PRELIMINARY TRIALS ON PRESERVATION OF BUCK SEMEN IN GLYCEROL CONTAINING DILUENTS

By

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THESIS

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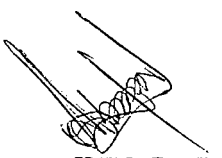
DECLARATION

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



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CERTIFICATE

Certified that this thesis entitled "PRELIMINARY TRIALS ON PRESERVATION OF BUCK SEMEN IN GLYCEROL CONTAINING DILUENTS" is a record of research work done independently by Sri.Aswini Kumar Sarmah, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

Dr. E. Mathai,
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DEDICATED TO THE MEMORY

OF

MY BELOVED TEACHER

Dr. C. K. SURENDRA VARMA RAJA

&

GRAND FATHER

RAJESWAR SARMAH

INTRODUCTION

INTRODUCTION

The commanding impact of social transformation of the weaker sections on the rural community in our country has long been recognised. No doubt, the care and management of goats, rightly called "poor man's cow", will form one of the most important facets of the livestock production in the economic uplift of landless labourers and socio-economically backward communities in our country. A variety of products are available from the goat enterprise, of which meat and milk are of major economic importance. The increased emphasis placed on enhancing milk and meat production, warrant the need for better understanding on the methods of improving the production potentials of goats.

The most important and predominant factor involved in the low production performances of our goat population can be attributed to poor genetic make up. Though, selective breeding and grading up of the local animals can bring about improvement in the genetic potential of the indegenous stock, crossing the indegenous breeds with Saanen and Alpine breeds to evolve cross breds having the adaptability and disease resistance of Indian goats and better production potential

of the exotic breeds is envisaged for faster improvement in shorter period of time.

Kerala alone possesses 1.683 million goats out of 71 millionsin this country. The goat husbandry is one of the most important livestock enterprises in Kerala, especially among rural community. Though Malabari is the only goat breed popular in Kerala, a large number of non-descript goats are being reared by farmers. By adopting intensive breeding programmes, the non-descript goat population of the state, can be transformed to a cross bred population possessing better production potential.

The technique of artificial insemination has occupied foremost place in the development of technology in the field of animal reproduction and proved to be the most ideal biological tool for rapid propagation of superior germ plasm for improvement in the reproductive efficiency and production performance. But artificial insemination in goats has not yet developed to the same stage of perfection as that in the case of cattle, even in most of the advanced countries of the world. In India, the application of the technique of artificial insemination for goat breeding is not popular under field conditions, though attempts have been made on

a limited scale in the organized goat farms. An all India Co-ordinated Research Project on Goats for increased milk production was started in 1971 at the National Dairy Research Institute, Karnal with two additional units, one in Kerala and another in Assam. Alpine and Saanen are the two exotic breeds which are being utilised for evolving cross bred goats in Kerala. Artificial Insemination can be the most suited technique that has to be adopted in large scale for the improvement of goats through breeding. As a preliminary step two artificial insemination centres for goats were commissioned in Kerala Agricultural University for the benefit of local farmers, to get their goats inseminated.

It is imperative to have a better understanding on the different semen characteristics of goats, before large scale implementation of artificial insemination programme for goat breeding. It is also necessary to evolve suitable extenders for long term preservation of superior quality semen. Though glycerol has been accepted as an important ingredient in semen extenders for long term preservation of semen in bulls, the effects of incorporation of glycerol, either at room temperature or at refrigeration temperature on the preservation and storage of buck semen in Tris diluent and Reconstituted Skim milk diluent have not been well

understood. Further, reports on different aspects of freezing of buck semen are scanty, though there is great scope for perfecting this innovation for goat breeding.

The present study was undertaken to find out the normal semen characteristics of bucks, the suitable level of glycerol incorporation in skim milk and Tris diluents, the effects of glycerolisation at room temperature and refrigeration temperature and to assess the effect of freezing, on buck semen.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Normal semen characteristics of buck semen

Normal semen characteristics were reported in Jamunapari and Barbari bucks by Patel (1967), Sahni and Roy (1969), Prasad et al. (1970), Mittal and Pandey (1972), Saxena and Tripathi (1980) and Singh and Sinha (1982), in Black Bengal and Saanen bucks by Sinha and Singh (1982) and Bordoloi and Sharma (1983). But similar reports on Alpine X Malabari cross bred bucks are scanty. Comparative studies on the quality and quantity of successive ejaculates from Malabari and Alpine X Malabari breeds are meagre.

Number of reports are available on the reaction time during the process of semen collection from bucks (Shukla and Bhattacharya, 1953; Sharma et al., 1957; Kurian and Raja, 1965; Krishnan, 1967; Patil, 1970; Sinha and Singh, 1982). Significant variations in the reaction time between years and between seasons are reported by Shukla and Bhattacharya (1953). In summer the reaction time was found to be less, and was found independent of the sperm production. In a study involving three beetal bucks, Sharma et al. (1957) observed individual variation in reaction time, which

ranged from 62.6 to 159.4 seconds, whereas Kurian and Raja (1965) reported an average reaction time of 35 seconds in Malabari bucks. According to Krishnan (1967), the reaction time for Malabari and Saanen bucks were 40.6 seconds and 53.4 seconds respectively. In an elaborate study, Patil (1970) reported a wide variation in reaction time, which ranged from 25 to 150 seconds with a mean of 49.37 ± 2.45 seconds in Malabari bucks. They observed negative correlation between reaction time and volume of semen. Sinha and Singh (1982) reported an average reaction time of 60.53 ± 1.223 seconds and 64.46 ± 1.233 seconds for Black Bengal and Saanen bucks respectively and concluded that the difference in reaction time of Black Bengal and Saanen bucks might be due to genetic causes.

Mann (1980) studied the characteristics of semen from West African dwarf bucks for one year and concluded that there wereconsiderable variation in semen traits among bucks and between ejaculates from the same buck. In contrast Mohan et al. (1980) could observe significant difference between bucks for all semen characteristics, in Pashmina bucks.

Significant variation in semen volume was observed between breeds (Eaton and Simmons, 1952), between bucks

(Mittal and Pandey, 1972) and between ejaculates (Mohan et al., 1980 and Saxena and Tripathi, 1980). The ejaculate volume was found to be 0.2 to 1.2 ml in Beetal bucks (Sharma et al., 1957), 0.4 to 1.2 ml in Malabari bucks (Kurian and Raja, 1965; Patil, 1970), 0.67 ± 0.03 ml in Native Zambian and 1.34 ± 0.05 ml in Boer bucks (Igboeli, 1974), 0.85 ± 0.04 ml in Kambing Katjang - Jamunapari (Koh, 1975), 0.77 ± 0.26 ml in West African dwarf bucks (Mann, 1980), 0.62 ± 0.02 ml in Pashmina bucks (Mohan et al., 1980), 0.37 ± 0.03 ml and 0.86 ± 0.09 ml in Jamunapari bucks (Saxena and Tripathi, 1980; Singh et al., 1982), 0.446 ± 0.011 ml in Black Bengal and 0.720 ± 0.016 ml in Saanen bucks (Sinha and Singh, 1982), 1.01 ± 0.04 ml in Barbari bucks (Singh et al., 1982) and 1.48 ml, 0.88 ml and 0.88 ml in Anglo-Nubian, Marota and Moxoto bucks, respectively (Vinha, 1982).

In a study involving Toggenburg and American bucks, Eaton and Simmons (1952) reported that the semen volume varied significantly from year to year. Sharma et al. (1957) found an increase in volume with an increase in body size. Kurian and Raja (1965) reported that the interval between collections did not markedly effect the semen volume. Prasad et al. (1970) observed significantly higher semen volume in adults than in nine month old Barbari bucks, but Patil (1970)

found no influence of age on ejaculate volume in Malabari bucks.

Significant effect of season on the semen volume was noted by Eaton and Simmons (1952), Shukla and Bhattacharya (1953) and Kang and Chung (1976). A higher volume was noted during post-monsoon season and lower volume during winter (Patil, 1970). Vinha (1975) reported that the values were greatest in Autumn and lowest in summer. Kang and Chung (1976) opined that ejaculate volume increased with decreasing day length and vice versa. Negative correlations between ejaculate volume and sperm concentration and motility were noted, but the ejaculate volume was positively correlated with percentage of abnormal sperms, pH and sperm motility (Kang and Chung, 1976). A significant correlation between ejaculate volume and sperm number was observed, by saxena and Tripathi (1980).

Colour of normal semen depends upon the concentration of the spermatozoa lesser the sperm number, the more watery and translucent the semen looks like. Various authors noted wide variation in colour of buck semen. Patil (1970) noted milky yellow to thick creamy yellow colour of semen in Malabari bucks. Igboeli (1974) observed that colour of ejaculate varied from creamy white to yellow in Native

Zambian and Boer bucks and reported that the colour changed between breeds, bucks and ejaculates. Creamy to light yellow colour for semen was noted in Black Bengal and Saanen Breeds by Sinha and Singh (1982). Singh et al. (1982) observed varying colours like watery, yellow, milky and creamy in Jamunapari and Barbari breeds of bucks.

In Malabari bucks, pH of semen was reported to vary from 6.4 to 6.7 with a mean value of 6.47 ± 0.16 (Patil, 1970). In cross bred Kambing Katjang-Jamunapari, the average pH was 6.8 (Koh, 1975). Kang and Chung (1976) reported the range of pH from 6.8 to 7.2 for Korean Native bucks. The average pH of the semen in West African dwarf buck was found to be 6.93 (Mann, 1980), in Pashmina 6.7 to 7.1 with a mean of 6.84 ± 0.02 (Mohan et al., 1980) and in Black Bengal and Saanen bucks, 6.79 ± 0.009 and 6.72 ± 0.004 respectively (Sinha and Singh, 1982).

Highly significant variation in pH and number of sperms were observed between years by Shukla and Bhattacharya (1953). But Patil (1970) did not observe significant variation in the pH of semen due to age of the animal. Though significant variation was not found in the pH of semen due to different seasons in bucks (Patil, 1970; Kang and Chung, 1976); Mohan et al.(1980) reported significant differences among

bucks. According to Sinha and Singh (1982), there was a significant effect of breeds on the variation of pH of the semen.

In the case of Malabari bucks, mass activity of sperms was reported to be in the range of '++' to '++++' (Patil, 1970). Mohan et al. (1980) reported an average motility score of 4.19 ± 0.09 out of 5 in Pashmina bucks, whereas Saxena and Tripathi (1980) noted forward motility of 3.78 ± 0.01 out of 5 in Jamunapari bucks. The average mass motility of Black Bengal and Saanen bucks was 4.439 ± 0.065 and 4.507 ± 0.048 respectively (Sinha and Singh, 1982). Mohan et al. (1980) noted a significant difference between collections for mass activity in Pashmina bucks.

The initial motility of sperms in Malabari buck semen was observed to be 60 to 90 per cent (Kurian and Raja, 1965) and 40 to 85 per cent with a mean of 66.14 ± 1.34 per cent (Patil, 1970). Igboeli (1974) observed initial motility of 52.3 ± 1.3 and 53.2 ± 1.2 per cent in Native Zambian with body weight 27.1 ± 1.8 kg and Boer bucks with body weight 54.1 ± 2.0 kg respectively. The initial motility of sperms was observed to be 85.00 ± 0.76 per cent in Katjang-Jamunapari cross bred bucks (Koh, 1975), 77.28 ± 7.75 per cent in African dwarf bucks (Mann, 1980), 60.62 ± 0.04 per cent in Pashmina

bucks (Mohan et al., 1980), 72.62 ± 1.06 per cent in Jamunapari bucks(Saxena and Tripathi, 1980), 74.00 ± 0.40 and 78.30 ± 2.48 per cent in Jamunapari and Barbari bucks, respectively (Singh et al., 1982), and 76.22, 68.33 and 62.75 per cent in Anglo-Nubian, Marota and Moxoto bucks respectively (Vinha, 1982).

Significantly higher sperm motility was found in adults in comparison to nine months old Barbari bucks (Prasad et al., 1970), whereas Patil (1970) reported that age had no influence on initial motility in Malabari bucks. Kurian and Raja (1965) reported that interval between collections did not markedly effect semen characters. A significant difference between collections for initial motility was noted in Pashmina bucks (Mohan et al., 1980).

According to Eaton and Simmons (1952), all characters, except motility, were influenced by season; but Shukla and Bhattacharya (1953) observed highly significant variations in initial motility due to seasons. Patil (1970) reported high initial motility during post-monsoon (October, November) and South-west monsoon (June, July, August and September), and lowest in winter (December, January and February) in Malabari bucks. Vinha (1975) obtained highest initial motility of 86.87 per cent during spring season and lowest

motility of 67.76 per cent in winter. In Korean native bucks, highest initial sperm motility of 83.3 per cent was observed from November to January and lowest motility of 55.1 per cent from July to September by Kang and Chung (1976) and reported that initial sperm motility was positively correlated with pH.

The livability of spermatozoa is generally used as a criterion for testing the fertility of semen. Lasley et al. (1942) and Lasley and Bogart (1943) reported that the semen samples containing less than 50 per cent live sperms were of doubtful fertility. In Malabari bucks the percentage of live spermatozoa was recorded in the range of 85 to 95 and 20 to 86.6 per cent (Kurian and Raja, 1965; Patil, 1970). The percentage of live sperm, in the semen of Native Zambian and Boer breeds, was found to be 87.2 ± 1.0 and 87.7 ± 1.0 respectively by Igboeli (1974). In the case of West African dwarf bucks, percentage of live sperm was found to be 84.93 (Mann, 1980). The live sperm percentage was observed to be 80.63 ± 0.29 in Pashmina bucks (Mohan et al., 1980), 77.65 ± 1.04 (Saxena and Tripathi, 1980) and 80.90 (Singh et al., 1982) in Jamunapari bucks, 85.45 ± 0.41 and 85.21 ± 0.402 in Black Bengal and Saanen breeds respectively (Sinha and Singh, 1982) and 83.80 ± 0.26 in Barbari breeds (Singh et al., 1982).

Prasad et al. (1970) stated that there was a higher percentage of live sperms in adult Barbari bucks in comparison to nine month old bucks. No significant seasonal variation in the percentage of live sperm was observed in Malabari bucks (Patil, 1970). It was reported that the percentage of live sperm is positively correlated to initial motility and pH of semen (Patil, 1970).

Dussardier and Szumowski (1952) observed 2.75 per cent total abnormal sperms, of which two per cent were bent tails and 0.3 per cent detached head in buck semen. The percentage of sperm abnormalities was observed to be 6 to 12 (Kurian and Raja, 1965) and 1.00 to 18.62 in Malabari bucks (Patil, 1970). An average of 3.11 ± 0.28 per cent sperm abnormalities in Kambing Katjang - Jamunapari cross bred bucks was reported by Koh (1975). In Jamunapari bucks abnormal sperms were observed to be 6.84 ± 0.60 per cent (Saxena and Tripathi, 1980). Sperm abnormalities in buck semen varied from 5 to 20 per cent (Hafez, 1980; Memon and Ott, 1981; and Mann, 1982). According to Sinha and Singh (1982) the average sperm abnormalities were 7.870 ± 0.214 per cent in Black Bengal and 6.196 ± 0.273 per cent in Saanen bucks. The percentages of abnormal sperms in Anglo-Nubian, Marota and Moxoto bucks, were 11.05, 11.21 and 16.36, respectively (Vinha, 1982). The average sperm abnormalities

in the ejaculates of Assam local, Beetal and Saanen bucks, were found to be in the order of 8.796 ± 0.644 per cent, 9.225 ± 0.818 per cent and 8.557 ± 1.060 per cent (Bordoloi and Sharma, 1983). The head abnormalities, mid piece abnormalities and tail abnormalities were 3.948 ± 0.477; 1.169 ± 0.093 and 3.547 ± 0.546 per cent, respectively in Assam local goats; 3.299 ± 0.464, 1.875 ± 0.269 and 3.750 ± 0.400 per cent in Beetal bucks; and 3.558 ± 0.507, 1.717 ± 0.334 and 3.240 ± 0.658 per cent in Saanen bucks (Bordoloi and Sharma, 1983).

According to Saxena and Tripathi (1980), the proportion of mid piece abnormalities (4.83 per cent) was significantly higher than that of head (0.88 per cent) or tail abnormalities (1.13 per cent) in Jamunapari bucks. They also observed that the proportion of tail abnormalities varied significantly between bucks.

Eaton and Simmons (1952) and Sinha and Singh (1982) noted significant effect of breed on percentage of abnormal sperms. No significant difference was observed in head, tail and total abnormalities of spermatozoa in different breeds of goat (Bordoloi and Sharma, 1983). However, they recorded significant differences in the mid piece abnormalities of spermatozoa due to breed.

There was practically no difference in the percentage of abnormal spermatozoa between first and second ejaculate (Arbeiter, 1964). On the contrary, Saxena and Tripathi (1980) noted significant variation in the proportion of mid piece abnormalities in successive ejaculates. The percentage of abnormal spermatozoa was not influenced by age of the animal (Patil, 1970); whereas, in Barbari bucks aged 4.5 ± 0.5, 9.0 ± 1.0 and 36.0 ± 6.0 months, the percentages of abnormal spermatozoa were 15.5 ± 2.1, 5.7 ± 0.7 and 4.5 ± 0.6, respectively (Prasad et al., 1970). A negative correlation between sperm concentration and percentage of abnormal sperms was reported by Patil (1970).

Eaton and Simmons (1952) observed significant influence of season on the sperm abnormality. According to Shukla and Bhattacharya (1953) there was significant variation in percentage of abnormal sperms due to seasons. Patil (1970) recorded the maximum sperm abnormalities during summer and minimum number in post-monsoon and south-west monsoon. Vinha (1975) observed significant seasonal variations in the percentage of sperm abnormalities, the incidence of abnormality being highest in spring and lowest in autum and winter. The incidence of sperm abnormality was higher in November to January than in July to September (Kang and Chung, 1976).

Williams and Savage (1925) expressed the view that the total sperm count could serve as a valuable index for the detection of male infertility. Bishop et al. (1954) and Cupps et al. (1954) stated that the measurement of sperm concentration alone might not be of much practical value in assessing the potential fertility of the semen samples used for routine insemination.

Number of sperms per ml of ejaculate was reported to be 5.424 billion in Beetal bucks (Sharma et al., 1957); 2 to 3 billions (Kurian and Raja, 1965) and 1.1 to 7.49 billions in Malabari bucks (Patil, 1970); 1.65 ± 0.02 billions in Native Zambian bucks, 2.70 ± 0.03 billions in Boer bucks (Igboeli, 1974), 3.975 ± 0.131 billion in Kambing Katjang-Jamunapari cross bred bucks (Koh, 1975); 3.22 ± 1.22 billion in West Africian dwarf bucks (Mann, 1980); 4.795 ± 0.293 billions and 2.293 ± 0.728 billions in Jamunapari bucks (Saxena and Tripathi, 1980 and Singh et al.,1982); 2.440 ± 0.041 billions and 2.780 ± 0.036 billions in Black Bengal and Saanen bucks respectively (Sinha and Singh, 1982); 1.92 ± 0.072 billions in Barbari bucks (Singh et al., 1982) and 1.559, 1.107 and 0.803 billions in Anglo-Nubian, Marota and Moxoto bucks respectively (Vinha, 1982).

Eaton and Simmons (1952), Sinha and Singh (1982) and

Vinha (1982) reported a significant effect of breed on sperm concentration. Eaton and Simmons (1952) stated that the difference in sperm concentration may be due to the difference in the weight of the buck. Patil (1970) found that the age had no influence on the concentration of spermatozoa in Malabari bucks. Mittal and Pandey (1972) observed a highly significant difference in sperm concentration between Barbari bucks, though differences in sperm concentration between weeks were not significant. Differences in sperm concentration, between bucks and between consecutive collections in Pashmina bucks, were significant (Mohan et al., 1980). Highly significant difference in sperm concentration due to variation of season was observed by Eaton and Simmons (1952) and Shukla and Bhattacharya (1953). The sperm concentration was found to be highest in spring (Eaton and Simmons, 1952). Sharma et al. (1957) observed high sperm concentration in winter and low in spring. Patil (1970) observed that the sperm concentration in the case of Malabari bucks was maximum during South-west monsoon and minimum during summer; whereas in Anglo-Nubian bucks highest sperm concentration was reported in summer and lowest in autumn (Vinha, 1975). Kang and Chung (1976) noted a lower sperm concentration from November to January and higher concentration from July to September.

Preservation of buck semen in glycerol containing diluents

Preservation of buck semen had been tried with varying results in Glucose phosphate diluent (Rosenberger, 1944; Wanger, 1949; Schmidt et al., 1950; Guha et al., 1951 and Hampel, 1951); in Egg yolk citrate extender (Konger, 1951; Achneit and Rosen Winkel, 1953; Blokhuis, 1957; Roy et al., 1959; Blokhuis, 1959; Knoblauch, 1962; Jelam and Nambiar, 1963; Nasim et al., 1964; Patel, 1967; Gonan, 1971; John and Raja, 1973; Koh and Ong, 1977; and Singh et al., 1982); in Yolk glycine diluent (Roy, 1957; Roy et al., 1959); in skim milk diluent (Dauzier, 1956; Blokhuis, 1959; and Ron and Aamdal, 1963); in Milk diluent (Jelam and Nambiar, 1963; Tiwari et al., 1968; Sahni and Roy, 1972; John and Raja, 1973 and Balakrishnan, 1979); in IVT diluent (Ron and Aamdal, 1963 and Balakrishnan, 1979); in Cornell University extender (Koh and Ong, 1977; Balakrishnan, 1979); in Coconut milk extender (Pillai, 1971; Sahni and Roy, 1972); and in Tris diluent (Balakrishnan, 1979; Mathew et al., 1982; and Singh et al., 1982). The glycerol containing diluents for preservation of buck semen

was also tried by several workers (Fraser, 1962; Anderson, 1969; Samouilidis and Hahn, 1972; Sahni and Roy, 1972; Rossouw, 1974; Corteel, 1974; Founger, 1976; Nimkar, 1977; Gonzalez, 1978; Singh et al., 1982). However, the study of viability of buck spermatozoa at different periods of storage in extenders with varying levels of glycerol appears to be scanty. Moreover, changes in the morphology of buck spermatozoa, during preservation and storage, have not been studied elaborately.

Although inclusion of glycerol in extender for the freezing of spermatozoa remains the standard procedure, very little has been known about the mechanism underlying the uptake and metabolism of glycerol by spermatozoa. White et al. (1954) observed that glycerol did not affect the motility and respiration of spermatozoa in a fructose containing medium, but increased markedly the oxygen uptake of washed sperm suspensions, resulting in the formation of lactic acid. O'Dell et al. (1956) showed that glycerol was taken up readily and utilized by bovine spermatozoa, with the production of carbondioxide during anaerobic incubation. Further, they concluded that spermatozoa, separated from the seminal plasma showed a higher uptake and a higher metabolic utilization of glycerol than those in the presence of seminal

plasma. Mann and White (1956) showed that glycerol was utilized aerobically by suspensions of washed spermatozoa, but there was little or no utilization of glycerol under anaerobic conditions. It was also noted that the disappearence of glycerol was accompanied by an increase in the oxygen uptake and accumulation of lactic acid, which appears to be an intermediary product in the oxydative breakdown of glycerol. This breakdown was a function of live spermatozoa and could be abolished by heating or by the addition of spermicidal agents. Mann and White (1957) observed that in ram spermatozoa, glycerol was oxidized to form fructose. Glycerol break down was essentially an oxidative function accompanied by the formation of fructose and its subsequent utilization. Glycerol stimulated respiration and exerted a "sparing effect" on aerobic glycolysis, but had no marked effect on anaerobic glycolysis. About one-sixth of the glycerol exidatively utilized was recoverable as fructose after a three hours incubation at 37°C, and nearly one-half of it was recovered as lactic acid (White, 1957). It was observed that addition of glycerol to bull semen in Egg yolk-citrate extender resulted in a marked increase in oxygen uptake (Vandemark et al., 1957). According to Pickett and Merilan (1959) a combination of glycerol and

fructose maintained a greater degree of motility and livability of bovine spermatozoa, at the end of 105 minute of incubation at 38°C, than did either fructose or glycerol alone. They found that addition of one per cent each of glycerol and fructose was apparently optimum for maintenance of spermatozoan motility and livability, as the low concentration of glycerol in the presence of fructose could be converted largely to carbon dioxide without any accompanying increase in toxic metabolic substances.

Bartlett and Vandemark (1961) opined that Amine buffers like Tris had been used effectively for maintaining physiological pH and that acted by direct titration of hydrogen ions without immediate reduction in carbon-dioxide content. Further, the capacity to produce equivalent extra and intra cellular pH changes suggested its intracellular penetration. Between Tris-phosphate diluent and Glucose-yolk-citrate diluent, Lopatko (1971) observed no significant difference in sperm survival rate during preservation of ram semen. Balakrishnan (1979) reported an average motility of 75.39, 62.97, 49.90, 37.42 and 19.30 per cent respectively after 24, 48, 72, 96 and 120 hours of storage for buck semen diluted with Tris at a rate of 1:10 and preserved at 5°C. Deka and Rao (1980) studied the preservability of ram

semen and found that the percentages of progressively motile spermatozoa after 72 hours of storage at 5°C were 47.33 ± 8.20, 58.53 ± 7.04 and 57.37 ± 7.06 in Egg yolk-citrate (EYC), Tris-egg yolk-glucode and cow's milk extenders respectively. The difference in motility percentage was significant between extenders and between preservation times. Tris diluent was found to be superior to milk extender in assessing sperm motility under microscope. Mathew et al. (1982) studied 18 combinations of 0.2 molar Tris diluent with six varying levels of egg yolk at three levels of pH at the temperature of 3° to 5°C and 6° to 8°C for buck semen and found that diluents containing 20 per cent and 25 per cent egg-yolk were superior on seventh day of preservation at 3° to 5°C with an average motility of 58.55 per cent and 61.29 per cent respectively. The pH levels were not significant in maintaining motility. Further, Tris diluent with five per cent egg yolk with pH seven was found to be superior to diluents containing higher percentage of egg-yolk for the preservation at 6° to 8°C. Hill et al. (1958), Sahni and Roy (1969) and Balakrishnan (1979) reported that milk based diluents were superior to the egg-yolk containing diluents for the preservation of buck semen.

Survival of bovine spermatozoa in 0.2 and 0.25 molar

Tris-buffered yolk glycerol (TYG) extender was superior to that in a standard citrate-yolk-glycerol (CYG) extender at 5°C, -25°C and -85°C (Davis et al., 1963). They noted that semen, with 62 per cent average initial motility in 0.2 and 0.25 molar Tris-yolk-glycerol, respectively maintained 50 and 48 per cent average motility after eight days of storage at 5°C. When bovine spermatozoa were preserved in 20 per cent yolk-glycerol extenders with 0.15, 0.20 and 0.25 molar Tris and adjusted with citric acid to pH of 6.5, 6.75 and 7.00, optimum sperm survival was found in the 0.2 molar extender with a pH of 6.75 (Davis et al., 1963). It was also found that in 0.2 molar Tris extender with a pH of 6.75, average motility was 42 per cent after eight days of storage at 5°C, compared with 35 per cent in Cornell University extender (CUE) and 18 per cent in a CYG extender. Foote (1970) reported that 0.2 molar Tris-yolk extender buffered to pH 6.5 or 6.75 was superior to 0.2 molar with pH 6.25 and 0.25 molar with pH of 6.25, 6.50 and 6.75 Tris extender in preserving motility of bull spermatozoa. Further, inclusion of 6.4 per cent glycerol in the extender at room temperature was as satisfactory as glycerolation at 5°C for maintaining sperm motility. In a study to compare the efficacy of three diluents viz. EYC, TYG and citric acid

whey, for preservation of buck semen at 5°C, Singh et al. (1982) reported that EYC extender was more efficient than the other two dilutors. The effect of dilutors was not significant, but the effect of period of preservation was highly significant on percentage of motility and live sperms.

For preservation of sperms, skim milk diluent was reported to be as effective as EYC diluent (Florentino, 1952; Thacker and Almquist, 1953; Almquist et al., 1954; Salisbury, 1957; and Kim and Lee, 1970). Marion and Olson (1952) and Collins (1953) stated that survival of bovine spermatozoa in diluents prepared from skim milk powder depended mainly on the degree of heating in original manufacture of the milk powder. Collins (1953) observed no statistical difference between brands of milk powder but there was highly significant difference between lots of milk powder. Dauzier (1956) reported that goat semen stored in skim milk diluent for periods below eight hours gave a conception rate of 64 per cent, though good motility was maintained satisfactorily for longer periods. Hill et al. (1958) using reconstituted skim milk as ram semen diluent obtained satisfactory motility for a period of 15 days at 1:10 dilution. Melrose et al. (1958) found that

skim milk powder was as effective as skim milk when used as extender. Blokhuis (1959) carried out fertility trials with buck semen diluted in skim milk and obtained 55.5 per cent conception rate. Fisher and Kandera (1960) did not observe any significant difference in the conception rate between semen extended with skim milk powder and whole milk,whereas, Ahmed (1963) found that heated skim milk was inferior to heated homogenised whole milk as a diluent for ram semen. The motility percentages of buck spermatozoa, diluted with dried milk, were 47 and 13 after a storage period of 48 hours and 160 hours respectively (Ron and Aamdal, 1963). According to Pavolvic and Vardin (1968) there were favourable results with skim milk diluent, for ram semen. They also noted that the conception rate was highest, when semen was diluted 11 times with reconstituted unheated skim milk extenders. Petruzzi and Tarantini (1974) reported that ram semen diluted in powdered or homogenised pasteurised cows milk with or without egg yolk gave better motility after storage for 96 hours at 5°C.

Willet at Ohms (1956) used EYC, Yolk-citrate-glucose and non-fat milk solids each containing 0, 1.25, 2.5, 5 and 10 per cent glycerol, as extender for bull semen to study the preservability at 5°C. They concluded that when

glycerol concentration increased, the motility decreased markedly in EYC; and to a lesser extent in yolk-citrate-glucose; whereas motility increased in non-fat milk solids. According to Albright et al. (1958), addition of semen directly to the completely formulated milk and egg yolk diluents at 32°C, containing 7.5 per cent glycerol, spermatozoa generally showed lower motility immediately after dilution; but motility declined at a slower rate during storage in the same diluents without glycerol. Melrose et al. (1958) reported that the addition of egg yolk or glycerine to a nine per cent solution of skim milk powder did not enhance conception rate. Using skim milk and skim milk with 10 per cent glycerol as diluents, O'Conor and Smith (1959), obtained an increase of conception rate of 4.7 and 10.6 per cent with semen used on the second and third day, respectively. Albright et al. (1960) could not observe significant difference in sperm motility at seven days of storage at 5°C either when 7.5 per cent glycerol was added step wise, at 5°C or 7.5 per cent glycerol was present in the diluent prior to semen addition at 32°C. When 10 or 13 per cent glycerol was incorporated in skim milk diluent, an increase in spermatozoan livability, at the end of 14 days storage at 5°C, was obtained in comparison to the

motility percentage in non-glycerol containing diluent (Almquist, 1962). According to Almquist and Wickersham (1962) the step wise addition of 13 per cent glycerol at 5°C, maintained highly significant motility during 14 days of storage, in comparison to the motility in direct addition of semen at room temperature. Jones (1965) used a combination of dimethyl sulphoxide, glycerol and reconstituted skim milk for the preservation of ram spermatozoa and found that incorporation of eight per cent reconstituted skim milk was better than seven or 11 per cent.

Tomar et al. (1964) reported that skim milk diluent was very good for preserving normal morphology of heads of spermatozoa. The dilutors and the storage periods had not exerted significant deleterious effect on abnormalities of bovine spermatozoa, however, Chaturvedi et al. (1978) found that acrosomal abnormalities increased with the advancement of storage period in all dilutors used. Rao and Rao (1979) studied the incidence of sperm head and tail abnormalities in fresh and stored bull semen in three different diluents viz. EYC, Tris and Citric acid whey; and noticed no significant difference in the incidence of head abnormalities between fresh and stored semen. But a significant increase was observed in case of tail abnormalities. However, no

significant difference was found either in the head or in the tail abnormalities between the diluents.

Zheltobryukh (1972) recorded the occurrence of acrosome abnormalities as 21.4 and 25.7 per cent, respectively, in fresh, undiluted and freshly diluted ram semen samples with hypertonic diluent. In a study on ram semen, collected by electro ejaculation, and diluted in egg yolk glucose diluent, Watson (1975) observed that the percentage of normal acrosomes before chilling, after chilling to 5°C and one hour after glycerolation were 98, 98 and 90 respectively. Watson and Martin (1975) concluded that, although the presenee of glycerol in the diluent improved the survival of ram spermatozoa, increasing the concentration produced significant deterioration of the acrosomes. Tasseron et al. (1977) found that the damage of the acrosome, in fresh ram semen extended with saline was 8.0 ± 0.4 per cent; whereas in fresh semen extended with raffinose-sodium citrate-yolk containing five per cent glycerol used immediately and used after cooling, were 24.8 ± 1.3 and 33.6 ± 1.8 per cent respectively. They suggested the necessity of improving the diluents since about 50 per cent of the acrosomes were damaged by dilution and cooling. Grocken and Asti (1980)

found that the proportion of acrosome deformities in fresh ram semen was 2.9 per cent, which increased after glycerolisation to 20.7 per cent, however, there was no relationship between the proportion of deformed spermatozoa and motility.

Deep freezing of buck semen

Smith and Polge (1950) first reported the successful recovery of motile goat spermatozoa after slow freezing to -79°C. Subsequently, Barker (1957), Liess and Ostrowski (1960) and Valchos and Tsakaloff (1963) found that the miscroscopic evaluations and fertility trials with frozen semen were too poor to be of any practical application. However, Fraser (1962) conducted a freezing experiment on buck semen extended in sterile skim milk to investigate an optimum level of glycerol, a suitable equilibration period and a satisfactory rate of freezing. On the basis of post-thawing motility he reported the best result with 6 to 9 per cent glycerol. The equilibration period provided was 8 to 24 hours. Temperature was reduced from 30°C to 5°C in 30 minutes, then from 5°C to -10°C at the rate of 1°C per minute, from -10°C to -17°C at the rate of 2°C per minute and from -17°C to -79°C at the rate of 4°C per

minute. Although, no mention was made about the percentage of motile spermatozoa after freezing, the frozen semen was tested for fertility through an insemination trial on six does, five of which conceived at the first insemination. Anderson (1969) observed best results of post-thawing motility when buck semen was frozen in straws using skim milk diluent, containing four per cent glycerol. Bonefert (1969) reported that the best period for freezing the semen of white German goats was September to December and post-thawing motility was found to be 50 to 60 per cent in the age group of 2 to 3 years. Paggi (1971) after a study involving raffinose egg yolk-glycerol diluent reported 65 per cent post-thawing motility. Samouilidis and Hahn (1972) observed a post-thawing motility of 60 to 65 per cent and 30 to 45 per cent following storage in medium sized and fine straws respectively in Laciphos containing diluent; and post-thawing motility of 60 to 70 per cent in Tris diluent frozen in medium straws. Sahni and Roy (1972) observed post-thawing motility of 30.0 $\pm$ 9.4, 38.8 $\pm$ 3.46 and 28.5 $\pm$ 6.32 per cent in yolk-citrate diluent with 3, 6 and 9 per cent glycerol respectively. The results with milk diluent were similar, except in 3 per cent level of glycerol; where the post-thawing revival was only

13.7 ± 5.56 per cent and 6 per cent glycerol level was significantly superior to 3 and 9 per cent glycerol levels. For varying equilibration periods, freezing rate or diluents did not significantly affect sperm survival. It was also observed that the semen of Jamunapari could stand deep freezing better than that of Barbari bucks. Rossouw (1974) pointed out that buck semen equilibrated for 2.5 hours after dilution in the rate of 1:4 with semen and any one of the three diluents viz. Tris, Egg yolk-phosphate-glycerol extender containing 11 per cent lactose or 18.5 per cent raffinose, the post-thawing motilities were 40, 19 and 8 per cent, respectively. When semen was diluted and frozen with Tris extender containing 0, 3, 8 and 15 per cent glycerol, motility on thawing was 0, 35, 27 and 13 per cent respectively. Post-thawing sperm motility was 37, 29, 12 and 0 per cent when the equilibration times were 2.5, 5, 10 and 20 hours respectively and 48 , 49 and 38 per cent when frozen semen was thawed at 40°C, 90°C and at room temperature respectively. Corteel (1974) reported that when semen samples were diluted in skim milk diluent with or without glucose, percentage of motile spermatozoa was higher in samples without plasma in comparison to those with plasma, both before and after freezing. Corteel and Barit (1975)

observed 44.4 and 43.7 per cent post-thawing motility in unwashed and washed spermatozoa, respectively. After freezing and storage for 3 to 90 days, there was an additional loss of 16.6 and 0 per cent motility for unwashed and washed spermatozoa respectively, whereas the reduction in motility was 22 and 1.3 per cent respectively after storage for 91 to 190 days. By using Tris-yolk-fructose-citric acid-glycerol extender for freezing buck semen by pellet method, 49.53 per cent post-thawing motility was noted by Hukeri et al. (1977). By using Tris-yolk-fructose citric acid-glycerol diluent for the freezing of buck semen at -196°C with final glycerol concentration of 6.4 per cent, Nimkar (1977) recorded the percentage of motile spermatozoa as 44.09, 44.08, 44.20, 43.23 and 44.07 respectively at 0, 7, 14, 21 and 28 days. Gonzalez (1978) collected semen from four Anglo Nubian bucks and diluted in Laciphos - 271 extender with a sperm concentration of 200 to 300 million before freezing and obtained 100 to 200 million live sperms after thawing. When ram semen with 80 per cent initial motility was used for freezing in egg yolk-Tris-Lactose diluent, the post-thawing motility of 39 per cent could be obtained (Zamfirescu et al., 1979). In freezing experiment conducted on ram semen to know the efficacy of Tris, Lactose

and raffinose based diluents and four thawing temperature ie. 37°C, 45°C, 60°C and 75°C, spermatozoa survived best in Tris-glucose-yolk-glycerol extender and motility was maximum when thawed at 37°C (Fukui, 1979). From an experiment carriéd out on ram semen involving Tris -citric acid-yolk-glucose, Tris-citric acid-yolk; or in glycero-phosphate-EDTA-glucose-glycerol-yolk-Tris and glycero-phosphate-EDTA-glucose-glycerol-yolk extenders at glycerol levels of 3.5, 4.5 and 7 per cent and equilibration times of 2,3 to 4 and 10 to 12 hours, Saxena et al. (1979) obtained best post-thawing motility of 45 to 48 per cent in semen samples diluted 1:10 with Tris-citric acid-glucose-yolk having 7 per cent glycerol with equilibration time of 10 to 12 hours. Vivanco and Valera (1980) recorded 38 and 29 per cent post-thawing motility for ram semen, when semen samples were subjected to rapid freezing in Tris fructose and Tris-glucose extenders. Ram semen diluted in Lactose-yolk-Tris, Milk-yolk-Tris, Sodium citrate-yolk and Saccharose-glucose-yolk-Tris diluents with 3.5 per cent glycerol, and then frozen in liquid nitrogen vapour in ampules, medium sized and thin straws, the highest post-thawing motility of 31 to 46 per cent was obtained with the Lactose diluent (Zamfirescu et al., 1980). An average post-thawing motility of 60 per cent was obtained by Harnath et al.

(1982), when washed buck spermatozoa were frozen, after six hours equilibration period, in Tris-fructose-citric acid-yolk diluent containing 6.4 per cent glycerol in 0.5 ml straws. Salamon and Rittar (1982) noted that buck spermatozoa could tolerate a relatively wide range in concentration of Tris; and glucose and fructose were more suitable components than lactose and raffinose in the diluent.

MATERIALS AND METHODS

MATERIALS AND METHODS

From among the breeding bucks maintained at the "All India Co-ordinated Research Project on Goats for Milk Production" attached to the College of Veterinary and Animal Sciences, Kerala Agricultural University, Mannuthy, Trichur; six adult bucks (3 Malabari and 3 Alpine X Malabari) in the age group of 3 to 5 years were utilised for this study. These bucks were kept under identical management and feeding regime. They did not show any signs of systemic or reproductive diseases throughout the period of study extending from July '82 to September '83.

For the study of normal characteristics of buck semen, two consecutive ejaculates were collected twice in a week from each buck by artificial vagina method (Perry, 1969). Reaction time with respect to each ejaculate was noted by means of a stop watch. Immediately after collection, volume and colour of each sample were noted and kept in a water bath at 37°C.

The density, pH, mass activity and motility of each sample were assessed as per the standard procedures (Roberts, 1971). Nigrosin-eosin stained smears were prepared and utilized to determine the percentage of live sperms (Campbell et al., 1956). Percentage of different types of sperm

abnormalities were found out and classified as described by Blom (1972). The sperm concentration was estimated using Spectronic-20 (Perry, 1969).

To study the effect of addition of different levels of glycerol at room temperature and at refrigeration temperature in reconstituted skim milk as well as in Tris-egg-yolk-fructose diluent, on the preservability of sperms at 5°C, semen samples were collected from the selected bucks twice a week at regular intervals. Immediately after collection of each sample, preliminary evaluation was conducted. Those samples which had over 70 per cent motility alone were utilized for the study. Such semen samples from the bucks of same genetic group were pooled before dilution. From each genetic group a total of eight semen samples were utilized for room temperature glycerolisation and four samples for refrigeration temperature glycerolisation.

I. Glycerol addition at room temperature

a) Skim milk extender

To prepare the basic diluent 9 g spray-dried skimmed milk powder (Anik spray, Hindustan lever Limited, Bombay) was diluted to make up the final volume of 100 ml, by adding

double glass distilled water. The milk so obtained, was heated at 92°C for 5 minutes,and then cooled and filtered, using sterile cotton wool. Penicillin and Streptomycin were added at the rate of 1000 i.u. and 1000 microgram, respectively per ml of the diluent. In addition 1.25 g fructose was added and stirred well with the help of a magnetic stirrer (Table 1). Then it was kept at 5°C in the refrigerator till further use. Before mixing with semen sample, the extender was brought to room temperature and split into four parts. Glycerol at 37°C was added to the second, third and fourth parts of the diluent at the proportion of one, three and severn per cent. The first part was used without adding glycerol (Table 2).

b) Tris-Extender

The four combinations of Tris diluents with different levels of glycerol were prepared and used for the present study (Table 3).

After preparation, the four parts of Tris diluents were kept at refrigeration temperature. Prior to use the diluents were brought to the room temperature.

To study the effect of glycerolisation at room temperature, on the preservability of sperms, half of the pooled

semen sample was diluted seperately at the ratio of 1:10 with four parts of reconstituted skim milk extenders and the other half with four parts of Tris diluents, each having different levels of glycerol, and preserved at 5°C. For dilution of the semen, split sampling technique was adopted.

II. Addition of Glycerol at refrigeration temperature

To study the effect of glycerolisation at refrigeration temperature on preservability of sperms, the pooled semen samples were mixed with the first half of reconstituted skim milk and Tris diluent both without glycerol, at the ratio of 1:5 using split sample method at room temperature (30° to 32°C) and transferred to the refrigerator. The second half of both the diluents were prepared with the concentration of 0, 2, 6 and 14 per cent glycerol; and then kept at refrigeration temperature. The respective glycerolated parts were mixed to the semen containing non-glycerolated parts at 5°C in three steps of 20, 30 and 50 per cent at 10 minutes interval. Thus the final concentration of 0, 1, 3 and 7 per cent glycerol was obtained. The diluted samples were preserved in the refrigerator.

a) Skim milk extender

Basic skim milk solutions were prepared as in table 1

and the non-glycerolated and glycerolated parts were prepared as per table (4).

b) Tris extender

The basic solution of Tris was prepared as shown in table (5). The non-glycerolated and glycerolated parts of Tris extender were prepared as in table (6).

After the addition of different levels of glycerol, at room temperature and refrigeration temperature, for the study on preservability of semen at 5° to 7°C in Tris and skim milk diluents, one drop of diluted semen was taken from sample and motility assessment was carried out (Laing, 1979). Wet smears were also prepared from each sample and air dried for 5 to 10 minutes. Then the smears, prepared from Tris diluent, were fixed for 10 to 15 minutes in buffered formal saline (Campbell et al., 1960). Smears were washed gently in tap water for one minute. After air-drying, the smears were stained in Giemsa stain by immersing the slides for 4 to 6 hours (Hancook, 1952; Watson, 1975). Stained slides were washed in distilled water and air dried.

The dried smears, prepared from skim milk diluent, were immersed in isopropyl alcohol for 20 to 25 minutes to aid in

defattening and then allowed to air-dry. Then stained in Giemsa stain as in the case of smears prepared from Tris diluted semen.

Later, the stained smears were examined under oil immersion objective of the microscope. One hundred sperms were counted from each slide for morphological abnormalities and acrosome defects. The motility scoring, sperm abnormalities and acrosome defects were studied at 0, 24, 48, 72, 96, 120 and 144 hours of storage.

To study the effect of deep-freezing on semen, separate collections were made from all the selected bucks twice a week at regular intervals. From each buck four ejaculates having atleast 70 per cent motility were utilized. Each ejaculate was extended with the Tris-egg yolk-Fructose and reconstituted skim milk diluents.

Tris extender

The composition used in part IV, table 6 was utilized for freezing of buck semen.

Skim milk extender

Reconstituted skim milk diluent was prepared as mentioned in part IV, table 4 and used for the present study.

The diluted semen as well as glycerolated part (equal in volume) were transferred to the refrigerator for gradual cooling in small sterile conical flask. The glycerolated portion was added to the semen containing portion in three stages as it was done in preservation studies under refrigeration temperature. The glycerolated semen was maintained at 5°C for a minimum of six hours as equilibration period. During this period French medium straws of different colours, filling clips, a bundle of sterilized cotton wool, polyvenyle alcohol sealing powder in a dish, towel, water bath with 500 ml of clean water with few drops of dettol were kept in the refrigerator for cooling to 5°C. After two hours of equilibration period the straws were filled gently with semen by applying negative pressure through the end with factory seal. Then the open end of the straws were dried with cotton wool, sealed with sealing powder and again wiped to take away the excess powder sticking outside. The straws were immersed in the cooled water bath at 5°C for 45 minutes. The straws were then taken out of the water bath and dried by rolling them in a pre-cooled towel. Then the straws were transported to Bull station, Dhoni for freezing. While transportation care was taken to maintain the temperature at 5°C and to avoid shaking. The straws were arranged on the pre-cooled freezing rack with the help of a freezing ramp in the

cold handling unit of the freezing laboratory. A wide-mouthed liquid nitrogen refrigerator (LNR-320-Union Carbide) was used for freezing. A freezing grill was placed 30 cm below its brim. Care was taken to see that the liquid nitrogen level touched the grill. The freezing rack with the straws were transferred to the LNR-320 quickly over the grill after six hours of equilibration period. Goblet was also kept along with the semen. The straws were frozen in the liquid nitrogen vapour, at 4 cm above the liquid nitrogen level. After eight minutes of freezing in the liquid nitrogen vapour, the straws were collected by quick movement of hand into the pre-cooled goblet and the goblet was plunged into the liquid nitrogen. Later, the goblet was shifted to LNR-250 filled with liquid nitrogen for storage. Immediately after freezing two straws of each sample were thawed at room temperature for 30 to 40 seconds. Post-thaw motility with respect to each sample at 37°C was recorded under a phase contrast microscope.

The data on normal semen characteristics were subjected to arc sine $\sqrt{\ }$ transformation before analysis on the basis of Heirarchial classification. Retransformed mean values were presented in the tables. The co-efficient of correlation, among seminal attributes for first and second ejaculates over

breeds, were worked out using standard procedure. The data on preservation of buck spermatozoa were analysed as a 2 x 2 x 4 CRD factorial experiment separately for room temperature and refrigeration temperature glycerolisation. Proportion values were subjected to arc sine transformation before analysis. Retransformed mean values were presented in the tables . Comparison between room temperature and refrigeration temperature glycerolisation were made using student 't' test. When the variance ratio was found significant then Cochran's 't' was used for comparison between the methods of glycerolisation. For freezing of buck semen, data were analysed as a 2 x 2 CRD factorial experiment (Snedecor and Cochran, 1967).

Table 1. Composition of basic solution for skim milk extender

Ingredients	Quantity
Skim milk powder (g)	9
Distilled water (ml)	to 100
Fructose (g)	1.25
Penicillin (i.u.)	100000
Streptomycin (g)	0.10

Table 2. Ingredients of I to IV parts of skim milk extender

Composition	Part I	Part II	Part III	Part IV
Basic solution (ml)	100	99	97	93
Glycerol (ml)	Nil	1	3	7
Total (ml)	100	100	100	100

Table 3. Composition of I to IV parts of Tris extender

Composition	Part I	Part II	Part III	Part IV
Tris (hydroxymethyl) aminomethane (g)	2.42	2.42	2.42	2.42
Citric acid (g)	1.34	1.34	1.34	1.34
Fructose (g)	1.00	1.00	1.00	1.00
Egg yolk (ml)	20.00	20.00	20.00	20.00
Glycerol (ml)	Nil	1.00	3.00	7.00
Distilled water (ml)	80.00	79.00	77.00	73.00
Penicillin (i.u)	100000	100000	100000	100000
Streptomycin (g)	0.10	0.10	0.10	0.10

Table 4. Composition of I to IV parts of skim milk diluent for glycerolation at refrigeration temperature

Composition		Part I	Part II	Part III	Part IV
Non-glycerolated part					
Basic solution	(ml)	50.00	50.00	50.00	50.00
Glycerolated part					
Basic solution	(ml)	50.00	49.00	47.00	43.00
Glycerol	(ml)	Nil	1.00	3.00	7.00
Total	(ml)	100.00	100.00	100.00	100.00

Table 5. Composition of basic solution of Tris diluent for addition of glycerol at refrigeration temperature

Ingredients		Part I	Part II	Part III	Part IV
Tris (hydroxymethyl) aminomethane	(g)	2.42	2.42	2.42	2.42
Citric acid	(g)	1.34	1.34	1.34	1.34
Fructose	(g)	1.00	1.00	1.00	1.00
Distilled water	(ml)	80.00	78.00	74.00	66.00

Table 6. Composition of four parts of Tris diluents for glycerolation at refrigeration temperature

Composition		Part I	Part II	Part III	Part IV
First half (non-glycerolated parts)					
Basic solution	(ml)	40.00	39.00	37.00	33.00
Distilled water	(ml)	Nil	1.00	3.00	7.00
Egg yolk	(ml)	10.00	10.00	10.00	10.00
Penicillin	(i.u.)	50000	50000	50000	50000
Streptomycin	(g)	0.05	0.05	0.05	0.05
Second half (glycerolated parts)					
Basic solution	(ml)	40.00	39.00	37.00	33.00
Glycerol	(ml)	Nil	1.00	3.00	7.00
Egg yolk	(ml)	10.00	10.00	10.00	10.00
Penicillin	(i.u.)	50000	50000	50000	50000
Streptomycin	(g)	0.05	0.05	0.05	0.05

RESULTS

RESULTS

Normal characteristics of buck semen

The mean reaction time, for Malabari and Alpine X Malabari bucks, is given in table 7. The overall average reaction time was found to be 98.86 ± 4.309 seconds. On statistical analysis the reaction time, for the first ejaculate was not significantly correlated with volume, density, mass activity, motility, live sperms, pH, concentration and total sperm abnormalities (Table 21). However, significant positive correlation ($P < 0.05$) was observed between reaction time and mass activity for second ejaculate (Table 22). Highly significant ($P < 0.01$) difference between ejaculates within buck was observed for reaction time (Table 23).

The data on mean semen volume are presented in table 8. The overall average volume of semen was found to be 0.64 ± 0.015 ml. There was significant positive correlation ($P < 0.05$) between volume and density for the first ejaculate as shown in table 21. It was also observed that semen volume had a significant negative correlation ($P < 0.05$) with motility percentage in first ejaculate (Table 21). On further analysis, the semen volume was found to be significant ($P < 0.05$) between breeds. Highly significant ($P < 0.01$)

difference was also noted for semen volume between ejaculates within buck (Table 23).

In the present study, the colour of semen of Malabari and Alpine X Malabari cross bred bucks was found to be milky yellow, thin creamy, thick creamy yellow or creamy white.

The average density score of buck semen is presented in table 9. The mean density score of 3.52 ± 0.030 out of four was observed for bucks. A highly significant ($P < 0.01$) positive correlation between density and sperm concentration was found in the present study for both first and second ejaculates (Table 21) and (Table 22). Statistical analysis further revealed that there was highly significant difference ($P < 0.01$) between bucks within the breed for density of semen (Table 23).

The overall mean pH of semen was 6.74 ± 0.016 (Table 10). On analysis there was significant ($P < 0.05$) difference in pH of semen between bucks within breed (Table 23).

The overall average mass activity of buck semen was found to be 3.66 ± 0.034 out of four (Table 11). There was a significant ($P < 0.05$) positive correlation between mass motility and concentration for both the first and second ejaculates as shown in table 21 and 22. Analysis of data

further showed highly significant difference for mass activity between breeds and between bucks within breed (Table 23).

The data pertaining to mean motility percentage of spermatozoa are presented in table 12. The overall initial motility was 80.64 ± 0.344 per cent. The initial motility was positively correlated ($P < 0.05$) with the live sperm percentage for the first ejaculate (Table 21). Whereas, highly significant positive correlation ($P < 0.01$) was observed between initial motility and live sperm percentage for the second ejaculate in Malabari and Alpine X Malabari buck semen (Table 22). A significant ($P < 0.05$) difference between Malabari and Alpine X Malabari bucks was obtained for initial motility (Table 23).

The data on average live sperms percentage are presented in table 13. The overall mean for live sperm percentage was found to be 90.64 ± 0.317. Significant positive correlation of live sperm percentage with sperm motility percentage was observed for first ($P < 0.05$) and second ejaculates ($P < 0.01$) in Malabari and cross bred bucks (Table 21 and 22).

The sperm concentration for buck semen is shown in table 14. The overall mean concentration was recorded to be 3.39 ± 0.061 billion per ml. Statistical analysis revealed

significant difference in sperm concentration between breeds ($P < 0.05$) and between bucks within breed ($P < 0.01$) as in table 23.

The overall mean value for total sperm abnormalities was 1.40 ± 0.265 per cent (Table 20). Statistical analysis showed significant ($P < 0.05$) difference in sperm abnormalities between breeds. It was also observed that there was highly significant ($P < 0.01$) difference for the percentage of sperm abnormalities between bucks within breed (Table 23).

The average abnormality percentages of head, mid piece and tail abnormalities of buck spermatozoa are shown in tables 15, 16 and 17. The overall average percentages of head, mid piece and tail abnormalities were 0.03 ± 0.012, 0.13 ± 0.023 and 0.70 ± 0.229 respectively. The mean values on percentages of protoplasmic droplets in buck spermatozoa are presented in table 18. An overall mean of 0.30 ± 0.069 per cent of protoplasmic droplet was observed. The overall mean for detached head abnormality was found to be 0.24 ± 0.034 per cent (Table 19).

Preservation of buck semen in glycerol containing diluents

The data on percentage of motility at zero hour of storage are presented in table 24. The overall mean motility

of buck semen glycerolised at room temperature and refrigeration temperature was found to be 79.63 and 80.41 per cent respectively. On analysis the motility was significant ($P < 0.01$) between breeds, when glycerolisation was done at refrigeration temperature (Table 31).

The data on preservation of buck semen at 24 hours of storage are shown in table 25. The overall average motility was found as 73.75 and 77.32 per cent at room temperature and refrigeration temperature glycerolisation respectively. Statistical analysis revealed that there was significant ($P < 0.01$) difference for motility percentage between diluents, both in room temperature and refrigeration temperature glycerolisation. The interaction between diluents and levels of glycerol was found to be significant in both cases of glycerolisation. However, motility percentage was significantly ($P < 0.01$) different between breeds for glycerolisation at refrigeration temperature (Table 31).

At 48 hours of storage the overall mean motility in extenders glycerolised at room temperature and refrigeration temperature was recorded as 65.50 and 70.26 per cent respectively (Table 26). On analysis the motility percentage was found to be significant ($P < 0.01$) between diluents at the two methods of glycerolisations. Similarly interaction

between diluents and levels of glycerol was significant ($P < 0.01$) for both the cases of glycerolations. There was significant difference ($P < 0.01$) for motility percentage in levels of glycerol at refrigeration temperature glycerolisation (Table 31).

The data on percentage of mean motility at 72 hours are in table 27. The overall mean motility of buck semen at room temperature and refrigeration temperature glycerolisation was found as 51.68 and 63.28 per cent respectively. Statistical analysis showed that there was significant ($P < 0.01$) difference for motility percentage between breeds and between diluents at room temperature glycerolisation; whereas, at refrigeration temperature glycerolisation significant ($P < 0.01$) difference was noticed between diluents alone. The interaction between diluent and levels of glycerol was found to be significant in motility percentage for both the glycerolisations. It was also noticed that motility percentage varied significantly among the levels of glycerol for refrigeration temperature glycerolisation (Table 31).

The overall mean motility percentage of 39.52 and 51.55 were noticed when glycerolisation was done at room temperature and refrigeration temperature, respectively, at 96 hours of storage (Table 28). On statistical analysis significant

difference ($P < 0.01$) was obtained for motility percentage between diluents for both the types of glycerolisations. The interaction between diluents and levels of glycerol was also found significant ($P < 0.01$) in motility percentage for both the cases. There was significant ($P < 0.05$) difference between breeds for room temperature glycerolisation. Significant ($P < 0.01$) difference was noticed in levels of glycerol for motility percentage for refrigeration temperature glycerolisation (Table 31).

The mean motility percentage of buck semen at 120 hours of storage was 26.54 and 39.06 per cent at room temperature and refrigeration temperature glycerolisation respectively (Table 29). On analysis the motility percentage was significant ($P < 0.01$) between diluents at both the temperature of glycerolisation. Similarly interaction between diluents and levels of glycerol was noted as significant ($P < 0.01$) in both the cases. But there was a significant ($P < 0.01$) difference for levels of glycerol in motility percentage when glycerolisation was done at refrigeration temperature (Table 31).

The data on mean motility percentage at 144 hours of storage are presented in table 30. The overall average motility of 19.23 and 31.62 per cent was noted at room

temperature and refrigeration temperature glycerolisation respectively. Statistical analysis revealed that there was significant ($P < 0.01$) difference between Tris and Skim milk diluents in mean motility percentage both at room temperature and refrigeration temperature glycerolisation. The interaction between diluents and levels of glycerol was also found significant ($P < 0.01$) for motility percentage in both types of glycerolations. However, the interaction between breed and diluent for motility percentage was significant ($P < 0.05$) in refrigeration temperature glycerolisation. There was significant ($P < 0.01$) difference in levels of glycerol for refrigeration temperature glycerolisation in mean motility percentage (Table 31).

The mean motility percentage of buck spermatozoa was found to vary significantly between room temperature and refrigeration temperature glycerolisation through out the period of storage.

The mean percentage of total abnormality of buck spermatozoa at zero hour of storage are presented in table 32. The overall mean for total abnormality percentage was 5.42 at room temperature glycerolisation and 3.14 at refrigeration temperature glycerolisation. On analysis percentage of total abnormality was found to be significant ($P < 0.01$) among

the levels of glycerol at both types of glycerolisations. However, significant difference ($P < 0.01$) for total abnormality percentage was observed between diluents at refrigeration temperature glycerolisation alone (Table 39).

The data on percentages of total sperm abnormality at 24 hours were 6.57 and 4.09 at room temperature and refrigeration temperature glycerolisation respectively (Table 33). Statistical analysis showed significant difference ($P < 0.05$) for total spermatozoan abnormality at room temperature glycerolisation among the levels of glycerols; whereas highly significant difference ($P < 0.01$) was noted at refrigeration temperature glycerolisation. In addition there was significant difference ($P < 0.01$) between diluents for total sperm abnormality at refrigeration temperature glycerolisation (Table 39).

The mean values for total sperm abnormality percentage at 48 hours of storage are presented in table 34. The overall mean for total sperm abnormalities was recorded as 6.76 and 4.23 per cent at room temperature and refrigeration temperature glycerolisation respectively. On analysis significant difference ($P < 0.01$) was observed for total sperm abnormality between breeds, between diluents and among the levels of glycerol at refrigeration temperature glycerolisation;

whereas that was not the case at room temperature glycerolisation (Table 39).

The mean values at 72 hours of storage for total sperm abnormalities were 6.91 and 4.47 per cent at room temperature and refrigeration temperature glycerolisation, respectively (Table 35). For total sperm abnormalities, significant difference ($P < 0.05$) was noted among the levels of glycerol at room temperature glycerolisation; and highly significant ($P < 0.01$) difference was observed at refrigeration temperature glycerolisation. When glycerolisation was done at refrigeration temperature there was highly significant difference between diluents (Table 39).

The mean values for total abnormality percentage of buck spermatozoa at 96 hours of storage are presented in table 36. An average total sperm abnormalities of 7.00 per cent was observed at room temperature glycerolisation as against, 4.45 per cent at refrigeration temperature glycerolisation. The data on analysis showed significant difference ($P < 0.01$) for total sperm abnormality between diluents and among the levels of glycerol at refrigeration temperature glycerolisation (Table 39).

The mean values of total sperm abnormality of 7.33 and

4.62 per cent were recorded at room temperature and refrigeration temperature glycerolisation, respectively at 120 hours of storage (Table 37). The variation in total sperm abnormality was found to be significant ($P \leq 0.01$) for Tris and Skim milk diluents and for different levels of glycerol at refrigeration temperature glycerolisation; whereas, significant ($P < 0.05$) difference due to levels of glycerol was noticed for room temperature glycerolisation (Table 39).

The average total sperm abnormality of 7.39 and 4.81 per cent was observed at room temperature and refrigeration temperature glycerolisation respectively at 144 hours of storage (Table 38). Statistical analysis revealed the significant difference ($P < 0.01$) in total sperm abnormality between diluents and the levels of glycerol at refrigeration temperature glycerolisation (Table 39).

The mean total abnormality percentage of buck spermatozoa was found to very significantly between room temperature and refrigeration temperature glycerolisation through out the storage period.

The data on percentages of head abnormality of buck spermatozoa at zero hour, 24 hours and 48 hours of storage are presented in tables 40, 41 and 42 respectively. The

average head abnormality percentages of 0.69 and 0.40 were observed at room temperature and refrigeration temperature glycerolisation, respectively, at zero hour of storage. The corresponding values were 0.63 and 0.37 per cent at room temperature and refrigeration temperature glycerolisation, respectively, at 24 hours of storage, 0.60 and 0.47 per cent at room temperature and refrigeration temperature glycerolisation, respectively, at 48 hours of storage.

The mean values for head abnormality percentage of buck spermatozoa at 72 hours of storage are shown in table 43. The mean values for head abnormality percentage were 0.57 and 0.49 at room temperature and refrigeration temperature glycerolisation respectively. Statistical analysis showed the significant difference ($P < 0.01$) for head abnormality percentage among the levels of glycerol at refrigeration temperature glycerolisation only (Table 47).

At room temperature and refrigeration temperature glycerolisation the average percentage of head abnormality were 0.64 and 0.54 respectively at 96 hours of storage (Table 44). Data on analysis revealed significant difference ($P < 0.01$) for head abnormality among the levels of glycerol at refrigeration temperature glycerolisation (Table 47).

The average values for head abnormality percentage

of buck spermatozoa at 120 hours of storage were noted as 0.61 and 0.54 at room temperature and refrigeration temperature glycerolization, respectively (Table 45). Significant difference ($P < 0.05$) for head abnormality percentage was observed between Tris and Skim milk diluents and among the levels of glycerol at refrigeration temperature glycerolisation at 120 hours of storage (Table 47).

The head abnormality percentage of 0.67 was noted at room temperature glycerolisation, as against, 0.59 per cent at room temperature glycerolisation at 144 hours of storage (Table 46). The incidence of head abnormality percentage was found to be significant ($P < 0.01$) among the levels of glycerol at refrigeration temperature glycerolisation (Table 47).

The data on average mid piece abnormality percentage of buck spermatozoa at zero hour of storage are presented in table 48. The occurrence of mid piece abnormality was noted to be 1.14 and 0.86 per cent at room temperature and refrigeration temperature glycerolisation respectively. On analysis, significant difference ($P < 0.01$) was observed between breeds and among the levels of glycerol for mid piece abnormality percentage at refrigeration temperature glycerolisation (Table 55).

The mean value of 1.35 per cent mid piece abnormality was noted at room temperature glycerolisation as against, 1.01 per cent at refrigeration temperature glycerolisation at 24 hours storage (Table 49). No significant difference could be found for mid piece abnormality percentage either at room temperature or refrigeration temperature glycerolisation (Table 55).

The average mid piece abnormality percentages of 1.48 and 1.04 were worked out at room temperature and refrigeration temperature glycerolisation respectively at 48 hours of storage (Table 50). Statistical analysis showed significant difference ($P < 0.05$) for mid piece abnormality percentage between diluents at room temperature glycerolisation (Table 55).

The data on average mid piece abnormality percentage of buck spermatozoa at 72 hours of storage are shown in table 51. The average mid piece abnormality percentage of 1.68 was noted at room temperature glycerolisation; while the corresponding values was 1.22 per cent at refrigeration temperature glycerolisation. Significant difference ($P < 0.01$) could be obtained between diluents for mid piece abnormality percentage at room temperature glycerolisation (Table 55).

Average mid piece abnormality percentage at 96 hours of storage can be read from table 52. At room temperature and refrigeration temperature glycerolisation the average mid piece abnormality percentages were 1.55 and 1.09 respectively. Significant difference ($P < 0.05$) could be obtained between breeds for mid piece abnormality percentage at refrigeration temperature glycerolisation at 96 hours of storage (Table 55).

An average mid piece abnormality of 1.73 per cent was observed at room temperature glycerolisation as against, 1.35 per cent at refrigeration temperature glycerolisation at 120 hours of storage (Table 53). On analysis no significant difference could be find out either at room temperature or at refrigeration temperature glycerolisation (Table 55).

The mean abnormality percentages of 1.78 and 1.56 were noted at room temperature and refrigeration temperature glycerolisation respectively at 144 hours of storage (Table 54). Statistical analysis showed significant difference between diluents ($P < 0.01$) at refrigeration temperature glycerolisation and between breeds ($P < 0.05$) at room temperature glycerolisation (Table 55).

The data on tail abnormality percentage of buck spermatozoa at zero hour of storage are presented in table 56. The mean tail abnormality percentage of 3.43 and 1.98 were noted at room temperature and refrigeration temperature glycerolisation respectively. The data on analysis showed significant difference between diluents at room temperature glycerolisation ($P < 0.05$) and at refrigeration temperature glycerolisation ($P < 0.01$). The tail abnormality percentage was found to be significant ($P < 0.01$) among the levels of glycerol at room temperature glycerolisation and between breeds ($P < 0.05$) for glycerolisation at refrigeration temperature. However, Breed X diluent X level of glycerol interaction was also found to be significant ($P < 0.05$) at refrigeration temperature but it was not at room temperature glycerolisation (Table 63).

An average tail abnormality percentage of 4.50 was noted at room temperature glycerolisation as against, 2.79 per cent at refrigeration temperature glycerolisation at 24 hours of storage (Table 57). Statistical analysis revealed significant difference ($P < 0.01$) between diluents for tail abnormality percentage at refrigeration temperature glycerolisation (Table 63).

Average tail abnormality percentage at 48 hours of storage are shown in table 58. Mean tail abnormality

percentages of 4.61 and 2.73 were noted at room temperature and refrigeration temperature glycerolisation respectively. There was significant difference ($P < 0.01$) for tail abnormality percentage between diluents at refrigeration temperature glycerolisation (Table 63).

The mean tail abnormality percentages of buck spermatozoa at 72 hours of storage were 4.52 and 2.74 per cent at room temperature and refrigeration temperature glycerolisation respectively (Table 59). On analysis significant difference ($P < 0.05$) could be found among the levels of glycerol at room temperature glycerolisation and between diluents ($P<0.01$) at refrigeration temperature glycerolisation (Table 63).

Mean values for tail abnormality percentage for buck spermatozoa at 96 hours of storage are in table 60. Average tail abnormality percentage of 4.72 was noted at room temperature glycerolisation as against, 2.79 per cent at refrigeration temperature glycerolisation. Statistical analysis revealed that there was significant difference ($P < 0.05$) among the levels of glycerol at room temperature glycerolisation and between diluents ($P < 0.05$) at refrigeration temperature glycerolisation (Table 63).

At room temperature and refrigeration temperature glycerolisation, average tail abnormality were 5.01 and 2.62

per cent respectively at 120 hours of storage (Table 61). Significant difference for tail abnormality percentage was obtained among the levels of glycerol at room temperature glycerolisation ($P < 0.01$) and at refrigeration temperature glycerolisation ($P < 0.05$).

The data on mean values for tail abnormality percentage of buck spermatozoa at 144 hours of storage are tabulated (Table 62). The mean values for tail abnormality percentage were 5.04 and 2.64 at room temperature and refrigeration temperature glycerolisation respectively. The data on analysis revealed significant difference ($P < 0.01$) for tail abnormality percentage between diluents at refrigeration temperature glycerolisation (Table 63).

The mean values for the percentage of acrosome defect of buck spermatozoa at zero hours of storage are mentioned in table 64. The mean values for acrosome defect was 0.87 per cent at room temperature glycerolisation as against, 0.29 per cent at refrigeration temperature glycerolisation. Percentage of acrosome defect showed significant difference between breeds ($P < 0.05$) at refrigeration temperature glycerolisation and among the levels of glycerol ($P < 0.05$) at room temperature glycerolisation (Table 71).

The data on acrosome defect of buck spermatozoa at 24 hours of storage are presented in table 65. The overall mean of acrosome defect was noted as 2.76 per cent at room temperature glycerolisation, and 1.30 per cent at refrigeration temperature glycerolisation. The data on analysis gave a significant difference ($P < 0.01$) among the levels of glycerol for acrosome defect at the two types of glycerolisations. There was significant difference between diluents at room temperature glycerolisation ($P < 0.01$) and at refrigeration temperature glycerolisation ($P < 0.05$). In addition, significant difference ($P < 0.01$) for acrosome defect was observed at diluent X level of glycerol interaction for room temperature glycerolisation (Table 71).

The mean values for acrosome defect of buck spermatozoa at 48 hours of storage are shown in table 66. Average acrosome defects were 3.62 and 2.79 per cent at room temperature and refrigeration temperature glycerolisation respectively. Statistical analysis showed significant difference for acrosome defect between diluents at room temperature glycerolisation ($P < 0.05$) and at refrigeration temperature glycerolisation ($P < 0.01$). However, significant difference ($P < 0.01$) for acrosome defect was noted among the levels of glycerol at room temperature glycerolisation also (Table 71).

Average values for percentage of acrosome defect of buck spermatozoa at 72 hours of storage are arranged in table 67. Acrosome defect was 4.47 per cent at room temperature glycerolisation and 3.92 per cent at refrigeration temperature glycerolisation. Statistical analysis revealed significant difference for acrosome defect among the levels of glycerol both at room temperature and refrigeration temperature glycerolisation ($P < 0.01$) and between diluents ($P < 0.01$) at refrigeration temperature glycerolisation. There was significant difference ($P < 0.05$) between breeds at refrigeration temperature glycerolisation (Table 71).

Mean values for acrosome defect were 5.19 and 4.61 per cent at room temperature and refrigeration temperature glycerolisation respectively at 96 hours of storage (Table 68). Significant difference ($P < 0.01$) could be obtained for acrosome defect among the levels of glycerol at both the glycerolisations (Table 71).

The data on acrosome defect showed 6.14 and 4.84 per cent as mean for room temperature and refrigeration temperature glycerolisation respectively at 120 hours (Table 69). On analysis significant difference ($P < 0.01$) could be observed among the levels of glycerol at the two types of glycerolisations (Table 71).

The mean values on acrosome defect were 7.08 and 6.15 per cent at room temperature and refrigeration temperature glycerolisation respectively at 144 hours (Table 70). Statistical analysis revealed significant difference ($P < 0.05$) for acrosome defect among the levels of glycerol at both the glycerolisations. Breed and diluent interaction was found to be significant ($P < 0.01$) for acrosome defect at room temperature glycerolisation. There was significant difference ($P < 0.05$) between breeds at refrigeration temperature glycerolisation (Table 71). Acrosome defects of buck spermatozoa stored in Tris and skim milk diluents are shown in Fig. 1 and 2.

Deep freezing of buck semen

The data on percentage of post-thawing motility after test freezing are given in table 72. The overall percentage of post-thawing motility of buck semen was 37.755. The mean post-thawing motility for Malabari buck semen was 36.087 per cent, with 41.649 per cent in Tris and 30.524 per cent in skim milk extenders. For Alpine X Malabari bucks the mean post-thawing motility was 39.424 per cent, with 47.36 per cent in Tris and 31.613 per cent in skim milk diluents. In Tris diluent the mean post-thawing motility was 44.443 per cent

whereas, in skim milk diluent it was 31.068 per cent only.

On analysis there was highly significant ($P < 0.01$) difference in post-thawing motility between Tris and skim milk diluents (Table 72).

Table 7. Mean reaction time (seconds)

Sl. No.	Malabari			Alpine X Malabari			Grand Total
	E1	E2	Total	E1	E2	Total	
1	51.80	136.65	94.23	57.65	176.65	117.15	105.69
2	80.10	119.35	99.73	68.15	156.90	112.53	106.13
3	55.35	124.45	89.90	52.35	107.00	79.68	84.79
Total	62.41	126.81	94.61	59.38	146.85	103.11	98.86
S.E.	6.480	8.942	5.811	4.441	8.768	6.326	4.309

Table 8. Mean volume (ml.)

Sl. No.	Malabari			Alpine X Malabari			Grand total
	E1	E2	Total	E1	E2	Total	
1	0.42	0.53	0.47	0.62	0.79	0.70	0.59
2	0.64	0.66	0.65	0.66	0.62	0.64	0.64
3	0.53	0.55	0.54	0.71	0.96	0.83	0.68
Total	0.53	0.58	0.55	0.66	0.79	0.72	0.64
S.E.	0.024	0.022	0.017	0.027	0.031	0.015	0.015

E1 : First ejaculate E2 : Second ejaculate

Table 9. Density score

Sl. No.	Malabari			Alpine X Malabari			Grand total
	E1	E2	Total	E1	E2	Total	
1	3.67	3.62	3.65	3.77	3.62	3.70	3.67
2	3.15	3.05	3.10	3.57	3.45	3.51	3.30
3	3.80	3.82	3.81	3.42	3.33	3.36	3.58
Total	3.54	3.50	3.52	3.59	3.45	3.52	3.52
S.E.	0.069	0.070	0.035	0.051	0.048	0.035	0.030

Table 10. Hydrogen ion concentration (pH)

Sl. No.	Malabari			Alpine X Malabari			Grand total
	E1	E2	Total	E1	E2	Total	
1	6.69	6.77	6.73	6.58	6.73	6.65	6.69
2	6.86	6.87	6.86	6.73	6.78	6.76	6.81
3	6.60	6.65	6.62	6.80	6.84	6.82	6.72
Total	6.71	6.76	6.74	6.70	6.78	6.74	6.74
S.E.	0.041	0.033	0.026	0.029	0.024	0.019	0.016

Table 11. Mass activity score

Sl. No.	Malabari			Alpine X Malabari			Grand total
	E1	E2	Total	E1	E2	Total	
1	3.65	3.77	3.71	3.85	3.92	3.88	3.80
2	2.92	3.07	3.00	3.72	3.75	3.73	3.36
3	3.80	3.90	3.85	3.80	3.75	3.77	3.81
Total	3.45	3.58	3.52	3.79	3.80	3.80	3.66
S.E.	0.081	0.074	0.055	0.052	0.048	0.035	0.034

Table 12. Mean motility percentage

Sl. No.	Malabari			Alpine X Malabari			Grand total
	E1	E2	Total	E1	E2	Total	
1	79.50	81.25	80.37	82.50	84.25	83.37	81.87
2	76.00	78.85	77.42	81.40	82.50	81.95	79.68
3	78.25	81.25	79.75	80.75	81.25	81.00	80.37
Total	77.91	80.45	79.18	81.55	82.66	82.10	80.64
S.E.	0.859	0.665	0.553	0.404	0.436	0.322	0.334

Table 13. Live spermatozoa percentage

Sl. No.	Malabari			Alpine X Malabari			Grand total
	E1	E2	Total	E1	E2	Total	
1	91.85	90.00	90.92	92.35	93.05	92.70	91.81
2	88.42	88.34	88.38	90.85	90.80	90.82	89.60
3	89.80	91.42	90.61	91.30	89.52	90.41	90.51
Total	90.02	90.08	89.97	91.36	91.12	91.31	90.64
S.E.	0.852	0.805	0.584	0.506	0.531	0.356	0.317

Table 14. Mean sperm concentration (10^9)

Sl. No.	Malabari			Alpine X Malabari			Grand total
	E1	E2	Total	E1	E2	Total	
1	3.83	3.72	3.77	3.76	3.50	3.63	3.70
2	2.81	2.56	2.69	3.22	3.08	3.15	2.92
3	3.91	4.14	4.02	3.14	2.96	3.05	3.54
Total	3.51	3.47	3.49	3.37	3.18	3.28	3.39
S.E.	0.137	0.147	0.098	0.101	0.098	0.070	0.061

Table 15. Percentage of head abnormality

Sl. No.	Malabari			Alpine X Malabari			Grand total
	E1	E2	Total	E1	E2	Total	
1	Nil	0.05	0.02	Nil	0.05	0.02	0.02
2	Nil	0.10	0.05	0.05	0.05	0.05	0.05
3	0.05	Nil	0.02	Nil	0.05	0.02	0.02
Total	0.01	0.05	0.03	0.01	0.05	0.03	0.03
S.E.	0.017	0.028	0.017	0.017	0.028	0.017	0.012

Table 16. Percentage of mid piece abnormality

Sl. No.	Malabari			Alpine X Malabari			Grand total
	E_1	E_2	Total	E_1	E_2	Total	
1	Nil	0.05	0.02	0.10	0.05	0.07	0.05
2	Nil	Nil	Nil	0.40	0.35	0.37	0.18
3	0.25	0.15	0.20	0.10	0.10	0.10	0.15
Total	0.08	0.06	0.07	0.20	0.16	0.18	0.12
S.E.	0.043	0.033	0.027	0.052	0.054	0.037	0.023

Table 17. Percentage of tail abnormality

Sl. No.	Malabari			Alpine X Malabari			Grand total
	E_1	E_2	Total	E_1	E_2	Total	
1	1.05	0.75	0.90	0.40	0.65	0.52	0.71
2	0.85	0.75	0.80	0.55	0.75	0.65	0.72
3	0.50	0.70	0.60	0.70	0.75	0.72	0.66
Total	0.80	0.73	0.76	0.55	0.71	0.63	0.70
S.E.	0.171	0.121	0.104	0.107	0.117	0.079	0.229

Table 18. Percentage of protoplasmic droplet

Sl. No.	Malabari			Alpine X Malabari			Grand total
	E_1	E_2	Total	E_1	E_2	Total	
1	0.75	0.45	0.60	0.10	0.05	0.07	0.33
2	0.30	0.05	0.17	0.15	0.10	0.12	0.15
3	0.60	0.70	0.65	0.20	0.20	0.20	0.42
Total	0.55	0.40	0.47	0.13	0.11	0.13	0.30
S.E.	0.215	0.160	0.133	0.050	0.042	0.035	0.069

Table 19. Percentage of detached head

Sl. No.	Malabari			Alpine X Malabari			Grand total
	E1	E2	Total	E1	E2	Total	
1	0.15	0.15	0.15	0.10	0.05	0.07	0.11
2	Nil	0.40	0.20	0.25	0.20	0.22	0.21
3	0.70	0.45	0.57	0.20	0.30	0.25	0.41
Total	0.28	0.33	0.30	0.18	0.18	0.18	0.24
S.E.	0.089	0.070	0.057	0.056	0.050	0.037	0.034

Table 20. Percentage of total abnormality

Sl. No.	Malabari			Alpine X Malabari			Grand total
	E1	E2	Total	E1	E2	Total	
1	1.95	1.45	1.70	0.65	0.90	0.77	1.23
2	1.15	1.30	1.22	1.35	1.45	1.40	1.31
3	2.10	2.00	2.05	1.15	1.40	1.27	1.66
Total	1.73	1.58	1.65	1.05	1.26	1.14	1.40
S.E.	0.302	0.228	0.188	0.135	0.129	0.093	0.265

Table 21. Co-efficient of correlation among the different seminal attributes of the first ejaculate of buck semen

	Reaction time	Volume	Density	Mass activity	Motility	Concentration	pH	Live Sperms	Total sperm abnormality
Reaction time	1.000	0.2429	0.1294	-0.0323	0.1842	0.0470	-0.0329	0.0569	-0.1028
Volume		1.0000	0.4973*	-0.1090	-0.4527*	0.3385	-0.6222	-0.2795	-0.0246
Density			1.0000	0.3803	-0.2573	0.8782**	-0.2836	-0.1571	00.0238
Mass activity				1.0000	0.2819	0.5484*	-0.0892	0.1603	-0.2163
Motility					1.0000	-0.2862	0.3644	0.4685*	-0.1903
Concentration						1.0000	-0.3506	-0.1373	-0.0433
pH							1.0000	0.1606	0.2345
Live sperms								1.0000	-0.2318
Total sperm abnormality									1.0000

* Significant at 5 per cent level. ** Significant at 1 per cent level.

Table 22. Co-efficient of correlation among the different seminal attributes of the second ejaculate of buck semen

	Reaction time	Volume	Density	Mass activity	Motility	Concentration	pH	Live sperms	Total sperm abnormality
Reaction time	1.0000	-0.0103	0.0606	0.4958*	0.3475	-0.0567	0.0322	0.0771	0.0174
Volume		1.0000	-0.0842	-0.1064	0.0151	-0.0587	-0.1814	-0.1522	-0.1400
Density			1.0000	0.3291	-0.4272	0.8079**	-0.2072	-0.2965	0.2704
Mass activity				1.0000	-0.1534	0.5188*	-0.0851	-0.2481	0.2717
Motility					1.0000	-0.4385	0.2736	0.7864**	-0.1243
Concentration						1.0000	-0.1982	-0.3629	0.3233
pH							1.0000	0.1159	0.0746
Live sperms								1.0000	-0.0762
Total sperm abnormality									1.0000

* Significant at 5 per cent level ** Significant at 1 per cent level

Table 23. Normal characteristics of buck semen

ANOVA

Source	df	Mean sum of squares								
		Reaction time	Volume	Density	Mass activity	Motility	Concentration	pH	Live sperms	Total abnormality
Between breeds	1	4335.00	1.768*	0.001	4.676**	528.067*	2.891*	0.002	58.838	30.192*
Between bucks within breed	4	8834.58	0.352	3.360**	4.285**	76.617	12.049**	0.426*	55.669	57.817**
Between ejaculates within buck	6	64231.78**	0.165**	0.111	0.095	42.200	0.415	0.065	13.605	2.717
Error	228	2807.85	0.035	0.169	0.189	23.218	0.689	0.059	23.750	16.396
Total	239									

*Significant at 5 per cent level ** Significant at 1 per cent level

Table 24. Mean motility percentage of buck semen at zero hour storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	79.35	79.90	79.98	79.27	79.63	81.30	79.51	80.41	80.41	80.41
1	79.35	79.61	79.98	78.97	79.48	81.30	79.51	80.41	80.41	80.41
3	79.65	79.90	79.98	79.58	79.78	81.30	79.51	80.41	80.41	80.41
7	79.35	79.91	79.68	79.58	79.63	81.30	79.51	80.41	80.41	80.41
Mean	79.43	79.83	79.91	79.35	79.63	81.30	79.50	80.41	80.41	80.41

Table 25. Mean motility percentage of buck semen at 24 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	72.51	73.26	77.85	67.63	72.89	77.81	75.73	79.65	73.78	76.76
1	72.47	72.93	77.22	67.93	72.69	77.81	76.32	79.65	74.39	77.08
3	74.19	74.53	77.02	71.61	74.36	79.65	76.92	79.65	76.92	78.30
7	74.62	75.44	76.29	73.76	75.03	77.85	76.33	76.64	77.55	77.09
Mean	73.46	74.05	77.09	70.26	73.75	78.29	76.33	78.91	75.68	77.32

B1 - Malabari
B2 - Alpine X Malabari
D1 - Tris diluent
D2 - Skim milk diluent

Table 26. Mean motility percentage of buck semen at 48 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	65.34	63.55	74.18	54.08	64.45	68.36	65.88	74.39	59.43	67.13
1	65.36	64.09	73.01	55.97	64.72	69.64	68.96	75.65	62.57	69.30
3	65.49	66.02	70.75	60.59	65.76	72.59	71.94	74.99	69.45	72.26
7	66.49	67.62	69.17	64.92	67.06	71.94	72.53	73.78	70.67	69.06
Mean	65.67	65.33	71.79	58.92	65.50	70.65	69.86	74.71	65.60	70.26

Table 27. Mean motility percentage of buck semen at 72 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	53.04	48.03	66.80	34.21	50.54	56.49	57.21	71.31	41.77	56.85
1	52.55	47.03	65.11	34.49	49.79	63.43	64.01	72.53	54.41	63.72
3	53.79	49.76	62.94	40.52	51.77	65.17	66.36	70.67	60.69	65.77
7	56.96	52.22	59.76	49.38	54.59	66.98	66.31	70.03	63.18	66.65
Mean	54.09	49.26	63.67	39.58	51.68	63.06	63.51	71.14	55.05	63.28

Table 28. Mean motility percentage of buck semen at 96 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	38.92	37.55	59.11	19.41	38.24	45.89	47.32	64.42	29.22	46.60
1	39.91	33.93	55.64	19.99	36.89	50.61	51.96	66.27	36.18	51.28
3	41.79	38.63	51.86	29.08	40.21	53.22	53.84	65.02	41.85	53.53
7	44.91	40.70	47.99	37.70	42.81	55.09	54.46	63.16	46.24	54.77
Mean	41.37	37.68	53.66	26.23	39.52	51.20	51.89	64.72	38.27	51.55

Table 29. Mean motility percentage of buck semen at 120 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	25.32	26.09	45.59	10.03	25.71	31.56	32.05	50.00	16.10	31.81
1	24.11	25.12	39.21	12.53	24.62	39.28	38.04	56.28	22.45	38.67
3	28.32	27.24	35.22	20.91	27.78	44.22	41.68	53.77	32.46	42.94
7	28.46	27.75	27.91	28.30	28.10	44.95	41.20	48.74	37.48	43.08
Mean	26.53	26.54	36.86	17.38	26.54	39.93	38.19	52.20	26.70	39.06

Table 30. Mean motility percentage of buck semen at 144 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	17.05	20.24	35.52	6.31	18.62	24.92	24.11	44.35	9.14	24.52
1	18.12	20.13	30.59	9.84	19.12	30.50	29.83	50.00	13.58	30.17
3	17.41	19.77	23.09	14.44	18.57	37.01	34.59	47.48	24.91	35.79
7	18.93	22.38	18.43	22.92	20.63	37.90	34.90	43.09	29.97	36.40
Mean	17.86	20.61	26.65	12.77	19.23	32.47	30.77	46.23	18.66	31.62

Table 31. Mean motility percentage of buck semen at 0, 24, 48, 72, 96, 120 and 144 hours of storage

ANOVA

Source	Glycerolisation at room temperature								Glycerolisation at refrigeration temperature							
		Mean sum of square								Mean sum of square						
	df	0 h	24h	48h	72h	96h	120h	144h	df	0 h	24h	48h	72h	96h	120h	144h
Breeds	1	2.65	4.73	1.35	245.29**	149.47*	0.003	127.88	1	26.89**	28.83**	3.90	1.15	2.51	16.63	17.63
Diluents	1	5.10	634.58**	1937.85**	6227.56**	8500.22**	5197.98**	3289.65**	1	0.00001	78.52**	522.37**	1476.02**	3769.05**	3675.94**	4758.87**
Breed X Diluent	1	2.73	0.19	0.33	44.46	19.61	14.65	0.08	1	0.00001	1.77	3.68	0.09	0.02	9.76	54.08
Levels of glycerol	3	0.25	17.52	16.35	46.99	72.37	37.73	15.39	3	0.00001	3.44	38.84**	109.54**	68.11**	157.55**	193.06**
Breed X levels of glycerol	3	0.12	0.19	5.68	1.00	10.52	3.68	1.78	3	0.00001	0.69	2.43	0.92	1.19	4.50	1.82
Diluent X levels of glycerol	3	0.61	41.34**	145.55**	280.49**	546.71**	897.34**	959.17**	3	0.00001	17.57**	48.49**	138.37**	90.01**	195.87**	249.44**
Breed X diluent X levels of glycerol	3	0.25	0.77	7.53	1.18	17.53	4.08	11.89	3	0.00001	0.74	1.93	0.50	2.54	2.10	0.78
Error	112	4.31	9.65	10.53	18.11	33.73	55.34	53.34	48	2.03000	3.04	4.63	9.48	6.96	7.79	8.50
Total	127								63							

* Significant at 5 per cent level

** Significant at 1 per cent level

Table 32. Total abnormality percentage of buck spermatozoa at zero hour storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	4.51	4.56	4.21	4.87	4.53	2.48	2.33	2.58	2.23	2.40
1	5.34	5.09	5.16	5.27	5.22	3.18	2.94	3.43	2.71	3.06
3	5.66	5.45	5.27	5.85	5.56	3.56	3.32	4.09	2.84	3.44
7	6.76	6.12	6.38	6.49	6.44	3.71	3.79	4.32	3.21	3.75
Mean	5.54	5.29	5.23	5.60	5.42	3.21	3.07	3.57	2.75	3.14

Table 33. Total abnormality percentage of buck spermatozoa at 24 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	5.76	6.03	5.73	6.06	5.89	3.36	3.58	3.84	3.12	3.47
1	6.57	6.25	6.59	6.23	6.41	3.95	4.21	4.58	3.61	4.08
3	6.65	6.49	6.74	6.41	6.57	4.34	4.46	4.99	3.85	4.40
7	7.33	7.66	7.59	7.39	7.49	4.32	4.60	5.11	3.85	4.46
Mean	6.57	6.59	6.65	6.51	6.57	3.98	4.20	4.62	3.60	4.09

Table 34. Total abnormality percentage of buck spermatozoa at 48 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	6.19	5.87	6.12	5.94	6.03	5.84	3.24	3.97	3.12	3.53
1	6.81	6.22	6.63	6.39	6.51	4.46	4.10	4.86	3.76	4.28
3	7.12	6.93	7.29	6.76	7.03	4.73	4.34	5.11	3.98	4.53
7	7.53	7.56	7.69	7.39	7.54	4.72	4.60	5.37	3.99	4.66
Mean	6.90	6.63	6.92	6.61	6.76	4.42	4.05	4.81	3.70	4.23

Table 35. Total abnormality percentage of buck spermatozoa at 72 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	6.35	5.73	5.94	6.14	6.04	3.85	3.87	3.98	3.74	3.86
1	6.49	6.43	6.12	6.81	6.46	4.49	4.36	4.87	3.40	4.42
3	7.73	7.04	7.02	7.75	7.38	4.83	4.60	5.37	4.10	4.71
7	8.10	7.57	7.78	7.89	7.83	5.09	4.83	5.47	4.47	4.96
Mean	7.15	6.67	6.69	7.13	6.91	4.55	4.41	4.90	4.07	4.47

Table 36. Total abnormality percentage of buck spermatozoa at 96 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	6.51	6.15	6.24	6.41	6.33	3.85	3.85	4.24	3.48	3.85
1	7.19	6.48	6.51	6.98	6.74	4.34	4.21	4.86	3.72	4.27
3	7.28	6.95	7.12	7.12	7.12	4.73	4.49	4.91	4.24	4.61
7	8.01	7.72	7.97	7.76	7.86	4.86	5.35	5.62	4.61	5.10
Mean	7.19	6.81	6.95	7.06	7.00	4.43	4.46	4.91	4.00	4.45

Table 37. Total abnormality percentage of buck spermatozoa at 120 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	6.02	6.47	6.59	5.90	6.24	3.85	3.72	4.24	3.36	3.79
1	7.63	7.27	7.53	7.38	7.45	4.58	4.71	5.24	4.09	4.65
3	7.89	7.55	7.96	7.49	7.72	4.71	4.75	5.36	4.21	4.77
7	8.37	7.60	8.78	7.21	7.98	5.35	5.34	5.99	4.74	5.35
Mean	7.45	7.21	7.69	6.98	7.33	4.61	4.63	5.19	4.09	4.62

Table 38. Total abnormality percentage of buck spermatozoa at 144 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	5.76	6.88	7.30	5.38	6.31	4.18	4.34	4.95	3.61	4.26
1	7.19	7.71	8.07	6.86	7.45	4.68	4.86	5.34	4.22	4.77
3	7.61	8.19	8.54	7.27	7.89	4.91	4.96	5.84	4.10	4.93
7	8.32	7.64	7.94	8.01	7.98	5.06	5.59	6.11	4.58	5.32
Mean	7.19	7.59	7.96	6.85	7.39	4.70	4.93	5.55	4.12	4.81

Table 39. Total abnormality percentage of buck spermatozoa at 0, 24, 48, 72, 96, 120 and 144 hours of storage

ANOVA

Source	Glycerolisation at room temperature								Glycerolisation at refrigeration temperature							
		Mean sum of square								Mean sum of square						
	df	0 h	24h	48h	72h	96h	120h	144h	df	0 h	24h	48h	72h	96h	120h	144h
Breeds	1	3.15	0.03	3.04	9.21	5.97	2.18	6.28	1	0.81	1.61	4.56**	0.64	0.02	0.02	1.49
Diluents	1	7.34	0.78	3.98	7.66	0.54	19.76	46.97	1	29.99**	34.47**	39.88**	21.18**	25.65**	36.49**	58.68**
Breeds X diluents	1	3.85	0.49	4.42	5.59	0.52	0.002	38.02	1	1,77	0.16	1.01	0.06	0.03	0.03	1.81
Levels of glycerol	3	31.95**	18.55*	17.72	27.72*	16.95	23.78*	23.53	3	15.03**	7.17**	8.60**	7.02**	8.62**	12.70**	5.66**
Breeds X levels of glycerol	3	0.88	1.07	0.71	0.78	0.13	2.56	5.76	3	0.23	0.05	0.40	0.11	0.74	0.12	0.27
Diluents X levels of glycerol	3	1.27	1.15	0.24	1.02	0.84	3.30	7.51	3	1.29	0.28	0.19	1.26	0.26	0.06	0.42
Breeds X diluents X levels of glycerol	3	1.36	0.09	0.83	0.78	0.23	4.14	2.19	3	0.32	0.29	0.47	0.23	0.28	0.11	0.07
Error	112	6.87	6.52	7.30	9.21	6.59	8.34	13.08	48	1.82	0.99	0.49	0.75	0.64	0.84	1.31
Total	127								63							

* Significant at 5 per cent level

** Significant at 1 per cent level

Table 40. Head abnormality percentage of buck spermatozoa at zero hour storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	0.68	0.47	0.54	0.61	0.57	0.39	0.32	0.39	0.32	0.35
1	0.74	0.65	0.85	0.55	0.69	0.39	0.56	0.56	0.39	0.47
3	0.60	0.69	0.65	0.64	0.64	0.47	0.39	0.47	0.39	0.43
7	1.06	0.69	0.95	0.78	0.86	0.39	0.32	0.32	0.39	0.35
Mean	0.76	0.62	0.74	0.65	0.69	0.41	0.39	0.43	0.37	0.40

Table 41. Head abnormality percentage of buck spermatozoa at 24 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	0.55	0.59	0.63	0.52	0.57	0.32	0.25	0.32	0.25	0.28
1	0.70	0.60	0.80	0.52	0.65	0.39	0.25	0.39	0.25	0.32
3	0.59	0.62	0.75	0.47	0.60	0.39	0.39	0.47	0.32	0.39
7	0.75	0.67	0.77	0.64	0.71	0.39	0.66	0.56	0.47	0.52
Mean	0.64	0.62	0.74	0.53	0.63	0.37	0.37	0.43	0.32	0.37

Table 42. Head abnormality percentage of buck spermatozoa at 48 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	0.59	0.50	0.59	0.50	0.54	0.39	0.47	0.56	0.32	0.43
1	0.67	0.53	0.71	0.49	0.60	0.56	0.47	0.47	0.56	0.52
3	0.94	0.60	0.92	0.63	0.76	0.47	0.47	0.47	0.47	0.47
7	0.59	0.47	0.63	0.42	0.52	0.47	0.47	0.47	0.47	0.47
Mean	0.69	0.52	0.71	0.51	0.60	0.47	0.47	0.49	0.45	0.47

Table 43. Head abnormality percentage of buck spermatozoa at 72 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	0.39	0.42	0.47	0.35	0.41	0.46	0.39	0.32	0.55	0.42
1	0.55	0.60	0.65	0.51	0.58	0.32	0.39	0.39	0.32	0.35
3	0.65	0.59	0.69	0.55	0.62	0.77	0.25	0.56	0.39	0.47
7	0.70	0.76	1.00	0.51	0.73	0.86	0.75	0.84	0.77	0.80
Mean	0.57	0.59	0.69	0.48	0.57	0.58	0.43	0.51	0.49	0.49

Table 44. Head abnormality percentage of buck spermatozoa at 96 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	0.68	0.51	0.59	0.59	0.59	0.32	0.25	0.32	0.25	0.28
1	0.72	0.47	0.54	0.63	0.59	0.39	0.56	0.66	0.39	0.52
3	0.62	0.76	0.76	0.62	0.69	0.75	0.77	0.66	0.86	0.76
7	0.55	0.89	0.73	0.68	0.71	0.88	0.56	0.77	0.66	0.71
Mean	0.64	0.64	0.65	0.63	0.64	0.56	0.54	0.59	0.51	0.54

Table 45. Head abnormality percentage of buck spermatozoa at 120 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	0.56	0.42	0.65	0.35	0.49	0.32	0.39	0.47	0.25	0.35
1	0.71	0.53	0.61	0.63	0.62	0.47	0.66	0.77	0.39	0.56
3	0.55	0.84	0.84	0.55	0.69	0.56	0.56	0.56	0.56	0.56
7	0.63	0.70	0.76	0.57	0.66	0.98	0.56	0.86	0.66	0.76
Mean	0.61	0.61	0.71	0.52	0.61	0.56	0.54	0.66	0.45	0.54

Table 46. Head abnormality percentage of buck spermatozoa at 144 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	0.53	0.42	0.63	0.35	0.48	0.39	0.25	0.39	0.25	0.32
1	0.69	0.76	0.86	0.60	0.73	0.56	0.66	0.56	0.66	0.61
3	0.83	0.55	0.68	0.69	0.68	0.56	0.77	0.66	0.66	0.66
7	1.10	0.64	0.87	0.78	0.86	0.77	0.96	1.10	0.64	0.86
Mean	0.78	0.59	0.77	0.59	0.67	0.56	0.63	0.66	0.54	0.59

Table 47. Head abnormality percentage of buck spermatozoa at 0, 24, 48, 72, 96, 120 and 144 hours of storage

ANOVA

Source	Glycerolisation at room temperature								Glycerolisation at refrigeration temperature							
		Mean sum of square								Mean sum of square						
	df	0 H	24h	48h	72h	96h	120h	144h	df	0 h	24h	48h	72h	96h	120h	144h
Breeds	1	7.50	0.23	11.90	0.21	0.02	0.003	14.01	1	0.13	0.001	0.001	6.03	0.09	0.09	0.92
Diluents	1	3.26	17.47	17.06	20.50*	0.14	15.87	12.25	1	1.16	4.65	0.52	0.09	1.29	10.08*	3.23
Breeds X diluents	1	1.15	0.16	2.06	0.66	0.62	1.45	0.90	1	3.23	2.07	4.65	1.04	6.03	0.18	6.33
Levels of glycerol	3	5.55	1.49	4.87	8.83	1.67	3.47	10.35	3	1.16	3.62	0.34	9.16**	12.63**	6.78*	12.28**
Breeds X levels of glycerol	3	3.36	0.59	0.98	0.30	7.76	4.76	4.02	3	1.16	2.41	0.34	4.66	3.25	3.36	1.72
Diluents X levels of glycerol	3	2.55	1.01	0.61	2.54	1.05	2.53	2.15	3	0.82	0.17	1.55	2.29	2.04	1.53	2.54
Breeds X diluents X levels of glycerol	3	4.11	1.34	0.79	2.86	1.83	3.03	0.38	3	1.51	1.03	2.92	3.45	1.84	1.38	1.51
Error	112	3.58	4.68	4.89	3.62	4.85	4.83	4.52	48	1.77	1.29	2.15	2.09	1.62	1.98	1.92
Total	127								63							

* Significant at 5 per cent level ** Significant at 1 per cent level

Table 48. Mid piece abnormality percentage of buck spermatozoa at zero hour storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	0.93	1.15	1.26	0.87	1.06	0.75	0.47	0.64	0.56	0.60
1	0.97	1.10	1.08	0.98	1.03	1.06	0.39	0.64	0.73	0.66
3	1.18	1.03	1.01	1.21	1.11	1.08	0.77	0.98	0.80	0.92
7	1.58	1.21	1.40	1.37	1.39	1.40	1.22	1.34	1.34	1.34
Mean	1.16	1.12	1.19	1.10	1.14	1.07	0.68	0.88	0.85	0.86

Table 49. Mid piece abnormality percentage of buck spermatozoa at 24 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	1.22	1.47	1.20	1.49	1.34	0.64	1.22	0.86	0.96	0.91
1	1.37	1.46	1.30	1.54	1.42	1.08	0.75	0.96	0.86	0.91
3	1.43	1.47	1.23	1.64	1.45	1.08	1.26	1.04	1.31	1.17
7	1.18	1.24	1.50	0.95	1.06	0.96	1.20	0.94	1.22	1.07
Mean	1.30	1.41	1.30	1.40	1.35	0.93	1.09	0.95	1.08	1.01

Table 50. Mid piece abnormality percentage of buck spermatozoa at 48 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	1.19	1.36	1.18	1.37	1.27	0.88	0.77	0.66	1.00	0.82
1	1.53	1.59	1.26	1.89	1.56	1.28	0.86	1.02	1.11	1.06
3	1.33	1.48	1.30	1.52	1.40	1.43	1.05	1.03	1.59	1.20
7	1.72	1.74	1.46	2.01	1.73	1.11	1.17	1.17	1.11	1.14
Mean	1.44	1.54	1.30	1.69	1.48	1.17	0.94	0.92	1.19	1.04

Table 51. Mid piece abnormality percentage of buck spermatozoa at 72 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	B2	Mean
0	1.81	1.69	1.35	2.20	1.75	1.17	0.86	0.94	1.08	1.01
1	1.78	1.51	1.38	1.94	1.65	1.40	1.40	1.56	1.26	1.40
3	1.89	1.48	1.39	1.99	1.68	1.46	1.59	1.59	1.46	1.52
7	1.76	1.59	1.40	1.97	1.68	0.94	1.06	0.92	1.08	1.00
Mean	1.81	1.57	1.38	2.02	1.68	1.23	1.21	1.23	1.22	1.22

Table 52. Mid piece abnormality percentage of buck spermatozoa at 96 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	1.60	1.51	1.72	1.40	1.55	0.75	1.22	1.08	0.86	0.97
1	1.42	1.77	1.47	1.71	1.59	0.94	1.22	1.31	0.86	1.07
3	1.31	1.54	1.51	1.34	1.42	1.08	1.31	1.31	1.08	1.20
7	1.68	1.64	1.66	1.67	1.66	0.84	1.43	1.20	1.04	1.12
Mean	1.50	1.61	1.59	1.52	1.55	0.90	1.29	1.22	0.96	1.09

Table 53. Mid piece abnormality percentage of buck spermatozoa at 120 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	1.53	1.97	1.76	1.73	1.74	1.19	1.29	1.40	1.08	1.24
1	1.51	1.92	1.66	1.76	1.71	1.72	1.20	1.46	1.43	1.24
3	1.62	1.76	1.44	1.95	1.69	1.34	1.34	1.59	1.11	1.34
7	1.79	1.79	1.96	1.63	1.79	1.31	1.49	1.36	1.43	1.40
Mean	1.61	1.86	1.70	1.76	1.73	1.38	1.32	1.45	1.26	1.35

Table 54. Mid piece abnormality percentage of buck spermatozoa at 144 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	1.23	1.86	1.56	1.50	1.53	1.43	1.34	1.56	1.22	1.38
1	1.61	2.14	2.01	1.73	1.86	1.51	1.72	1.92	1.34	1.61
3	1.78	2.01	2.01	1.78	1.89	1.86	1.59	1.86	1.59	1.72
7	1.76	1.98	1.84	1.93	1.87	1.59	1.56	1.82	1.34	1.57
Mean	1.59	1.99	1.85	1.72	1.78	1.59	1.55	1.79	1.37	1.56

Table 55. Mid piece abnormality percentage of buck spermatozoa at 0, 24, 48, 72, 96, 120 and 144 hours of storage

ANOVA

Source	Glycerolisation at room temperature								Glycerolisation at refrigeration temperature							
	Mean sum of square								Mean sum of square							
	df	0 h	24h	48h	72h	96h	120h	144h	df	0 h	24h	48h	72h	96h	120h	144h
Breeds	1	0.35	2.39	1.92	9.27	2.29	9.44	25.39*	1	24.08**	3.41	6.62	0.05	19.16*	0.34	0.18
Diluents	1	1.79	2.02	26.69*	65.67**	0.68	0.63	2.37	1	0.13	2.27	9.44	0.01	8.55	3.66	15.02**
Breeds X diluents	1	0.10	0.83	10.79	1.87	5.98	0.19	0.47	1	3.23	10.00	0.61	1.46	12.40	4.71	0.03
Levels of glycerol	3	5.88	2.33	6.86	0.28	1.74	0.33	4.77	3	15.16**	2.18	3.62	7.82	1.10	0.78	1.71
Breeds X levels of glycerol	3	3.41	0.45	0.24	0.66	1.97	1.76	1.98	3	2.32	5.14	1.79	1.33	0.92	2.31	0.78
Diluents X levels of glycerol	3	3.71	10.66	1.86	0.70	2.47	4.71	0.89	3	0.37	0.99	3.41	1.36	0.52	1.65	0.41
Breeds X diluents X levels of glycerol	3	1.25	4.41	1.18	5.85	1.33	1.68	0.39	3	5.88	0.62	1.15	3.54	0.95	3.02	4.67
Error	112	3.12	4.11	4.92	4.81	4.16	3.56	4.64	48	2.43	2.91	2.61	4.18	3.28	3.30	1.76
Total	127								63							

* Significant at 5 per cent level

** Significant at 1 per cent level

Table 56. Tail abnormality percentage of buck spermatozoa at zero hour storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	2.48	2.98	2.39	3.08	2.72	1.46	1.82	1.69	1.59	1.64
1	3.29	3.33	2.84	3.80	3.31	1.79	2.23	2.35	1.69	2.00
3	3.79	3.71	3.58	3.92	3.75	2.03	2.30	2.69	1.69	2.16
7	3.98	4.13	3.90	4.20	4.05	1.90	2.42	2.84	1.69	2.15
Mean	3.36	3.52	3.15	3.74	3.43	1.79	2.19	2.37	1.86	1.98

Table 57. Tail abnormality percentage of buck spermatozoa at 24 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	4.01	4.08	4.14	4.16	4.04	2.54	2.33	2.82	2.08	2.44
1	4.61	4.01	4.39	4.22	4.31	2.79	3.42	3.26	2.94	3.09
3	4.53	4.42	4.47	4.33	4.48	2.94	2.78	3.46	2.32	2.86
7	5.12	5.31	5.43	4.99	5.21	2.94	2.64	3.51	2.14	2.79
Mean	4.56	4.44	4.63	4.37	4.50	2.80	2.78	3.26	2.36	2.79

Table 58. Tail abnormality percentage of buck spermatozoa at 48 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	4.38	4.02	4.31	4.02	4.20	2.69	2.08	2.78	2.00	2.38
1	4.58	4.07	4.58	4.07	4.32	2.53	2.84	3.36	2.08	2.68
3	4.65	4.77	4.89	4.53	4.71	2.77	2.90	3.81	2.00	2.84
7	5.21	5.28	5.51	4.99	5.24	3.15	2.92	3.65	2.48	3.03
Mean	4.70	4.52	4.81	4.41	4.61	2.78	2.68	3.39	2.13	2.73

Table 59. Tail abnormality percentage of buck spermatozoa at 72 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	4.14	3.55	4.04	3.64	3.84	2.14	2.71	2.84	2.03	2.42
1	4.02	4.21	4.14	4.16	4.11	2.78	2.58	2.96	2.42	2.68
3	5.03	4.87	4.86	5.03	4.95	2.60	2.96	3.24	2.35	2.78
7	5.47	5.05	5.15	5.36	5.26	3.26	2.93	3.65	2.58	3.09
Mean	4.64	4.40	4.52	4.52	4.52	2.68	2.79	3.16	2.34	2.74

Table 60. Tail abnormality percentage of buck spermatozoa at 96 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	4.09	4.18	3.82	4.46	4.14	2.96	2.54	2.96	2.54	2.75
1	4.77	4.28	4.49	4.56	4.52	3.10	2.35	2.84	2.58	2.71
3	5.33	4.53	4.73	5.12	4.92	2.82	2.28	2.96	2.16	2.55
7	5.64	5.04	5.43	5.24	5.34	3.04	3.32	3.56	2.82	3.18
Mean	4.94	4.51	4.60	4.84	4.72	2.98	2.61	3.07	2.52	2.79

Table 61. Tail abnormality percentage of buck spermatozoa at 120 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	3.88	4.18	4.08	3.98	4.03	2.48	1.60	1.85	2.20	2.02
1	5.32	4.78	5.15	4.94	5.05	2.46	2.75	2.96	2.27	2.60
3	5.63	4.87	5.54	4.96	5.25	2.81	2.98	3.20	2.60	2.89
7	5.89	5.72	5.93	5.69	5.81	2.90	3.18	3.57	2.55	3.04
Mean	5.15	4.88	5.15	4.87	5.01	2.66	2.59	2.85	2.40	2.62

Table 62. Tail abnormality percentage of buck spermatozoa at 144 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	4.18	4.79	5.32	3.72	4.49	2.48	2.94	3.08	2.35	2.71
1	4.91	5.02	5.23	4.71	4.97	2.58	2.43	2.79	2.23	2.50
3	4.91	5.98	5.88	5.01	5.43	2.49	2.54	3.32	1.83	2.52
7	5.32	5.32	5.43	5.22	5.32	2.71	2.98	3.10	2.60	2.84
Mean	4.82	5.27	5.46	4.65	5.04	2.56	2.72	3.07	2.24	2.64

Table 63. Tail abnormality percentage of buck spermatozoa at 0, 24, 48, 72, 96, 120 and 144 hours of storage

ANOVA

Source	Glycerolisation at room temperature								Glycerolisation at refrigeration temperature							
		Mean sum of square								Mean sum of square						
	df	0 h	24h	48h	72h	96h	120h	144h	df	0 h	24h	48h	72h	96h	120h	144h
Breeds	1	2.15	0.81	1.92	3.72	11.15	4.23	11.17	1	10.74*	0.02	0.52	0.58	6.58	0.24	1.19
Diluents	1	27.14*	4.31	9.71	0.002	3.26	4.29	36.37	1	37.34**	39.28**	77.91**	33.39**	14.69*	10.56	34.88**
Breeds X diluents	1	11.39	0.25	1.94	4.13	0.005	0.52	30.89	1	1.19	0.02	5.02	0.10	0.87	4.65	0.08
Levels of glycerol	3	27.09**	14.92	12.97	27.45*	15.56*	31.41**	10.19	3	4.29	3.67	3.86	3.79	3.44	11.09*	1.33
Breeds X levels of glycerol	3	1.47	1.91	1.49	1.93	2.08	3.07	3.19	3	0.18	2.10	2.27	2.41	2.39	4.88	0.92
Diluents X levels of glycerol	3	2.31	0.17	0.26	1.33	2.03	0.56	5.57	3	3.92	2.54	1.94	0.47	0.80	4.48	2.97
Breeds X diluents X levels of glycerol	3	2.38	0.40	1.85	3.12	0.49	0.91	3.10	3	5.58*	0.65	1.56	0.55	0.47	0.27	2.00
Error	112	6.24	5.99	5.25	9.32	5.11	5.93	9.44	48	1.71	3.25	1.65	2.29	2.42	3.47	1.66
Total	127								63							

* Significant at 5 per cent level

** Significant at 1 per cent level

Table 64. Percentage of acrosome defect of buck spermatozoa at zero hour storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	0.43	0.55	0.47	0.51	0.49	0.25	0.25	0.25	0.25	0.25
1	0.43	0.64	0.55	0.51	0.53	0.39	0.25	0.32	0.32	0.32
3	0.64	0.87	0.56	0.99	0.77	0.39	0.25	0.32	0.32	0.32
7	1.02	0.87	0.86	1.03	0.94	0.39	0.25	0.32	0.32	0.32
Mean	0.61	0.73	0.60	0.74	0.87	0.35	0.25	0.29	0.29	0.29

Table 65. Percentage of acrosome defect of buck spermatozoa at 24 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	1.90	1.88	1.73	2.05	1.89	0.66	0.66	0.77	0.56	0.66
1	2.47	2.53	2.69	2.32	2.49	1.19	0.97	1.06	1.12	1.08
3	3.35	3.21	3.75	2.83	3.28	1.66	1.46	1.93	1.22	1.56
7	3.52	3.58	4.28	2.89	3.55	2.33	1.93	2.42	1.86	2.13
Mean	2.77	2.76	3.03	2.51	2.76	1.39	1.21	1.47	1.14	1.30

Table 66. Percentage of acrosome defect of buck spermatozoa at 48 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	2.97	2.95	3.17	2.76	2.96	2.21	2.11	2.76	1.63	2.16
1	3.48	3.39	3.49	3.38	3.44	2.73	2.78	3.45	2.14	2.76
3	3.96	3.79	4.21	3.56	3.87	3.14	2.92	3.54	2.56	3.03
7	4.41	4.21	4.69	3.94	4.31	3.29	3.26	4.05	2.58	3.28
Mean	3.69	3.57	3.87	3.39	3.62	2.83	2.75	3.43	2.21	2.79

Table 67. Percentage of acrosome defect of buck spermatozoa at 72 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	3.68	3.48	3.68	3.47	3.58	3.84	2.71	3.43	3.07	3.25
1	4.51	4.46	4.45	4.52	4.48	3.95	3.71	4.10	3.57	3.83
3	4.89	4.69	5.15	4.45	4.79	4.33	4.09	4.58	3.85	4.21
7	5.29	4.94	5.25	4.98	5.11	4.57	4.35	4.98	3.97	4.46
Mean	4.57	4.38	4.61	4.34	4.47	4.17	3.69	4.25	3.61	3.92

Table 68. Percentage of acrosome defect of buck spermatozoa at 96 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	4.32	3.95	3.99	4.27	4.13	3.85	3.45	3.34	3.97	3.65
1	5.05	4.87	4.82	5.12	4.96	4.49	4.47	4.24	4.73	4.48
3	5.74	5.53	5.62	5.65	5.63	5.12	5.11	5.24	4.99	5.11
7	6.03	6.29	6.17	6.17	6.17	5.23	5.36	5.36	5.23	5.20
Mean	5.27	5.13	5.12	5.28	5.19	4.66	4.57	4.51	4.72	4.61

Table 69. Percentage of acrosome defect of buck spermatozoa at 120 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	4.55	5.15	4.71	4.99	4.85	4.24	4.15	4.05	4.33	4.19
1	5.83	5.97	5.89	5.90	5.91	4.49	4.71	4.22	4.99	4.59
3	6.58	6.91	6.85	6.63	6.74	5.23	4.96	4.84	5.35	5.09
7	7.49	6.91	7.45	6.95	7.20	5.71	5.35	5.59	5.47	5.07
Mean	6.07	6.22	6.19	6.09	6.14	4.89	4.78	4.66	5.03	4.84

Table 70. Percentage of acrosome defect of buck spermatozoa at 144 hours storage

Glycerol level (%)	Glycerolisation at room temperature					Glycerolisation at refrigeration temperature				
	B1	B2	D1	D2	Mean	B1	B2	D1	D2	Mean
0	5.22	6.45	6.19	5.45	5.82	6.04	5.31	5.64	5.70	5.67
1	6.86	7.50	7.72	6.66	7.18	6.23	5.72	6.08	5.86	5.97
3	7.36	8.08	8.14	7.29	7.71	6.74	5.85	6.59	5.98	6.29
7	8.15	7.25	8.03	7.36	7.69	6.85	6.61	7.24	6.24	6.73
Mean	6.86	7.31	7.50	6.67	7.08	6.46	5.86	6.38	5.95	6.15

Table 71. Percentage of acrosome defect of buck spermatozoa at 0, 24, 48, 72, 96, 120 and 144 hours of storage

ANOVA

Source	Glycerolisation at room temperature								Glycerolisation at refrigeration temperature							
		Mean sum of square								Mean sum of square						
	df	0 h	24h	48h	72h	96h	120h	144h	df	0 h	24h	48h	72h	96h	120h	144h
Breeds	1	5.89	0.003	1.02	2.33	1.03	1.02	8.24	1	4.65*	3.43	0.26	8.09*	0.23	0.39	8.12*
Diluents	1	7.63	26.33**	16.89*	4.48	1.34	0.38	27.65	1	0.0001	11.36*	72.41**	14.54**	1.00	3.84	4.23
Breeds X diluents	1	1.99	2.33	0.09	6.52	0.81	3.21	77.21**	1	0.001	0.43	10.63	2.14	0.09	3.84	1.29
Levels of glycerol	3	17.29*	57.83**	25.38**	28.00**	42.01**	50.31**	33.27*	3	0.520	40.85**	11.67	9.89**	17.28**	9.66**	4.64*
Breeds X levels of glycerol	3	2.95	0.20	0.09	0.20	1.02	2.99	8.82	3	0.520	0.46	0.16	2.18	0.48	0.45	0.45
Diluents X levels of glycerol	3	3.83	11.81**	1.26	1.46	0.38	1.23	0.28	3	0.001	2.24	0.44	0.49	1.61	1.02	1.12
Breeds X diluents X levels of glycerol	3	0.31	2.78	1.08	0.19	1.31	2.28	1.99	3	0.001	0.19	9.34	0.42	0.32	0.09	1.05
Error	112	4.73	2.50	3.71	4.03	3.01	3.89	10.67	48	0.780	2.40	3.69	1.46	0.85	1.51	1.45
Total	127								63							

* Significant at 5 per cent level ** Significant at 1 per cent level

Table 72. Percentage of post-thawing motility of buck semon

Diluents	Malabari	Alpine X Malabari	Mean
Tris diluent	41.649	47.236	44.443
Skim milk ,,	30.524	31.613	31.068
Mean	36.087	39.424	37.755

ANOVA

Source	df	MSS	F
Breeds	1	33.417	3.878
Diluents	1	536.602	62.270**
Breeds X Diluents	1	15.178	1.761
Error	8	8.617	
Total	11		

** Significant at 1 per cent level

Fig. 1. Acrosome defects of buck spermatozoa stored in Tris diluent

Fig. 2. Acrosome defects of buck spermatozoa stored in reconstituted skim milk diluent

DISCUSSION

DISCUSSION

Normal characteristics of buck semen

Two hundred and forty ejaculates, 20 first ejaculates and 20 second ejaculates, each from three Malabari and three Alpine X Malabari bucks collected have been used to study the normal characteristics of semen.

In the present study, the overall mean reaction time is found to be 98.86 ± 4.309 seconds. For Malabari and Alpine X Malabari bucks the reaction time is found to be 94.61 ± 5.811 and 103.11 ± 6.326 seconds respectively. These values are within the range of reaction time reported by Sharma *et al.* (1957). According to Kurian and Raja (1965), Krishnan (1967) and Patil (1970) the average reaction time for Malabari bucks was 35 seconds, 40.60 seconds and 49.37 seconds, respectively. However, present observation on reaction time is higher than the reported values for the same breed. In the present study, the reaction time for first ejaculate is 62.41 ± 6.480 seconds and for second ejaculate 126.81 ± 8.942 seconds in Malabari bucks. The reaction time in Alpine X Malabari for first and second ejaculates are found to be 59.38 ± 4.441 and 146.85 ± 8.768 seconds respectively (Table 7). There is significant difference between ejaculate within buck in both the genetic groups (Table 23). This is in accordance to the observation

made by Mann (1980). Significant difference in reaction time between breeds was reported by Mohan et al. (1980) and Sinha and Singh (1982). However, in the present study, difference between breed in reaction time could not be observed (Table 23). A significant positive correlation is observed between reaction time and mass activity in second ejaculates. This shows that time lapse between ejaculate increases the mass motility of second ejaculate.

The mean volume of semen is found to be 0.64 ± 0.015 ml in the present observation. For Malabari and Alpine X Malabari bucks, the volume is 0.55 ± 0.017 and 0.72 ± 0.015 ml respectively (Table 8). These values compare favourably with the earlier reports of Sharma et al. (1957); Kurian and Raja (1965); Koh (1975); Mohan et al. (1980); Sinha and Singh (1982). However, higher average values for semen volume have been reported by Igboeli (1974), Singh et al. (1982) and Vinha (1982). In the present study, the mean volume is observed to vary between breeds ($P < 0.05$). Similar reports are made by Eaton and Simmons (1952) and Vinha (1982). There is significant difference ($P < 0.01$) between ejaculates within buck also. This is in accordance with the observation made by Mohan et al. (1980); and Saxena and Tripathi (1980). The first ejaculate volume is

found to have significant positive correlation ($P < 0.05$) with density and negative correlation ($P < 0.05$) with motility percentage (Table 21).

In the present study, colour of buck semen varies from milky yellow to creamy white. This observation compares favourably with the reports of earlier workers (Patil, 1970; Igboeli, 1974; Sinha and Singh, 1982; Singh et al., 1982).

The overall density score of buck semen is found to be 3.52 ± 0.030 out of four (Table 9). The density score varies significantly ($P < 0.01$) between bucks within breed (Table 23), which shows significant positive correlation ($P < 0.01$) with sperm concentration, both in first and second ejaculates (Table 21 and 22). This shows that the density score is a good indicator for sperm concentration in buck semen.

The present study shows that the overall mean pH of buck semen is 6.74 ± 0.016. Mean values for pH of Malabari and Alpine X Malabari buck semen are found as 6.74 ± 0.026 and 6.74 ± 0.019 respectively (Table 10). These values are well within the range of values reported by Patil (1970), Mohan et al. (1980) and Sinha and Singh (1982). Semen pH is found to vary significantly due to bucks within breed. This observation is in agreement with earlier report of Mohan et al. (1980).

The overall mass activity score in the present investigation, is 3.66 ± 0.034 out of four. For Malabari and Alpine X Malabari, the mean mass activity is found as 3.52 ± 0.055 and 3.80 ± 0.035 respectively (Table 11). These values are within the range of mass activity reported by Patil (1970) and Saxena and Tripathi (1980). However, higher values have been reported by Mohan et al. (1980) and Sinha and Singh (1982). In the present study, mass activity is found to vary significantly ($P < 0.01$) between breeds and between bucks within breed. However, no significant difference is observed between ejaculates, eventhough Mohan et al. (1980) observed significant difference between ejaculates. Mass activity is found to be positively correlated ($P < 0.05$) with sperm concentration, both in first and second ejaculates (Table 21 and 22). This denotes that, mass activity score can be considered as a direct indication of the sperm concentration.

The overall mean initial motility percentage in buck semen is observed to be 80.64 ± 0.334, which is within the range reported by Kurian and Raja (1965), Patil (1970), Mann (1980), Saxena and Tripathi (1980), Singh et al. (1982) and Vinha (1982). In Malabari bucks, motility percentage is observed to be 79.18 ± 0.553 which is in close relation to the values reported by Kurian and Raja (1965) and Patil (1970).

The difference in motility percentage varies significantly ($P < 0.05$) between breeds (Table 23). This is in accordance with the findings of earlier workers (Singh et al., 1982; Vinha, 1982). Though Mohan et al. (1980) reported significant difference in initial sperm motility of Pashmina bucks between collections, in the present study, there is no significant difference between ejaculates in Malabari and Alpine X Malabari bucks. In the present observation, initial motility is found to have significant positive correlation with the live sperm percentage, both in first and second ejaculates (Table 21 and 22). This clearly indicates that the percentage of initial motility can be considered as an important parameter to denote the live sperm count in buck semen.

The overall mean live sperm percentage of buck semen is found as 90.64 ± 0.317. In Malabari and Alpine X Malabari, the mean live spterm percentages are 89.97 ± 0.584 and 91.31 ± 0.356 respectively (Table 13). These values are within the range reported by Kurian and Raja (1965), Patil (1970), Igboeli (1974), Sinha and Singh (1982). In the present study undertaken, percentage of live sperm is found to have significant positive correlation with motility percentage both in first ejaculates ($P < 0.05$) and second

ejaculates ($P < 0.01$). Similarly, Patil (1970) also reported the significant correlation ($P < 0.05$) between percentages of live sperms and motile sperms.

The overall sperm concentration ($\times 10^9$) is observed to be 3.39 ± 0.061 per ml. In Malabari and Alpine X Malabari bucks, the mean sperm concentration ($\times 10^9$) is found to be 3.49 ± 0.098 and 3.28 ± 0.070 respectively (Table 14). In Malabari bucks, Kurian and Raja (1965) reported sperm concentration ($\times 10^9$) as 2 to 3 whereas Patil (1970) reported 1.12 to 7.49 billions with a mean of 3.534 ± 1.761 billions. There is significant difference in sperm concentration between breeds ($P < 0.05$) and between bucks within breed ($P < 0.01$) in the present observation (Table 23). Eaton and Simmons (1952), Sinha and Singh (1982) and Vinha (1982) have also reported significant effect of breed on sperm concentration. Mohan et al. (1980) have reported significant difference in sperm concentration between consecutive collections. However, in the present study, no significant difference could be seen between ejaculates; although the sperm concentration is less in the second ejaculates of both the breeds studied.

The average total sperm abnormalities of buck semen is observed to be 1.40 ± 0.265 per cent. In Malabari and

Alpine X Malabari bucks the total sperm abnormality percentages are 1.65 ± 0.188 and 1.14 ± 0.093 respectively (Table 20). Patil (1970) reported mean total abnormality of 4.34 ± 0.48 per cent in the range of 1.00 to 18.62 per cent in Malabari bucks. In the present study, the total sperm abnormality percentage varies significantly between breeds ($P < 0.05$) and between bucks within breed ($P < 0.01$). These observations are in accordance to the findings of Vinha (1982), Sinha and Singh (1982). In the study undertaken presently, there is no significant difference for total sperm abnormality percentage in first and second ejaculate. This observation is in par with the report of Arbeiter (1964).

The overall average percentages of abnormalities of head, midpiece and tail are found to be 0.03 ± 0.012, 0.129 ± 0.023 and 0.70 ± 0.229 respectively (Table 15, 16 and 17). The percentage of sperms with attachment of protoplasmic droplet is found to be 0.30 ± 0.069. The mean detached head abnormality is found to be 0.24 ± 0.034 per cent. Bordoloi and Sharma (1983) reported 3.948 per cent head abnormality in Assam local goats and 3.299 per cent in Beetal bucks and 3.558 in Saanen bucks. According to them, percentage of midpiece abnormality is 1.16 in Assam

local, 1.87 in Beetal bucks and 1.71 in Saanen bucks. Tail abnormality percentages have been 3.54, 3.75 and 3.24 in Assam local, Beetal and Saanen bucks respectively. In the present study, percentages of abnormality observed in different parts of the spermatozoa are lower than the values reported by Sinha and Singh (1982), Vinha (1982) and Bordoloi and Sharma (1983).

Preservation of buck semen in glycerol containing diluents

The overall mean percentage of initial (zero hour of storage) motility at room temperature glycerolisation is observed to be 79.63, which decreases to 19.23 per cent at 144 hours of storage. Similarly the mean initial motility percentage of 80.41 at refrigeration temperature glycerolisation declines to 31.62 per cent at 144 hours of storage (Table 24-30). During the entire period of storage, the motility percentage of buck spermatozoa at refrigeration temperature glycerolisation is found to be significantly superior to that at room temperature glycerolisation. The present observation is in agreement with the report of Almquist and Wickersham (1962), who also observed that step wise addition of glycerol at 5°C maintained superior motility in comparison to the motility obtained by direct

addition of semen to the completely formulated diluent at room temperature. However, Albright et al. (1960) reported no significant difference in sperm motility at seven days storage at 5°C, when glycerol was added step wise at 5°C or added at room temperature (32°C).

At room temperature glycerolisation, mean motility percentage in Tris diluent is found to be 79.91 at zero hour and 26.65 at 144 hours of storage; and in skim milk diluent initial motility is noted to be 79.35 per cent and at 144 hours 12.77 per cent. At refrigeration temperature glycerolisation, mean motility percentage is found to be 80.41 at zero hour and 46.23 at 144 hours in Tris, while in skim milk diluent, mean motility percentage of 80.41 at zero hour is found to decline gradually to 18.66 per cent at 144 hours of storage. In the present study highly significant difference ($P < 0.01$) is noted in the motility percentage between diluents from 24 hours to 144 hours irrespective of glycerolisation method (Table 31). This clearly shows, that Tris diluent is significantly superior to skim milk diluent in the preservation of buck semen both at room temperature and at refrigeration temperature glycerolisation. The present observation is in accordance to the findings of Balakrishnan (1979) and Deka and Rao (1980).

The mean motility at room temperature glycerolisation for Malabari and Alpine X Malabari buck semen are found to be 79.43 and 79.83 per cent respectively at zero hour. The motility gradually reduces to 17.86 and 20.61 per cent at 144 hours of storage. Similarly at refrigeration temperature glycerolisation, the average percentages are found to be 81.30 and 79.50 at zero hour and 32.47 and 30.77 at 144 hours for Malabari and Alpine X Malabari breeds respectively. On analysis, there is significant difference between breeds in motility percentage at zero and 24 hours of storage at refrigeration temperature glycerolisation; whereas, at room temperature glycerolisation the difference is found to be significant at 72 and 96 hours of storage.

At room temperature glycerolisation, the percentage of motility at zero level of glycerol (control group) decreases from 79.98 to 35.52 in Tris diluent and 79.27 to 6.31 per cent in skim milk diluent. Similarly, at refrigeration temperature glycerolisation in the control group the motility percentage is found to decline from 80.41 to 44.35 in Tris diluent and 80.41 to 9.14 per cent in skim milk diluent (Table 24-30). In both the types of glycerolisation, Tris diluent in control group is found to be superior to skim milk diluent. The difference observed in motility percentage

between the two control groups may be due to step wise addition of the diluent to the partially diluted semen at refrigeration temperature glycerolisation. It is further observed that skim milk diluent with zero per cent glycerol can maintain above 50 per cent motility of buck spermatozoa up to 48 hours of storage (Table 26); whereas, Tris diluent can maintain the same motility percentage up to 96 hours of storage (Table 28). The declins in motility due to preservation in Tris diluent observed in this study is comparatively less than that reported by Balakrishnan (1979). However, it is in agreement with the report of Mathew et al. (1982), who obtained an average of 58 per cent motility for seven days of storage. However, the present observation of motility percentage in skim milk diluent without glycerol is in agreement with the report of Ron and Aamdal (1963).

The percentage of motility is found to vary significantly due to different levels of glycerol at refrigeration temperature glycerolisation during storage from 48 hours to 144 hours (Table 31). But, level of glycerol does not show any significant influence on the percentage of motility at room temperature glycerolisation. The effect of interaction between diluents and levels of glycerol is found to have highly significant effect on the percentage of motility

at 24 to 144 hours of storage at room temperature and refrigeration temperature glycerolisation (Table 31).

With one per cent glycerol, percentage of motility at zero hour is found to be 79.98 and 78.97 in Tris and skim milk diluent respectively, while at 144 hours of storage only 30.59 and 9.84 per cent could be observed at room temperature glycerolisation. Whereas, at refrigeration temperature glycerolisation in Tris and skim milk diluents, the percentage of initial motility is found to be 80.41 and the final motility at 144 hours of storage is 50.00 and 13.58. From the present study, it is observed that the buck spermatozoa can be preserved up to 96 hours with more than 50 per cent motility in Tris diluent containing one per cent glycerol in room temperature glycerolisation and up to 120 hours at refrigeration temperature glycerolisation (Table 28 and 29). Similarly, at room temperature and refrigeration temperature glycerolisation in skim milk containing one per cent glycerol, the sperm can be preserved successfully (with above 50 per cent motility) up to 48 hours and 72 hours of storage respectively (Table 26 and 27).

At three per cent glycerol, the motility percentages are 79.98 and 79.58 in Tris and skim milk diluents respectively at zero hour, on room temperature glycerolisation,

then the motility reduces to 23.09 and 14.44 per cent at 144 hours of storage. Similarly, at refrigeration temperature glycerolisation in Tris and skim milk diluents containing three per cent glycerol, the initial motility of 80.41 per cent declines to 47.48 and 24.91 per cent respectively (Table 24 - 30). The present study shows that buck spermatozoa can be preserved successfully in Tris diluent with three per cent glycerol up to 96 hours and 120 hours of storage at room temperature and refrigeration temperature glycerolisation respectively (Table 28 and 29). In the same way, skim milk diluent with three per cent glycerol can preserve above 50 per cent motility up to 48 hours at room temperature glycerolisation and 72 hours at refrigeration temperature glycerolisation (Table 26 and 27).

With seven per cent glycerol in Tris and skim milk, the motility percentages at zero hour are found to be 79.68 and 79.58, which reduce to 18.43 and 22.92 per cent at 144 hours of storage at room temperature glycerolisation. At refrigeration temperature glycerolisation in Tris and skim milk diluents the initial motility of 80.41 per cent declines to 43.09 and 29.97 per cent respectively at 144 hours of storage (Table 24-30). The present study reveals that above 50 per cent motility can be maintained in Tris diluent

with seven per cent glycerol up to 72 hours and 96 hours of storage at room temperature and refrigeration temperature glycerolisation respectively (Table 27 and 28). Whereas, skim milk diluent can preserve up to 48 hours at room temperature glycerolisation and up to 72 hours at refrigeration temperature glycerolisation (Table 26 and 27).

From the present study, it is observed that through out the period of storage the motility percentage of buck spermatozoa is higher in diluents with seven per cent glycerol, followed by three and one per cent in skim milk diluents at both the types of glycerolisation. Whereas in case of Tris diluent, at room temperature glycerolisation, higher motility percentage is noted with zero per cent glycerol followed by 1, 3 and 7 per cent; but in case of refrigeration temperature glycerolisation higher motility percentage is noted with one per cent glycerol, followed by 3, zero and 7 per cent. The present observation of maximum percentage of motility at 144 hours of storage in Tris diluent with one per cent glycerol is in par with the observation made by Pickett and Merilan (1959). Although, using Tris as a diluent with 6.4 per cent glycerol, Davis et al. (1963), Davis et al. (1963) and Foote (1970) observed above 40 per cent motility of bovine spermatozoa at eight days of

storage, Singh et al. (1982) using the same diluent observed 13.30 ± 0.78 and 5.00 ± 0.63 per cent motility for Jamunapari and Barbari bucks respectively at 96 hours of storage. However, in the present study, Tris with seven per cent glycerol shows a higher percentage of motility than that reported by Singh et al. (1982).

Willett and Ohms (1956) have observed that motility percentage of bovine spermatozoa increases significantly as the glycerol concentration increased in skim milk diluent. Similar increase is observed in the present observation also. The present study in skim milk diluent regarding motility percentage is in accordance with the finding of Albright et al. (1958) who found that, in general, the addition of 7.5 per cent glycerol reduced the decline in motility with increasing age. Almquist and Wickersham (1962) noted that when glycerol is added slowly to the partially diluted semen at 5°C or added in low concentrations, sufficient time is allowed for establishment of osmotic equilibrium before sperm motility is affected. In par with the observation made, in the present study also; 1, 3 and 7 per cent glycerol in Tris and skim milk diluent shows comparatively lower motility at room temperature glycerolisation in comparison to the refrigeration temperature glycerolisation with the

same level of glycerol. O'Dell et al. (1959) observed that lactic acid accumulation in skim milk diluent in presence of 10 per cent glycerol was approximately 25 per cent less ($P < 0.01$) than that of skim milk diluent without glycerol.

Nahas (1961) observed that Tris had a good buffering capacity and was relatively non toxic to living cells. Tris in addition to its better buffering capacity, can readily diffuse into sperm cells and serve as an intra cellular buffer (Bartlett and Vandemark, 1961). The presence of fructose in this extender might serve as an additive nutrient besides maintaining osmotic pressure and thus have a beneficial influence on motility percentage. Lecithin fraction of egg yolk (Blackshaw, 1954) prevents the spermatozoa from undergoing cold-shock. All these factors may contribute for the beneficial effects of Tris diluent over skim milk diluent both at room temperature and at refrigeration temperature glycerolisation.

In the present study, at room temperature glycerolisation for both Tris and skim milk diluents, total abnormality percentages are found to be higher than that at refrigeration temperature glycerolisation. This is akin to the reports of Almquist and Wickersham (1962), who noted

that when glycerol is added slowly to the partially diluted semen at 5°C, or in low concentrations, sufficient time is allowed for establishment of osmotic equilibrium before sperm ultrastructure is affected. Present study reveals, that as the days of storage advance total sperm abnormalities increase in both the diluents, irrespective of glycerolisation methods. This is in agreement with the reports of Chaturvedi et al. (1978) and Rao and Rao (1979), who observed an increase in the incidence of sperm abnormalities during the storage. There is no significant difference between diluents, at room temperature glycerolisation as observed in the present study, Rao and Rao (1979) also could not observe significant difference in sperm abnormalities among the diluents used.

In the present study, it is noted that there is significant difference in percentage of abnormality among the levels of glycerol at zero hour of storage ($P < 0.01$) and at 24 hours, 72 hours and 120 hours of storage ($P < 0.05$) at room temperature glycerolisation (Table 39). However, at refrigeration temperature glycerolisation there is significant difference ($P < 0.01$) among the levels of glycerol at all intervals of storage (Table 39). With both methods of glycerolisation, in Tris and skim milk diluents,

the total abnormality percentages are seen lower in control groups and the abnormalities tend to increase as the levels of glycerol increase. This may be attributable to osmotic and electrolyte changes that could have taken place between the spermatozoa and the diluent as indicated by Rao and Rao (1979).

From the tables 40-46, it can be noted that changes in head abnormality through out the storage period are maintained below 0.22 per cent. The present finding, of head abnormalities is in close agreement with the report of Rao and Rao (1979) who stated that the osmotic and electrolyte changes which take place between the sperm and extender during preservation did not produce any recognizable changes in the shape of head to render them abnormal. Lagerlof (1934), Blom (1950), Rao (1971) have reported that the abnormalities of sperm head arise in the testes, but not outside the genital tract.

The overall mean mid piece abnormality percentages are found to be 1.14 and 0.86 at zero hour of storage at room temperature and refrigeration temperature glycerolisation respectively. These values gradually increase correspondingly to 1.78 and 1.56 per cent at 144 hours of storage (Table 48-54). Although, there is an increase of mid piece

abnormality percentage as the days of storage advance in both the diluents and glycerolisation methods, significant difference in the interaction between diluents and levels of glycerol is not observed through out the period of storage.

The overall mean percentages of tail abnormality at room temperature and refrigeration temperature glycerolisation are found to be 3.43 and 1.98 respectively at zero hour of storage and 5.04 and 2.64 respectively at 144 hours of storage (Table 56-62). On analysis of the data significant difference between breeds at zero hour of storage ($P < 0.05$) at refrigeration temperature glycerolisation, is observed. Significant difference between diluents at zero hour of storage ($P < 0.05$) at room temperature glycerolisation and at zero, 24, 48, 72 and 144 hours of storage ($P < 0.01$) and 96 hours ($P < 0.05$) at refrigeration temperature glycerolisation are also observed (Table 63). However, Chaturvedi et al. (1978) and Rao and Rao (1979) have not reported significant difference in the incidence of tail abnormality percentage between diluents, though a significant increase was recorded during storage. Blom (1950) and Rao and Rao (1979) reported that tail abnormalities of spermatozoa might increase after ejaculation and

during storage. In the present study, tail abnormality percentages are found to be higher at all hours of storage at room temperature glycerolisation in comparison to that at refrigeration temperature glycerolisation. This is in accordance to the report of Almquist and Wickersham (1962). There is significant difference in tail abnormality percentage among the levels of glycerol at zero and 120 hours of storage ($P < 0.01$) and 72 and 96 hours of storage ($P < 0.05$) at room temperature glycerolisation and 120 hours of storage ($P < 0.05$) at refrigeration temperature glycerolisation (Table 63). This may be attributable to the effect of glycerol at room temperature glycerolisation; as the level of glycerol increase abnormality percentage also increase.

From the present study, it is observed that the overall mean percentages of acrosome defect of 0.87 and 0.29 at room temperature and refrigeration temperature glycerolisation respectively, at zero hour of storage, gradually increase to 7.08 and 6.15 per cent in the same order at 144 hours of storage (Table 64-70). Comparison between room temperature and refrigeration temperature glycerolisation on acrosome defect is found to be significant at all hours of storage. At refrigeration temperature glycerolisation, there is significant difference between breeds ($P < 0.05$) at zero, 72 and 144 hours of storage. Though there is significant

difference at both the methods of glycerolisation on acrosome defect, it is not observed at all the intervals of storage, irrespective of glycerolisation methods (Table 71). The present study reveals the increase of acrosomal abnormalities with the advancement of storage period in both the diluents at both the methods of glycerolisation, which supports the report of Chaturvedi et al. (1978), who also noted the increase of acrosomal abnormalities with the advancement of storage period in all the diluents used. However, Watson (1975) noted normal acrosome percentages of 98, 98 and 90 before chilling, after chilling to 5°C and one hour after glycerolisation in ram semen. The lower values of acrosome defect in the present study may be attributable to low susceptibility of buck spermatozoa to cold conditions under preservation in comparison to ram semen. In the present study, there is significant difference in percentages of acrosome defect among the levels of glycerol at almost all the hours of storage irrespective of glycerolisation methods (Table 71). Increase of acrosomal abnormalities with the increase in glycerol level, in the present study support the report of Watson and Martin (1975), who observed that although the presence of glycerol in the diluent improved the survival of ram spermatozoa, increasing

the concentration of glycerol produced significant deterioration. The present observation on acrosome defect of the buck spermatozoa are in agreement with the findings of Groken and Asti (1980), who have reported that the proportion of acrosome deformities increased after glycerolisation in ram semen.

Deep freezing of buck semen

Post-thawing motility of buck semen has been reported to be 50-60 per cent (Bonfert, 1969); 30-45 per cent (Samouilidis and Hahn, 1972); 30-40 per cent (Sahni and Roy, 1972); 40 per cent (Rossouw, 1974); 44.4 per cent (Corteel and Barit, 1975); 40.53 per cent (Hukeri et al., 1977) and 44.09 per cent (Nimkar, 1977); 60 per cent (Harnath et al., 1982). In the present study, the overall average post thawing motility is found to be 37.75 per cent. Post thawing motility percentages in Malabari and Alpine X Malabari are 36.08 and 39.42, respectively. However, the variation between breeds in post-thawing motility percentage is statistically not significant. However, Sahni and Roy (1972) had observed that semen of Jamunapari could withstand deep freezing better than that of Barbari bucks.

The average post-thawing motility of buck semen in Tris

and skim milk diluent is found as 44.44 and 31.06 per cent respectively. The variation, on analysis shows significant difference ($P < 0.01$) between diluents, Tris diluent being superior to skim milk diluent. Milk diluent is reported to be inferior in preserving buck semen by Sahni and Roy (1972). Suitability of Tris containing diluent for freezing has been reported by Hukeri et al. (1977), Nimkar (1977), Fukui (1979), Saxena et al. (1979), Vivanco and Valera (1980), Zamfirescu et al. (1980), Salamon and Ritar (1982) and Harnath et al. (1982). In the present study also, it is observed that Tris diluent in comparison to skim milk diluent is more suited for the freezing of buck semen.

SUMMARY

SUMMARY

Normal characteristics of buck semen, desirable level of glycerol incorporation in Tris and reconstituted skim milk diluent, effect of glycerolisation at room temperature and refrigeration temperature and effect of freezing on buck spermatozoa were subjected to systematic study.

A total of 240 ejaculates, from 3 Malabari and 3 Alpine X Malabari bucks maintained at AICRP on Goats for Milk Production, Kerala Agricultural University, were utilised to study the normal characteristics of buck semen.

To study the effect of glycerolisation in Tris and skim milk diluents on preservability of buck spermatozoa at 5°C, the semen samples from Malabari and Alpine X Malabari bucks with more than 70 per cent initial motility were pooled separately. From the two genetic groups, eight pooled samples each were utilized for room temperature glycerolisation and four each for refrigeration temperature glycerolisation. The pooled samples were diluted 1:10 with Tris and skim milk diluents using split sample technique with each one having 0, 1, 3 and 7 per cent of final glycerol concentration. In the case of refrigeration temperature glycerolisation, semen samples were initially diluted with 50 per cent of the non-glycerolated parts of the diluent and later

glycerolated parts were added step wise at 5°C. After dilution all the samples were stored at 5°C in the refrigerator. One drop of diluted semen was taken from each sample and motility assessment carried out from zero hour to 144 hours, at 24 hour intervals of storage. At the same intervals, smears of diluted semen were also prepared and stained with Giemsa stain and examined for various types of sperm abnormalities.

To study the effect of deep freezing on buck spermatozoa, four ejaculates each from six bucks were utilized. The semen samples were diluted in Tris and skim milk diluents, each having seven per cent glycerol. The post-thawing motility with respect to each sample was assessed.

The overall average reaction time was found to be 98.86 ± 4.309 seconds. There was significant positive correlation between reaction time and mass activity in case of second ejaculates. It clearly showed that time lapse between ejaculates increased the mass motility of buck semen.

The mean semen volume was found to be 0.55 ± 0.017 and 0.72 ± 0.015 ml in Malabari and Alpine X Malabari bucks respectively. Significant difference in semen volume was observed between breeds and between ejaculates within buck.

The colour of buck semen varied from milky yellow to creamy white.

The overall density score of buck semen was 3.52 ± 0.030 out of four. The density score was found to vary significantly between bucks within breed. Significant positive correlation between density score and sperm concentration, both in first and second ejaculates was observed.

Mean values for pH of Malabari and Alpine X Malabari semen were 6.74 ± 0.026 and 6.74 ± 0.019 respectively. Semen pH was found to vary significantly due to bucks within breed.

The overall mass activity score was 3.66 ± 0.034 out of four. The Malabari and Alpine X Malabari bucks the average mass activity score was 3.52 ± 0.055 and 3.80 ± 0.035 respectively. Significant variation in mass activity between breeds and between bucks within breed was noted. Mass activity was positively correlated with sperm concentration, both in first and second ejaculates.

The mean motility percentage of Malabari and Alpine X Malabari buck semen was found to be 79.18 ± 0.553 and 82.10 ± 0.322 respectively. The difference in motility percentage varied significantly between breeds. Initial motility was found to have significant positive correlation with the live

sperm percentage, both in first and second ejaculates. Hence, initial motility can be considered as an indicator of live sperm percentage in buck semen.

The overall mean live sperm percentage was 90.64 ± 0.317 in the present study.

In Malabari and Alpine X Malabari bucks, the mean sperm concentration (X 10^9) was 3.49 ± 0.098 and 3.28 ± 0.070 respectively. Significant difference in sperm concentration was noted between breeds and between bucks within breed. Sperm concentration was slightly higher in the first ejaculate than in the second ejaculate.

The average total sperm abnormalities was 1.40 ± 0.265 per cent. Average total sperm abnormalities of 1.65 ± 0.183 and 1.14 ± 0.093 per cent were noted in Malabari and Alpine X Malabari bucks respectively. There was significant variation between breeds&between bucks within breed for total sperm abnormality percentage.

The overall average percentages of abnormalities of head, mid piece, tail, protoplasmic droplet and detached head were 0,03 ± 0.012, 0.13 ± 0.023, 0.70 ± 0.229, 0.30 ± 0.069 and 0.24 ± 0.034 respectively.

The mean motility percentage of buck spermatozoa was

found to vary significantly between room temperature and refrigeration temperature glycerolisation through out the period of storage. However, refrigeration temperature glycerolisation was significantly superior to room temperature glycerolisation in the preservation of motile spermatozoa. The overall mean percentage of initial motility at room temperature glycerolisation was 79.63, which decreased to 19.23 per cent at 144 hours of storage; similarly, the mean motility percentage of 80.41 at refrigeration temperature glycerolisation declined to 31.62 per cent at 144 hours of storage. At room temperature glycerolisation, mean motility percentage in Tris diluent was 79.91 at zero hour and 26.65 at 144 hours of storage; and in skim milk diluent initial motility was 79.35 per cent and at 144 hours 12.77 per cent. Tris diluent was significantly superior to skim milk diluent in the preservation of buck semen both at room temperature and refrigeration temperature glycerolisation.

Comparative evaluation of motility percentages up to 144 hours of storage showed that above 50 per cent motility could be maintained up to 96 hours and 48 hours respectively in Tris and skim milk diluents with zero per cent glycerol both at room temperature and refrigeration temperature glycerolisation.

With one per cent glycerol level, above 50 per cent motility was maintained in room temperature and refrigeration temperature glycerolisation up to 96 hours and 120 hours respectively in Tris diluent; whereas it was only up to 48 hours and 72 hours respectively in skim milk diluent.

Buck spermatozoa could be preserved with above 50 per cent motility in Tris diluent with three per cent glycerol up to 96 hours and 120 hours of storage at room temperature and refrigeration temperature glycerolisation respectively. Similarly, skim milk diluent with three per cent glycerol could preserve motility percentage above 50 per cent up to 48 hours at room temperature glycerolisation and 72 hours at refrigeration temperature glycerolisation.

It was observed that above 50 per cent motility could be preserved in Tris diluent with 7 per cent glycerol up to 72 hours and 96 hours of storage at room and refrigeration temperature glycerolisation respectively. Whereas, skim milk diluent with the same concentration of glycerol could preserve 50 per cent motility up to 48 hours at room temperature glycerolisation and up to 72 hours at refrigeration temperature glycerolisation.

In the present investigation, throughout the period of storage, the motility percentage of buck spermatozoa was

higher in diluents with seven per cent glycerol, followed by three and one per cent in skim milk diluents at both the types of glycerolisation. Whereas, in case of Tris diluent, at room temperature glycerolisation, higher motility percentage was noted with zero per cent glycerol, followed by one, three and seven per cent. From the present study it could be concluded that skim milk diluent with seven per cent glycerol at refrigeration temperature glycerolisation could be used for preservation of buck spermatozoa with above 50 per cent motility up to 72 hours of storage; and there was no advantage in addition of glycerol at room temperature in skim milk diluent. Similarly, Tris diluent with one per cent glycerol was found to be suitable for preservation of buck spermatozoa at 5°C in both the methods of glycerolisation.

At room temperature glycerolisation for both Tris and skim milk diluents, total abnormality percentages were higher than that at refrigeration temperature glycerolisation. Significant difference in total abnormality percentage was noted among the levels of glycerol at zero hour, 24 hours, 72 hours and 120 hours of storage, at room temperature glycerolisation. However, at refrigeration temperature glycerolisation, there was significant difference in abnormality among the levels of glycerol at all intervals of storage. With both methods of glycerolisation in Tris and skim milk diluents, the total

abnormality percentages were lower in control groups, and higher as the level of glycerol increased.

The average head abnormality percentages of 0.69 and 0.40 were observed at room temperature and refrigeration temperature glycerolisation, respectively, at zero hour of storage. There was an increase of mid piece abnormality percentage as the days of storage advanced in both Tris and skim milk diluents irrespective of glycerolisation methods. Significant difference was observed between room temperature and refrigeration temperature glycerolisation in tail abnormality percentage from zero hour to 144 hours of storage. Tail abnormality was higher at all hours of storage at room temperature glycerolisation in comparison to that at refrigeration temperature glycerolisation.

The overall mean percentages of acrosome defect of 0.87 and 0.29 at room and refrigeration temperature glycerolisation respectively, at zero hour of storage, gradually increased to 7.08 and 6.15 per cent at 144 hours of storage. The present study revealed an increase in acrosomal defects with the advancement of storage period in Tris and skim milk diluents, at both the methods of glycerolisation.

The overall average post-thawing motility was found to be 37.75 per cent. Post-thawing motility of 36.08 and

39.42 per cent were observed in Malabari and Alpine X Malabari buck semen respectively. The average post-thawing motility in Tris and skim milk diluent was 44.44 and 31.06 per cent. The variation showed significant difference between diluents, Tris diluent being superior to skim milk diluent.

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PRELIMINARY TRIALS ON PRESERVATION OF BUCK SEMEN IN GLYCEROL CONTAINING DILUENTS

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ABSTRACT

A systematic study was carried out on normal characteristics and preservation of buck semen in chilled and frozen conditions.

A total of 240 ejaculates from 3 Malabari and 3 Alpine X Malabari bucks were utilized for the study on normal semen characteristics. The overall average reaction time was 98.86 ± 4.309 seconds. Significant positive correlation between reaction time and mass activity was observed. The mean semen volume was found to be 0.55 ± 0.017 and 0.72 ± 0.015 ml in Malabari and Alpine X Malabari bucks respectively. Semen volume was significantly higher in cross bred bucks. The colour of buck semen varied from milky yellow to cremy white. The overall density score of buck semen was 3.52 ± 0.030 out of four. Mean values for pH of Malabari and Alpine X Malabari semen were 6.74 ± 0.026 and 6.74 ± 0.019 respectively. Mass activity varied significantly between bucks. Significant difference was noted in motility percentage between breeds. Initial motility was having significant positive correlation with the live sperm percentage. The overall mean live sperm percentage was 90.64 ± 0.317. Significant difference in sperm concentration was observed between bucks. Average total sperm abnormalities of 1.65 ± 0.183 and 1.14 ± 0.093 per cent were noted in Malabari and Alpine X Malabari bucks respectively.

Effect of room temperature and refrigeration temperature glycerolisation of Tris and reconstituted skim milk diluents, each having 0, 1, 3 and 7 per cent glycerol, on preservability of buck spermatozoa was studied. Motility and abnormality assessment were made at zero hour to 144 hours, at 24 hours interval. Skim milk diluent with seven per cent glycerol at refrigeration temperature glycerolisation preserved above 50 per cent sperm motility up to 72 hours of storage. No added advantage could be observed in the addition of glycerol at room temperature in skim milk diluent. Tris diluent with one per cent glycerol was found to be suitable for preservation of buck spermatozoa at 5°C, in room temperature and refrigeration temperature glycerolisation. Refrigeration temperature glycerolisation was found to be significantly superior to room temperature glycerolisation in preserving motility percentage in both the Tris and skim milk diluents.

In both the diluents, total abnormality percentages at room temperature glycerolisation were significantly higher than that at refrigeration temperature glycerolisation. With both methods of glycerolisation in Tris and skim milk, the total abnormality percentages were higher as the level of glycerol increased. The present study revealed an increase in acrosomal defects with the advancement of storage period. The occurence of acrosomal defects was significantly higher in room temperature glycerolisation.

Four ejaculates each from six bucks were diluted in Tris and skim milk diluents each with seven per cent glycerol, to study the effect of deep freezing and post-thawing motility. The average post-thawing motility in Tris and skim milk diluent was 44.44 and 31.06 per cent respectively. Tris diluent was found significantly superior to skim milk diluent for freezing buck spermatozoa.