranjan *et al.* (2009) in Marwari buck reported a live sperm count of 83.17 ± 1.01 per cent at 72 h of preservation in 10 per cent egg yolk level at refrigeration temperature. But Sarmah (1983) observed much lower values.

5.2.3.3 Sperm abnormality

Mean sperm abnormality was 5.34 ± 0.11 per cent. However, Sarmah (1983) obtained 6.76 per cent sperm abnormality at 72 hours of preservation in Tris diluent.

5.2.3.4 Acrosome integrity

The mean percentage of sperms with intact acrosome was 85.09 ± 0.44 which is lower than the values recorded by Sarmah (1983).

5.2.4 Effect of duration of chilling on semen quality

There was significant difference between semen characteristics at various storage periods. Motility, viability and acrosome integrity of sperm decreased with increase in storage periods. Sperm abnormality increased with duration of storage. Similar results were obtained by Balakrishnan (1979), Singh *et al.* (1982), Sarmah (1983), Simon (1999) and Ranjan *et al.* (2009).

Major percentage of sperm abnormality was due to significant increase in tail defects as the period of storage increased (Rao and Rao, 1979).

Watson (1975) opined that the increase in acrosomal abnormalities might be due to progressive disruption of acrosomal membrane during preservation by chilling.

5.3 SEMEN QUALITY DURING CRYOPRESERVATION

5.3.1 Initial extension (Stage I)

5.3.1.1 Sperm motility

The mean percentage of sperm motility was 78.50 ± 0.50 which is higher than the values recorded by Simon (1999) and Prasanth (1995). However, Ranjini (1998) obtained 82.00 ± 0.61 per cent sperm motility after washing and initial extension.

Memon *et al.* (1985) reported that the percentage of sperm motility was higher in samples devoid of seminal plasma because of the removal of an egg yolk coagulating factor present in the seminal plasma. Deka and Rao (1987) opined that there was no significant reduction in sperm motility by centrifugation. According to Azeredo *et al.* (2001) seminal plasma removal caused a significant reduction in the percentage of motile spermatozoa.

5.3.1.2 Sperm viability

The mean percentage of live sperms was 84.67 ± 0.91 which is slightly lower than the value recorded by Ranjini (1998).

5.3.1.3 Sperm abnormality

The mean sperm abnormality was 3.82 ± 0.12 which is in agreement with the report of Ranjini (1998). The increase in sperm abnormality compared to fresh semen may be due to mechanical damage caused by centrifugation.

5.3.1.4 Acrosome integrity

The mean percentage of sperms with intact acrosome was 85.68 ± 0.72 per cent. The average percentage of intact acrosomes after washing of semen and initial extension was 86.05 ±1.25 (Ranjini, 1998).

5.3.2 Cooling to 5°C (Stage II)

5.3.2.1 Sperm motility

Mean percentage of sperm motility was 73.67 ± 0.21 which is lower than the value obtained by Ranjini (1998).

5.3.2.2 Sperm viability

The mean percentage of sperm viability was 79.19 ± 0.48. However, Ranjini (1998) obtained a sperm viability of 83.00 ± 0.72 per cent after cooling to 5°C.

5.3.2.3 Sperm abnormality

The mean percentage of sperm abnormality was 4.75 ± 0.15. This value is in agreement with the values obtained by Ranjini (1998).

The increase in sperm abnormalities from that of stage I might be due to cold shock as the samples were kept at 5°C.

5.3.2.4 Acrosome integrity

The mean percentage of sperms with intact acrosome was 80.84 ± 1.73 which is slightly lower than the values reported by Ranjini (1998).

5.3.3 Glycerolisation and equilibration (Stage III)

5.3.3.1 Sperm motility

Mean sperm motility was 63.00 ± 0.63 per cent. However, Ranjini (1998) obtained a sperm motility percentage of 70.50 ± 0.96 after glycerolisation and equilibration. Significant reduction in percentage motility after glycerolisation was probably due to the interaction between spermatozoa and glycerol (Prasanth and Mathai, 1996).

5.3.3.2 Sperm viability

Mean percentage of live sperms was 69.25 ± 1.48. This is much lower than the value reported by Ranjini (1998).

5.3.3.3 Sperm abnormality

The mean percentage of sperm abnormality was 5.58 ± 0.51. As per the report of Ranjini (1998) the percentage of sperm abnormality after glycerolisation and equilibration was 5.50 ± 0.43.

5.3.3.4 Acrosome integrity

The mean percentage of sperms with intact acrosome was 77.61 ± 1.09. This is slightly lower than the value recorded by Ranjini (1998).

5.3.4 Freezing and thawing (Stage IV)

5.3.4.1 Sperm motility

The mean post thaw motility was 37.67 ± 0.49. This abrupt reduction in motility from that of stage III might be due to formation of intracellular ice crystals or increased solute concentration in the media or due to their interaction (Arthur, 1996). This value is much lower than those obtained by Corteel and Baril (1975), Cetinkaya *et al.* (1980), Deka and Rao (1985), Sinha *et al.* (1987), Sinha *et al.*(1991), Das and Rajkonwar (1995) and Mendez *et al.* (1995). Average post thaw motility of Malabari crossbred buck semen diluted in Tris diluent containing six per cent glycerol and packed in 0.5 ml French straws was 42.0 ± 1.84 (Prasanth and Mathai, 1996). Mendez *et al.* (1995) reported that semen frozen in 0.5 ml straws and thawed at 37°C for 15 s was having an average post thaw motility of 62.7 per cent compared to 59.0 per cent for similarly treated semen frozen in 0.25 ml straws. (1995) Ranjini (1998), Sivaselvam *et al.* (2000), Tambing *et al.* (2002), Afsal (2003), Baruah *et al.* (2003), Zamfirescu and Nadolu (2007) and Apu *et al.*(2009) obtained higher values. However, Simon (1999) obtained a post thaw motility of 35.42 ± 0.71, 34.45 ± 0.74,

33.17 ± 1.14 at zero, fifteen and thirty days of freezing respectively. Azeredo *et al.* (2001) recorded a post thaw motility of 28.32 per cent in Saanen bucks. Purdy (2006) obtained lower values.

The variable results recorded by different workers could be attributed to the difference in composition of the extenders, equilibration time and methods of freezing and preservation of the semen (Das and Rajkonwar, 1995).

5.3.4.2 Sperm viability

The mean sperm viability was 60.36 ± 0.77 which is in agreement with that of Simon (1999) but much higher than that recorded by Ranjini (1998). Deshpande and Mehta (1991) reported that the percentage of live sperms after 24 hours of freezing was 44.50 ± 2.98 at six per cent glycerol level. Mean percentage of live spermatozoa in the freeze thawed Boer buck semen was 52.61 ± 1.56 (Afsal, 2003).

5.3.4.3 Sperm abnormality

The mean percentage of sperm abnormality was 6.34 ± 0.22 which is lower than that reported by Ranjini (1998) and is almost in agreement with that of Simon (1999).

5.3.4.4 Acrosome integrity

The mean percentage of intact acrosomes was 76.06 ± 1.41 which is in full agreement with that obtained by Ranjini (1998) but is lower than the values recorded by Simon (1999). The mean percentage of intact acrosomes after freezing in Tris hydroxymethyl aminomethane-citric acid-egg yolk-glycerol diluent were 69.29 ± 4.61 and 66.07 ± 5.06 for washed and unwashed semen respectively (Memon *et al.*, 1985). The percentage of intact acrosomes in Beetal buck semen after freezing in Tris, skim milk and EYC extenders was found to be 79.01 ± 0.44, 55.80 ± 0.38, 51.90 ± 0.54 per cent respectively (Sinha *et al.*, 1991). Singh and Purbey (1996) reported that the percentage of intact acrosomes in buck semen diluted in Tris extender and frozen in

liquid nitrogen declined from 88.76 ± 0.43 in pre freeze to 68.90 ± 0.82 in post thaw semen.

5.3.5 Effect of processing and freezing on semen quality

Significant difference was observed between sperm motility, viability, abnormality and acrosome integrity at various stages of processing and freezing except acrosome integrity of stage III and stage IV. There was progressive reduction in semen quality from stage I to stage IV which may be due to the stress involved in the complex procedures of processing and freezing.

5.4 EFFECT OF METHODS OF PRESERVATION ON SEMEN QUALITY

There was significant difference ($p<0.01$) between semen quality of frozen semen and chilled semen at various storage periods.

Significant reduction in motility percentage after freezing was due to the death of weak spermatozoa from thermal shock and internal ice formation (Prasanth and Mathai, 1996). During the physical change in the media, the sperms are subjected to high stress and ice formation leading to mechanical injury in certain cases (Mathew, 1984). Azeredo *et al.* (2001) reported that removal of seminal plasma decreased motility and vigour rates in frozen samples. Cryopreservation induces detrimental effects in terms of sperm ultra structural, biochemical and functional damage resulting in a reduction in motility, membrane integrity and fertilizing ability (Dorado *et al.*, 2007).

Some ultra structural damages were observed in cryopreserved spermatozoa such as partial and integral plasmatic membrane loss, nuclear and mitochondrial degeneration (Zamfirescu and Nadolu, 2007) which may also contribute to the reduction in semen quality of frozen-thawed semen compared to fresh/chilled semen.

5.5 ARTIFICIAL INSEMINATION

5.5.1 Heat signs

Predominant behavioural signs observed using "Buck jar" technique were bleating (79.55 per cent), wagging of tail (65.91 per cent), frequent urination (45.45 per cent) and flehmen reaction (13.64 per cent). Similar results were reported by Goel and Agrawal (1994), Joseph (2003), Afsal (2003) and Nuti (2007) and Ramya *et al.* (2008).

The major clinical signs were oedema, moistness and hyperaemia of vulva and vagina and mucus discharge and opening of cervical os which is comparable with the observations made by Joseph (2003), Afsal (2003) and Nuti (2007).

5.5.2 Intensity of heat

Overall mean intensity of heat score was 2.91 ± 0.10 which is lower than that recorded by Joseph (2003) in superovulatd Malabari does.

5.5.3 Depth of penetration of AI gun in to cervix

The depth of penetration of AI gun ranged from 6 – 36 mm with an average of 20.07 ± 1.35 mm. It was reported that if the cervix had been penetrated at least 13 mm there was good chance for sperms to negotiate the remainder of the cervical canal. In no way, the depth of penetration should exceed 38 mm to avoid perforation of the uterine wall or entry into one of the uterine horns (Nuti, 2007).

5.5.4 Correlation between intensity of heat and depth of penetration of AI gun

No significant correlation was obtained between intensity of heat and depth of penetration of AI gun. Similar results were obtained by Naqvi *et al.* (1998) which showed that the degree of cervical penetration was not influenced by the stage of oestrus.

5.6 CONCEPTION RATE

Fertility rates of stored goat semen was influenced by factors like natural oestrus, synchronised and induced oestrus, characteristics of individual animal, season of semen production, physiological condition of females, age and parity, time of insemination, volume of inseminate and number of spermatozoa, site of semen deposition and superovulation (Leboeuf *et al.* 2000).

5.6.1 AI using chilled semen

Overall conception rate in does inseminated using chilled semen was 72.73 per cent.

5.6.1.1 Group I (0-24 h)

The conception rate was 81.82 per cent which is much higher than the values obtained by Balakrishnan (1979) and Mathai and Nair (1981). Senthilkumar (2002) observed conception percentage of 45.45 after insemination with chilled semen in Malabari crossbred buck. Molina *et al.* (2007) reported a conception rate of 62.4 per cent using fresh cooled semen from Payoya goats. This higher value may be due to the fact that in the present study semen was deposited by AI gun which facilitated deep penetration compared to glass pipette.

According to Ritar and Salamon (1983) depth of deposition semen into the genital tract influences fertility rates, which improve with increasing depth of insemination. However, Roca et al. (1997) in their study in Murciano - Granadina goats reported that fertility rates were indistinguishable between uterus and cervical deposition. The reason for this was attributed to increased number of sperms per dose of semen inseminated. So the higher conception rate in the present study might be due to increased depth of penetration as well due to increased number of spermatozoa per dose.

5.6.1.2 Group II (24-48 h)

The conception rate was 63.64 per cent which is comparable with the values obtained by Bargohain *et al.* (1985). This was much higher than the values obtained by Mathai and Nair (1981).

5.6.1.3 Group III (48-72 h)

Conception rate was 72.73 per cent. This is much higher than those reported by Mathai and Nair (1981).

5.6.1.4 Effect of duration of chilling on conception rate

In the present study, conception rate was maximum when chilled semen stored up to 24 hours was used for AI. Beyond 24 hours, the conception rate decreased. This is in agreement with the observations made by Mathai and Nair (1981) who reported that conception rate for inseminations done with chilled semen of bucks stored up to 24 hours was 42.78 per cent and for semen stored beyond 24 hours it was only 29.26 per cent.. This is mainly due to the reduction in semen quality with increasing period of storage (Roca *et al.,* 1997). However, higher conception rate was obtained with semen stored for 48-72 hours compared with semen stored for 24-48 hours. No significant difference was observed between conception rates at various storage periods. This shows that the number of progressively motile spermatozoa per dose of semen inseminated is more important than duration of storage in determining the conception rate. Increased depth of penetration provided in this study might have contributed to this. The does used for fertility trials were maintained by farmers under various managemental regimes. Individual variations in the reproductive and nutritional status of does as well as the lower number of animals used in the present study might have contributed to this variation. Pregnancy loss due to embryonic death may also be a causative factor for this variation

5.6.2 Group IV (AI using frozen semen)

Conception rate was 27.27 per cent which is similar to that obtained by Zamfirescu and Nadolu (2007) for Saanen bucks. Cetinkaya *et al.* (1980) in Angora goats, Sinha *et al.* (1987) in Jamnapari, Barbari and Black Bengal bucks, Prasanth (1995) in Malabari x Alpine cross bred bucks, Zamfirescu and Nadolu (2007) in Alpine bucks, Molina *et al.* (2007) in Payoya goats and Dorado *et al.* (2007) in Florida goats, Angora goats, Cashmere goats, Beetal goats, Bengal goats and Murciano-Granadina goats obtained higher values. However, Naqvi *et al.* (1998) obtained overall lambing rate of 22.7 per cent in Malpura ewes from deposition of frozen-thawed semen into the cervix or uterus using the transcervical technique even though the mean post-thaw motility of semen was 78.70 per cent. Afsal (2003) recorded a conception rate of 21.68 per cent in oestrus synchronized crossbred Malabari does inseminated using frozen semen.

The major reason for reduced conception rate in the present study might be due to the low freezability of the ejaculates from the Malabari cross bred buck used which may be responsible for the low post thaw motility. Irrespective of the process used for freezing, differences have been observed between males regarding the freezability and fertility of semen (Leboeuf *et al.*, 2000). Reduction in conception rate can also be attributed to the low post thaw motility obtained in the present study compared to other workers. Eppleston *et al.* (1994) reported that there was a linear increase in fertilization rate of ova as the number of motile spermatozoa inseminated increased. In addition only single insemination was carried out in the present study as none of the animals were presented for AI 24h later. Prasanth (1995) obtained a conception rate of 47.36 per cent by practising double insemination using 0.5 ml straws. Another causative factor for low freezability which resulted in low post thaw motility is the use of 0.25 ml French straws in manual freezing in the present study instead of 0.5 ml straws used by other workers as suggested by Mendez *et al.* (1995). In addition, transcervical insemination was practised in the study. However, the best method to improve the fertility of frozen thawed semen was intrauterine insemination by laparoscopy (Moore et al., 1989; Salamon and Maxwell, 1995b and Baldassarre and Karatzas, 2004). Fertility trials in a large population of does are required to obtain more accurate results.

Differences in managemental practices, methods of processing and freezing and skill of inseminators may have also contributed to the variation in fertility rates by different investigators (Prasanth and Mathai, 1996).

5.6.3 Effect of methods of preservation on conception rate

Overall conception rate in does inseminated using chilled semen was 72.73 per cent while that of frozen semen was 27.27 per cent. There was significant difference ($p<0.01$) in conception rate between chilled and frozen semen.

Lower conception rate of frozen semen compared to that of chilled semen obtained in the present study might be due to the fact that cryopreservation induces detrimental effects in sperm cells, resulting in a reduction of motility, membrane integrity and fertilizing ability (Purdy, 2006). In the present study even after 72 hours of refrigeration storage, there was 42.81 ± 0.95 per cent sperm motility compared to 37.67 ± 0.49 per cent post thaw motility which may also be responsible for the difference in conception rate between frozen and chilled semen.

An additional problem with frozen-thawed semen is the reduced viability of spermatozoa in the female reproductive tract, characterised by longevity often half that of fresh spermatozoa (Salamon and Maxwell, 1995b). According to Maxwell and Watson (1996), frozen storage of semen advances the maturation of sperm membranes, thus increasing the proportion of capacitated and acrosome reacted cells in comparison with fresh semen. Such 'capacitated cells' have a short life span and can not withstand further 'ageing' in the reproductive tract. If those capacitated cells are not exposed to oocytes within a short time, fertilization will not be achieved. So, determination of ideal time for insemination is very much important in increasing the fertility rate of frozen semen. In Angora goats, Ilgaz and Sevinc (1983) obtained higher kidding rates when inseminations were carried out 12 h after the beginning of oestrus compared to inseminations at the beginning of oestrus or 24 h after the beginning of oestrus. In the present study timing of insemination in relation to ovulation could not be precisely assessed as the animals were maintained by individual farmers. None of the animals were presented for a second insemination after 24 hours of first AI.

The sperm plasma membrane is richly endowed with unsaturated fatty acids which may cause peroxidative damage. Excessive production of reactive oxygen species is associated with reduction in the motility and fertilizing capacity of stored spermatozoa which can be prevented by antioxidants. Antioxidants may delay the membrane destabilisation associated with sperm ageing and thus improve the fertility of frozen but not fresh spermatozoa (Maxwell and Watson, 1996).

Freezing and thawing of ram semen causes ultra structural, biochemical and functional damage to a significant proportion of spermatozoa which include reduction in percentage of intact acrosomes and motile spermatozoa and morphological changes in the mitochondrial sheath resulting in loss of protein. Biochemical changes include increased release of glutamic oxaloacetic transaminase (GOT) due to cryogenic damage and loss of lipoproteins, amino acids especially acidic, hydrophobic and neutral amino acid residues associated with the plasma membrane. Freeze-thawing also caused inactivation of the acrosome enzymes hyaluronidase and acrosin. A decrease in sulphydryl groups in the cell membrane was also reported with a consequent destabilisation of its protein structure. Reduction or cessation of adenosine tri and diphosphate (ATP and ADP respectively) synthesis in the damaged mitochondria and a decrease in acrosomal proteolytic activity may be the causes of disturbance in the functional integrity of spermatozoa after freeze thawing (Salamon and Maxwell, 1995a).

Lower fertility of frozen semen samples may be as a result of decrease in the number of normal sperm in these samples (Gravance *et al.*, 1997). Nuti (2007) opined that minimum safe numbers of motile spermatozoa for A. I. in goats using frozen semen was 180 million per dose before preservation. It has been shown that the number of spermatozoa capable of fertilization is 2.5 times less in the thawed than in the fresh semen (Salamon and Maxwell, 1995a).

Methods used to improve the fertility after insemination using frozen semen include increased concentration of spermatozoa in the inseminate, treatment with hormones and double and deep cervical insemination (Salamon and Maxwell, 1995a). The number of motile spermatozoa inseminated was more important than whether single or double inseminations were performed (Salamon, 1977). According to

Salamon (1971) the second insemination significantly improved lambing. Injection of ewes with relaxin, oxytocin and prostaglandins may relax the cervix, which would permit deep cervical or uterine deposition of frozen-thawed semen, or help to increase the contractility of the genital tract and thus improve the sperm transport (Salamon and Maxwell, 1995a). Efficient method of oestrus detection and insemination at ideal time in relation to ovulation may improve the fertility of frozen semen. Synchronisation of oestrus and fixed time insemination also can be practised (Leboeuf *et al.,* 2000).

Since pregnancy diagnosis was done after a period of two months of insemination, this lower conception rate also can be attributed to embryonic death.

5.7 EFFECT OF INTENSITY OF HEAT ON PREGNANCY STATUS

No significant correlation was noticed between intensity of heat and pregnancy status of does inseminated using chilled semen in the present study. But significant ($p<0.05$) correlation was observed between intensity of heat and pregnancy status of does inseminated using frozen semen. This shows that since cryopreservation results in severe reduction in semen quality, fertilizing ability and viability in the female reproductive tract, determination of optimal time for insemination in relation to ovulation (Leboeuf *et al.,* 2000) will give an added advantage in increasing the conception rate, when single insemination is practised. However, fertility trials using large number of females are required to draw definite conclusions using frozen semen.

5.8 EFFECT OF DEPTH OF PENETRATION OF AI GUN ON PREGNANCY STATUS

No significant correlation was observed between depth of penetration of AI gun and pregnancy status of does inseminated using chilled semen. However, significant correlation was observed between depth of penetration of AI gun and pregnancy status of goats inseminated using frozen semen. A major factor limiting the fertility of frozen- thawed semen is the inability of the spermatozoa to traverse the cervical folds (Roca *et al.,* 1997). So the depth of insemination in to the reproductive

tract has got greater significance in determining the conception rate of does inseminated using frozen semen. Eppleston *et al.* (1994) reported that while using frozen semen the fertility was increased by 7 – 12 per cent for each cm increase in the depth of insemination. Baldassarre and Karatzas (2004) opined that "the more damaged the sperm is, the deeper semen needs to be deposited in order to achieve good fertilization rates".

Summary

6. SUMMARY

A study was carried out to find out the factors affecting the conception rate on artificial insemination in goats using normal ejaculates collected thrice weekly at an interval of two to three days from an adult Malabari crossbred buck maintained at Artificial Insemination Centre, under the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy. The semen was diluted in such a way that each dose contained 200 million motile sperms before preservation. After preliminary evaluation, the semen samples for chilling were diluted in Tris-yolk buffer, packed in 0.25 ml straws and preserved at 3 - 5°C for 72 hours. The semen samples for freezing were extended 10 times with Tris buffer, subjected to centrifugation and the seminal plasma was removed. The pellet formed was extended (1: 1) with non glycerolated Tris extender. After cooling to 5°C it was further diluted to 1:3 with glycerolated fraction of extender, filled in 0.25 ml French straws and equilibrated for four hours. Manual freezing was performed in a styroform box in liquid nitrogen vapour for 4.5 minutes and the straws were stored in liquid nitrogen container.

A total of 44 adult healthy does brought to the AI Centre for insemination were selected for the study after detecting heat using buck jar technique. Selected does were at random allotted to four groups with eleven animals in each group. Does in first, second and third group were inseminated using chilled semen having motility over 35 per cent during 0-24 h, 24-48 h and 48-72 h of preservation respectively. Insemination of animals in fourth group was carried out using frozen semen with a minimum of 35 per cent post thaw motility. Transcervical insemination was carried out in the does in oestrus by speculum method. The depth of penetration of AI gun was assessed using another sheath as yardstick. Pregnancy diagnosis was performed at two months after AI by ultrasonography or at three months by abdominal palpation.

Average volume of semen was 1.25 ± 0.97 ml. The colour of all the ejaculates was found to be creamy with a yellowish tinge. The mean density and mass activity were DDDD and ++++ respectively. Mean pH and sperm concentration were 6.89 ± 0.21 and 2781.82 ± 51.69 millions per ml respectively. The mean percentage of initial

motility, sperm viability, abnormality and intact acrosomes was 82.77 ± 0.33, 90.14 ± 0.53, 2.28 ± 0.12 and 92.27 ± 0.21 respectively.

The percentage of sperm motility at 24, 48 and 72 h of preservation at refrigeration temperature was 70.25 ± 0.60, 61.13 ± 0.72 and 42.81 ± 0.95 respectively. The live sperm percentage was 83.42 ± 1.27 at 24 h, 78.84 ± 1.47 at 48 h and 74.57 ± 1.53 at 72 h of preservation. The percentage of sperm abnormalities increased to 2.97 ± 0.13 at 24 h, 4.12 ± 0.15 at 48 h and 5.34 ± 0.11 at 72 h of preservation. The percentage of intact acrosomes was 90.27 ± 0.18 at 24 h, 87.71 ± 0.37 at 48 h and 85.09 ± 0.44 at 72 h of refrigeration storage. There was significant difference between sperm motility, viability, abnormality and acrosome integrity at various storage periods.

The percentage of sperm motility decreased from 78.50 ± 0.50 at the end of initial extension to 37.67 ± 0.49 after cryopreservation. The mean live sperm percentage was 84.67 ± 0.91 at the end of initial extension which after freezing and thawing decreased to 60.36 ± 0.77. The mean percentage of sperm abnormalities increased form 3.82 ± 0.12 to 6.34 ± 0.22 after freezing. The mean percentage of intact acrosomes was 85.68 ± 0.72 at the end of initial extension which on cryopreservation showed a decrease to 76.06 ± 1.41. There was significant difference between sperm motility, sperm viability, sperm abnormality and acrosome integrity at various stages of processing and freezing. Also significant difference ($p<0.01$) was noticed between semen quality of frozen semen and that of chilled semen at 24, 48 and 72 h of storage.

Predominant behavioural signs of heat observed using "Buck jar" technique were bleating (79.55 per cent), wagging of tail (65.91 per cent), frequent urination (45.45per cent) and flehmen reaction (13.64 per cent) .Average intensity of heat score 2.91 ± 0.10. The major clinical signs were oedema, moistness and hyperaemia of vulva and vagina and mucus discharge and opening of cervical os.

The depth of penetration of AI gun ranged from 6-36 mm with an average of 20.07 ± 1.35 mm. No significant correlation was observed between intensity of heat and depth of penetration of AI gun.

Overall conception rate in does inseminated using chilled semen was 72.73 per cent. Conception rate in Group I, II and III were 81.82, 63.64 and 72.73 per cent respectively. No significant difference was observed between conception rates at various storage periods. The conception rate in does inseminated using frozen semen was 27.27 per cent.

There was significant difference ($p<0.01$) in conception rate in does inseminated using frozen semen and chilled semen at various periods of storage. Conception rate was maximum when the intensity of heat score was four. Maximum conception rate was observed when the depth of penetration of AI gun was in the range of 30-40 mm.

The present study revealed that semen quality and conception rate was maximum when refrigeration storage of semen was below 24 hours. Thereafter, semen quality and conception rate decreased. The number of progressively motile spermatozoa per dose of semen inseminated was more important than type or duration of storage in determining the conception rate. The results of the present study showed that fertility of chilled semen is much higher compared to that of frozen semen. No significant correlation was observed between intensity of heat and depth of penetration of AI gun with overall pregnancy status. Intensity of heat and depth of penetration of AI gun showed significant correlation with pregnancy status of does inseminated using frozen semen. The present study revealed that liquid storage of buck semen under refrigeration conditions is a viable alternative for propagation of germplasm of superior bucks with low freezability as it ensures better conception rates.

Satisfactory fertility on artificial insemination in goats requires attention to each step of the procedures for semen storage. The careful management of males that produce semen is the first step toward achieving acceptable fertility after artificial

insemination. The second important consideration is the choice of short or long term in vitro storage of spermatozoa. The sperm cells are easily damaged after ejaculation, and the seminal plasma modifies their invitro viability. More investigations are needed on the inhibition of the bulbourethral gland lipase to avoid washing of spermatozoa before frozen storage. Another promising area of investigation is the pretreatment of spermatozoa to preserve membrane integrity during the freeze-thawing process. The third important consideration is the organisation of the artificial insemination, which should be performed close to the time of ovulation in the females. Accurate and careful detection of oestrus and control of oestrus and ovulation are necessary to reach a satisfactory fertility level. As long as the viability and fertility of frozen-thawed semen is not improved, it is desirable to develop a more efficient method for liquid storage.

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FACTORS AFFECTING CONCEPTION RATE ON ARTIFICIAL INSEMINATION IN GOATS

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ABSTRACT

With the objective of evaluating the factors affecting conception rate on artificial insemination in goats, a study was carried out at Artificial Insemination Centre, under the department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur using 22 ejaculates collected thrice weekly from an adult Malabari crossbred buck. Semen samples for chilling were diluted in Tris-yolk buffer and preserved at 3-5°C for 72 hours. Cryopreservation was done in Tris yolk glycerol extender after the removal of seminal plasma. The semen was diluted and packed in 0.25 ml straws and each dose contained 200 million progressively motile sperms before processing.

A total of 44 adult healthy does brought to the AI Centre for insemination were selected for the study after detecting heat using buck jar technique. Selected does were at random allotted to four groups with eleven animals in each group. Does belonging to Group I, II and III were inseminated using chilled semen having motility over 35 per cent and stored for 0-24 h, 24-48 h and 48-72 h respectively. Animals of Group IV were inseminated using frozen semen having a minimum of 35 per cent post thaw motility. Transcervical insemination was carried out in the does in oestrus by speculum method. The depth of penetration of AI gun was assessed using another sheath as yardstick. Pregnancy diagnosis was performed at two months of gestation by ultrasonography or at three months of gestation by abdominal palpation.

Average volume, density and mass activity of buck semen were 1.25 ± 0.97 ml, DDDD and ++++ respectively. Colour of the semen was creamy with a yellowish tinge. Average semen pH and sperm concentration were 6.89 ± 0.21 and 2781.82 ± 51.69 millions per ml respectively. The mean percentage of initial motility, sperm viability, abnormality and intact acrosomes was 82.77 ± 0.33, 90.14 ± 0.53, 2.28 ± 0.12 and 92.27 ± 0.21 respectively.

The percentage of sperm motility at 24, 48 and 72 h of preservation at refrigeration temperature was 70.25 ± 0.60, 61.13 ± 0.72 and 42.81 ± 0.95 respectively. The live sperm percentage dropped to 83.42 ± 1.27 at 24 h, 78.84 ± 1.47 at 48 h and 74.57 ± 1.53 at 72 h of preservation by chilling. The percentage of sperm

abnormalities increased to 2.97 ± 0.13 at 24 h, 4.12 ± 0.15 at 48 h and 5.34 ± 0.11 at 72 h of preservation. The percentage of intact acrosomes was 90.27 ± 0.18 at 24 h, 87.71 ± 0.37 at 48 h and 85.09 ± 0.44 at 72 h of refrigeration storage. There was significant difference between sperm motility, viability, abnormality and acrosome integrity at various storage periods.

The percentage of sperm motility of 78.50 ± 0.50 at the end of initial extension decreased to 37.67 ± 0.49 after cryopreservation. The mean live sperm percentage was 84.66 ± 0.91 at the end of initial extension which after freezing and thawing dropped to 60.36 ± 0.77. The mean percentage of sperm abnormalities increased from 3.82 ± 0.12 to 6.34 ± 0.22 at the end of cryo preservation. The mean percentage of intact acrosomes was 85.68 ± 0.72 at the end of initial extension which showed a decrease to 76.06 ± 1.41 after cryopreservation. There was significant difference ($p<0.01$) between semen quality of frozen semen and chilled semen at various storage periods.

Predominant behavioural signs observed using "Buck jar" technique were bleating, wagging of tail, frequent urination and flehmen reaction with an average intensity of heat score 2.91 ± 0.10. The major clinical signs were vulval oedema, moistness and hyperaemia of vagina, mucus discharge and opening of cervical os. Average depth of penetration of AI gun was 20.07 ± 1.35 mm.

Overall conception rate in does inseminated using chilled semen was 72.73 per cent. Conception rate in Group I, II and III were 81.82, 63.64 and 72.73 per cent respectively, which did not differ significantly. The conception rate in does inseminated using frozen semen was 27.27 per cent.

The study indicated that progressive motility and fertility of buck semen decrease on freezing causing a significant ($p<0.01$) reduction in conception rates compared to chilled semen. Intensity of heat and depth of penetration of AI gun have significant correlation with pregnancy status of does inseminated using frozen semen. The study revealed that liquid storage of buck semen under refrigeration is a viable alternative for propagation of germplasm of superior bucks with low freezability as it ensures better conception rates.