EFFECT OF FREQUENCY OF EJACULATION ON SEMEN CHARACTERISTICS AND LIBIDO IN CROSS - BRED BUCKS

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "EFFECT OF FREQUENCY OF EJACULATION ON SEMEN CHARACTERISTICS AND LIBIDO IN CROSS-BRED BUCKS" 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.

Mannuthy,
17-11-1983.

METILDA JOSEPH.

CERTIFICATE

Certified that this thesis entitled "EFFECT OF FREQUENCY OF EJACULATION ON SEMEN CHARACTERISTICS AND LIBIDO IN CROSS-BRED BUCKS" is a record of research work done independently by Dr. Metilda Joseph under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Dedicated to my

Beloved Parents

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Introduction

INTRODUCTION

India ranks first among the countries of the World in goat population. According to the livestock census (FAO, 1979) there are 71 million goats in India constituting about 19 per cent of the total world population.

Goats popularly known as the "poor man's cow", play a significant role in the rural economy of our State. Certain special inherant traits such as small size, inquisitive feeding habits, feed conversion efficiency, continuous breeding under tropical conditions, high prolificacy, and short generation interval have made them ideally suited for the peculiar farming situations existing in our State.

The emphasis on improving the production potential of the native goats by cross-breeding with exotic breeds has opened up new avenues. In this context, artificial insemination programme for exploiting the full reproductive potential of both the exotic and cross-bred bucks has

received much attention. There are established norms on the frequency of collection from rams of temperate regions, where sheep are considered seasonal breeders. However, there are no scientific reports on the frequency of collection from goats either from temperate regions or from tropical countries. Therefore a study has been undertaken to evaluate the effect of different collection frequencies on seminal attributes and libido of cross-bred bucks. It is further envisaged to estimate the Daily Sperm Production (DSP) and epididymal sperm reserves of bucks in order to assess their reproductive potential in terms of the maximum daily yield of spermatozoa. Thus this study will help to fix the optimum number of collections, which can be taken from adult bucks without detrimentally affecting their libido, semen quality and fertility.

Review of Literature

REVIEW OF LITERATURE

Semen is variable in composition not only between different species, but also between individuals of the same species. Age, size, breed, season, nutrition and frequency of ejaculation are the major factors which influence the semen characteristics. It is essential to have an insight into the normal semen picture of bucks to comprehend the effect of frequency of ejaculation on semen characteristics and libido in bucks.

Prasad et al. (1970 a) reported the mean semen volume of Barbari bucks to be 0.92 ml, with an initial motility of 4.17. The live sperm percentage and the mean concentration in million per ml was 68.3 and 2783 respectively.

In an extensive study on sex libido and semen characteristics of Malabari bucks, Patil (1972) reported that the mean reaction time was 49.37 $\pm$ 2.5 seconds. He further observed that the colour of the buck semen ranged from milky yellow to thick creamy yellow with a mass

activity ranging from ++ to +++ and a mean spermatozoan motility of 66.14%. The average volume, pH and concentration (X 10^6/ml) were found to be 0.5 ml, 6.47 and 3534 respectively. The mean time taken for methylene blue reduction was 4.52 minutes. While the average live sperm percentage was 81.38, abnormal spermatozoa varied from 1 to 18.62% with a mean of 4.34 $\pm$ 0.48.

Igboeli (1974) studied characteristics of semen obtained from Zambian and Boer goats by electro ejaculation. The ejaculate volume averaged 0.67 ml and 1.34 ml respectively in these 2 breeds. He further reported that the mean percentage motility, sperm concentration (X 10^9/ml) and percentage of live spermatozoa for the above 2 breeds were respectively 52.3 $\pm$ 1.3, and 53.2 $\pm$ 1.2; 1.65 $\pm$ 0.02 and 2.70 $\pm$ 0.03 and 87.2 $\pm$ 1 and 87.7 $\pm$ 1.0. Ejaculate colour ranging from creamy white to yellow varied considerably between breeds, within breeds and between ejaculates of the same buck.

Vinha and Megale (1974) studied the semen quality of Anglo-Nubian, Marota and Moxoto goats. While the ejaculate volume in ml averaged 1.48, 0.88 and 0.88 for Anglo - Nubian, Marota and Moxoto goats respectively, the sperm concentration (X 10^6/cmm) were respectively 1.559, 1.107

and 0.803 and the spermatozoan motility percentage were respectively, 75.22, 68.33 and 62.75. The percentage of abnormal spermatozoa for Anglo-Nubian buck was 11.05 as against 11.21 and 16.36 for Marota and Moxoto respectively.

The volume, pH, concentration ($X10^6$/ml), motility and percentage of abnormality of semen from Katjang x Jamnapari bucks were reported to be 0.85 ± 0.04 ml; 6.8 ± 0, 3974.8 ± 130.8, 85 ± 0.76% and 3.11 ± 0.28 respectively (Koh, 1975). The mean values of semen volume, sperm concentration ($X\ 10^6$/ml), sperm motility and percentage of abnormal sperms for Angora goats were respectively 0.98 ml; 3674, 86% and 2.3 (Cetin Kaya, 1980).

Hulet and Shelton (1980) opined that the volume, concentration ($X\ 10^9$/ml), motility percentage and percentage of abnormal spermatozoan of goats ranged from 0.1 - 1.5 ml, 2 - 6, 60 - 80 and 11 respectively.

The overall mean values of various seminal attributes such as volume, pH, motility (score scale 1 - 5), percentage of initial motility, sperm concentration ($X\ 10^8$/ml), total sperm count per ejaculate ($X\ 10^8$) and percentage of live sperm in Pashmina goats were reported to be 0.62 ±0.02 ml,

6.84 ± 0.02, 4.19 ± 0.04, 60.62 ± 0.38 , 35.21 ± 1.18, 21.51 ± 0.92 and 80.63 ± 0.29 respectively (Mohan et al. 1980).

Saxena and Tripathi (1980) carried out a detailed study on semen characteristics in Jamnapari bucks and reported that the ejaculate volume, initial sperm motility, sperm concentration (X 10^6/ml) , sperm number per ejaculate (X 10^6), percentage live sperm and percentage of abnormal sperms were 0.37 ± 0.03 ml, 72.62 ± 1.06%, 4795.00 ± 292.97, 1837.00 ± 214.44, 77.65 ± 1.04 and 6.84 ± 0.06 respectively. He further stated that the percentage of midpiece abnormality (4.83) was significantly higher than that of head (0.88) and tail (1.13).

Mann (1981) investigated the seminal characteristics of African Dwarf goats and found that 95% of the ejaculates were ivory coloured and creamy in consistancy. He further reported that the mean pH, volume, sperm concentration (X 10^9/ml), sperm number per ejaculate (X 10^9), progressive motility , percentage of abnormal and percentage of dead sperms to be 6.93, 0.77 ± 0.26 ml, 3.22 ± 1.22, 2.46 ± 1.18, 77.28 ± 7.75%, 13.45 ± 8.77 and 15.07 respectively.

Memon et al. (1982) while comparing the different semen collection methods for bucks, reported the mean volume, concentration (X 10^9/ml), mass activity, motility and pH of semen collected by artificial vagina to be 0.36 ± 0.08 ml, 4.62 ± 0.45, 4.0 ± 0.25, 80.5 ± 2.03 and 6.15 ± 0.08 respectively.

Muhuyi et al. (1982) reported the mean semen volume of 0.64, 0.61, 0.8, 1.21 and 0.74 ml respectively for pure-bred Alpine, La Mancha, Nubian, Saanen and Toggenberg bucks. They further reported that the sperm cell concentration (X 10^9/ml) for the respective breeds were 2.99, 2.36, 3.21, 3.57 and 4.7. The overall mean motility for these 5 breeds was 59%.

Sinha and Singh (1982) studied the semen characteristics of Black Bengal and Saanen bucks. They reported that the mean reaction time, volume, mass activity, sperm concentration (X 10^6/ml), live sperm percentage, abnormal sperm percentage and pH of semen from Black Bengal bucks were 60.53 ± 1.223 seconds, 0.446 ± 0.611 ml, 4.439 ± 0.065, 2440.151 ± 40.885, 85.45 ± 0.414, 7.870 ± 0.214 and 6.79 ± 0.009 respectively. The corresponding values in Saanen breed were 64.46 ± 1.233 seconds, 0.720 ± 0.016 ml, 4.507 ± 0.048, 2780 ± 35.68, 85.21 ± 0.402, 6.196 ± 0.273 and 6.72 ± 0.004 respectively.

Zerfas and Steinbach (1982) reported the mean volume, concentration (X 10^9/ml) and motility of semen from Alpine bucks to be 0.8 ml, 3.6 and 64% respectively. The corresponding values for Boer bucks were respectively 0.7 ml, 4.0 and 66%.

The spermatozoan abnormality percentage in the semen of Assam local goats, Beetal and Sannen breeds were respectively 8.796, 9.9225 and 8.577 (Bordoloi and Sharma, 1983). Percentage of live spermatozoa in the above 3 breeds were further reported to be 83.68, 83.43 and 77.45 respectively.

Proper sexual preparation and frequent semen collections are the key factors in obtaining maximum number of usable sperms per unit of time (Hale and Almquist, 1960). The period of spermatogenesis and the passage of sperms to the epididymis are remarkably constant, but frequency of collection influences the evacuation of stored sperms in the epididymis (Koefoed-Johnson, 1960). Spermatozoa available for ejaculation come from extragonadal sperm reserves of cauda epididymis and ampulla which are replenished by the spermatozoa produced every day. Hence stocks of spermatozoa available for release at ejaculation are limited for a given day, thus the total sperm per ejacu-

lation is decreased by increasing the frequency of collection (Bielanski and Wierzbowski, 1961 a; Salamon, 1962).

Frequent semen collection is somewhat analogous to a water reservoir used sufficiently to prevent much water loss via the overflow. Likewise fewer sperms are lost in the urine and by other means like epididymal resorption when semen is collected frequently. A practical time schedule for collection is one in which collections are frequent enough to obtain majority of sperms available for ejaculation without completely depleting the extragonadal sperm reserves, so that the ejaculated semen will contain significant number of spermatozoa (Foote, 1969).

Carefully controlled studies in dairy bulls (Almquist and Hale, 1956; Boyd and VanDemark, 1957; Willet and Ohms, 1957; Hafs et al., 1958, 1959; Hale and Almquist, 1960; Amann and Almquist, 1962) indicate that bulls may be ejaculated as often as once daily for long periods with high sperm output in the ejaculates. Almquist and Amann (1976) and Almquist (1982) observed that there was no significant deterioration of semen quality in Holstein bulls with a frequency of 6 ejaculations per week.

Unlike bulls, rams and goats can be ejaculated many

times a day with moderate number of sperms per ejaculate for several weeks before severely depleting the epididymal sperm reserve. This is because of the larger epididymal sperm reserve and smaller ejaculate volume (Foote, 1980). This report is consistanc with the observation of Kuznecova et al. (1933) who stated that it is extremely difficult to exhaust spermatozoan reserves of rams completely.

Gunn (1936) observed that semen could be collected by electro-ejaculation as frequently as eight times in 10 days without altering semen characteristics of ram. Mckenzie and Berliner (1937) reported that rams gave 13 to 24 ejaculates in nine hours, with some decrease in the number of sperms in successive ejaculates.

Lambert and Mckenzie (1940) opined that rams could stand three and in some cases even more matings daily during the breeding season without apparent deterioration of their breeding efficiency.

Comstock et al. (1943) were unable to demonstrate any relationship between semen quality and frequency of collection in Shorpshire and Hampshire rams. Chang (1945) studied the semen characteristics of 4 adult Suffolk and Romney rams during normal breeding season in Britain. Collections were taken at frequencies of 1, 2 and 6 per day over a period of 10 days. It was reported, that as

number of collections per day rose, the mean volume and spermatozoan number per ejaculate fell rapidly and spermatozoan concentration fell slightly. The total volume, total number of spermatozoa and reaction time increased, semen quality however, remained unchanged.

Swanson and Blackwell (1955) found that in rams, the ejaculates obtained at the rate of one per day was superior to those obtained at the frequency of 4 per day in both quality and in the number of sperms ejaculated.

Harrington et al. (1956) showed that in rams collections of two ejaculates daily, one ejaculate per day and one ejaculate every other day ranked in that order in the number of sperms ejaculated per day. However, motility was slightly higher for the semen collected every other day.

Salamon (1959) collected 20 to 30 ejaculates per day from a Romney ram and found that the number of spermatozoa per ejaculate decreased from 46 x 10^8 to 2 x 10^8 on first day and from 2.5 x 10^8 to 0.6 x 10^8 on the second day. However, there was not much of a change in motility.

Kastyak (1962) investigated the semen quality of ram which were ejaculated on a schedule of once, twice and

thrice daily over a period of 25 days. There was no significant difference in the semen volume between rams ejaculated once daily and those ejaculated thrice daily. However, the number of spermatozoa decreased slowly during the experimental period, the rate of decrease depending on the frequency of ejaculation. Semen volume, sperm concentration, the number of spermatozoa per ejaculate and fructose concentration of semen also showed decreasing trend in rams irrespective of the frequency of ejaculation. The volume of second ejaculate was as a rule larger and that of third ejaculate smaller than that of the first ejaculate. Sperm concentration and total sperms in the ejaculateswere largest at the first mount and showed a decreasing trend in each subsequent mounts. When semen was collected at short intervals longivity of sperm was highest in second ejaculate. When there was an interval of 7 hours between mounts longivity of sperm fell in successive ejaculates.

Salamon (1962, 1964) reported that during a 5-day period, rams can be ejaculated as often as 16 times per day. It was found that volume, density and number of sperms declined on successive ejaculations within days. Motility and percentage of abnormal sperms were not affected by frequent ejaculations. Fertility was not

altered if, sperm numbers were not below 120 - 125 million per insemination. Reaction time increased linearly to a highly significant degree with successive days of collection and with successive ejaculates within days. Rams with longer reaction time tended to produce relatively high volume of semen.

Bielanski (1964) obtained 26 billion sperms per week from rams ejaculated on an average of 3.8 times daily. Lunca (1964) recommended collecting 2 to 3 or 3 to 4 ejaculates per day, depending on the capability of ram. Amir (1966) also reported that in Awassi rams, the sex libido, sperm concentration and semen volume decreased with successive ejaculates.

Danov et al. (1967) opined that in rams with four successive ejaculations every other day, ejaculate volume and sperm concentration decreased considerably, sperm resistance and motility decreased insignificantly, sperm survival fell between first 2 ejaculates and seminal pH increased. The semen characteristics of each of the four ejaculates were well within normal physiological limits required for insemination.

Salamon and Lightfoot (1967) opined that both undiluted and diluted ram semen showed greater susceptibility to cold

shock with successive ejaculates. Sumner et al. (1963) reported that sex libido in rams declined after several matings with one female, but the same was restored on exposure to anotner female in heat. Foote (1969) opined that an average of 20 ejaculations per week will be optimum for rams in that they maintain good libido and ejaculate as much as 25 billion sperms per week.

Sharma et al. (1969) studied the effect of frequency of collection at the rate of 1,2 and 3 times daily for one week in Bikaneri rams. Ejaculate volume, sperm concentration per ml, sperm per ejaculate and percentage of live spermatozoa declined significantly ($P/\_0.01$) with increase in frequency of collection. The difference between rams was significant for the first three seminal attributes studied. It was concluded that there was no substantial advantage in increasing the frequency of collection from 2 to 3 per day. In a further study, semen was collected once daily, from 3 Bikaneri rams for 3 consecutive weeks. It was observed that there was no significant difference in seminal attributes between weeks except that the number of spermatozoa per ejaculate showed a significant decrease ($P/\_0.01$) in second and third week of collection. They further recorded that daily semen collection from Bikaneri and Mandya rams for 20 and 40

days respectively had no adverse effect on the semen quality. The conception rate to single insemination with diluted semen (1:5) of Bikaneri rams was 35.7% as against 63% with the semen of Mandya rams.

Male animals offered frequent opportunities for copulation usually lose libido before the quality of ejaculate declines to a degree that would affect fertility (Roberts, 1971).

Brukner and Bauer (1972) opined that ejaculation frequency significantly affected semn volume, but not the quality. The mean semen volume, sperm concentration (X 10^6/ml) and percentage of motile spermatozoa for the first and second ejaculates were 1.18 and 1.05 ml, 3985 and 3683, and 67 and 67.2 respectively. Based on the results of their study, they recommended twice daily collection at 1 hour interval.

Shevalei (1972) reported that there was no significant reduction in fertility of cross-bred Merino rams, if semen was collected at an ejaculation frequency of 7 - 12 per day for a period of 10 days.

Honmode and Tiwari (1974) reported that there was a progressive decline in semen volume, sperm concentration and libido in Malpura and Chokla rams when collections were

made at frequency of once daily for a continuous period of 50, 80 and 110 days. However, they opined that seminal attributes were excellent during the entire period of study and the semen could be used for insemination. The lambing rates for these 3 groups were respectively 63.8, 54.7 and 68.3% for Malpura breed as against 67.7, 80.9 and 52.9% in Chokla breed.

Colas (1975) found that in young Romanov rams ejaculate volume and sperm concentration increased regularly independent of ejaculation frequency. He recommended a collection frequency of 2 x 1 from 6 - 10 months, 2 x 2 from 11 to 17 months and 3 x 2 from 18 months onwards in this breed.

Mikus and Pilko (1975) reported that when rams were used for collection once daily ejaculate volume, seminal pH, MBR time and sperm concentration(X 10^6/ml) averaged 2.15 ml, 6.83, 43 seconds and 3.08 respectively. The corresponding values when they were ejaculated 11 times daily were 0.82 ml, 7.44, 225 seconds and 1.03. For rams mating 1,2,3,4,5 or 6 ewes daily, the conception rate of the mated ewes was 76.1, 74.8, 78.4, 67.3, 70 and 60% respectively.

Sahni and Tiwari (1975) reported that when semen was collected from Rambouillet rams once daily for periods

ranging from 20 to 36 days in early autumn, a deterioration in semen quality with marked increase in the number of refusals was observed. However, when semen collection frequency was reduced to once on alternate days, the semen of optimum quality suitable for artificial insemination was obtained for a period of 54 days each during spring and autumn.

Sixteen Suffolk rams were ejaculated 14 - 32 times within 8 hour and it was observed that while ejaculate volume and concentration declined in successive ejaculations, the percentage of abnormal sperms did not. It was concluded that after the 8th ejaculate, the number of spermatozoa per ejaculate fell below that required for fertilization (Jennings & McJeeny, 1976).

Tomkins and Bryant (1976) studied the effect of high mating pressure (90 ejaculates) and low mating pressure (45 ejaculates) on semen quality of rams over a 3 day period each in summer and autumn. It was found that real decline of quality of semen was greater with high mating pressure and that the volume, concentration and percentage of abnormal sperms showed a seasonal variation.

The number of services in 30 minutes and time taken

for 1st, 2nd and 3rd consecutive services in 51 rams averaged 3.76 services and 0.7, 4.14 and 16.81 minutes respectively (Dhillon et al., 1979).

Simpson and Edey (1979) reported that the semen quality and quantity fell during first two weeks of paddock mating in rams, but there was a steady recovery through the remaining of the mating period and within 2 weeks of removal of rams from the paddock.

Twentyfour, 18 month old German Mutton Merino full or half brothers were subjected over a two year period, to two collection frequencies of 3 or 5 single mounts weekly (control) and 3 double or treble mounts per week (experimental). An increase from 3 to 6 collections per week had no significant influence on ejaculate volume. Sperm concentration in the second ejaculate was significantly lower in experimental group (4.51×10^6/ml), whereas forward motility was significantly higher in the experimental groups. An increase to 9 collections weekly from three single collections resulted in decrease in ejaculate volume and sperm concentration. However, motility was observed to be significantly higher in experimental animals (Menger and Hamad, 1981).

Synnott et al. (1981) reported that in 14 rams with continuous mating schedule for 6 days, the number of matings per day averaged 7, 11.5 and 12.1 in the presence of 1, 4 and 8 estrus ewes respectively. At all times the rams preferred certain ewes and not more than 5 ewes were mated per day by each ram, regardless of the number available. Mittal (1982) reported that breed and season had no effect on increasing the ejaculation frequency in Marwari and Magra rams.

Unlike rams, the tropical goats are non-seasonal breeders. There are only a few reports on the effect of frequency of collection on seminal attributes in goats. Roberts (1956) stated that bucks can be collected 2 - 6 times or more daily. Blokhuis (1962) reported that males showing particularly good libido may if necessary, serve once or twice daily without deterioration of semen quality. Iritani et al. (1964) reported that when 3 ejaculates were collected in succession on each day, the egg yolk coagulating enzyme activity increased from first to the third ejaculate.

Tewari et al. (1968) collected semen once daily for 54 days from Barbari and Saanen bucks and reported that the

mean ejaculate volume was 0.52 ± 0.06 ml and 0.997 ± 0.055 ml respectively. Sperm concentration (X 10^6/ml) sperm number per ejaculate (X 10^6) and percentage of live spermatozoa for two breeds were respectively, 2035.62 and 2393, 1049.31 and 2369 and 86.12 and 84.65. There was significant difference in ejaculate volume, sperm concentration and total sperms per ejaculate between breeds. He further reported that in Jamnapari, Sannen X Jamnapari and Barbari bucks with a frequency of 2 collection per day for 54 days, the mean ejaculate volume, sperm concentration (X 10^6/ml) sperm per ejaculate (X 10^6), percentage of live sperm and initial motility were respectively 1.36, 1.37 and 0.94 ml, 3728.3, 4935 and 3 650, 3003, 3494 and 2028; 85.3, 89 and 86 and 4.6 ± 0.09, 5.0 ± 0.0 and 4.2 ± 0.11. The overall conception rate in group of Barbari goats as a result of insemination carried out over a period of 40 days was 79%.

Prasad et al. (1970 b) studied the semen quality of Barbari bucks on once daily collection schedule for 8 weeks. They did not observe any significant deterioration in semen characteristics over this period of study. The conception rate of does inseminated with semen from these bucks was 83.63%.

Mittal and Pandey (1972) reported that semen

collection schedule of once daily over a period of 5 weeks did not affect semen picture and fertility of Barbari and Jamnapari bucks. He reported a fertility rate of 70.8 and 80% respectively for Barbari and Jamnapari breeds. Patil (1973) observed that collection frequency of 4 to 5 times daily did not cause any deterioration in semen quality of goats.

Foote (1980) opined that volume, sperm concentration (X 10^9/ml) total sperm per ejaculate (X 10^9), total sperm/ week (X 10^9), percentage motility and abnormality percentage of goat semen with an ejaculation frequency of 7 - 20 per week were respectively in the range of 0.5 - 1.5 ml, 3 - 6, 1.5 -6, 25 - 35, 60 - 80 and 5 - 20.

Tris buffered diluents containing egg yolk was reported to be superior for preservation of both buck semen (Fougner,1976) and ram semen(Fukui,1979, Sexana et al., 1979; Deka and Rao,1980). Mathew (1983 b) opined that buck semen could be preserved in tris yolk extender for 7 days at 6 - 8°C with good motility.

The recorded conception rates of goats with artificial insemination ranged from 30 to 90% (Roy et al.,1959; Blokhuis,1962; Knoblauch,1962; Bonfert and Thier,1963; Lunca,1964; Ricordean,1964; Lyngset et al., 1965; Sahni

and Roy,1967; De-Saint-Seine, 1969; Gruttemeiner, 1969; Cetinkaya, 1980). The conception rates for sheep and goats during mid breeding season in temperate zones was reported to be about 85% (Hulet and Shelton,1980). Mathai et al. (1980) reported a conception rate of 40% in Malabari and Malabari cross-bred does based on kidding.

Spermatozoa are produced continuously throughout the year, irrespective of the sexual activity of the ram. When rams are neither allowed to mate nor used for collection of semen, spermatozoa are usually voided through urine (Lino et al.,1967). The capacity of seminiferous tubules to produce spermatozoa varies markedly between species and between individuals within species. Spermatogenesis and Daily Sperm Production (DSP) are influenced by a variety of factors such as age, size of testis, environmental conditions, nutrition, breeding season, density of animal population and even social stress (Gartner et al., 1973).

Daily Sperm Production can be defined as the total number of spermatozoa produced per day, by the two testes. Daily sperm output (DSO) is total number of ejaculated spermatozoa collected over a period of time expressed on a per day basis. Daily Sperm Production apparently exceeds

the number of sperms which can be recovered in ejaculated semen, as a portion of the spermatozoa produced is lost through urine, by resorption in the epididymis and in the collection equipment (Amann, 1970).

Ortavant (1958) indicated that Daily Sperm Production and Daily Sperm Output of rams are not equal. Bielanski and Wierzbowski (1961 a) reported that the mean daily spermatozoan loss through urine in sexually rested ram was 281 x 10^6. Lino et al. (1967) recovered 6.2 x 10^9 sperms daily from the urine of sexually rested rams, which was 88% of Daily Sperm Output.

The Daily Sperm Production can be estimated either by determination of sperm output at varying ejaculation frequencies (Edwards, 1940; Chang, 1945; Boyd and VanDemark, 1957; Ortavant, 1958; Amann and Almquist, 1961b; Sengar and Sharma, 1965) or by direct determination using testicular homogenates (Amann and Almquist, 1962; Kennelly and Foote, 1964; Singh et al., 1965).

Daily Sperm Production has been estimated by several depletions following an initial depletion during a 13 day period, in bulls (VanDemark, 1956; Boyd & VanDemark, 1957; Frederick, 1958; Bielanski and Wierzbowski, 1963; VanDemark

et al., 1964) in buffalo-bulls (Sengar and Sharma, 1965) in boars (Kaplan, 1966; 1967) in rams (Bielanski and Wierzbowski, 1961 a) and in Stallions (Bielanski and Wierzbowski, 1961 b).

Daily Sperm Output of bulls was reported to range from 1.9 x 10^9 to 3.9 x 10^9 (Boyd and VanDeMark, 1957; Sayed and Oloufa, 1957; Federick, 1958; Almquist, 1969; Amann and Almquist, 1976). In contrast, Willet and Ohms (1957) recorded that 2 year old bulls produced 32.9 x 10^9 sperms/week. Many other workers have also reported that the DSO of bulls was $\sim$ 4.5 billion (Almquist et al., 1958; Hafs et al., 1959; Amann and Almquist, 1960; Amann and Almquist, 1962; Hahn et al., 1969).

Reports on the estimation of Daily Sperm Output are few in buffalo-bulls. Sayed and Oloufa (1957) found that it is $\sim$ 1000 (X 10^6). On the other hand, Sengar and Sharma (1965) reported that the Daily Sperm Output of buffalo-bulls was 2.48 x 10^9.

The earliest report on estimation of Daily Sperm Output in sheep was by Chang (1945) who recorded the value to be 8,600 (X 10^6). Ortavant (1958) recorded that the Daily Sperm Output in ram was 5500 millions.

Fielden and Barker (1964) conducted depletion trials of goats and reported that the number of services to exhaustion, total ejaculate volume and sperm production ($X\ 10^9$) ranged from 4.0 - 11.7, 1.7 - 11.4 ml and 6.3 - 21.0 respectively. A rest period of 14 days between trials appeared to be adequate for recovery.

Estimation of Daily Sperm Production based on the counts of elongated spermatids or spermatozoa in testicular homogenates has been carried out by various workers (Ortavant, 1958; Amann and Almquist, 1961a; Singh et al., 1965; Verma et al., 1966; Orgebin-Crist, 1968). Daily Sperm Production can be calculated by dividing the number of spermatids in testicular homogenates by a time divisor which represents the number of days of production these reserves represent (Almquist and Amann, 1961; Amann and Almquist, 1962). This time divisor for bulls was 3.27 days representing the VI to VIII stages of an eight stage cycle of seminiferous epithelium (Amann & Almquist, 1962). A time divisor of 3.56 days was calculated for rams from the data of Ortavant (1958), representing VI to VIII stages of the cycle of seminiferous epithelium (Amann, 1970).

Ortavant (1956) reported that the spermatozoan reserve per gram of testis in bull was 99 millions and the Daily Sperm Production per gram of testis has worked out to be 30.27 millions. The Daily Sperm Production in bulls based on

testicular homogenate studies was found to range from 16.8 to 19.39 million/g of testis (Amann and Almquist, 1960; Almquist and Amann, 1961; Amann and Almquist, 1962; Ortavant et al., 1964, Hafs et al., 1968; Macmillan and Hafs, 1968; Almquist & Amann, 1969). Amann and Almquist (1976) reported that the Daily Sperm Production of bulls averaged 3.42 billions. In buffalo-bulls Verma et al. (1965) recorded Daily Sperm Production to be 32 x 10^6/g of testis.

Though there are numerous reports on the estimation of Daily Sperm Production in rams, there appears to be only a few reports on the same in goats. In ram, Ortavant (1952) reported the Daily Sperm Production to be 27 x 10^6/g of testis. Subsequently Ortavant and Thibault (1956) estimated the DSP of rams to be 24 x 10^6/g of testis. Ortavant (1959) further reported that the DSP of ram was 12.2 x 10^6/g of testicular parenchyma.

Abdou et al. (1978) recorded significant correlation between paired testicular weight and testicular spermatid number in Awassi rams of different age groups. They reported that, as the paired testicular weight increased from 164.9 to 315.2 g, the testicular spermatid number (X 10^9) increased from 15.9 to 55.8.

Carew and Egbunike (1981) reported that Maradi goats with an average paired testis weight of 102.92 ± 7.05 g

produced 752 million sperms daily and 15.8 millions per gram of testicular parenchyma.

Daily Sperm Production rates of bulls obtained by quantitative testicular histology and direct canulation of testicular effluents from rete testis and vas deferens. (Amann and Almquist,1962; Swierstra,1966 and Amann & Almquist,1976) are not comparable to those recorded either by depletion studies or testicular homogenate method. Daily Sperm Production of bulls based on canulation of rete testis or vas deferens was found to range between 2.96 to 3.81 billions (Amann & Almquist,1976).

Daily Sperm Production of Merino rams based on direct canulation has been estimated to be 16 x 10^6 sperms per g of testis (Voglmayr et al.,1966). Subsequent studies (Voglmayr et al.,1967; Setchell et al.,1969) have recorded higher values for rams. However, Voglmayr (1968) opined that a Daily Sperm Production of 18 x 10^6 sperms/g testis was typical for Merino rams.

Extragonadal sperm reserves include the sperm stored in epididymis, vasdeferons and ampulla. Epididymal sperm reserves can be determined either by direct count (Polovceva, 1938; Chang,1945; Novoseljcev, 1951; Ortavant, 1958; Kennelly, 1960; Amann & Almquist, 1961a; Singh, 1961, 1962;

Singh et al., 1965; Swierstra, 1971; Gebauer et al., 1974 and Amann and Almquist, 1976) or by estimation from the number of sperm obtained in depletion trials (Walton and Edwards, 1938; Almquist and Hale, 1956; Almquist et al., 1958; Hale and Almquist, 1960). The first approach had been considered more accurate and hence adopted more extensively. Recently a catheterization technique enabling collection of epididymal semen from the cauda has been developed to study the epididymal sperm reserves (Voylmayr et al., 1977).

There are many reports on estimation of epididymal sperm reserves in bull by direct count (Bialy and Smith, 1958; Ortavant, 1958; Kennelly, 1960; Almquist and Amann, 1961). Almquist and Amann (1961) reported that the sperm counts of caput, corpus and cauda averaged 19.4, 4.7 and 37.6 billion respectively for sexually rested bulls, 22.6, 5.2 and 26.4 billions for bulls which were collected 6 - 7 times weekly and 16.2, 3.0 and 13.7 billions for sexually rested bulls depleted just before slaughter. The extragonadal sperm reserves averaged 72.6 billions for these 3 groups of bulls. MacMillian and Hafs (1968) reported the epididymal sperm reserves in caput and corpus together and cauda separately in bulls aged 12 months to be 8.6×10^9 and 8.1×10^9 respectively.

Amann and Almquist (1976) found that the extragonadal sperm reserves (X 10^9) in caput, corpus cauda and vasdeferens including ampulla of sexually rested bulls were respectively 20, 5, 39 and 8.

Verma et al. (1965) reported that the mean epididymal sperm reserves in caput, corpus and cauda epididymis of buffalo-bulls were 2.34 (25.03%), 0.75 (8.03%) and 6.26 (66.92%) billions respectively. Sharma and Gupta (1978) found that the number of spermatozoa in caput, corpus and cauda epididymis averaged 5.42, 0.75 and 11.45 billions respectively with a total epididymal sperm reserve of 36.2 billions in buffalo-bulls.

Data on epididymal sperm reserves of rams and goats are also few in literature. Polovceva (1938) reported that the total sperm reserve per epididymis (X 10^9) was 40.528 with the sperm reserves in caput, corpus and cauda to be respectively 4.589 (11.3%), 5.129 (12.7%) and 30.817 (76%). Chang (1945) estimated the spermatozoan reserves (X 10^9) in the caput, corpus and cauda epididymis of suffolk rams to be 8.627 (16.3%), 4.220 (9%), 52.770 (73%) respectively with a total reserve of 65.932 per epididymis.

Ortavant (1952) recorded the total epididymal reserves of one side (X 10^9) in Illede France rams to be 40.4 with

the sperm content of 5.6 (14%), 3.5 (9%) and 31.3 (77%) in the caput corpus and cauda respectively. Ortavant & Thibault (1956) found that the total epididymal sperm reserves of one side was 62.50 (X 10^9) in rams. Further Ortavant (1958) estimated the spermatozoan reserves in caput, corpus and cauda to be 11.51 (14.3%), 5.720 (7.1%) and 63.11 (78.5%) with a total reserve of 80.36 per epididymis.

Dott and Skinner (1967) recorded the total reserves per epididymis (X 10^9) in rams to be 27.13 of which the proportions in the caput, corpus and cauda were 4.655 (16.8%), 6.709 (25.7%) and 15.773 (57.5%) respectively. Abdou et al. (1978) reported that the epididymal sperm reserves (X 10^9) of 1 - 2.5 year old rams ranged from 11.1 - 98.8. There was correlation between epididymal reserves and paired epididymal weight. Fielden and Barker (1964) observed that the extragonadal sperm reserves in goats ranged from 34.54 x 10^9 to 64.26 x 10^9. A significant correlation between extragonadal sperm reserves and sperm output estimated by depletion trials was also reported.

Jindal and Panda (1980) reported that in adult male goats, the mean spermatozoan reserves (X 10^9) in caput, corpus and cauda epididymis of one side were 1.9 $\pm$ 0.16

(21.5%),0.66 ± 0.04 (7.5%) and 6.18 ± 0.9 (70.8%). The testis weight was found to be correlated (0.89) with the epididymal weight. Similarly the testis weight and epididymal weights were correlated with the epididymal sperm reserves. Bhatt and Chauhan (1982) recorded sperm reserves per epididymis (X 10^9) of sexually mature local goats to be 10.82 ± 0.82. The proportion of spermatozoa (X 10^9) in the caput, corpus and cauda were respectively 1.73 ± 0.14 (16.85%), 0.77 ± 0.07 (7.13%) and 8.31 ± 0.71 (76.77%) respectively.

Materials and Methods

MATERIALS AND METHODS

Nine adult Malabari cross-bred bucks, aged two to three years, belonging to All India Co-ordinated Research Project (AICRP) on Goats for Milk, Mannuthy were selected for the study. The bucks, maintained under identical feeding and management were randomly allotted to three experimental groups of three bucks each.

Group I : One ejaculate was taken daily for a continuous period of three months.

Group II : Two successive ejaculates were taken daily for a continuous period of three months.

Group III : Three successive ejaculates were collected daily for a continuous period of three months.

Semen was collected around 9 a.m. on all days using Artificial Vagina (A.V.) for goats (Mathew, 1983 a). Clean dry 2 ml graduated collection vials having 0.1 ml precision were used to obtain semen samples.

Adult male bucks were used as teasers. Two false mounts were given for stewing the bucks before taking

collection. Teasers were changed for obtaining second and third collections, whenever necessary.

Sex libido was evaluated from the reaction time and refractory period of individual bucks. For the purpose of this study, time interval between the approach of buck to the teaser and ejaculation was taken as reaction time and this included the time taken for two false mounts also. The time interval between two successive ejaculations which included the time taken for two false mounts before the second ejaculation was taken as the refractory period.

Immediately after collection the semen vials were transferred to a water-bath maintained under 37°C.

The semen characteristics such as colour, volume, density, mass activity, motility, percentage of dead and abnormal sperms were estimated by standard procedures (Blom, 1950; Roberts, 1971). For determination of pH B.D.H. pH indicator paper strips in the range of 5.3 to 7 and 7 to 8.5 were used. Concentration was estimated spectrometrically using Bausch and Lomb spectronic 20" photocolorimeter by standard procedure (Perry, 1969).

Methylene Blue Reduction test.

The procedure adopted for Methylene Blue Reduction

(MBR) was in partial modification to the test suggested by Beck and Salisbury (1943). The procedure for the preparation of the reagents and the test are furnished below.

Reagents

i. Methylene blue solution.

The solution was prepared by dissolving 50 mg of methylene blue in 100 cc of 3.6% sodium citrate solution.

ii. Tris egg yolk diluent (0.2 Molar Tris having pH 7).

Procedure.

In a 10 ml test-tube 0.4 ml of Tris yolk diluent and 0.1 ml of fresh semen were taken and mixed thoroughly. To this was added 0.05 ml of methylene blue solution and mixed well. After sealing the test-tube with 1 cm layer of mineral oil, it was kept in a hot water bath at 115°F. The time taken for the disappearance of blue colour due to reduction of methylene blue to leucomethylene blue was taken as the MBR time.

Sperm viability test.

Semen was diluted at the rate of 1:10 using Tris egg yolk diluent (Mathew, 1983 b). From the diluted semen

0.1 ml each was placed in two, 10 ml test-tubes and incubated for 30 minutes in a water bath at 46.5°C. The spermatozoan motility was then assessed every 10 minutes for 30 minutes. Motility of the extended samples preserved at 6 - 8°C was also assessed at 24 hour intervals, until 96 hrs. of preservation or total cessation of motility whichever was earlier.

Fertility Trial.

Twenty does in heat were inseminated with semen collected from bucks ejaculated thrice daily. Pooled semen samples from bucks after a continuous period of two months study, were diluted in Tris yolk diluent, giving a final concentration of 100 million sperms per dose of semen (0.01 ml). Fertility was assessed both on basis of conception rate and kidding rate.

The data on the effect of frequency of ejaculation on semen characteristics, libido and fertility of bucks were analysed statistically (Snedecor and Cochran, 1967).

Daily Sperm Production.

The procedure adopted by Boyd and VanDemark (1957) was followed for estimation of Daily Sperm Production in the

present study. Six healthy, Malabari cross-bred bucks, aged 2 - 3½ years, were divided into two groups of three each and randomised in a 3 x 3 Latin square design. Ten ejaculates were collected using A.V. for goats in rapid succession from each of the bucks at intervals of one, four and seven days following an initial partial exhaustion with 10 ejaculates. Thus 40 ejaculates were collected from each buck in a 13 day period. The 10 ejaculates collected each day from each buck were pooled and the total volume was found out. The concentration of pooled ejaculate was estimated photocolorimetrically and the total number of sperms in ejaculates was worked out by multiplying the concentration of sample with the total volume.

A graph was drawn by plotting the independant variable (time interval) in the X-axis and the dependant variable (concentration) in Y-axis. The residual sperm count and daily sperm production were worked out using regression of time interval on sperm count (Snedecor and Cochran, 1967).

Testicular and Epididymal sperm reserves.

The method suggested by Verma et al.(1965) was partially modified to estimate the Daily Sperm Production (DSP) and epididymal sperm reserves. Three adult cross-bred Malabari

bucks aged 2½ to 3½ years were slaughtered after two to three weeks of sexual rest. The reproductive organs were harvested soon after slaughter and transported to the laboratory wrapped up in a moist cloth. The weight of the testes with and without tunica albuginea and weight of the epididymes were duly recorded.

The testes were homogenized in a tissue homogenizer for two minutes and the volume of homogenized mass was made upto 500 ml using normal saline. Similarly the caput, corpus and cauda epididymes were homogenized seperately for three minutes and the volume was made upto 200 ml in volumetric flasks. Ampicillin was added at the rate of 500 microgram/ml of the homogenized tissue to prevent bacterial multiplication.

The testicular homogenates were rediluted at the rate of 1:10 in normal saline containing 0.2% eosin at 36 hrs. of preservation and the sperm concentration was estimated haemocytometrically. Similarly epididymal homogenates were filtered through a coarse sieve to get rid of the fibrous tissue materials at 72 hours of preservation. While the cauda and caput epididymal samples were rediluted at the rate of 1:10, the dilution rate of corpus epididymal homogenate was only 1:5. Spermatozoan concentration in the

epididymal homogenate was also estimated haemocytometrically. The total number of spermatozoa in the testes and epididymes was worked out in billions.

Results

RESULTS

In order to understand the effect of different ejaculation frequencies on seminal attributes, libido and fertility in cross-bred bucks, a study was carried out using nine Malabari cross-bred bucks belonging to A.I.C.R.P. on Goat for Milk, Mannuthy. The animals were randomly allotted to three experimental groups viz: group I (G1), group II (G2) and group III (G3) based on ejaculation frequencies of once daily, twice daily and thrice daily respectively for a period of three months. The entire study was conducted during summer months. The results on the effect of frequency of ejaculation on seminal attributes and libido of cross-bred bucks are presented in tables 1 to 17. Analysis of the data was done using nested hierarchal design and critical difference was found out wherever necessary using Duncun's Multiple range test.

Libido.

The mean reaction time in seconds for group I, II & III were 58.44 $\pm$ 38.2210, 80.208 $\pm$ 90.4439 and 93.8514 $\pm$ 67.5135 respectively. Statistical analysis revealed that there was

no significant difference in reaction time between groups. However, significant difference was noticed between bucks within group ($P < 0.01$) and between months within group ($P < 0.01$). The mean reaction time during 1st month (M1), second month (M2), and third month (M3) for G1, G2 and G3 bucks are furnished in table 2. Analysis using critical difference test revealed that there was no significant difference between months in group I. On the other hand in group II bucks heterogenisity was observed between M2 & M3. Similarly, M1 & M2, M1 & M3 and M2 & M3 were homogenous in group III bucks.

The refractory period in the case of group II bucks was found to be 283.4179 ± 643.9361 seconds. In group III bucks, the first refractory period averaged 332.3418 ± 223.9995 seconds as against the second refractory period of 431.1636 ± 288.6819 seconds (Table 3). Analysis using paired 't' test revealed that there was no significant difference between refractory period of group II bucks and 1st refractory period of group III bucks. However, in group III, the 1st refractory period was significantly different from second refractory period ($P < 0.01$).

Colour.

The semen of group I bucks was thick creamy to creamy during 1st month of study, which gradually changed to creamy

and thin creamy in the 2nd and 3rd month. In group II bucks the colour of the semen was creamy to thin creamy yellow and thin creamy yellow to yellowish milky for the first and second ejaculates respectively beyond one week of study. In contrast, the colour of the 1st ejaculate became thin creamy yellow beyond three or four days of study in group III bucks. In the latter part of the study, even the first ejaculate in some cases became yellowish milky. Second ejaculates of group III bucks were initially thin creamy yellow then becoming yellowish milky or rarely thin yellowish milky. Third ejaculates of this group were yellowish milky in the beginning which became thin yellowish milky within a few days of study.

Volume.

The mean volume of semen of group I bucks was found to be 0.4870 ± 0.1624 ml as against 0.4978 ± 0.1488 ml in group II and 0.4288 ± 0.0910 ml in group III. Analysis of data revealed that there was no significant difference between groups. However, there was significant difference between bucks within group ($P/\_0.01$) and between months within group ($P/\_0.01$). The mean semen volume during M1, M2 and M3 for group I, II and III are furnished in table 4. While M2 & M3 were homogenous M1 & M3 and M1 & M2 were heterogenous in group I bucks. In contrast, there was no significant difference

in semen volume between months in group II and III bucks (Table 4).

The mean volume of 1st ejaculate (E1) in group II bucks was recorded to be 0.5174 ± 0.1873 ml as against 0.4798 ± 0.1862 ml for the second ejaculate (E2). Analysis revealed that there was no significant difference between 1st and 2nd ejaculates (Table 20). In group III bucks the volume showed a decreasing trend with successive ejaculations. First ejaculate volume averaged 0.4525 ± 0.1397 ml as against 0.4335 ± 0.1520 ml for 2nd ejaculate and 0.3955 ± 0.1453 ml for the 3rd ejaculate (E3). Analysis of the data revealed that there was significant difference between ejaculates ($P/\_0.01$). While E1 & E2 were found to be homogenous, E1 & E3 and E2 & E3 showed heterogenisity.

Density.

The numerical scores on the density of semen of group I, II and III bucks were respectively 3.3611 ± 0.3438, 2.6676 ± 0.2791 and 2.2209 ± 0.3332. Analysis revealed that there was significant difference between groups ($P/\_0.01$), between bucks within groups ($P/\_0.01$) and between months within groups ($P/\_0.01$). Analysis revealed that there was significant difference in density between M1 & M2; M1 & M3 and M2 & M3 in group I. However, in group II bucks only M1 & M2

were found to be heterogenous. Similarly heterogenisity was observed only between M1 & M3 and M2 & M3 in group III bucks (Table 5). There was also significant difference ($P/\_0.01$) in density between ejaculates in both group II and group III bucks (Tables 20 & 22).

Mass activity.

The numerical scores on the mass activity of semen samples of bucks belonging to 1st, 2nd and 3rd group were 3.6392 ± 0.6249, 3.3645 ± 0.5193 and 2.8573 ± 0.5145 respectively. Statistical analysis showed that there was significant difference in mass activity between groups ($P/\_0.01$) and between months within groups ($P/\_0.01$). Though group I and group II were homogenous, heterogenisity was observed between G1 & G3 and G2 & G3. The mass activity was found to decrease as the ejaculation frequency increased. Analysis of the data revealed that while M1 & M2 were homogenous, M1 & M3 and M2 & M3 were heterogenous in group I bucks. There was no significant difference between months in group II bucks. In contrast, heterogenosity was observed between M1 & M2, M1 & M3 and M2 & M3 in group III bucks (Table 6). The 1st and 2nd ejaculates in group II were found to be heterogenous with respect to mass activity (table 20). Further, heterogenisity was observed between E1 & E3 and E2 & E3 in group III bucks (Table 22).

Motility.

The mean percentage motility of spermatozoa in group I, II and III were 82.1795 ± 5.9695, 79.9652 ± 7.9659 and 76.0796 ± 9.8700 respectively. Though there was no significant difference in motility between groups, there was significant difference between bucks within group ($P \leq 0.05$) and between months within groups ($P \leq 0.01$) (Table 7). The percentage motility of spermatozoa for M1, M2 and M3 were respectively 83.4066 ± 3.7917, 83.0769 ± 4.1345 and 80.0549 ± 8.3498 in group I bucks as against 80.2747 ± 6.9541, 80.6868 ± 7.5099 and 78.9340 ± 9.3812 in group II and 77.4222 ± 9.2679, 75.6792 ± 10.9601 and 75.1375 ± 9.2461 in group III. In group I bucks M1 & M2 were homogenous while M1 & M3 and M2 & M3 were heterogenous. In contrast M1 & M2, M1 & M3 and M2 & M3 were nomogenous in group II bucks. But in group III heterogenisity was observed between M1 & M3 (Table 7).

The mean motility percentage of 1st and 2nd ejaculates in group II bucks were 80.8029 ± 9.9143 and 79.5387 ± 11.2574, which did not show any significant difference on analysis (Table 20). In case of group III bucks percentage motility of E1, E2 and E3 averaged 78.3891 ± 11.6198, 76.9158 ± 15.2461 and 72.6423 ± 18.5358 respectively. Analysis revealed that, while E1 & E2 were homogenous, E1 & E3 and E2 & E3 showed heterogenisity (Table 22).

pH.

The mean values of pH for group I, II & III have worked out to be 6.8652 ± 0.0853, 6.9610 ± 0.1106 and 7.0864 ± 0.1243 respectively. Statistical analysis revealed that there was no significant difference between groups. But bucks within groups (P/_0.01) and months within groups (P/_0.01) were found to influence pH significantly. There was significant difference in pH between months in group I bucks. On the other hand, in group II, heterogenisity was observed only between M1 & M2 and M1 & M3. Similarly in group III bucks also heterogenisity was observed between M1 & M2 and M1 and M3 (Table 8).

The pH of 1st and 2nd ejaculates in group II bucks were respectively 6.9147 ± 0.1427 and 7.0030 ± 0.1076 (Table 20), which on statistical analysis showed a significant difference (P/_0.01). In group III bucks pH of 1st, 2nd and 3rd ejaculates averaged 7.0512 ± 0.138, 7.0903 ± 0.1320 and 7.1607 ± 0.1280 respectively. Analysis of data revealed that there was significant difference (P/_0.01) in pH between ejaculates of group III bucks (Table 22).

Concentration.

The sperm concentration (X 10^6/cmm) of group I bucks averaged 2.8317 ± 0.3058 as against 1.6851 ± 0.3730 in group II bucks and 1.3003 ± 0.3171 in group III bucks. Statistical

analysis of the data revealed that there was significant difference between groups ($P/\_ 0.01$) between bucks within group ($P/\_ 0.01$) and between months within group ($P/\_ 0.01$). Critical difference test showed that G2 & G3 were homogenous while G1 & G2 and G1 and G3 showed heterogenisity. The mean spermatozoan concentration ($X\ 10^6$/cmm) for M1, M2 & M3 in group I bucks were respectively 3.0280 ± 0.9009, 2.8312 ± 0.6784 and 2.6084 ± 0.7954. Analysis has shown that there was no significant difference in concentration between months, M1 & M2, M1 & M3 and M2 & M3 being homogenous. The concentration ($X\ 10^6$/cmm) during the 1st, 2nd and 3rd month of study in group II bucks were respectively, 2.1879 ± 1.4153, 1.7304 ± 0.3205 and 1.7010 ± 0.3459. Analysis revealed that M1 & M2 and M1 & M3 were heterogenous groups. The sperm concentration ($X\ 10^6$/cmm) in M1, M2 and M3 in group III bucks were 1.3676 ± 0.3551, 1.3244 ± 0.2807 and 1.2397 ± 0.3061 respectively. Analysis showed that M1 & M2, M1 & M3 and M2 & M3 were homogenous (Table 9).

The concentration ($X\ 10^6$/cmm) of 1st ejaculate averaged 1.9655 ± 0.5355, while that of the 2nd ejaculate was only 1.4638 ± 0.4115 in group II (Table 20). In group III bucks concentration ($X\ 10^6$/cmm) of E1, E2 and E3 were respectively 1.5525 ± 0.4790, 1.3215 ± 0.3950 and 1.0254 ± 0.3080 (Table 22). Statistical analysis revealed that both in

group II and group III, there was significant differe ce in co ncentration between ejaculates ($P/\_ 0.01$).

Dead percentage.

The mean values for percentage of dead sperms in the semen of group I, II, and III bucks were 9.2485 ± 3.9017, 8.8773 ± 4.7096 and 8.9239 ± 4.4881 respectively. Analysis of the data showed that there was no significant difference between groups and between bucks within groups. However there was significant difference in dead sperm percentage between months within groups. Statistical analysis revealed that M1 & M2, M1 & M3 and M2 & M3 were homogenous in group I and group III bucks, while in group II M1 & M2, M1 & M3 and M2 & M3 showed heterogenisity (Table 10). Similarly, percentage of dead sperms did not show any significant difference between ejaculates both in group II and III bucks (Tables 20 & 22).

Methylene Blue Reduction time.

Methylene Blue Reduction time showed an increasing trend on increasing the ejaculation frequency. The mean MBR time in seconds were 133.0886 ± 61.4200, 299.7969 ± 116.0773 and 312.5348 ± 104.1285 respectively for groups I, II & III. Analysis of the data proved that there was significant difference between groups ($P/\_ 0.01$) and between months ($P/\_ 0.01$)

within groups. However, there was no significant difference in MBR time between bucks within groups. In group I bucks, there was no significant difference in MBR time during 1st, 2nd and 3rd month of study. In contrast, there was heterogenisity between M1 & M2 and M2 & M3 in group II bucks. Similarly in group III also heterogenisity was observed between M1 & M3 and M2 & M3 (Table 11).

The mean MBR time in seconds for the 1st and 2nd ejaculate in group II bucks were 188.2119 ± 102.4402 and 264.2531 ± 182.8060 (Table 20). Analysis revealed that there was significant difference in MBR time between ejaculates in group II bucks ($P < 0.01$). For group III bucks, the mean values for E1, E2 & E3 were respectively 259.0777 ± 151.5950, 293.8542 ± 152.3510 and 404.1493 ± 197.3870 seconds. Analysis of the data showed that E1 & E2, E1 & E3 and E2 & E3 were heterogenous (Table 22).

Percentage of spermatozoan abnormalities.

The percentage of head abnormality and tail abnormality, proximal droplets and total abnormality were recorded to be respectively, 0.4918 ± 0.4997, 1.2732 ± 1.8003, 0.1366 ± 0.4032, 1.8689 ± 1.3139 in group I as against 0.5625 ± 0.7244, 2.1393 ± 1.8137, 0.2115 ± 0.3941 and 3.3306 ± 4.8591 in group II and 0.5095 ± 0.6750, 1.9881 ± 1.9834, 0.2352 ± 0.5146 and

2.6734 ± 2.1503 in group III bucks. The percentage of distal protoplasmic droplets in group I, II and III bucks were respectively 0.7104 ± 1.9438, 2.3525 ± 5.4651 and 0.6052 ± 1.2711 (Table 16). Analysis showed that there was no significant difference in total sperm abnormality percentage between groups. However, both bucks within groups and months within groups were found to influence the percentage of head, tail and total abnormalities. In contrast, only bucks within group ($P/\_0.01$) was found to influence the percentage of proximal and distal protoplasmic droplets (Tables 14 &16). Percentage of head & tail abnormality, proximal droplets, total abnormality and distal droplets for M1, M2 and M3 for G1, G2 and G3 are furnished in tables 12 to 16. In group I bucks, heterogenisity was observed only between M1 & M3 with regard to total abnormality. In contrast, significant difference in abnormality percentage between M1 & M2 and M2 & M3 was noticed in group II. Similarly heterogenisity was observed between M1 & M2 and M1 & M3 in group III bucks. The mean values of sperm abnormality percentage in the different ejaculates of group II and group III bucks are furnished in table 21 and 23 respectively. Analysis revealed that there was no significant difference between ejaculates in both these groups.

Sperm viability on incubation at 46.5°C and on preservation at 6 - 8°C.

The percentage of motile sperms at 0 hr., 10 minutes,

20 minutes and 30 minutes of incubation at 46.5°C and at 24 hrs., 48 hrs., 72 hrs., and 96 hrs. of preservation at 6 - 8°C are furnished in table 17. Analysis of the data revealed that there was no significant difference between groups in sperm viability on incubation for 30 minutes and preservation for 72 hrs. even though, the initial motility was significantly different ($P/\_0.05$). In contrast, sperm viability under chilled storage conditions showed a significant difference between groups ($P/\_0.05$) at 96 hrs. (Table 17). The percentage of spermatozoan motility of group I, II and III at 96 hrs. were respectively 34.5082 ± 25.2274, 35.1818 ± 24.6637, and 22.7193 ± 24.8040. Analysis revealed that while G1 & G2 were homogenous, G1 & G3 and G2 & G3 showed heterogenisity (Table 17). Correlation coefficients were worked out for 30 minutes incubation at 46.5°C and 24, 48, 72 and 96 hrs. preservation at 6 - 8°C (Table 18). Analysis revealed that there was significant correlation between sperm viability at 30 minutes incubation and sperm motility at 96 hrs. preservation under refrigeration temperature ($P/\_0.01$).

Fertility.

Out of the 20 does inseminated using the semen from group III bucks, 15 were followed up. While conception rate

was worked out to be 73.37%, kidding rate was recorded to be only 66.67% (Table 19).

Daily Sperm Production.

The data on estimation of DSP by depletion studies (DSO) are furnished in table 24. The total volume of semen, concentration of spermatozoa (X 10^6/ml) and total ejaculated spermatozoa (X 10^6) on days of initial depletion were respectively 6.2333 ± 2.7544, 2250 ± 1129.7256 and 12391.3300 ± 3972.4810. The corresponding values in the ejaculates taken at 1, 4 and 7 days intervals were respectively 3.3833 ± 1.1286, 1685 ± 536.4384 and 5383.6667 ± 1683.3520; 4.7833 ± 0.9368, 1671.6667 ± 549.0507 and 7619.3333 ± 1168.3360; and 7.7417 ± 1.9294, 1706.6667 ± 308.1091 and 12990.3333 ± 3011.7828. The data on the total spermatozoa ejaculated on initial depletion and also at intervals of 1, 4 and 7 days were plotted in a graph with time interval on the X-axis and sperm number (X 10^6) in the Y-axis. A linear relationship was noticed in the curve obtained by plotting these data. The daily sperm production (X 10^6) and residual sperm reserve (X 10^6) were worked out using regression of time interval on sperm counts and were found to be respectively 1267.98 and 3593.3233.

The data on the weight of testis, testicular sperm

reserve and daily sperm production by testicular homogenate method are furnished in table 25. The mean of paired testicular weight without tunica albuginea in grams and the total testicular sperm reserve (X 10^9) were respectively 171.6017 ± 6.9358 and 13.5100 ± 0.7234. The Daily Sperm Production (X 10^9) as per buck and DSP per g of testis (X 10^6) were worked out to be 3.7949 ± 0.2032 and 22.1974 ± 0.3775 respectively (Table 25).

Epididymal sperm reserves.

The mean weight in grams of caput, corpus and cauda and whole epididymis were respectively 10.13 ± 2.22, 1.67 ± 0.51, 5.64 ± 1.30 and 17.44 ± 3.75. The spermatozoan reserves (X 10^9) in caput, corpus and cauda epididymis were respectively 3.39 ± 0.07 (26.26%), 0.83 ± 0.08 (6.45%) and 8.64 ± 1.17 (67.18%) with an overall epididymal reserve of 23.72 ± 1.95 (Table 27).

The data on sperm reserves in cauda, estimated sperm production for 90 days based on testicular homogenate study and the ejaculated spermatozoa in group I, II and III for 90 days are furnished in table 28. The estimated total spermatozoa available for ejaculation for the entire period of study was 358.791 as against the total ejaculated spermatozoa of 124.11, 150.99 and 150.54 for group I, II & III bucks respectively.

The DSP based on testicular homogenate study, sperm reserves in caput and corpus, sperm reserves in cauda and transit time through epididymis are furnished in table 29. The total transit time of spermatozoa through epididymis was found to be 6.78 days with a transit time of 2.23 days through caput & corpus and 4.55 days through cauda.

Tables

Table 1

Effect of frequency of ejaculation on seminal attributes and libido in cross-bred bucks.

Sl. No.	Seminal attributes	Group I (G1) (one ejaculate daily)	Group II (G2) (two ejaculate daily)	Group III (G3) (three ejaculate daily)
1.	Reaction time (seconds)	58.4400±38.2210(273)	80.2080±90.4439(273)	93.8514±67.5135(276)
2.	1st refractory period (seconds)	..	283.4179±643.9361(268)	332.3418±223.9995(276)
3.	2nd refractory period (seconds)	..	..	431.1635±6319(276)
4.	Volume (ml)	0.4870±0.1624(270)	0.4978±0.1488(270)	0.4283±0.0910(270)
5.	Density (D)	3.3611±0.3438(270)	2.6676±0.2791(273)	2.2209±0.3332(276)
6.	Mass activity (+)	3.6392±0.6249(273)	3.3645±0.5193(273)	2.8573±0.5145(276)
7.	Motility (%)	82.1795±5.9695(273)	79.9652±7.9559(273)	76.0796±9.8700(276)
8.	pH	6.8652±0.0853(273)	6.9610±0.1106(273)	7.0864±0.1243(276)
9.	Concentration (million/cmm)	2.8317±0.8058(201)	1.9851±0.3730(202)	1.3003±0.3171(228)
10.	Dead (%)	9.2485±3.9017(169)	8.8778±4.7096(174)	8.9239±4.4831(170)
11.	Methylene blue reduction time(seconds)	133.0886±61.4200(158)	229.7969±116.0773(96)	312.5348±104.1285(60)
12.	Total spermatozoan abnormality (%)	1.8689±1.8139(183)	3.3306±4.8591(183)	2.0734±2.1503(183)
13.	Motility on 30 minutes of incubation (%)	36.8196±22.9183(31)	42.2545±26.5052(55)	35.5203±23.9359(57)
14.	Motility after 96 hrs. of preservation(%)	34.5082±25.2274(61)	35.1818±24.6037(55)	22.7193±24.8040(57)

Figures in parenthesis denote number of observations.

Table 2

Effect of frequency of ejaculation on reaction time of cross-bred bucks.

Reaction time in seconds												F value		
Groups			Months within group I			Months within group II			Months within group III			Between groups.	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3 M1	G2 M2	G3 M3			
.4400	80.2080	93.8514	50.7262	62.8571	61.7302	77.7363	68.6044	94.2857	68.7282	95.4457	117.3804			
±	±	±	±	±	±	±	±	±	±	±	±	0.5179	12.1406 **	4.2453 **
.2210	90.4439	67.5135	27.8348	42.9171	41.3212	75.9611	77.5380	112.2366	49.6741	68.1383	73.9169			
273)	(273)	(276)	(91)	(91)	(91)	(91)	(91)	(91)	(92)	(92)	(92)			

G1 & G2, G1 & G3 and G2 & G3 are homogenous

G1 M1 & G1 M2, G1 M1 & G1 M3 and G1 M2 & G1 M3 are homogenous

G2 M1 & G2 M2 and G2 M1 & G2 M3 are homogenous
G2 M2 & G2 M3 are heterogenous.

G3 M1 & G3 M2, G3 M1 & G3 M3 and G3 M2 & G3 M3 are homogenous.

Figures in parenthesis denote number of observations.

Table 3

Effect of frequency of ejaculation on refractory period of bucks ejaculated twice and thrice daily.

Refractory period (seconds)	Group II (G2)				Group III (G3)				tn-1
	M1	M2	M3	Mean	M1	M2	M3	Mean	
1st	234.9888 ± 232.6169 (91)	206.1111 ± 178.4441 (89)	406.4667 ± 1065.5147 (88)	283.4179 ± 643.9361 (268)	282.8667 ± 167.8926 (91)	328.7391 ± 218.5349 (92)	375.3226 ± 228.5283 (93)	332.3418 ± 223.9995 (276)	0.9320
2nd	..	..	..	..	367.5055 ± 221.8956 (92)	416.3043 ± 258.2372 (92)	506.7391 ± 356.6152 (92)	431.1636 ± 288.6819 (276)	
tn-1								6.4439**	

Figures in parenthesis denote number of observations.

Table 4

Effect of frequency of ejaculation on volume of semen in crossbred bucks.

Volume (ml)												F value		
Groups (G)			Months within Group I			Months within Group II			Months within Group III			Between groups.	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3 M1	G3 M2	G3 M3			
0.4870 ± 0.1624 (270)	0.4978 ± 0.1488 (270)	0.4288 ± 0.0910 (270	0.4394 ± 0.1594 (90)	0.5144 ± 0.1652 (90)	0.5067 ± 0.1549 (90)	0.4945 ± 0.1754 (90)	0.5037 ± 0.1417 (90)	0.4954 ± 0.1267 (90)	0.4347 ± 0.1201 (90)	0.4346 ± 0.0479 (90)	0.4170 ± 0.0899 (90)	0.589	7.90**	6.15**

G1 & G2, G1 & G3 G2 & G3 are homogenous

G1 M1 & G1 M2 and G1 M1 & G1 M3 heterogenous G1 M2 & G1 M3 homogenous

G2 M1 & G2 M2, G2 M1 & G2 M3 and G2 M2 & G2 M3 are homogenous

C3 M1 & G3 M2, G3 M1 & G3 M3 and G3 M2 & G3 M3 are homogenous

Figures in parenthesis denote number of observations.

Table 5

Effect of frequency of ejaculation on density of semen in cross-bred bucks.

Density (D) (Score 1 - 4)												F value		
Groups			Months in group I			Months in group II			Months in group III			Between groups.	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3 M1	G3 M2	G3 M3			
3.3611 ± 0.3438 (270)	2.6676 ± 0.2791 (273)	2.2209 ± 0.3332 (276)	3.4670 ± 0.3807 (90)	3.3870 ± 0.2782 (90)	3.2278 ± 0.3238 (90)	2.6291 ± 0.3235 (91)	2.7170 ± 0.2392 (91)	2.6566 ± 0.2902 (91)	2.7700 ± 0.3757 (92)	2.2522 ± 0.2910 (32)	2.1335 ± 0.3126 (92)	29.4353**	7.1146**	5.7905**

G1 & G2, G1 & G3 and G2 & G3 are heterogenous

G1 M1 & G1 M2, G1 M1 & G1 M3 and G1 M2 & G1 M3 are heterogenous

G2 M1 & G2 M2, heterogenous G2 M1 & G2 M3 and G2 M2 & G2 M3 homogenous

G3 M1 & G3 M2 homogenous G3 M1 & G3 M3 heterogenous G3 M2 & G3 M3 heterogenous

Figures in parenthesis denote number of observations.

Table 6

Effect of frequency of ejaculation on mass activity of semen in cross-bred bucks.

Mass activity (+) (Score 0 - 4)												F value		
Groups			Months within group I			Months within group II			Months within group III			Between groups	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3 M1	G2 M2	G3 M3			
3.6392 ± 0.6249 (273)	3.3645 ± 0.5593 (273)	2.8573 ± 0.5145 (276)	3.7747 ± 0.5124 (91)	3.7363 ± 0.5758 (91)	3.4066 ± 0.7068 (91)	3.4010 ± 0.5747 (91)	3.3626 ± 0.6110 (91)	3.3237 ± 0.6850 (91)	3.0256 ± 0.4789 (92)	2.8487 ± 0.4500 (92)	2.6976 ± 0.6134 (92)	17.6520**	1.5836	5.7045**

G1 & G2 homogenous
G1 & G3 heterogenous
G2 & G3 heterogenous

G1 M1 & G1 M2 homogenous
G1 M1 & G1 M3 heterogenous
G1 M2 & G1 M3 heterogenous

G2 M1 & G2 M2 homogenous
G2 M1 & G2 M3 homogenous
G2 M2 & G2 M3 homogenous

G3 M1 & G3 M2 heterogenous
G3 M1 & G3 M3 heterogenous
G3 M2 & G3 M3 heterogenous

Figures in parenthesis denote number of observations.

Table 7

Effect of frequency of ejaculation on spermatozoan motility in cross-bred bucks.

Motility %												F value		
Groups (G)			Months within group I			Months within group II			Months within group III			Between groups	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3 M1	G3 M2	G3 M3			
82.1795 ± 5.9695 (273)	79.9652 ± 7.9659 (273)	76.0796 ± 9.8700 (276)	83.4066 ± 3.7917 (91)	83.0769 ± 4.1345 (91)	80.0549 ± 8.3493 (91)	80.2747 ± 6.9541 (91)	80.6868 ± 7.5099 (91)	78.9340 ± 9.3812 (91)	77.4222 ± 9.2679 (92)	75.6792 ± 10.9601 (92)	75.1375 ± 9.2461 (92)	4.0947	2.9507*	4.2412**

G1 & G2 homogenous
G1 & G3 homogenous
G2 & G3 homogenous

G1 M1 & G1 M2 homogenous
G1 M1 & G1 M3 heterogenous
G1 M2 & G1 M3 heterogenous

G2 M1 & G2 M2 homogenous
G2 M1 & G2 M3 homogenous
G2 M2 & G2 M3 homogenous

G3 M1 & G3 M2 homogenous
G3 M2 & G3 M3 homogenous
G3 M1 & G3 M3 heterogenous

Figures in parenthesis denote number of observations.

Table 8

Effect of frequency of ejaculation on pH of semen in cross-bred bucks

pH												F value		
Groups (G)			Months within group I			Months within group II			Months within group III			Between groups.	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3 M1	G3 M2	G3 M3			
6.8652 ± 0.0853 (273)	6.9610 ± 0.1106 (273)	7.0864 ± 0.1243 (276)	6.8319 ± 0.0758 (91)	6.8714 ± 0.0981 (91)	6.8923 ± 0.0934 (91)	6.9396 ± 0.0828 (91)	6.9709 ± 0.1193 (91)	6 9725 ± 0.1232 (91)	7.0530 ± 0.1292 (92)	7.0954 ± 0.1222 (92)	7.1108 ± 0.1152 (92)	4.1985	14.4915 **	10.8068 **

C & G2 homogenous
G1 & G3 homogenous
G2 & G3 homogenous

G1 M1 & G1 M2 heterogenous
G1 M1 & G1 M3 heterogenous
G1 M2 & G1 M3 heterogenous

G2 M2 & G2 M3 homogenous
G2 M1 & G2 M2 heterogenous
G2 M1 & G2 M3 heterogenous

G3 M1 & G3 M2 heterogenous
G3 M1 & G3 M3 heterogenous
G3 M2 & G3 M3 homogenous

Figures in parenthesis denote numoer ofobservations.

Table 9

Effect of frequency of ejaculation on concentration of semen in cross-bred bucks.

Concentration (million/cmm)												F value		
Groups (G)			Months within group I			Months within group II			Months within group III			Between groups	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3 M1	G3 M2	G3 M3			
2.8317 ± 0.8058 (201)	1.6851 ± 0.3730 (202)	1.3003 ± 0.3171 (228)	3.0280 ± 0.9009 (67)	2.8312 ± 0.6784 (75)	2.6084 ± 0.7954 (59)	2.1879 ± 1.4153 (66)	1.7304 ± 0.3205 (75)	1.7010 ± 0.3459 (61)	1.3676 ± 0.3551 (80)	1.3244 ± 0.2807 (80)	1.2397 ± 0.3061 (68)	11.6299 **	11.6740 **	6.2630 **

G1 & G2 heterogenous
G1 & G3 heterogenous
G2 & G3 homogenous

G1 M1 & G1 M2 homogenous
G1 M1 & G1 M3 homogenous
G1 M2 & G1 M3 homogenous

G2 M1 & G2 M2 heterogenous
G2 M1 & G2 M3 heterogenous
G2 M2 & G2 M3 homogenous

G3 M1 & G3 M2 homogenous
G3 M1 & G3 M3 homogenous
G3 M2 & G3 M3 homogenous

Figures in parenthesis denote number of observations.

Table 10

Effect of frequency of ejaculation on percentage of dead sperms in the semen of cross-ored bucks.

Percentage of dead sperms												F value		
Groups (G)			Months within group I			Months within group II			Months within group III			Between groups.	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3 M1	G3 M2	G3 M3			
9.2485 ± 3.9017 (169)	8.8778 ± 4.7096 (174)	8.9239 ± 4.4881 (170)	9.5926 ± 4.3023 (54)	8.8421 ± 2.8334 (57)	9.3276 ± 4.4144 (58)	11.2575 ± 4.8151 (54)	7.6200 ± 2.4785 (61)	8.0881 ± 4.3065 (59)	10.2004 ± 6.3132 (56)	7.4990 ± 2.0419 (62)	9.1769 ± 3.3021 (52)	0.7827	0.2776	15.8709**

G1 & G2 homogenous
G1 & G3 homogenous
G2 & G3 homogenous

G1 M1 & G1 M2 homogenous
G1 M1 & G1 M3 homogenous
G1 M2 & G1 M3 homogenous

G2 M1 & G2 M2 heterogenous
G2 M1 & G2 M3 heterogenous
G2 M2 & G2 M3 heterogenous

G3 M1 & G3 M2 homogenous
G3 M1 & G3 M3 homogenous
G3 M2 & G3 M3 homogenous

Figures in parentnesis denote number of observations.

Table 11

Effect of frequency of ejaculation on Methylene Blue Reduction time of semen in cross-bred bucks.

Methylene Blue Reduction time (seconds)												F value		
Groups (G)			Months within group I			Months within group II			Months within group III			Between groups.	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3 M1	G3 M2	G3 M3			
133.0886 ± 61.4200 (158)	229.7969 ± 116.0773 (96)	312.5348 ± 104.1285 (60)	124.8654 ± 38.7931 (52)	127.2419 ± 35.3440 (62)	151.0455 ± 98.7708 (44)	246.1324 ± 116.4053 (34)	194.1892 ± 100.4080 (37)	260.2800 ± 127.5909 (25)	259.5260 ± 92.6594 (15)	296.6872 ± 92.4764 (25)	372.1010 ± 101.2776 (20)	17.3870**	1.6574	4.3823**

G1 & G2 heterogenous
G1 & G3 heterogenous
G2 & G3 homogenous

G1 M1 & G1 M2 homogenous
G1 M1 & G1 M3 homogenous
G1 M2 & G1 M3 homogenous

G2 M1 & G2 M3 homogenous
G2 M1 & G2 M2 heterogenous
G2 M2 & G2 M3 heterogenous

G3 M1 & G3 M2 homogenous
G3 M1 & G3 M3 heterogenous
G3 M2 & G3 M3 heterogenous

Figures in parenthesis denote number of observations.

Table 12

Effect of frequency of ejaculation on percentage of head abnormality in the semen of cross-bred bucks.

Spermatozoan head abnormality (%)												F value		
Groups (G)			Months within group I			Months within group II			Months within group III			Between groups	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3 M1	G3 M2	G3 M3			
0.4918	0.5625	0.5095	0.2903	0.6406	0.4386	0.5873	0.5082	0.5917	0.7069	0.3642	0.4611			
±	±	±	±	±	±	±	±	±	±	±	±	0.1507	2.7846*	1.9108*
0.4997	0.7244	0.6750	0.5244	1.0057	0.6818	0.8159	0.5664	0.7729	0.8004	0.5472	0.6178			
(183)	(183)	(183)	(63)	(61)	(59)	(63)	(61)	(59)	(60)	(63)	(60)			

G1 & G2 homogenous
G1 & G3 homogenous
G2 & G3 homogenous

G1 M1 & G1 M3 homogenous
G1 M2 & G1 M3 homogenous
G1 M1 & G1 M2 heterogenous

G2 M1 & G2 M2 homogenous
G2 M1 & G2 M3 homogenous
G2 M2 & G2 M3 homogenous

G3 M1 & G3 M2 heterogenous
G3 M1 & G3 M3 heterogenous
G3 M2 & G3 M3 homogenous

Figures in parenthesis denote number of observations.

Table 13

Effect of frequency of ejaculation on percentage of tail abnormality in the semen of cross-bred bucks.

Spermatozoan tail abnormality (%)												F value		
Groups (G)			Months within group I			Months within group II			Months within group III			Between groups.	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3M1	G3 M2	G3 M3			
1.2732 ± 1.8903 (183)	2.1393 ± 1.8137 (183)	1.9881 ± 1.9834 (183)	1.7774 ± 1.694 (62)	0.9844 ± 1.9880 (64)	1.7018 ± 1.9362 (57)	2.0794 ± 1.5918 (63)	2.2131 ± 2.2279 (61)	2.1271 ± 1.5442 (59)	2.2956 ± 2.1928 (60)	1.8865 ± 2.0116 (62)	1.1179 ± 1.7053 (60)	1.1179	0.5192**	2.5261**

G1 & G2 homogenous
G1 & G3 homogenous
G2 & G3 homogenous

G1 M1 & G1 M2 homogenous
G1 M1 & G1 M3 heterogenous
G1 M2 & G1 M3 heterogenous

G2 M1 & G2 M2 homogenous
G2 M1 & G2 M3 homogenous
G2 M2 & G2 M3 homogenous

G3 M1 & G3 M2 neterogenous
G3 M1 & G3 M3 heterogenous
G3 M2 & G3 M3 homogenous.

Figures in parerthesis denote number of observations.

Table 14

Effect of frequency of ejaculation on percentage of proximal protoplasmic droplets in the semen of cross-bred bucks.

Proximal protoplasmic droplets (%)												F value		
Groups (G)			Months within group I			Months within group II			Months within group III			Between groups.	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3 M1	G3 M2	G3 M3			
0.1366	0.2115	0.2352	0.1774	0.1719	0.0526	0.2258	0.2459	0.1610	0.3329	0.1235	0.2497			
±	±	±	±	±	±	±	±	±	±	±	±	0.1009	5.1131**	1.5269
0.4032	0.3941	0.5146	0.5287	0.3803	0.2253	0.4215	0.3722	0.3878	0.6242	0.2580	0.5714			
(183)	(183)	(183)	(62)	(64)	(57)	(63)	(61)	(59)	(60)	(63)	(60)			

G1 & G2 homogenous
G1 & G3 homogenous
G2 & G3 homogenous

G1 M1 & G1 M2 homogenous
G1 M1 & G1 M3 homogenous
G1 M2 & G1 M3 homogenous

G2 M1 & G2 M2 homogenous
G2 M1 & G2 M3 homogenous
G2 M2 & G2 M3 homogenous

G3 M1 & G3 M2 homogenous
G3 M1 & G3 M3 homogenous
G3 M2 & G3 M3 homogenous

Figures in parenthesis denote number of observations.

Table 15

Effect of frequency of ejaculation on total spermatozoan abnormalities in the semen of cross-bred bucks.

Total Spermatozoan abnormality (%)												F value		
Croups (G)			Months within group I			Months within group II			Months within group III			Between groups.	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3 M1	G3 M2	G3 M3			
1.8689	3.3306	2.6734	1.6290	1.8281	2.1754	2.8175	4.2951	2.8814	3.3598	2.3375	2.3226			
±	±	±	±	±	±	±	±	±	±	±	±	1.0405	9.3646**	2.1284**
1.8139	4.8591	2.1503	1.8398	1.4752	2.0970	1.8929	7.9475	1.8484	2.5998	2.1200	1.4178			
(183)	(183)	(183)	(62)	(64)	(57)	(03)	(61)	(59)	(60)	(03)	(60)			

G1 & G2 homogenous
G1 & G3 homogenous
G2 & G3 homogenous

G1 M1 & G1 M3 heterogenous
G1 M2 & G1 M3 homogenous
G1 M1 & G1 M2 homogenous

G2 M1 & G2 M3 homogenous
G2 M1 & G2 M2 heterogenous
G2 M2 & G2 M3 heterogenous

G3 M1 & G3 M2 heterogenous
G3 M1 & G3 M3 heterogenous
G3 M2 & G3 M3 homogenous

Figures in parenthesis denote number of observations.

Table 16

Effect of frequency of ejaculation on percentage of distal protoplasmic droplets in the semen of cross-bred bucks.

Distal protoplasmic droplets (%)												F value		
Groups (G)			Months within group I			Months within group II			Months within group III			Between groups.	Between bucks within group	Between months within bucks
G1	G2	G3	G1 M1	G1 M2	G1 M3	G2 M1	G2 M2	G2 M3	G3 M1	G3 M2	G3 M3			
0.7104 ± 1.9438 (183)	2.3525 ± 5.4651 (183)	0.6052 ± 1.2711 (183)	0.5484 ± 1.0816 (62)	0.4531 ± 1.4577 (64)	1.1754 ± 3.1460 (57)	2.5476 ± 5.9472 (63)	1. 7787 ± 3.4982 (61)	2.7373 ± 6.5360 (59)	0.5802 ± 0.9482 (60)	0.3754 ± 0.8519 (63)	0.8717 ± 1.7870 (60)	0.8894	34.3067**	0.6950

G1 & G2 homogenous
G1 & G3 homogenous
G2 & G3 homogenous

G1 M1 & G1 M2 homogenous
G1 M1 & G1 M3 homogenous
G1 M2 & G1 M3 homogenous

G2 M1 & G2 M2 homogenous
G2 M1 & G2 M3 homogenous
G2 M2 & C2 M3 homogenous

G3 M1 & G3 M2 homogenous
G3 M1 & G3 M3 homogenous
G3 M2 & G3 M3 homogenous

Figures in parenthesis denote number of observations.

Table 17

Effect of frequency of ejaculation on viability of spermatozoa in cross-bred bucks on incubation and preservation.

Groups	Initial motility	Viability of spermatozoa (%)						
		Incubation at 46.5°C			Preservation at 6 - 8°C.			
		10 mts.	20 mts.	30 mts.	24 hr.	48 hr.	72 hr.	96 hr.
Group I (G1)	84.3442± 4.3278 (61)	60.885± 17.2038 (61)	53.7049± 23.1771 (61)	36.8196± 22.9183 (61)	70.1639± 17.004 (61)	56.0820± 23.6095 (61)	45.4590 24.3336 (61)	34.5082 ± 25.2274 (61)
Group II (G2)	84.1071± 2.1547 (55)	66.0714± 20.9049 (55)	53.625± 26.2398 (55)	42.2545± 26.5652 (55)	66.8181± 21.4598 (55)	58.7818± 25.4650 (55)	47.5636± 25.9270 (55)	35.1818± 24.6637 (55)
Group III (G3)	82.7193± 3.6681 (57)	70.5263± 11.7140 (57)	56.1403± 19.5945 (57)	35.5263± 23.9859 (57)	71.3157± 16.4351 (57)	57.7719± 24.1705 (57)	39.7719± 28.3366 (57)	22.7193± 24.8040 (57)
F Value between groups	3.9 *	0.7684	0.3762	0.5004	1.2644	0.1977	0.873	3.9061 *

G1 & G2 homogenous
G2 & G3 homogenous
G1 & G3 heterogenous

G1 & G2 homogenous
G1 & G3 heterogenous
G2 & G3 heterogenous

Figures in parenthesis denote number of observations.

Table 18

Coefficient of correlation (r) of sperm viability on incubation and preservation.

No. of observations	Correlation between	Coefficient of correlation (r)
173	30 minutes incubation & 24 hrs. of preservation	0.7028 **
173	30 minutes incubation & 48 hrs. of preservation	0.5366 **
173	30 minutes incubation & 72 hrs. of preservation	0.7750 **
173	30 minutes incubation & 96 hrs. of preservation	0.7430 **

Table 19

Fertility rate of does inseminated with semn from bucks ejaculated thrice daily.

No. of does inseminated	Number of cases followed up	Conception		Kidding		Remarks
		No	%	No	%	
20	15	11	73.37	10	66.67	One doe has aborted at 4th month of pregnancy.

Table 20

Seminal attributes of bucks ejaculated twice daily.

Sl. No.	Seminal attributes	1st ejaculate	2nd ejaculate	Difference (t n-1)
1.	Volume (ml)	0.5174 ± 0.1873 (273)	0.4798 ± 0.1862 (272)	1.2538
2.	Density (D)	2.8815 ± 0.3719 (270)	2.51310± 0.4122 (269)	11.0157 **
3.	Mass activity (+)	3.4834 ± 0.7180 (271)	3.2278 ± 0.7473 (270)	4.2288 **
4.	Motility (%)	80.8029 ± 9.9143 (269)	79.5387 ±11.2574 (271)	1.6386
5.	pH	6.9147 ± 0.1427 (265)	7.0030 ± 0.1076 (267)	12.0748 **
6.	Concentration (10^6/cmm)	1.9655 ± 0.5355 (210)	1.4638 ± 0.4115 (199)	11.0806 **
7.	Dead (%)	8.5838 ± 4.4093 (173)	9.0882 ± 7.9091 (170)	0.7663
8.	Methylene blue reduction time (seconds)	188.2119 ± 102.4402	264.2581 ± 182.8060	2.7 **
9.	Total abnormal spermatozoan (%)	2.64 ± 1.1966 (179)	3.0057 ± 3.0105 (179)	1.1908

Figures in parenthesis denote number of observations.

Table 21

Percentage of spermatozoan abnormalities in bucks ejaculated twice daily.

Ejaculate No	Spermatozoan abnormality %			Total	Distal droplet
	Head	Tail	Proximal droplet		
1st ejaculate (E1)	0.4805±0.7293 (179)	2.00±2.1180 (179)	0.2857±0.7593 (179)	2.64±1.9657 (179)	2.5337±7.2845 (179)
2nd ejaculate (E2)	0.6325±1.0257 (179)	2.2743±0.7966 (179)	0.2126±0.6040 (179)	3.0057±3.0105 (179)	1.8857±5.7032 (179)
t n-1 value	1.5578	1.1691	1.8533	1.1908	0.6899

Figures in parentnesis denote number of observations.

Table 22

Seminal attributes of bucks ejaculated thrice daily.

Sl. No.	Seminal attributes	1st ejaculate (E1)	2nd ejaculate (E2)	3rd ejaculate (E3)	F Value: Between bucks	F Value: Between ejaculates within bucks	
1.	Volume (ml)	0.4525 ± 0.1397 (276)	0.4335 ± 0.1520 (276)	0.3955 ± 0.1453 (276)	8.9225*	6.8370**	E1 & E2 homogenous, E2 & E3 heterogenous E1 & E3 heterogenous
2.	Density (D)	2.5146 ± 0.4550 (274)	2.2564 ± 0.4650 (274)	1.8909 ± 0.4130 (275)	0.5410	63.2831**	E1 & E2 heterogenous E1 & E3 heterogenous D2 & E3 heterogenous
3.	Mass activity (+)	3.0018 ± 0.6970 (275)	2.9539 ± 0.8030 (271)	2.6588 ± 0.8150 (274)	1.6242	5.9722**	E1 & E2 homogenous E1 & E3 heterogenous E2 & E3 heterogenous
4.	Motility (%)	78.3891 ± 11.6198 (275)	76.9058 ± 15.2461 (273)	72.6423 ± 18.5358 (274)	4.9096	3.5896**	E1 & E2 homogenous E2 & E3 heterogenous E1 & E3 heterogenous
5.	pH	7.0512 ± 0.1380 (269)	7.0903 ± 0.1320 (267)	7.1607 ± 0.1280 (270)	7.4980 7	62.4806**	E1 & E2 heterogenous E1 & E3 heterogenous E2 & E3 heterogenous
6.	Concentration ($X\ 10^6$)/cmm	1.5525 ± 0.4790 (213)	1.3215 ± 0.3950 (211)	1.0254 ± 0.3080 (205)	0.8507	44.0219**	E1 & E2 heterogenous E1 & E3 heterogenous E2 & E3 heterogenous
7.	Dead (%)	8.3810 ± 4.1741 (168)	8.6790 ± 7.3151 (162)	9.3048 ± 6.8106 (165)	0.8132	1.0004	E1 & E2 homogenous E1 & E3 homogenous E2 & E3 homogenous
8.	MBR time (seconds)	259.0777 ± 151.5950 (103)	293.8542 ± 152.3510 (96)	404.1493 ± 197.3870 (67)	0.8481	14.6206 **	E1 & E2 heterogenous E1 & E3 heterogenous E2 & E3 heterogenous

Figures in parenthesis denote the number of observations.

Table 23

Percentage of spermatozoan abnormalities in bucks ejaculated thrice daily.

Ejaculate No.	Spermatozoan abnormality %				
	Head	Tail	Proximal droplets	Total	Distal droplets
1st ejaculate (E1)	0.4469 ± 0.7429 (179)	2.25 ± 3.6508 (179)	0.2582 ± 0.8592 (179)	2.7341 ± 3.6005 (179)	0.5722 ± 1.5098 (179)
2nd ejaculate (E2)	0.4011 ± 0.6419 (179)	1.8181 ± 2.2401 (179)	0.1944 ± 0.5502 (179)	2.3631 ± 2.4048 (179)	0.611 ± 1.6991 (179)
3rd ejaculate (L3)	0.7356 ± 1.5541 (179)	2.0113 ± 2.6307 (179)	0.1944 ± 0.6438 (179)	2.9195 ± 3.2744 (179)	0.5856 ± 2.1133 (179)
F value between bucks	6.8961*	13.3256**	36.7815 /*	23.5966	**176.9278 **
Between ejaculates within bucks	1.8201	1.0246	0.5474	0.1454	0.1270

Figures in parenthesis denote number of observations.

E1 & E2 homogenous E1 & E2 homogenous E1 & E2 homogenous E1 & E2 homo. E1 & E2 homogenous
L1 & L3 " E1 & L3 " E1 & E3 " E1 & L3 " E1 & L3 "
E2 & E3 " E2 & E3 " E2 & E3 " E2 & L3 " E2 & E3 "

Table 24

Semen volume and total sperm counts of bucks on depletion,

Buck No.	Details of depletion trials											
	Initial			One day interval			Four day interval			Seven day interval		
	Total volume (ml)	Sperm concentration per ml ($\times 10^6$)	Total spermatozoa ($\times 10^6$)	Total volume (ml)	Sperm concentration per ml ($\times 10^6$)	Total spermatozoa ($\times 10^6$)	Total volume (ml)	Sperm concentration per ml ($\times 10^6$)	Total spermatozoa ($\times 10^6$)	Total volume (ml)	Sperm concentration per ml ($\times 10^6$)	Total spermatozoa ($\times 10^6$)
1	4.1	4300	17630	2.7	2680	7236	3.5	2440	8540	8	2140	17120
2	10.2	1560	15912	5.0	1450	7250	5.4	1450	7830	10	1520	15200
3	3.3	2820	9306	2.0	1820	3640	3.9	2300	8970	5.4	2060	11124
4	9.0	1520	13680	4.0	1500	6000	5.5	1200	6600	9.5	1440	13680
5	5.4	1900	10260	2.6	1760	4576	5.8	1360	7888	5.6	1560	8658
6	5.4	1400	7560	4.0	900	3600	4.6	1280	5888	8.0	1520	12160
Mean & S.E.	6.2333 ± 2.7544	2250 ± 1129.726	12391.3300 ± 3972.4810	3.3833 ± 1.1286	1685 ± 586.438	5383.6667 ± 1683.3520	4.7833 ± 0.9368	1671.6667 ± 549.0507	7619.3333 ± 1168.3360	7.7417 ± 1.9294	1706.6667 ± 308.1991	12990.3333 ± 3011.7828

Table 25

Testicular sperm reserves and Daily Sperm Production of bucks.

Buck No	Body Wt. (kg)	Weight of the testis (g)						Testicular sperm reserves ($X 10^9$)			Daily Sperm Production	
		With tunica albuginea			Without tunica albuginea						Per buck* ($X 10^9$)	Per g of testis ($X 10^6$)
		Rt	Lt	Paired	Rt	Lt	Paired	Rt	Lt	Total		
1	43	97.08	93.73	95.41	91.02	87.61	178.81	7.55	6.75	14.3	4.0169	22.4644
2	45	92.66	86.63	89.64	85.43	79.55	164.98	6.80	6.08	12.88	3.6180	21.9305
3	46	90.05	91.62	90.84	84.59	86.43	171.02	6.60	6.75	13.35	3.7500	21.9273
Mean & S.E.	44.67 ± 1.53	93.26 ± 3.55	90.66 ± 3.50	91.96 ± 3.04	87.01 ± 3.50	84.53 ± 4.35	171.60 ± 6.94	6.98 ± 0.50	6.53 ± 0.39	13.51 ± 0.72	3.7949 ± 0.2032	22.1974 ± 0.3775

* Testicular sperm reserve divided by 3.56.

Table 26

Comparison of the Daily Sperm Production estimated by depletion trials and testicular homogenate method.

Buck No.	Daily Sperm Production ($X 10^6$)		$\frac{DSO}{DSP} \times 100$
	Based on testicular homogenate method	Based on depletion trials (DSO)	
1	4016.85	1247.33	31.05
2	3617.98	1325.00	36.62
3	3750.00	1647.33	43.93
Mean	3794.94	1406.55	37.06

Table 27

Epididymal sperm reserve of bucks

Buck No	Weight of epididymis(g)										
	Caput			Corpus			Cauda			Mean	Total
	Rt	Lt	Mean	Rt	Lt	Mean	Rt	Lt	Mean		
1	7.20	7.95	7.58	1.020	1.82	1.42	4.52	3.75	4.14	13.13	26.26
2	12.13	10.96	11.55	1.580	1.10	1.34	6.21	6.49	6.35	19.24	38.47
3	11.48	11.05	11.27	2.070	2.45	2.26	6.21	6.64	6.43	19.95	39.90
Mean	10.27	9.99	10.13	1.560	1.82	1.67	5.65	5.63	5.64	17.44	34.88
	±	±	±	±	±	±	±	±	±	±	±
S.E.	2.67	1.76	2.22	0.530	0.75	0.51	0.98	1.63	1.30	3.75	7.50

(contd....)

Table 27 contd.

Buck No	Epididymal sperm reserves ($X 10^9$)									Mean	Total
	Caput			Corpus			Cauda				
	Rt	Lt	Mean	Rt	Lt	Mean	Rt	Lt	Mean		
1	3.33	3.6	3.47	0.82	0.88	0.85	7.2	7.43	7.32	11.63	23.26
2	3.40	3.26	3.33	0.75	0.74	0.75	10.2	8.83	9.52	13.59	27.18
3	3.33	3.43	3.38	0.92	0.88	0.90	10.3	7.88	9.09	13.37	26.74
Mean	3.35	3.43	3.39	0.83	0.83	0.83	9.23	8.05	8.64	12.86	25.72
	±	±	±	±	±	±	±	±	±	±	±
S.E.	0.04	0.17	0.07	0.09	0.08	0.08	1.76	0.71	1.17	1.13	1.95
			(26.26)			(6.45)			(67.18)		

Figures in parenthesis denote the values in percentage.

Table 28

Comparison of the estimated total spermatozoa available for ejaculation and sperm output based on ejaculation frequencies.

Reserve in cauda epididymis at the start of experiment. ($X 10^9$)	Total estimated sperm production during 90 days ($X 10^9$)	Total spermatozoa available for ejaculation ($X 10^9$)	Total spermatozoan output for 90 days			Difference between III & IV ($X 10^9$)	Difference between III & V ($X 10^9$)	Difference between III & VI ($X 10^9$)
			Group I ($X 10^9$)	Group II ($X 10^9$)	Group III ($X 10^9$)			
I	II	III	IV	V	VI			
17.280	341.511	358.791	124.110	150.990	150.545	234.681	207.801	208.248

Table 29

Transit time of spermatozoa through epididymis.

Buck No.	DSP ($X\ 10^9$)	Epididymal sperm reserves ($X\ 10^9$)			Transit time through epididymis * (days)		
		Caput + Corpus	Cauda	Total	Caput & corpus	Cauda	Whole epididymis
1	4.016	8.63	14.63	23.26	2.15	3.64	5.79
2	3.617	8.15	19.03	27.18	2.25	5.26	7.51
3	3.750	8.56	18.18	26.74	2.28	4.85	7.13
Mean	3.795	8.45	17.28	25.73	2.23	4.55	6.78

* Epididymal sperm reserves divided by DSP.

Discussion

DISCUSSION

The objective of the study was to assess the effect of different ejaculation frequencies on sex libido and seminal attributes of cross-bred bucks with a view to prescribe the maximum number of collections which can be taken from bucks without adversely affecting semen quality and libido. Nine adult Malabari cross-bred bucks were randomly allotted to 3 experimental groups in group I, group II and group III, from which ejaculations were taken at the rate of once daily, twice daily and thrice daily respectively. In order to assess the long term effect of higher ejaculation frequency, the duration of study was fixed as 90 days.

The effects of higher ejaculation frequency on reaction time, refractory period, colour, volume, density, mass activity, motility, pH, concentration, percentage of dead sperms, MBR time, percentage of sperm abnormality and sperm viability on incubation and preservation under chilled condition were studied. Estimations of Epididymal sperm

reserve and Daily Sperm Production by both depletion trials and testicular homogenate study were also carried out to assess the sperm reserves available for ejaculation.

Among the various seminal attributes and libido studied, ejaculation frequency was found to influence the colour, density, mass activity, concentration and MBR time. The thick creamy to creamy colour of the semen gradually changed to thin creamy and then to thick yellowish milky to thin yellowish milky with increasing frequency of collection and on progressive days of study. The change in colour could be attributed to drop in sperm cell concentration, which in turn resulted in the yellowish colour of the semen becoming prominent. There was a progressive decrease in the density score from 3.3611 $\pm$ 0.3438 in group I bucks to 2.6676 $\pm$ 0.2791 in group II bucks and 2.2209 $\pm$ 0.3332 in group III bucks. Similarly there was significant drop in the sperm cell concentration (X 10^6/cmm) from 2.8317 $\pm$ 0.8058 in group I to 1.6851 $\pm$ 0.3730 in group II and 1.3003 $\pm$ 0.3171 in group III. Though there was no significant drop in volume of semen with increasing ejaculation frequency, there was significant reduction in spermatozoan concentration. This is in confirmity with the reported findings in ram (Kastyak, 1962). In contrast drop in semen volume and sperm concentration were reported with increasing ejaculation frequency

in rams (Chang, 1945; Sharma et al., 1972). The total estimated spermatozoa available for ejaculation (X 10^9) for 90 days of study was estimated to be 358.791 which is far in excess of the spermatozoan output (X 10^9) of 150.54 for 90 days in bucks with an ejaculation frequency of 3 times daily. Still it was observed that there was significant drop in sperm concentration on increasing the ejaculation frequency from once to thrice daily. This might be attributed to the fact that a fairly high proportion of spermatozoan reserve available for ejaculation might have been lost either through epididymal resorption or voided through urine. Amann and Almquist (1962) reported that the epididymal resorption of sperms in bulls which were ejaculated as frequently as eight times weekly was 57% of the DSP. Lino et al. (1967) recovered 88% of daily sperm output, from urine of rams. Moreover, incomplete sexual stimulation due to short interval between collections and the use of teaser bucks as mounts might have also contributed to low sperm cell concentration in group II and III bucks. The observation of low spermatozoan concentration in the second ejaculates of group II and in the 2nd and 3rd ejaculates of group III bucks supports this hypothesis.

The numerical score of the mass activity of semen in group I, II and III were respectively 3.6392 ± 0.6249, 3.3645 ± 0.5193, and 2.8573 ± 0.5145, which on analysis was

found to be significantly different. The low score of mass activity with increasing collection frequency could be attributed to low sperm cell concentration in group II and III bucks. Similarly, there was significant difference in mass activity between ejaculates in group II and III possibly, on account of the differences in sperm cell concentration between ejaculates.

The MBR time in seconds of group I, II & III were found to be respectively 133.0886 ± 61.4200; 229.7969 ± 116.0773 and 312.5348 ± 104.1285 which showed significant difference between groups. MBR time was significantly more in group II and group III bucks. In the latter, there was significant increase in MBR time beyond 1st month of study. Similarly, MBR time was significantly higher in second ejaculate of group II bucks and the second and third ejaculates of group III bucks. The higher MBR time in group II and III bucks, the second ejaculate of group II and the second and third ejaculate of group III can be explained in terms of the low sperm cell concentration.

There was no significant difference between groups with respect to reaction time, refractory period, volume, percentage motility, pH, percentage of dead sperms, percentage of abnormal sperms and sperm viability on incubation for 30

minutes and preservation at 6 - 8°C for 72 hrs., even though bucks within groups and months within groups were found to influence most of the above parameters. In group II bucks the pH of 2nd ejaculate was found to be significantly higher than that of the 1st ejaculate. Similarly in group III bucks significant differences between ejaculates were noticed with respect of volume, percentage motility and pH.

The Daily Sperm Production (X 10^9) by depletion trials (DSO) and the residual sperm reserve (X 10^9) were estimated to be 1.2680 and 3.5933 respectively. This residual sperm of 3.5933 billions were seemed to become available for ejaculation, regardless of the interval or extent of depletion. Apparently this reserve became available within 24 hr. period, and the additional spermatozoa, that became available for ejaculation were dependant upon the number produced daily. There does not seem to have any reports on estimation of DSP by depletion trials in goats. The value presently recorded is found to be lower than the DSO reported in rams (Chang, 1945; Ortavant, 1958).

The average paired testicular weight in three cross-ored bucks was 171.6017 $\pm$ 6.9358 g. The Daily Sperm Production per buck (X 10^9) and DSP per gram of testis (X 10^6) have worked out to be 3.7949 $\pm$ 0.2032 and 22.1974 $\pm$ 0.3775 respectively.

Carew and Egbunike (1980) estimated the DSP in Maradi goats with a paired testicular weight of 103 g to be 752 millions. The DSP/g of testicular parenchyma in this case was found to be 15.8 millions. The higher value presently obtained could be attributed to the breed and age difference of bucks. The DSO estimated by depletion trials was found to be only 37.06% of the estimated DSP by testicular homogenate method (Table 26). This wide difference could be accounted by a sizable loss of spermatozoa by resorption in epididymis, elimination through urine and by wastage in the equipments used for collection. Almquist and Amann (1962) and Swierstra (1966) reported that only 42% and 25% respectively of the DSP in bulls was accounted by DSO on depletion trials. In contrast, 83% and 88% of the DSP were accounted in the DSO in Yorkshire and Lacombe boars respectively (Swierstra, 1968).

The mean spermatozoan reserves (X 10^9) in the caput, corpus and cauda of cross-bred bucks were found to be 3.3900 $\pm$ 0.0709 (26.26%), 0.8300 $\pm$ 0.0764 (6.45%) and 8.64 $\pm$ 1.1660 (67.18%) respectively with an overall epididymal sperm reserve of 25.72 $\pm$ 1.95. The values presently recorded are much higher than those reported in goats (Corteel, 1973; Jindal and Panda, 1981; Bhatt and

Chauhan, 1982). The higher values in the present study may be attributed to the difference in breed, age, paired testicular weight and paired epididymal weight. The average transit time of spermatozoa through epididymis in the present study was 6.78 days with 2.23 days in caput & corpus and 4.55 days in cauda. This finding is in agreement with that of Salisbury et al. (1978) who reported that the transit time in the caput & corpus to be 3.6 days and that in the cauda to be 2.4 days for 15 - 17 month old bulls.

The present findings clearly indicate that the semen of bucks ejaculated once daily over a period of 90 days did not undergo any quantitative and qualitative change. On the other hand, by increasing the frequency of collection to twice and thrice daily, a significant drop in spermatozoan concentration, density, mass activity, and an increase in MBR time were noticed. These changes could be attributed to a reduction in number of spermatozoa per unit volume. The drop in concentration took place inspite of the fact that the total sperm available for ejaculation was far in excess of the total sperms ejaculated even with three collections daily. This might possibly be on account of loss of a good proportion of the sperm reserves by epididymal

resorption and through urine. Moreover incomplete sexual stimulation due to use of teaser bucks as mounts and too short intervals between ejaculations might have also contributed to lower yield of spermatozoa in the second ejaculate of group II bucks and 2nd & 3rd ejaculates of group III bucks. The very fact that important seminal attributes such as volume, pH, motility, percentage of abnormal and dead sperms and spermatozoan viability were unaffected, clearly points out that there is no deterioration of the semen quality, with increasing collection frequency. The observation that the percentage of proximal and distal protoplasmic droplets in the semen of bucks ejaculated thrice daily did not vary between months and at the same time were well within the normal permissible limit goes to prove that extragonadal sperm reserve was not depleted. Moreover, the libido of bucks was also unaffected by increasing the frequency of collection to three times daily. Good fertility obtained with semen from bucks ejaculated thrice daily on a limited study also confirms the above finding. Since the total number of sperms harvested during the course of the study from group II and group III bucks are almost the same, there does not seem to be any definite advantage of increasing the frequency of collection from 2 to 3 times daily. But more number of spermatozoa can be

harvested for artificial insemination without significant deterioration of semen quality by increasing the frequency of collection from one to two times daily.

Summary

SUMMARY

The objective of the present study was to find out the maximum number of collections per day which can be taken from adult bucks without adversely affecting their libido and semen quality.

Nine adult Malabari cross-bred bucks, aged 2 to 3 years formed the materials for the study. The animals were randomly allotted to three experimental groups viz: group I, group II and group III with ejaculation frequencies of once daily, twice daily and thrice daily respectively. The effect of different ejaculation frequencies on libido and semen characteristics such as colour, volume, density, mass activity, motility, pH, concentration, percentage of dead sperms, percentage of abnormal sperms, Methylene Blue Reduction time and viability of spermatozoa on incubation at 46.5°C and preservation at 6 to 8°C were studied. The Daily Sperm Production of bucks was assessed both by depletion trials (DSO) and by testicular homogenate

study. The epididymal sperm reserves of sexually rested bucks were also estimated.

Among the various seminal attributes evaluated, ejaculation frequency was found to influence the colour, density, mass activity, concentration and MBR time. Frequency of ejaculation was not found to influence reaction time, refractory period, volume, motility, pH, percentage of dead sperms, percentage of abnormal sperms, and sperm viability on incubation at 46.5°C for 30 minutes and preservation at 6 to 8°C for 72 hrs. Bucks within groups were found to influence all seminal attributes except mass activity, percentage of dead sperms and MBR time. Similarly all the seminal attributes with the exception of proximal and distal protoplasmic droplets were influenced by months within groups. Libido was also influenced both by bucks within groups and months within groups.

The thick creamy to creamy colour of the semen gradually changed to thin creamy and then to thick yellowish to thin yellowish milky with increase in frequency of collection and also as the days of study progressed. The density of semen progressively decreased from 3.3611 $\pm$ 0.3438 in group I bucks to 2.6676 $\pm$ 0.2791 in group II and 2.2209 $\pm$ 0.3332 in group III bucks. The numerical score of mass

activity in group I, group II and group III bucks were respectively 3.6392 ± 0.6249, 3.3645 ± 0.5193, and 2.8573 ± 0.5145. Spermatozoan concentration (X 10^6/cmm) dropped significantly from 2.8317 ± 0.8058 in group I to 1.6851 ± 0.3730 in group II and 1.3003 ± 0.3171 in group III bucks. The MBR time in seconds of group I, II and III were found to be respectively 133.0886 ± 61.4200, 229.7969 ± 116.0773 and 312.5348 ± 104.1285.

The mean reaction time in seconds for group I, II and III bucks were 58.44 ± 38.2210, 80.2080 ± 90.4439 and 93.8514 ± 67.5135 respectively. Though there was no significant difference in reaction time between groups, both bucks within groups and months within group were found to influence reaction time. Refractory period in the case of group II bucks was found to be 283.4179 ± 643.9361 seconds, whereas in group III bucks, the first refractory period averaged 332.3418 ± 223.9995 seconds as against the second refractory period of 431.1636 ± 288.6819 seconds. There was no significant difference between refractory period of group II and group III bucks. But in group III bucks 1st and 2nd refractory periods showed significant difference ($P < 0.01$).

The mean volume of semen of group I bucks was found to be 0.4870 ± 0.1624 ml as against 0.4978 ± 0.1488 ml in group II

and 0.4288 ± 0.0910 ml in group III. Even though there was no significant difference in semen volume between groups, both bucks within groups and months within groups were seen influencing semen volume. The mean percentage motility of spermatozoa in group I, II and III were 82.1795 ± 5.9695, 79.9652 ± 7.9659 and 76.0796 ± 9.8700 respectively. Only bucks within groups and months within groups were observed to influence the mean percentage sperm motility.

The pH of semen in group I, II and III bucks were 6.8652 ± 0.0853, 6.9610 ± 0.1106 and 7.0864 ± 0.1243 respectively. The pH of semen was not found to be influenced by ejaculation frequency although both bucks and months within group were found to influence the pH of semen. The mean values for percentage of dead sperms in the semen of groupI, II and III bucks were 9.2485 ± 3.9017, 8.8778 ± 4.7096 and 8.9239 ± 4.4881 respectively. Even though the frequency of ejaculation was not found to influence the sperm abnormality, months within groups were found to affect the total sperm abnormality. Percentage of head abnormality, tail abnormality, proximal droplets and total abnormality were recorded to be respectively 0.4918 ± 0.4997, 1.2732 ± 1.8903, 0.1366 ± 0.4032, 1.8689 ± 1.8139 in group I as against 0.5625 ± 0.7244, 2.1393 ± 1.8137, 0.2115 ± 0.3941 and 3.3306 ± 4.8591 in group II and 0.5095 ± 0.6750, 1.9881 ± 1.9834, 0.2352 ±0.5146

and 2.6734 ± 2.1503 in group III bucks. The percentage of distal protoplasmic droplets in group I, II & III bucks were respectively 0.7104 ± 1.9438, 2.3525 ± 5.4651 and 0.6052 ± 1.2711. Only bucks within group was found to influence the percentage of proximal and distal droplets. There was no significant difference in sperm viability on incubation at 46.5°C for 30 minutes and preservation at 6 to 8°C for 72 hours between groups, eventhough, the initial motility was significantly different ($P/\_ 0.05$). The percentage motility at 96 hrs. of preservation at 6 to 8°C in group I, II & III bucks were respectively 34.5082 ± 25.2274, 35.1318 ± 24.6637 and 22.7193 ± 24.8040 which showed a significant difference ($P/\_ 0.05$). A positive correlation was observed between incubation for 30 mts. at 46.5°C and preservation for 96 hrs. at 6 - 8°C. A conception rate of 73.37% and a kidding rate of 66.67% was recorded using semen from bucks ejaculated thrice daily.

While in group II bucks only density, mass activity, pH, concentration and MBR time showed significant difference between ejaculates, in group III bucks all seminal attributes except percentage of dead sperms varied significantly between ejaculates.

The Daily Sperm Production ($X\ 10^9$) based on depletion trials (DSO) and the residual sperm reserves ($X\ 10^9$) were estimated to be respectively, 1.2680 and 3.5933. The Daily

Sperm Production per buck (X 10^9) and per gram of testis (X 10^6) based on testicular homogenate study were found to be 3.7949 ± 0.2032 and 22.1974 ± 0.3775 respectively. The DSO was found to be only 37.06% of the DSP.

The mean spermatozoan reserves (X 10^9) in caput, corpus and cauda of cross-bred bucks were found to be 3.3900 ± 0.0709 (26.26%), 0.8300 ± 0.0764 (6.45%) and 8.64 ± 1.1660 (67.18%) respectively with an overall epididymal sperm reserve of 25.72 ± 1.95. The average transit time of spermatozoa through epididymis was found to be 6.78 days with 2.23 days in caput & corpus and 4.55 days in cauda.

The present finding clearly indicated that the semen of bucks ejaculated once daily over a period of 90 days did not show any deterioration in quality. On the other hand by increasing the frequency of collection to twice and thrice daily, a significant drop in spermatozoan concentration, density, mass activity and an increase in MBR time were noticed. The deterioration of the above seminal attributes could be due to a reduction in number of spermatozoa per unit volume. The drop in concentration took place inspite of the fact that sperms available for ejaculation was far in excess of the total sperms ejaculated even with three collections daily. This might be possibly on account of loss of a good proportion of the sperm reserves by epididymal

resorption and through urine. Moreover, incomplete sexual stimulation due to use of teaser bucks as mounts and too short intervals between ejaculations might have also contributed to the lower yield of spermatozoa in the second ejaculate of group II bucks and 2nd and 3rd ejaculates of group III bucks. The observation that the percentage of proximal and distal protoplasmic droplets in the semen of bucks ejaculated thrice daily were well within the permissible limit and did not vary between months goes to prove that there was no depletion of the extragonadal sperm reserves. The very fact that important seminal attributes such as volume, pH, motility, percentage of abnormal and dead sperms and spermatozoan viability were unaffected clearly points out that there is no deterioration of semen quality with increasing frequency of collection. Moreover, libido of bucks was also unaffected by increasing the frequency of collection to three times daily. Good fertility obtained with semen from bucks ejaculated thrice daily on a limited study also confirmed the above findings. Since the total number of sperms harvested during the course of the study, from group II and group III were almost the same, there does not seem to have any definite advantage of increasing the frequency of collection from 2 to 3 times daily. But more number of spermatozoa can be harvested for artificial

insemination without significant deterioration of libido, semen quality and fertility by increasing the frequency of collection from one to two times daily.

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EFFECT OF FREQUENCY OF EJACULATION ON SEMEN CHARACTERISTICS AND LIBIDO IN CROSS - BRED BUCKS

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ABSTRACT

The objective of the study was to find out the optimum number of collections per day which can be taken from adult cross-bred bucks without adversely affecting their libido and semen quality. Ejaculation frequencies of once, twice and thrice daily for a continuous period of three months were adopted for the study. Epididymal sperm reserves and Daily Sperm Production were estimated to assess the sperm reserves of bucks. While colour, density, mass activity, concentration and MBR time were found to be influenced by ejaculation frequencies, volume, pH, percentage of dead sperms, percentage of abnormal sperms and sperm viability were unaffected. Frequency of ejaculation was not found to affect the libido of bucks. Bucks within group were found to influence all seminal attributes except mass activity, percentage of dead sperms and MBR time. Similarly all the seminal attributes with the exception of proximal and distal protoplasmic droplets were influenced by months within groups. A positive correlation between sperm viability at 46.5^{o}C for 30 minutes and preservation at 6 to 8^{o}C for 96 hrs. was observed. While in group II bucks only density, mass activity, pH, concentration and MBR time showed significant difference between ejaculates, in group III, all seminal attributes except percentage of dead sperms were observed to be significantly different between ejaculates.

The Daily Sperm Production per buck (X 10^9), per gram of testis (X 10^6), Daily Sperm Output (X 10^9) and dpididymal sperm reserves (X 10^9) were respectively 3.7949 $\pm$ 0.2032, 22.1974 $\pm$ 0.3775, 1.2680 and 25.72 $\pm$ 1.95. The average transit time of spermatozoa through epididymis was found to be 6.78 days. Increasing the frequency of collection from once daily to twice or thrice daily resulted in a significant drop in spermatozoan concentration, thus affecting seminal attributes such as colour, density, mass activity and MBR time. The very fact that other important seminal attributes such as volume, pH, motility, percentage of abnormal sperms, percentage of dead sperms, percentage of proximal and distal protoplasmic droplets and spermatozoan viability were unaffected, clearly points out that there is no deterioration of semen quality with increasing collection frequency. Similarly there was no deterioration in the sex libido and fertility of bucks even with three collections daily. However, there does not seem to be any definite advantage in increasing collection frequency from 2 to 3 times daily, as the total harvest of sperms from group II and group III were almost same. But increasing the frequency of collection from one to two times daily has definite advantage, as it yields more spermatozoa for artificial insemination and hence is recommended for adoption.