CHANGES IN SPERM MORPHOLOGY OF CROSSBRED BULLS DURING PRESERVATION

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "CHANGES IN SPERM MORPHOLOGY OF CROSSBRED BULLS DURING PRESERVATION" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

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Certified that this thesis, entitled "CHANGES IN SPERM MORPHOLOGY OF CROSSBRED BULLS DURING PRESERVATION" is a record of research work done independently by Sri. Pronab Kumar Duarah, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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DEDICATED TO THE MEMORY
OF
MY BELOVED FATHER
GOLAP CHANDRA DUARAH

INTRODUCTION

INTRODUCTION

The National Commission on Agriculture have targetted an annual milk production of 64.40 million tonnes by 2000 AD and to achieve this ambitious goal a much faster pace for intensive cattle development has been duly recommended. The most important and predominant factor responsible for the poor performance of our indigenous cattle is heredity. To overcome this, crossbreeding with exotic temperate breeds such as Jersey, Brown Swiss and Holstein Friesian has been accepted as a major tool. This offers a broad genetic basis from which, by a process of rigorous selection and appropriate breeding system, new gene combinations can be made for specific purposes.

The breeding policy of our country suggests that the percentage of exotic inheritance has to be limited between 50 and 62.5 for achieving superior milk production traits. Further when adaptability and longevity are considered half-breds excel all other levels of crossbreds. To maintain this level of exotic inheritance crossbred bulls have to be extensively used for Artificial Insemination. The maximum utilisation of outstanding sires in artificial breeding depends partly upon the frequency with which they are ejaculated and partly upon the preservation of maximum fertility of spermatozoa. Several extenders ensuring high fertility of spermatozoa under chilled conditions for a

period of three to four days have been successfully evolved (Salisbury et al., 1941; Pursley and Herman, 1950; Almquist et al., 1954; Kerruish, 1956; Adler and Rasbech, 1956 and Foote, 1970).

There are reports to indicate that sperm abnormalities occur during preservation in different extenders. A negative correlation between abnormal sperm count and fertility was noted in the Holstein-Frisian-Ongole and Brown Swiss Ongole crossbred bulls by Rao and Rao (1979). Khan and Kharche (1983) reported on the semen characteristics and fertility of Jersey bulls in tropics. Alterations in the acrosomal structure has been reported to be the most important ultrastructural change of spermatozoa affecting fertility (Sattar, 1973). The relevance of sperm morphology in respect of fertility has been adequately stressed in several reports. However, studies on morphological changes of spermatozoa of crossbred bulls during preservation in different extenders which has a definite correlation to fertility appear to be scanty.

In the present study, an attempt has been made to assess the various morphological changes of spermatozoa of crossbred bulls in different extenders at various intervals during preservation.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Attempts made to evolve an accurate and objective test for assessing the potential fertility of a bull on the basis of some specific characteristics of a given semen sample have so far not been successful. The consensus of opinion prevailing is that a combination of characteristics such as ejaculate volume, initial motility, concentration and livability of spermatozoa and the magnitude of incidence of spermatozoan abnormality will serve the purpose of a single sure test to select bulls of high fertility from a mixed herd (Maule, 1962). Further, information regarding spermatozoan abnormality and mensuration during preservation and storage in different extenders, though available in exotic bulls, is very meagre in crossbred bulls. However, the various observations made are briefly reviewed.

The mean concentration of sperms for the first and second ejaculate in the exotic breeds of cattle has been shown as 1.259 and 1.281 million per cmm respectively (Salisbury, 1944). The concentration of spermatozoa per ml of semen in the case of Bos taurus bulls has been reported as 1108 millions by Blom (1950). The sperm count in Brown Swiss bulls has been found to be around 1.396 millions per cmm (Mathew, 1974). Rao and Rao (1975) found 1296 millions sperms per ml of semen in Bos taurus bulls. A sperm count of 1472 millions per ml of ejaculate has been reported in Jersey-Sindhi crossbred

bulls by Rao and Reddyya (1977). Rao and Rao (1978) have recorded 994.93 and 611.84 millions sperms per ml of semen in the case of Brown Swiss-Ongole and Holstein Friesian-Ongole crosses, respectively. Rao and Rao (1980) found the sperm concentration in the ejaculate of Ongole bulls as 765.90 million per ml. Raja (1981) reported a sperm concentration of 1599.64 ± 55.371 millions per ml of semen in case of Brown Swiss cross-bred bulls. He also found that there was no significant variation between bulls in respect of physical characteristics of semen and sperm concentration in Brown Swiss crossbred bulls.

The average percentage of live spermatozoa in the semen of Hariana bulls has been reported to be 77.9 by Bishop et al. (1954) and 70.1 by Bratton et al. (1956). The percentage of live sperm in the ejaculate of Hariana bulls has been recorded as 70.9 by Tomar et al. (1966) and 80.6 by Singh et al. (1967). The live sperm count in the ejaculate of Holstein Friesian-Ongole and Brown Swiss-Ongole bulls was reported as 65.20% and 67.22%, respectively by Rao and Rao (1979). Sarma and Tripati (1978) recorded 83.07 per cent live sperm in the semen of Jersey-Sahiwal crossbred bulls.

Significant seasonal variation in the percentage of live sperm per ejaculate has been observed in exotic and Hariana bulls by several workers (Tomar et al., 1966 and Rao and Rao, 1979).

Bishop et al. (1954), Brown (1959) and Almquist et al. (1963) have reported that the average percentage of initial motility of sperm in the ejaculate of Bostaurus bulls were 83%, 63% and 65% respectively. The initial motility of sperms in Brown Swiss-Ongole and Holstein Friesian-Ongole was observed to be 84.13% and 80.80% respectively (Rao and Rao, 1976) and in Jersey-Sahival, 71.17% (Saxena and Tripati, 1978). According to Raja (1981) the initial motility of sperms in Brown Swiss crossbred bulls was 66.24 ± 0.874%. He observed no significant variation in initial motility of sperms between Brown Swiss crossbred bulls.

The basic characters of a diluting fluid of semen sample are (a) Osmotic tension, (b) pH, (c) buffering capacity, (d) non-toxicity and (e) a correct balance of electrolytes and non-electrolytes, cations and anions (Anderson, 1945). Stability of the diluent even after prolonged storage, presence of substrates to support the reduced metabolism of the stored spermatozoa and absence of any detrimental or inhibiting effect of antibiotics are also essential features of diluting fluids. The diluent should be easy to prepare and cheap, permit the microscopical examination of spermatozoa in a clear fluid and render no difficulty in the cleaning of glasswares or other semen containers (Maule, 1962).

Salisbury et al. (1941) described egg yolk citrate as semen diluent which could give a clear picture for microscopic

assessment of motility without causing any adverse effect on sperm metabolism. Equal volumes of egg yolk and of either a 3.6 or a 2.9% solution of sodium citrate (dihydrate) were used for preparing this diluent. Although Salisbury et al. (1948) and Aschaffenburg (1950) showed that a 2.9% citrate concentration was isotonic with semen, Melrose and Stewart (1956) obtained equally satisfactory conception rates with equal volumes of either 2.9 or 3.6% sodium citrate solutions and egg yolk.

The use of a reduced quantity of yolk was studied by Stewart et al. (1950), Almquist (1951a) and Holt (1952) and reported that fertility was not affected even when the yolk concentration was reduced to 20%. Albright and Erb (1958) studied the desirability of the egg yolk-citrate (YC), whole milk (WM), skim milk (SM), and egg yolk-glycine (YG) and their combinations based on progressive motility of spermatozoa after diluting semen at the rate of 1:24 and storing at 5°C. It was found that YC, WM, SM and YG lowered sperm motility after extension at day 3 and day 7 of storage ($P < 0.01$) compared with their various combinations. Combinations of WM, YG and SMYG showed higher average motility on day 7 ($P < 0.01$) than did WMYC, SMYC, WMSM or YCYG. YCYG was superior to either YC or YG alone. WM, SM and WMYC had higher motility after extension ($P < 0.05$) than the same extender containing glycerol. They also reported that

addition of 2.9% glycine enhanced the ability of sperm to maintain progressive motility under storage at 5°C, when extended with WM, SM or YC.

Glycerol has a definite depressing effect on lactic acid production by spermatozoa when diluted with commonly used semen diluents such as heated skim milk or egg yolk-citrate. According to O'Dell et al. (1959) glycerol would possibly depress glycolysis by bull spermatozoa.

Foote and Bratton (1960) reported that a yolk-citrate-glucose-glycine-sulfanilamide-antibiotic extender, designated as CU-16, maintained 49 and 52 per cent of motile spermatozoa over a 12 day storage period at 5°C in two experiments.

Tomar and Desai (1961) found that egg yolk-glucose-glycine-sodium citrate and egg yolk-glucose-fructose-sodium citrate were superior than egg yolk-sodium citrate and egg yolk-glycine-sodium citrate in maintaining motility and livability of buffalo semen at 5°C and 7°C. They also reported that the presence of glucose, fructose and/or glycine is indispensable for the preservation of buffalo bull semen. The greater need of metabolizable sugars by the buffalo bull spermatozoa for their preservation might be due to the comparatively higher rate of fructolysis by them.

When 10 or 13% glycerol was incorporated in skim milk diluent by gradual addition to partially diluted semen at 5°C,

spermatozoan livability during 14 days storage at 5°C was higher than in the absence of glycerol. These glycerol levels prevented the sharp decline in motility observed between the 4th and 6th days of storage for semen diluted in skim milk diluent without glycerol (Alnquist, 1962). Foote (1962) reported that the addition of 1 to 100 ug of catalase per milliliter of yolk extender substantially improved the survival of bull sperm stored at 5°C, but they also found that additional amount of catalase did not improve sperm survival and tended to increase clumping of sperms. Kale (1963) devised five diluters for preservation of buffalo semen using varying proportions of whole milk, egg yolk, glucose, sodium bicarbonate and sodium citrate dihydrate. He observed that milk diluents in general were able to maintain a + 3 motility of spermatozoa over three days and that it could form a suitable media for semen diluents replacing egg yolk. Grewal et al. (1969) investigated on the comparative efficiency of different diluents for the preservation of semen of different genetic groups. They reported that half life (number of days for which 50 per cent motility is presented) was maintained for 3, 6, 8.8 and 8.3 days in egg yolk citrate diluent; 3, 5.8, 8 and 8 days in milk diluent; 4, 8.7, 11.1 and 10.7 days in glucose-sodium bicarbonate-sulphanozathine diluent; 4, 6.4, 9.4 and 9.8 days in milk with glycine diluent for buffalo, zebu, crossbred and Red Dane bulls, respectively. They also found that egg yolk-

citrate-milk-glycine diluent was best for buffalo bull semen whereas glucose-soda bicarb diluent was ideal for Zebu cross-bred and Red Dane bull semen. Abhi and Grewal (1971) studied the keeping quality of buffalo semen in three different diluents at three dilution rates. They found that at 4 to 6°C sodium citrate (EYC) diluent was much inferior to Foote's (CUE) and Glycine (EYG) diluents on all the three days of storage. They also found that Foote's (CUE) diluent was slightly superior to glycine on the 1st day of storage while both were of nearly equal value during 2nd day. However, the glycine was more efficient for storage period beyond 2nd day. There were no significant differences in motility during storage in dilution rate of 1:10 and 1:20 but there were significant differences between 1:10 and 1:50 and between 1:20 and 1:50 dilutions. They also reported highly significant differences between dilutors and dilutions and the effect of dilutions was independent of the diluents. Foulkes and Stewart (1977) found that the percentages of non-return rate of cows at 16 weeks were 66.4, 67.1 and 66.1 when semen frozen in the egg yolk-lactose, egg yolk-citrate and lipo-protein citrate diluent respectively were used.

Significant differences in motility of chilled goat semen stored for 12 days at 6°C diluted in CUE and egg yolk-citrate diluent have been reported by Koh and Ong (1977).

Park and Hunter (1977) studied the immunological aspect

of diluent containing egg yolk and reported that under routine conditions of artificial insemination, three to four exposures to extenders with or without egg yolk did not affect subsequent fertility adversely.

Leka and Rao (1980) reported that Tris -egg yolk-glucose and milk extenders were significantly superior to egg yolk-citrate extender in maintaining spermatozoan motility of ram semen upto 72 hr.

Watson (1981) studied the role of lipid and protein in the protection of ram spermatozoa at 5°C using egg yolk lipoprotein and reported that the phospholipid of low density lipoprotein fraction (LDF) of egg yolk provides the protection to the sperm cell membrane. The protein of the LDF was reported to solubilize the lipid and would bind it to the cell membrane.

Antiserum raised in rabbits against an egg yolk lipoprotein fraction, used in conjunction with alkaline-phosphatase-conjugated goat anti-rabbit IgG to demonstrate lipoprotein interaction with bovine spermatozoa showed that lipoprotein bound firmly to spermatozoa and was not removed by extensive washing, suggesting that the lipoprotein had become irreversibly associated with the sperm membrane (Cookson and Foulkes, 1984). Since skim milk is considerably cheaper, its use has appealed to many commercial organisations. Almquist et al. (1954), Kerruish (1956), Adler and Rashbach (1956),

and Melrose (1956) reported that heat treated fresh skim milk was as effective as yolk-citrate diluent. Melrose et al. (1956) reported that powdered skim milk was as effective as fresh skim milk as diluent. But they also cautioned about unexplainable differences in conception due to differences in preservation time and reported better results by antibiotic addition to skim milk diluent and advocated the need for using a powder from a reliable source. Platett et al. (1956) reported that bovine semen extended in egg yolk-citrate-glycerol, heated, homogenized whole milk-glycerol and heated skim milk-egg yolk-glycerol and stored at 5°C maintained a motility rating of one or above (0-4 scale) for 5, 6 and 9.5 days respectively. Rajamahendran et al. (1979) opined that citric acid whey and milk-egg yolk diluent have better preservability for buffalo spermatozoa than egg yolk citrate diluent.

Barnett and Van Demark (1961) reported that amino buffers like Tris had been used effectively for maintaining physiological pH and that they acted by direct titration of hydrogen ions without immediate reduction in carbondioxide content. Further, the capacity to produce equivalent extra and intracellular pH changes suggested its intracellular penetration. Between Tris phosphate diluent and Glucose-yolk-citrate diluent, Lopatko (1971) observed no significant difference in sperm survival rate during preservation of ram

semen. Balakrishnan (1979) reported an average motility of 75.39, 62.97, 49.90, 37.42 and 19.30 per cent, respectively, after 24, 48, 72, 96 and 120 hours of storage for buck semen diluted in Tris at a rate of 1:10 and preserved at 5°C. Mathew et al. (1984) studied 18 combinations of 0.2 molar Tris diluent with six varying levels of egg yolk at three levels of pH at the temperature of 3° to 5°C and 6°C to 8°C for buck semen and found that diluents containing 20 per cent and 25 per cent egg yolk were superior at seventh day of preservation at 3° to 5°C with an average motility of 58.55 per cent and 61.29 per cent respectively. The pH levels were not significant in maintaining motility. Further, Tris diluent with five per cent egg yolk with pH 7 was found to be superior to diluents containing higher percentage of egg yolk for preservation at 6° to 8°C.

According to Davis et al. (1963) survival of bovine spermatozoa in 0.2 and 0.25 molar Tris-buffered yolk glycerol (TYG) extender was superior to that in a standard citrate-yolk-glycerol (CYG) extender at 5°C, -25°C and -85°C. They noted that semen with 62 per cent average initial motility in 0.2 and 0.25 molar Tris-yolk-glycerol, respectively maintained 50 and 48 per cent average motility after eight days of storage at 5°C. When bovine spermatozoa were preserved in 20 per cent yolk-glycerol extenders with 0.15, 0.20 and 0.25 molar Tris and adjusted with citric acid to pH of 6.5,

6.75 and 7.00, optimum sperm survival was found in the 0.2 molar extender with a pH of 6.75. It was also found that in 0.2 molar Tris extender with a pH of 6.75, average motility was 62 per cent after eight days of storage at 5°C, compared with 38 per cent in Cornell University extender (CUE) and 18 per cent in a TYC extender.

Pesce (1970) found that a 0.20 molar Tris-yolk extender buffered to pH 6.50 or 6.75 was superior to 0.2 molar with pH 6.25 and 0.25 molar with pH of 6.25, 6.5 and 6.75 Tris extender in preserving motility of bull spermatozoa.

Edwin et al. (1975) also noticed that the motility rate and percentage of live sperm in cattle and buffalo semen preserved in Tris-yolk-glycerol extender for a period of 24, 48, 72, 96 and 120 hours of storage at 5 ± 1°C were higher than those preserved in egg yolk-citrate and sodium bicarbonate glucose extenders and the differences were highly significant.

There is divergence of opinion as to the importance of the presence of varying number of abnormal sperms seen in stained semen preparations. Lagerlof (1934) observed lowered fertility with more than 17% of abnormal spermatozoa in the ejaculate. Similar observations were made by Davis et al. (1940) and Anderson (1941).

May (1949) reported an average of 15.1% of abnormal sperm within a range of 6-26% in the case of normal fertile

exotic bulls. In the opinion of Hancock (1959), the presence of 10% or more of any single type of abnormality is often associated with reduced fertility.

Tomar et al. (1971) reported an overall average of 9.37 ± 0.66% abnormal sperm studied in different season in Hariana bulls.

Total abnormality of sperms in the ejaculates of Jersey, Guernsey, Jersey-Ongole and Brown Swiss-Sahiwal bulls were reported to be 10.65%, 11.46%, 38.95% and 43.26% respectively (Rao and Reddyya, 1974). Rao and Rao (1975) observed that the frequency of occurrence of head abnormalities, free loose head, proximal protoplasmic droplets, mid piece abnormalities and tail abnormalities in Jersey bulls was 11.74%, 3.75%, 2.20%, 1.20% and 15.47% respectively. The percentage of sperm abnormalities in respect of Hariana-Holstein Friesian, Hariana-Brown Swiss and Hariana-Jersey were observed to be 14.36, 13.09 and 16.34 respectively (Sharma et al., 1976). The mean percentage of abnormal sperm in Jersey-Sahiwal crossbred bulls was 18.93 (Saxena and Tripathi, 1979). The head abnormalities, free loose head, mid piece abnormalities, tail abnormalities and proximal protoplasmic droplets were 9.46%, 2.9%, 0.74%, 3.02% and 2.66%, respectively in the case of Brown Swiss-Ongole crosses and 19.66%, 6.13%, 0.92%, 3.03% and 5.06%, respectively in the case of Holstein Friesian-Ongole crosses (Rao and Rao, 1978). In Ongole bulls aged

3 years, the percentages of head abnormalities, free loose head, mid piece abnormalities, tail abnormalities and proximal protoplasmic droplets were reported to be 7.13, 2.76, 0.75, 2.87 and 1.74, respectively (Rao and Rao, 1980). In case of Brown Swiss crossbred bulls the average sperm head abnormalities, free loose head, middle piece abnormalities, tail abnormalities and proximal protoplasmic droplets were found to be 3.43 ± 0.300%, 4.91 ± 0.439%, 0.65 ± 0.45%, 6.47 ± 0.775% and 1.20 ± 0.148%, respectively (Raja, 1981). Red Dane-Kathi crossbred bulls had higher percentage of sperm abnormalities than Red Dane bulls (Bhatt and Chauhan, 1982).

In the case of Brown Swiss-Ongole, Brown Swiss, Jersey and Ongole bulls, Raju and Rao (1982) recorded respective percentages of 8.69, 6.75, 3.77 and 4.77 sperm head abnormalities; 6.96, 1.60, 0.83 and 3.28 free loose heads; 1.06, 0.60, 0.45 and 0.60 proximal protoplasmic droplets; 0.43, 0.33, 0.40 and 0.33 mid piece abnormalities and 6.31, 5.72, 5.50 and 4.95 tail abnormalities. Raja and Rao (1983) found significant variation between bulls in the incidence of head abnormalities, free loose head, mid piece abnormalities, tail abnormalities and proximal protoplasmic droplets in case of Brown Swiss crossbred bulls with 62.5% Brown Swiss inheritance.

Haq (1949) and Rollinson (1951) are in near agreement that the normal fertile bulls should not have more than

3 to 4% abnormal head, 4 to 10% abnormal mid piece, 0.5 to 2% abnormal tail and 0.5 to 6% free loose head. Van Dongen and Salisbury (1962) reported that during storage at 5°C and 27°C in egg yolk-citrate diluent, the spermatozoan head shape changed from an elongated to a more rounded form. They did not however find any significant differences between bulls in the total length, base width and maximum width and informed that storage at 27°C significantly increased the sperm head area. In contrast there was no change when stored at 5°C.

Tomar et al. (1964) studied the morphological characteristics of bovine spermatozoa during preservation and reported that the normal length and breadth of spermatozoan head of Hariana and Murrah bulls were 9.220 µ and 7.656 µ, and 5.271 µ and 5.003 µ, respectively. They further reported that the head length of spermatozoa of Hariana bull decreased significantly after preservation for 5 days at 5°C.

According to Healey (1969) the ultrastructure of bull spermatozoa was almost identical before and after freezing, but ram and chinchilla spermatozoa showed consistent damage to the outer membrane and acrosome complex. In the ram damage ranged from slight swelling of the acrosome to total removal of the cytoplasmic regions. In boar spermatozoa also, gross damage to the acrosome was observed during freezing.

Mukherjee and Kumar (1971) studied the morphology of spermatozoa of Hariana bulls in relation to fertility, and reported that of all the different characteristics only the head length showed highly significant correlation with the conception rate. They concluded that of the remaining characteristics, head breadth and mid piece length appeared to be of least importance in predicting the fertilising capacity of bull spermatozoa.

According to Toelsron et al. (1977) about 50% of ram spermatozoa were damaged only by dilution and cooling, the damage due to freezing and thawing consisted mainly of moderate bubbling and swelling of the acrosome.

According to Kumar et al. (1977) the correlation coefficients between spermatozoan head length, head breadth and head shape with conception rates were 0.33, -0.10 and 0.50 respectively and they opined that spermatozoan head shape might be a better criterion in predicting the fertilising capacity of buffalo bull spermatozoa.

Tomar et al. (1980) reported that there was no significant variation in sperm head biometry as a result of storage at 5°C for a period of 48 hours when extended in egg yolk-glucose-fructose-sodium bicarbonate and in D_2-CO_2 medium. It was also observed that the overall fertility of bull was not significantly altered from each other and concluded that

there was no relationship between fertility of bulls and the sperm head measurements.

Patil and Raja (1973) reported that the mean length, width and width at base of sperm head were 8.39, 4.37 and 2.45 microns respectively in the case of spermatozoa of Malabari buck. They also reported that, length from anterior cap to the greatest width, length and width of middle piece and length of tail were 3.73, 11.01, 1.06 and 41.35 microns respectively.

Khan et al. (1977) studied the comparative efficacy of EYC, EYCG (Egg-yolk-sodium citrate-glycine), SMC and skim milk diluent to preserve cytomorphological characteristics of buffalo spermatozoa. They found that EYCG, SMC and skim milk diluents were more efficient in preserving quantitative morphological characteristics of spermatozoa for a period of 72 hrs at 5°C than that of EYC diluent.

Breeddermen and Foote (1969) studied the effect of osmolarity of solution upon the cell volume of spermatozoa of bull and reported that spermatozoan volume decreased in hypertonic medium where as the volume increased in the hypotonic medium.

Wells and Awa (1979) reported the efficacy of Wells-Awa stain which effectively elucidated the acrosome of bull, ram, boar, stallion, and rabbit sperm. The stain, a combination

of eosin B and fast green FCF, was effective in revealing acrosomal characteristics of spermatozoa in both fresh and frozen semen samples. The presence or absence of the acrosome could be determined and degree of attachment and condition such as swollen or disintegrating, could be identified.

Watson (1975) reported the efficacy of Giemsa stain to detect changes in acrosome of frozen ram spermatozoa.

Dott (1969) found that when the acrosome is lost as a result of cold shock, the equatorial segment is raised above the level of its surroundings and a series of holes or indentations appear at the posterior border.

The incidence of acrosomal abnormalities increased with the advancement of storage period in all the four diluents viz., Russian dilutor (RD), Glucose citrate (GC), Russian dilutor-Catalase (RD-C) and Glucose citrate-Catalase (GC-C) when stored at ambient temperature (20-28°C) in dark place for 120 hr. The increase in abnormalities, however, was minimum in RD-C followed by RD, GC and GC-C dilutor (Chaturvedi et al., 1978). Rao and Rao (1979) studied the morphology of bovine spermatozoa during preservation and storage in three different diluents, i.e., egg yolk-citrate, Tris buffer and citric acid whey and observed no significant differences in the incidence of head abnormalities between fresh and stored semen, while a significant increase was

observed in the case of tail abnormalities. They could not find any significant differences in either the head or tail abnormalities between the diluents.

MATERIALS AND METHODS

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Five healthy crossbred bulls in the age group of three to seven years which were maintained under identical conditions of feeding and management in the artificial insemination centre attached to the College of Veterinary and Animal Sciences, Mannuthy formed the experimental animals for the study. Semen samples were collected from these bulls by artificial vagina method (Perry, 1969) during the period from May, 1985 to September, 1985. Collections were made once in a week from each bull and 72 samples so collected were utilized for the study. Immediately after collection these semen samples were subjected to routine evaluation for colour, volume, density, mass activity, pH and motility as per the standard procedure (Robert, 1971).

To determine the percentage of live sperms, nigrosin-eosin stained smears were prepared (Campbell et al., 1956).

Percentage of different types of sperm abnormalities was assessed by using Giemsa stain and was classified as described by Blom (1972). The concentration of sperm was estimated haemocytometrically.

To study the effect of preservation on morphology and motility of sperms, the semen samples were diluted in egg yolk-sodium citrate, fresh skim milk and Tris diluent at room temperature in the ratio of 1:20 and after gradual cooling stored in the refrigerator at 5°C.

Preparation of diluents

1. Egg yolk-sodium citrate diluent (EYC)

Egg yolk citrate diluent was prepared by adding 2.9 g of sodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$) to 100 ml of double distilled water and mixing it at a rate of one part egg yolk to three parts of buffer solution. To this solution 1000 units of crystalline penicillin and 1000 micrograms of dihydrostreptomycin per ml were added. The EYC diluent was prepared afresh for each collection.

2. Skim milk diluent

Cow' milk was centrifuged for 30 minutes for skimming. The fresh skim milk so obtained was heated at 92°C for five minutes, filtered and colled down gradually. Penicillin and streptomycin were added at the rate of 1000 units and 1000 microgrms per ml of diluent respectively.

3. Tris diluent

Tris diluent was prepared by adding 3.028 g tris (hydroxymethyl) aminomethane (0.20 M Tris at pH 6.76), 1.25 g fructose, 1.675 g citric acid monohydrate and 8 ml glycerol to 92 ml of glass distilled water. Then egg yolk was added at the rate of 1 part egg yolk to 4 parts of buffer solution and mixed properly with the help of magnetic stirrer. Then antibiotics were added at the rate of 1000 IU of penicillin and 1 mg of streptomycin per millilitre of the diluent.

Immediately after dilution wet smears were prepared from each of the sample and air dried for 5 to 10 minutes. Then the smears prepared from egg yolk citrate and Tris diluent were fixed for 10-20 minutes in buffered formal saline (Campbell et al., 1960). Smears were washed gently in tap water for one minute. After air-drying the smears were stained in Giemsa stain by immersing the slides for 3 to 4 hours (Hancock, 1952; Watson, 1975). Stained slides were washed in distilled water and air dried.

The dried smears prepared from skim milk diluent were immersed in isopropyl alcohol for 20 to 30 minutes for defattening, allowed to air dry and then stained with Giemsa stain as in the case of smears prepared from egg yolk sodium citrate and tris diluent. Later the stained smears were examined under oil immersion objective of a microscope. A minimum number of one hundred sperms were counted from each of the slide to study the morphological abnormalities and acrosome defects.

The sperm head biometry was studied with the help of an ocular micrometer eye piece whose calibration was done using a stage micrometer under oil immersion objective of a compound microscope.

The semen smears stained in Giemsa stain prepared before and after dilution in three different dilutors to study the

sperm abnormalities were used to measure the length and breadth of the sperm head. Like sperm abnormality sperm head biometry was also studied before dilution and immediately after dilution. To study the effect of preservation in the above three diluents, semen samples diluted and stored in the refrigerator were taken at 24, 48 and 72 hours of preservation and the motility was assessed under the high power objective of a microscope. Morphological study and sperm head biometry were also assessed during the above periods of preservation.

The data were analysed statistically to see whether there was any significant variation in motility, morphology and biometry of sperm head during preservation and to compare the efficacy of the different diluents in preserving characteristics of sperms towards normal. Data pertaining to different bulls were also analysed to assess any individual difference between the five crossbred bulls.

RESULTS

RESULTS

The results of investigation on the semen characteristics of crossbred bulls and the various changes observed during preservation in egg yolk-citrate, Tris and skim milk diluent at 0, 24, 48 and 72 hours of storage are presented in table 1 to 35.

The perusal of data presented in table 1 would reveal a mean sperm concentration of 1.53, 1.55, 1.60, 1.62 and 1.68 millions per cmm with an overall mean of 1.59 ± 0.025 millions. Analysis of data revealed no significant difference in sperm concentration between the bulls (Table 2).

The mean live sperm percentage was noted to be 84.45, 83.55, 82.75, 84.58 and 81.81 with an overall mean of 83.45 ± 0.522 (Table 3). On analysis, no significant difference was observed in live sperm percentage between the bulls (Table 4).

It could be seen from table 5 that the percentage of motile sperm averaged 83.00 ± 1.00 per cent immediately after dilution in EYC, Tris and skim milk diluent. The values decreased gradually upto 72 hours of storage with a mean of 57.10 ± 0.4, 58.00 ± 0.689 and 55.80 ± 1.11 per cent respectively in the above three diluents. On analysis (Table 6) highly significant ($P < 0.01$) difference in the percentage of motile sperm was observed between bulls and between different

storage periods. However, no significant difference in motility was observed during preservation between various diluents. Sperm motility was also not altered significantly by animal x diluent x storage period interaction.

The mean value for total sperm abnormalities before dilution was 16.68 ± 0.590 per cent (Table 7). The values after diluting in egg yolk-citrate were 17.48 ± 0.661, 17.78 ± 0.772, 18.46 ± 0.674 and 19.24 ± 0.558 per cent respectively at 0, 24, 48 and 72 hours of storage. The values obtained during preservation in Tris and skim milk at 0, 24, 48 and 72 hours of storage were 16.92 ± 0.595, 17.28 ± 0.655, 18.30 ± 0.540, 18.96 ± 0.620 and 17.66 ± 0.720, 17.86 ± 0.651, 19.24 ± 0.277 and 19.82 ± 0.572 respectively.

The mean percentage of free normal head (Fig.1) of bull spermatozoa before dilution was 2.20 ± 0.151 per cent (Table 8). The mean values during preservation in EYC diluent at 0, 24, 48 and 72 hours of storage were 2.26 ± 0.146, 2.28 ± 0.124, 2.30 ± 0.122 and 2.38 ± 0.139 per cent respectively. The mean values obtained during preservation in Tris and skim milk diluents at 0, 24, 48 and 72 hours of storage were 2.24 ± 0.116, 2.30 ± 0.1, 2.36 ± 0.12, 2.40 ± 0.109 and 2.32 ± 0.149; 2.30 ± 0.149, 2.36 ± 0.132 and 2.44 ± 0.102 per cent respectively. Statistical analysis revealed highly significant ($P < 0.01$) variation in free normal head between bulls. However, no significant difference

could be found between the diluents and between storage periods. Interaction between animal, diluent and storage periods was also not significant (Table 9).

The mean value of free abnormal head of bull spermatozoa was found to be 1.50 ± 0.132 per cent before dilution (Table 10). The mean values at 0, 24, 48 and 72 hours of preservation in EYC diluent were 1.60 ± 0.128, 1.64 ± 0.132, 1.69 ± 0.135, 1.73 ± 0.126 per cent respectively. The respective values obtained for Tris were 1.50 ± 0.094, 1.56 ± 0.092, 1.66 ± 0.124 and 1.70 ± 0.114 per cent and for skim milk diluent, 1.66 ± 0.102, 1.70 ± 0.114, 1.70 ± 0.114 and 1.78 ± 0.106 per cent (Table 10). On analysis highly significant ($P < 0.01$) variation was observed between the bulls for the incidence of free abnormal head. However, between the diluents and between the storage periods the variations were not significant. Animal x diluent x storage period interaction was also found to be non-significant (Table 11).

The average predilution percentage of detached acrosome (Fig. 2) of bull spermatozoa was 1.26 ± 0.180. The mean values for detached acrosome at 0, 24, 48 and 72 hours of preservation in EYC, Tris and skim milk diluent were 1.20 ± 0.198, 1.32 ± 0.192, 1.36 ± 0.180 and 1.42 ± 0.177 per cent; 1.26 ± 0.180, 1.34 ± 0.186, 1.44 ± 0.163 per cent and 1.48 ± 0.159 and 1.24 ± 0.163, 1.34 ± 0.163, 1.24 ± 0.163, 1.40 ± 0.176 and 1.42 ± 0.168 per cent respectively (Table 12).

Statistical analysis revealed highly significant ($P < 0.01$) difference in the incidence of detached acrosome between the bulls and between various storage periods. However, no significant difference was found between the diluents and animal x diluent x storage period interaction was also found to be nonsignificant (Table 13).

The average percentage of pear shaped head was 1.80 ± 0.130 before dilution. The mean values obtained at 0, 24, 48 and 72 hours of preservation in EYC diluent were 1.84 ± 0.128, 1.84 ± 0.092, 1.92 ± 0.128 and 1.94 ± 0.150 per cent respectively (Table 14). The corresponding values in Tris and skim milk diluent were 1.82 ± 0.12, 1.82 ± 0.139, 1.84 ± 0.14 and 1.86 ± 0.120 and 1.86 ± 0.143, 1.88 ± 0.146, 1.90 ± 0.130 and 1.90 ± 0.114 per cent respectively. Analysis of data showed highly significant ($P < 0.01$) variation between the bulls regarding the occurrence of pear shaped head. At the same time no significant difference could be obtained either between the diluents or between the storage periods. Interaction between animal, diluent and storage period was also not at variance (Table 15).

The average predilution percentage of spermatozoan abnormality viz., narrow at the base (Fig. 3) was noted as 1.22 ± 0.086. The average percentage of this abnormality during preservation at 0, 24, 48 and 72 hours of storage in EYC diluent was 1.24 ± 0.074, 1.28 ± 0.086, 1.26 ± 0.087 and

1.38 ± 0.08 respectively. The values obtained during the above storage periods in Tris and skim milk diluent were 1.28 ± 0.058, 1.32 ± 0.096, 1.38 ± 0.077 and 1.36 ± 0.074 and 1.39 ± 0.054, 1.36 ± 0.067, 1.38 ± 0.09 and 1.42 ± 0.073 per cent respectively (Table 16). On analysis, highly significant ($P < 0.01$) differences was noted between the bulls. However, no significant difference could be seen between the diluents and between the storage periods. Animal x diluent x storage period interaction was also found to be nonsignificant (Table 17).

The mean value for knobbed head defect (Fig. 4) of bull spermatozoa was found to be 0.80 ± 0.122 per cent before dilution. The values obtained during preservation in egg diluent at 0, 24, 48 and 72 hours of storage were 0.90 ± 0.122, 0.90 ± 0.122, 0.94 ± 0.107 and 0.90 ± 0.115 per cent respectively. Similar values in Tris and skim milk diluent were 0.86 ± 0.102, 0.86 ± 0.102, 0.90 ± 0.130 and 0.96 ± 0.14 and 0.92 ± 0.091, 0.94 ± 0.092, 0.94 ± 0.092 and 1.00 ± 0.077 per cent respectively (Table 18). On analysis of data, highly significant ($P < 0.01$) difference was found between the animals, but no significant difference could be noticed between the diluents and between the storage periods. Animal x diluent x storage period interaction for knobbed head defect was also noted to be nonsignificant (Table 19).

The predilution data on average percentage of abnormal

contour (Fig. 5) of bull spermatozoa was found to be 1.52 ± 0.08. The values for the same at 0, 24, 48 and 72 hours of preservation in EYC diluent were 1.56 ± 0.097, 1.56 ± 0.107, 1.66 ± 0.087 and 1.70 ± 0.083 per cent respectively. Values obtained while preservation in Tris and skim milk diluent for the above periods were 1.52 ± 0.115, 1.60 ± 0.104, 1.64 ± 0.097 and 1.70 ± 0.104 and 1.58 ± 0.086, 1.58 ± 0.115, 1.70 ± 0.114 and 1.68 ± 0.101 per cent respectively (Table 20). Highly significant ($P < 0.01$) difference was found between the animals for the incidence of abnormal contour of bull spermatozoa. Differences between the diluents and between the storage periods were not significant, so also the interaction between animal, diluent and storage period (Table 21).

The mean values for underdeveloped head of bull spermatozoa was found to be 0.82 ± 0.032 per cent before dilution. The mean values for the incidence during preservation in EYC at 0, 24, 48 and 72 hours of storage were 0.86 ± 0.087, 0.90 ± 0.083, 0.92 ± 0.080 and 1.04 ± 0.097 per cent respectively. The values obtained while preservation in Tris and skim milk diluent for the above periods were 0.82 ± 0.086, 0.82 ± 0.086, 0.88 ± 0.091 and 0.98 ± 0.101 and 0.82 ± 0.086, 0.86 ± 0.067, 0.96 ± 0.087 and 1.10 ± 0.089 per cent respectively (Table 22). Analysis of data revealed highly significant ($P < 0.01$) difference between bulls for the incidence of

underdeveloped head. But no significant difference could be found out between diluents and between storage periods. Animal x diluent x storage period interaction was also found to be nonsignificant for the abnormality (Table 23).

The data on average percentage of proximal protoplasmic droplet (Fig. 6) of bull spermatozoa was found to be 1.18 ± 0.156 before dilution. The mean values noted during preservation in EYC, Tris and skim milk diluent at 0, 24, 48 and 72 hours of storage were 1.22 ± 0.163, 1.26 ± 0.180, 1.30 ± 0.184 and 1.40 ± 0.173, 1.20 ± 0.181, 1.26 ± 0.203, 1.26 ± 0.196 and 1.30 ± 0.219 and 1.30 ± 0.181, 1.32 ± 0.193, 1.40 ± 0.194 and 1.48 ± 0.198 per cent respectively (Table 24). The incidence of proximal protoplasmic droplet vary significantly between bulls ($P < 0.01$). But no significant difference could be found out either between diluents or between storage periods. Animal x diluent x storage period interaction was also nonsignificant (Table 25).

The average predilution data on middle piece defect of the bull spermatozoa was found to be 0.90 ± 0.083 per cent. The values during preservation in EYC diluent at 0, 24, 48 and 72 hours of storage were 0.98 ± 0.086, 1.00 ± 0.089, 1.06 ± 0.087 and 1.10 ± 0.089 per cent respectively, while the corresponding values during preservation in Tris and skim milk diluent were 0.90 ± 0.070, 0.90 ± 0.070, 0.94±0.050 and 1.04 ± 0.50 and 0.94 ± 0.067, 0.94 ± 0.050, 1.04 ± 0.024

and 1.06 ± 0.040 per cent respectively (Table 26). Statistical analysis revealed highly significant ($P < 0.01$) variation between bulls but no significant difference could be observed between diluents and between storage periods for the defect. Animal x diluent x storage period interaction was also nonsignificant (Table 27). The average predilution data on simple bent tail (Fig. 7) was found to be 2.15 ± 0.106 per cent. Average percentage of simple bent tail during preservation in EYC diluent at 0, 24, 48 and 72 hours of storage were 2.26 ± 0.120, 2.28 ± 0.101, 2.66 ± 0.067 and 2.74 ± 0.150 respectively. Similarly the corresponding values while preservation in Tris and skim milk diluent were 2.22 ± 0.096, 2.20 ± 0.130, 2.68 ± 0.037 and 2.74 ± 0.050 and 2.38 ± 0.139, 2.36 ± 0.112, 3.04 ± 0.044 and 3.06 ± 0.037 per cent respectively (Table 28). Statistical analysis revealed highly significant ($P < 0.01$) difference between the animals. Highly significant difference was also noted for the above incidence between diluents and between storage periods. Animal x diluent x storage period interaction, however, found to be nonsignificant (Table 29).

The average percentage of coiled tail (Fig. 8) of bull spermatozoa was found to be 1.24 ± 0.102 before dilution. The mean values on coiled tail during preservation in EYC diluent at 0, 24, 48 and 72 hours of storage were 1.30 ± 0.447, 1.32±0.086, 1.40 ± 0.089 and 1.44 ± 0.092 per cent respectively.

The values during preservation in Tris and skim milk diluent for the above periods were 1.30 ± 0.094, 1.30 ± 0.094, 1.40 ± 0.089 and 1.44 ± 0.092 and 1.34 ± 0.102, 1.36 ± 0.092, 1.42 ± 0.086 and 1.40 ± 0.235 per cent respectively (Table 30). On analysis highly significant ($P < 0.01$) difference was found between the bulls and between the storage periods. No significant difference was noted between the diluents for the incidence of coiled tail. Animal x diluent x storage period interaction was also found to be nonsignificant (Table 31).

The average head length of bull spermatozoa before dilution was found to be 9.51 micron. The data on average head length during preservation in EYC diluent at 0, 24, 48 and 72 hours of storage was 9.50, 9.51, 9.51 and 9.50 micron respectively. The values obtained while preservation in Tris and skim milk diluent at 0, 24, 48 and 72 hours of storage was 9.50, 9.51, 9.50 and 9.50 and 9.50, 9.50, 9.50 and 9.49 micron respectively (Table 32). On analysis highly significant ($P < 0.01$) difference was found between the bulls but no significant difference was found between the diluents and between the storage period, for head length of bull spermatozoa. Similarly animal x diluent x storage period interaction was also nonsignificant for the character (Table 33). The average head breadth (maximum breadth) of bull spermatozoa before dilution was 4.72 micron. The data during preservation in EYC, Tris and skim milk diluent at 0, 24, 48

and 72 hours of storage were 4.74, 4.73, 4.72 and 4.72; 4.72, 4.72, 4.72 and 4.71 and 4.72, 4.72, 4.71 and 4.71 micron respectively (Table 34). On analysis highly significant ($P < 0.01$) difference could be found between the bulls but no significant difference was found between the diluents and between the storage periods. Animal x diluent x storage period interaction was also found to be non-significant (Table 35).

Table 1

Sperm concentration

Bull	Sperm concentration (10,000/cmm)												Mean
B_1	141	164	159	170	162	164	134	175	135	172	129	135	153.33
B_2	190	174	129	136	172	144	191	129	143	144	160	154	155.50
B_3	184	182	135	144	175	149	191	181	143	151	163	132	160.83
B_4	174	180	139	154	182	171	139	149	164	173	149	172	162.16
B_5	175	182	149	182	171	162	149	184	165	137	181	179	168.00
										Overall mean			159.96
										S.E.			2.589

Table 2

Concentration of spermatozoa

ANOVA

Source	DF	SS	MS	F
Treatment	4	1609.00	402.25	1.18
Error	55	18715.00	340.27	1.00

* significant at 5 per cent level

** significant at 1 per cent level

Table 3

Percentage of live spermatozoa

Bull												Mean
B_1	84.09	81.08	87.38	87.38	81.09	86.99	84.09	87.09	84.98	85.56	80.78	84.92	84.45
B_2	85.56	84.09	84.08	81.09	83.48	81.09	94.29	80.78	78.88	81.08	80.78	85.56	83.55
B_3	87.38	80.18	85.56	79.89	80.48	83.48	84.59	83.48	80.48	87.09	80.18	79.89	82.75
B_4	85.56	87.09	85.31	82.58	90.99	80.48	83.48	90.99	80.48	78.88	81.09	87.09	84.58
B_5	81.09	80.78	79.89	82.58	85.31	81.09	79.89	80.48	85.31	83.48	80.78	81.08	81.94

Overall mean 83.44

S.E. ±0.322

Table 4

Percentage of live sperm

ANOVA

Source	DF	SS	MS	F
Treatment	4	65.81	16.45	1.69
Error	55	539.70	9.81	1.00

Table 5

Mean motility percentage of bull spermatozoa at 0, 24, 48 and 72 hours of storage

Bull	Before dilution	Egg yolk-citrate				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	86.00	86.00	77.00	68.00	58.00	86.00	78.00	68.50	68.00	86.00	77.50	68.00	58.00
B_2	83.00	83.00	73.50	65.00	58.00	83.00	73.00	64.00	59.00	83.00	68.50	65.50	57.00
B_3	82.00	82.00	72.00	64.00	56.50	82.00	72.50	63.00	57.50	82.00	72.00	63.00	56.00
B_4	84.00	84.00	74.50	65.00	57.00	84.00	75.00	66.00	57.50	84.00	74.50	64.00	56.50
B_5	80.00	80.00	70.00	62.00	56.00	80.00	70.00	62.00	56.00	80.00	71.00	61.00	51.50
Mean	83.00	83.00	73.40	64.80	57.10	83.00	73.70	64.70	58.00	83.00	72.70	64.30	55.80
S.E.	1.00	1.00	1.176	0.969	0.4	1.00	1.33	1.157	0.689	1.00	1.53	1.178	1.113

Table 6

Motility percentage of spermatozoa

ANOVA

Source	DF	SS	MS	F	
Animal	4	2830.5	707.625	46.667	**
Diluent	2	30	15	0.989	
Period	4	79077	19769.25	1303.785	**
Animal x Diluent	8	22	2.75	0.181	
Animal x Period	16	197	12.312	0.812	
Diluent x Period	8	81.5	10.187	0.671	
Animal x Diluent x Period	32	108.5	3.390	0.223	
Error	675	10235	15.162		

Table 7

Total abnormality percentage of bull spermatozoa during preservation

Bull	Before dilution	EYC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	15.40	15.90	16.10	16.70	17.90	15.50	15.60	16.60	17.40	15.80	16.00	17.50	18.30
B_2	16.10	16.80	17.20	17.80	18.90	16.40	16.70	18.10	18.60	17.00	17.20	18.30	18.90
B_3	16.50	16.80	17.30	18.30	19.00	16.70	17.40	18.40	18.60	17.50	17.70	19.70	20.20
B_4	18.90	19.80	20.70	20.80	21.30	19.10	19.60	20.00	21.20	20.20	19.90	21.10	21.60
B_5	16.50	17.10	17.60	18.70	19.10	16.90	17.10	18.40	19.00	17.80	18.50	19.60	20.10
Mean	16.68	17.48	17.78	18.46	19.24	16.92	17.28	18.30	18.96	17.66	17.86	19.24	19.82
S.E.	0.590	0.661	0.772	0.674	0.558	0.595	0.655	0.540	0.620	0.720	0.651	0.277	0.572

Table 9

Percentage of free normal head of bull spermatozoa during preservation

Bull	Before dilution	EYC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72h	0 h	24 h	48 h	72 h
B_1	1.70	1.80	1.90	1.90	1.90	2.00	2.00	2.00	2.10	1.80	1.80	1.90	2.10
B_2	2.10	2.20	2.20	2.30	2.40	2.20	2.30	2.40	2.50	2.30	2.30	2.40	2.40
B_3	2.20	2.20	2.20	2.20	2.30	2.00	2.20	2.20	2.20	2.30	2.30	2.30	2.40
B_4	2.60	2.70	2.60	2.60	2.70	2.40	2.40	2.50	2.50	2.50	2.50	2.50	2.60
B_5	2.40	2.40	2.50	2.50	2.60	2.60	2.60	2.70	2.70	2.70	2.70	2.70	2.70
Mean	2.20	2.26	2.28	2.30	2.36	2.24	2.30	2.36	2.40	2.32	2.30	2.36	2.44
$S.E_x$	0.151	0.146	0.124	0.122	0.139	0.116	0.1	0.120	0.109	0.149	0.149	0.132	0.102

Table 9

Free normal head of bull spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	4	47.010	11.954	24.475**
Diluent	3	0.298	0.149	0.305
Period	4	3.751	0.937	1.920
Animal x Diluent	6	2.061	0.257	0.527
Animal x Period	16	1.961	0.121	0.248
Diluent x Period	8	0.338	0.026	0.053
Animal x Diluent x Period	32	1.299	0.040	0.083
Error	675	329.699	0.488	

** Significant at 1 per cent level

Table 19

Percentage of free abnormal head of bull spermatozoa during preservation

Bull	Before dilution	EYC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	1.60	1.60	1.60	1.70	1.60	1.60	1.60	1.60	1.70	1.70	1.70	1.70	1.60
B_2	1.40	1.40	1.50	1.50	1.50	1.30	1.40	1.40	1.50	1.50	1.50	1.50	1.60
B_3	1.30	1.30	1.30	1.30	1.40	1.30	1.30	1.60	1.40	1.40	1.40	1.40	1.50
B_4	2.00	2.00	2.10	2.10	2.10	1.80	1.80	2.00	2.00	2.00	2.00	2.00	2.10
B_5	1.60	1.70	1.70	1.80	1.80	1.60	1.70	1.90	1.90	1.70	1.90	1.90	1.90
Mean	1.56	1.60	1.64	1.68	1.72	1.50	1.56	1.66	1.70	1.66	1.70	1.70	1.78
S.E.	0.132	0.122	0.132	0.135	0.134	0.094	0.092	0.124	0.114	0.102	0.114	0.114	0.106

Table 11

Free abnormal head of mill spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	4	41.341	10.335	29.585**
Diluent	2	0.970	0.485	1.389
Period	4	2.861	0.715	2.047
Animal x Diluent	8	0.442	0.055	0.158
Animal x Period	16	0.952	0.059	0.170
Diluent x Period	8	0.442	0.055	0.158
Animal x Diluent x Period	32	0.563	0.018	0.048
Error	675	235.800	0.349	

** Significant at 1 per cent level

Table 12

Percentage of Detached Acrosome of bull spermatozoa during preservation

Bull	Before dilution	EYC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	1.00	1.00	1.10	1.10	1.20	1.00	1.10	1.10	1.20	1.00	1.00	1.10	1.20
B_2	0.90	0.90	1.00	1.00	1.10	0.90	0.90	1.20	1.20	0.90	0.90	1.10	1.10
B_3	1.10	1.10	1.10	1.20	1.30	1.10	1.20	1.30	1.30	1.10	1.10	1.20	1.20
B_4	1.40	1.40	1.40	1.50	1.40	1.40	1.60	1.60	1.70	1.40	1.40	1.60	1.60
B_5	1.90	2.00	2.00	2.00	2.10	1.90	1.90	2.00	2.00	1.80	1.80	2.00	2.00
Mean	1.26	1.28	1.32	1.36	1.42	1.26	1.34	1.44	1.48	1.24	1.24	1.40	1.42
S.E.	0.100	0.103	0.102	0.100	0.177	0.100	0.100	0.103	0.109	0.103	0.103	0.176	0.102

Table 13

Motility scores of bull spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	4	91.396	22.849	90.936**
Diluent	2	0.199	0.099	0.397
Period	4	4.461	1.115	4.438**
Animal x Diluent	8	0.373	0.046	0.185
Animal x Period	16	0.338	0.021	0.084
Diluent x Period	8	0.386	0.048	0.192
Animal x Diluent x Period	32	0.573	1.017	0.071
Error	675	169.600	0.251	

** Significant at 1 per cent level

Table 14

Percentage of pear shaped head during preservation

Bull	Before dilution	BKC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	1.90	1.90	2.00	1.90	2.10	1.80	1.90	1.90	1.90	1.90	2.00	1.90	1.90
B_2	1.70	1.80	1.90	1.90	2.00	1.80	1.80	1.90	2.00	1.80	1.80	1.90	1.90
B_3	1.80	1.80	1.80	2.00	1.90	2.00	2.00	2.00	1.90	1.90	1.90	2.00	2.00
B_4	2.20	2.20	2.00	2.30	2.30	2.10	2.10	2.10	2.10	2.30	2.30	2.30	2.20
B_5	1.40	1.40	1.50	1.50	1.40	1.40	1.30	1.30	1.40	1.40	1.40	1.50	1.50
Mean	1.80	1.84	1.84	1.92	1.94	1.82	1.82	1.84	1.86	1.86	1.88	1.90	1.90
S.E.	0.130	0.128	0.092	0.128	0.150	0.129	0.130	0.14	0.129	0.143	0.146	0.130	0.114

Table 15

Pear shape head of bull spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	4	47.499	11.874	25.634**
Diluent	2	0.356	0.133	0.301
Period	4	0.952	0.238	0.537
Animal x Diluent	8	0.933	0.116	0.262
Animal x Period	16	1.207	0.075	0.170
Diluent x Period	8	0.199	0.024	0.056
Animal x Diluent x Period	32	1.400	0.043	0.098
Error	675	298.609	0.442	

** Significant at 1 per cent level

Table 16

Percentage of Narrow at the base of bull spermatozoa during preservation

Bull	Before dilution	EEC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	1.30	1.30	1.30	1.10	1.30	1.30	1.20	1.20	1.20	1.30	1.30	1.30	1.30
B_2	1.20	1.20	1.20	1.20	1.30	1.20	1.20	1.20	1.20	1.30	1.20	1.30	1.30
B_3	1.50	1.50	1.60	1.60	1.70	1.50	1.70	1.60	1.60	1.50	1.60	1.70	1.70
B_4	1.10	1.10	1.20	1.20	1.30	1.20	1.30	1.30	1.40	1.20	1.20	1.30	1.40
B_5	1.00	1.10	1.10	1.20	1.30	1.20	1.30	1.20	1.40	1.20	1.40	1.30	1.40
Mean	1.22	1.24	1.28	1.26	1.38	1.28	1.32	1.30	1.36	1.30	1.36	1.36	1.42
S.E.	0.036	0.076	0.066	0.087	0.08	0.036	0.096	0.077	0.076	0.054	0.067	0.08	0.073

Table 17

Narrow at the base of bull spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	4	14.698	3.674	9.730**
Diluent	2	0.594	0.297	0.787
Period	4	2.952	0.738	1.954
Animal x Diluent	8	0.495	0.050	0.134
Animal x Period	16	1.251	0.078	0.208
Diluent x Period	8	0.351	0.043	0.116
Animal x Diluent x Period	32	0.715	0.022	0.0591
Error	675	254.900	0.377	

** significant at 1 per cent level

Table 19

Percentage of knobbed head of bull spermatozoa during preservation

Bull	Before dilution	EYC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	0.70	0.80	0.80	0.80	0.90	0.90	0.90	0.90	1.10	0.90	0.90	0.90	1.10
B_2	1.10	1.20	1.20	1.20	1.30	1.10	1.10	1.30	1.30	1.10	1.10	1.10	1.10
B_3	0.40	0.50	0.50	0.60	0.60	0.50	0.50	0.50	0.50	0.60	0.60	0.60	0.70
B_4	1.00	1.10	1.10	1.10	1.10	1.00	1.00	1.00	1.10	1.10	1.10	1.10	1.10
B_5	0.80	0.90	0.90	1.00	1.00	0.80	0.80	0.80	0.80	0.90	1.00	1.00	1.00
Mean	0.80	0.90	0.90	0.94	0.98	0.86	0.86	0.90	0.96	0.92	0.94	0.94	1.00
S.E.	0.122	0.122	0.122	0.107	0.115	0.102	0.102	0.130	0.14	0.091	0.092	0.092	0.077

Table 19

Knobbed head defect of bull spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	4	36.925	9.231	24.629**
Diluent	2	0.194	0.097	0.259
Period	4	2.631	0.657	1.755
Animal x Diluent	8	1.338	0.167	0.446
Animal x Period	16	1.007	0.062	0.168
Diluent x Period	8	0.192	0.024	0.064
Animal x Diluent x Period	32	1.008	0.032	0.084
Error	675	252.999	0.374	

** Significant at 1 per cent level

Table 20

Percentage of Abnormal contour of bull spermatozoa during preservation

Bull	before dilution	EEC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	1.40	1.30	1.30	1.50	1.90	1.30	1.40	1.40	1.40	1.30	1.30	1.40	1.40
B_2	1.70	1.70	1.60	1.80	1.80	1.60	1.70	1.80	1.80	1.60	1.70	1.70	1.70
B_3	1.70	1.70	1.70	1.80	1.90	1.70	1.80	1.80	1.90	1.70	1.80	1.90	1.90
B_4	1.50	1.70	1.70	1.80	1.80	1.20	1.80	1.80	1.90	1.80	1.80	2.00	1.90
B_5	1.30	1.30	1.30	1.40	1.50	1.20	1.30	1.40	1.50	1.50	1.30	1.90	1.80
Mean	1.52	1.56	1.56	1.66	1.70	1.32	1.60	1.64	1.70	1.58	1.58	1.70	1.68
S.E.	0.08	0.097	0.107	0.097	0.093	0.115	0.104	0.097	0.104	0.086	0.115	0.114	0.101

Table 21

Abnormal contour of bull spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	5	25.973	5.195	13.322**
Diluent	2	0.007	0.003	0.008
Period	4	3.900	0.950	1.940
Animal x Diluent	8	0.538	0.067	0.138
Animal x Period	10	2.026	0.120	0.259
Diluent x Period	8	0.151	0.019	0.038
Animal x Diluent x Period	32	0.502	0.015	0.032
Error	675	322.929	0.487	

** Significant at 1 per cent level

Table 22

Percentage of underdeveloped head of bull spermatozoa during preservation

Bull	Before dilution	EYC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	0.70	0.80	0.80	0.80	0.80	0.70	0.70	0.70	0.90	0.70	0.80	0.90	1.00
B_2	0.80	0.80	0.90	0.90	1.20	0.80	0.80	0.90	0.90	0.80	0.80	0.90	1.00
B_3	1.10	1.10	1.20	1.20	1.20	1.10	1.10	1.20	1.30	1.10	1.10	1.30	1.40
B_4	0.90	1.00	0.90	1.00	1.20	0.90	0.90	0.90	1.10	0.90	0.90	0.90	1.20
B_5	0.60	0.60	0.70	0.70	0.90	0.60	0.60	0.70	0.70	0.60	0.70	0.80	0.90
Mean	0.82	0.86	0.90	0.92	1.06	0.82	0.82	0.88	0.98	0.82	0.86	0.96	1.10
s_x	0.082	0.087	0.083	0.080	0.097	0.086	0.086	0.091	0.101	0.086	0.087	0.087	0.080

Table 23

Underdeveloped head of bull spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	4	21.379	5.344	6.696**
Diluent	2	0.356	0.177	0.219
Period	4	4.645	1.311	1.697
Animal x Diluent	8	0.335	0.035	0.044
Animal x Period	16	0.421	0.026	0.032
Diluent x Period	8	0.370	0.047	0.058
Animal x Diluent x Period	32	0.914	0.028	0.035
Error	675	540.1	0.809	

** Significant at 1 per cent level

Table 24

Percentage of proximal protoplasmic droplet of bull spermatozoa during preservation

Bull	Before dilution	EYC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	0.70	0.80	0.80	0.80	0.90	0.70	0.70	0.70	0.70	0.80	0.80	0.90	0.90
B_2	1.00	0.90	0.90	1.00	1.10	0.90	0.90	1.00	1.00	1.00	1.00	1.00	1.20
B_3	1.20	1.20	1.30	1.30	1.50	1.20	1.30	1.20	1.20	1.30	1.30	1.50	1.50
B_4	1.40	1.60	1.60	1.60	1.70	1.60	1.70	1.70	1.80	1.70	1.70	1.70	1.80
B_5	1.60	1.60	1.70	1.80	1.80	1.60	1.70	1.70	1.80	1.70	1.80	1.90	2.00
Mean	1.18	1.22	1.26	1.30	1.40	1.20	1.26	1.26	1.30	1.30	1.32	1.40	1.48
S.E.	0.156	0.168	0.180	0.184	0.173	0.181	0.203	0.196	0.219	0.181	0.193	0.194	0.198

Table 25

Proximal protoplasmic droplets of bull spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	6	93.791	24.698	66.445**
Diluent	2	1.218	0.609	1.639
Period	4	3.685	0.921	2.478
Animal x Diluent	8	0.447	0.055	0.150
Animal x Period	16	1.461	0.091	0.245
Diluent x Period	8	0.514	0.064	0.173
Animal x Diluent x Period	32	0.612	0.019	0.052
Error	675	250.899	0.371	

** Significant at 1 per cent level

Table 26

Percentage of middle piece defect of bull spermatozoon during preservation

Bull	Before dilution	EYC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	1.20	1.30	1.30	1.30	1.40	1.10	1.10	1.10	1.20	1.10	1.10	1.10	1.20
B_2	0.90	0.90	0.90	1.00	1.00	0.90	0.90	0.90	1.00	0.80	0.90	1.00	1.00
B_3	0.70	0.80	0.80	0.80	0.90	0.70	0.70	0.80	0.90	0.80	0.80	1.00	1.00
B_4	0.90	1.00	1.10	1.20	1.20	1.00	1.00	1.00	1.10	1.10	1.00	1.10	1.10
B_5	0.80	0.90	0.90	1.00	1.00	0.90	0.80	0.90	1.00	0.90	0.90	1.00	1.00
Mean	0.90	0.98	1.00	1.06	1.10	0.80	0.90	0.94	1.04	0.94	0.94	1.04	1.06
S.E.	0.083	0.086	0.089	0.087	0.089	0.070	0.070	0.050	0.050	0.067	0.059	0.024	0.040

Table 27

Middle piece defect of bull spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	6	13.225	3.306	8.836**
Diluent	2	0.650	0.325	0.869
Period	4	2.620	0.656	1.754
Animal x Diluent	8	0.749	0.093	0.250
Animal x Period	16	0.746	0.046	0.124
Diluent x Period	8	0.269	0.033	0.089
Animal x Diluent x Period	32	0.597	0.018	0.049
Error	675	252.000	0.374	

** Significant at 1 per cent level

Table 20

Percentage of single bent tail of bull spermatozoa during preservation

Bull	Before dilution	EYC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	2.10	2.10	2.00	2.50	2.70	2.10	1.90	2.70	2.70	2.10	2.10	3.10	3.10
B_2	2.10	2.50	2.40	2.70	2.90	2.40	2.40	2.80	2.80	2.60	2.60	3.10	3.10
B_3	2.00	2.50	2.20	2.60	2.60	2.00	2.00	2.70	2.70	2.10	2.10	3.10	3.10
B_4	2.60	2.90	2.60	2.90	2.90	2.50	2.60	2.60	2.80	2.80	2.60	3.10	3.10
B_5	2.10	2.10	2.20	2.60	2.60	2.10	2.10	2.60	2.60	2.30	2.40	2.80	2.90
Mean	2.18	2.25	2.23	2.66	2.74	2.22	2.20	2.68	2.74	2.38	2.36	3.04	3.06
S.E.	0.106	0.120	0.101	0.057	0.150	0.095	0.130	0.037	0.050	0.130	0.112	0.044	0.037

Table 29

Simple hank roll of bull spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	4	17.461	4.365	9.077**
Diluent	2	6.066	3.033	6.309**
Period	4	59.994	14.998	31.189**
Animal x Diluent	8	0.186	0.023	0.048
Animal x Period	16	7.458	0.466	0.969
Diluent x Period	8	3.253	0.406	0.845
Animal x Diluent x Period	32	2.095	0.065	0.136
Error	675	324.599	0.480	

** Significant at 1 per cent level

Table 30

Percentage of coiled tail of bull spermatozoa during preservation

Bull	Before dilution	EYC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	1.10	1.20	1.20	1.30	1.40	1.10	1.10	1.30	1.30	1.20	1.20	1.30	1.30
B_2	1.20	1.20	1.30	1.30	1.30	1.30	1.30	1.30	1.40	1.30	1.30	1.40	1.50
B_3	1.60	1.60	1.60	1.70	1.70	1.60	1.60	1.70	1.70	1.70	1.70	1.70	1.80
B_4	1.30	1.40	1.40	1.50	1.60	1.40	1.40	1.50	1.60	1.40	1.40	1.50	1.50
B_5	1.00	1.10	1.10	1.20	1.20	1.10	1.10	1.20	1.20	1.10	1.20	1.20	1.30
Mean	1.24	1.30	1.32	1.40	1.44	1.30	1.30	1.40	1.44	1.34	1.36	1.42	1.48
S.E.	0.102	0.447	0.086	0.089	0.092	0.094	0.096	0.089	0.092	0.702	0.092	0.086	0.215

Table 31

Coiled tail of bull spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	4	25.671	6.417	24.461**
Diluent	2	0.151	0.075	0.289
Period	4	4.179	1.044	3.981**
Animal x Diluent	8	0.287	0.035	0.137
Animal x Period	16	0.388	0.018	0.069
Diluent x Period	8	0.061	0.007	0.029
Animal x Diluent x Period	32	0.431	0.013	0.051
Error	675	177.100	0.262	

** Significant at 1 per cent level

Table 32

Average head length of bull spermatozoa (in micron) during preservation

Bull	Before dilution	EYC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	40 h	72 h	0 h	24 h	48 h	72 h
B_1	9.54	9.51	9.52	9.52	9.51	9.52	9.52	9.52	9.51	9.51	9.52	9.51	9.50
B_2	9.49	9.49	9.49	9.49	9.49	9.49	9.49	9.49	9.49	9.49	9.49	9.49	9.49
B_3	9.50	9.49	9.51	9.51	9.50	9.51	9.51	9.50	9.50	9.50	9.49	9.49	9.50
B_4	9.52	9.52	9.51	9.51	9.51	9.51	9.51	9.50	9.52	9.51	9.49	9.51	9.49
B_5	9.52	9.51	9.52	9.52	9.50	9.51	9.52	9.51	9.52	9.51	9.52	9.51	9.51
Mean	9.51	9.50	9.51	9.51	9.50	9.50	9.51	9.50	9.50	9.50	9.50	9.50	9.49

Table 33

Head length of bull spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	4	0.093	0.023	11.375**
Diluent	2	0.007	0.003	1.395
Period	4	0.015	0.003	1.395
Animal x Diluent	8	0	0	0
Animal x Period	16	0.007	0.0004	0.237
Diluent x Period	8	0	0	0
Animal x Diluent x Period	32	0.007	0.0002	0.119
Error	675	1.330	0.002	

** significant at 1 per cent level

Table 34

Average head breadth of bull spermatozoa (in micron) during preservation

Bull	Before dilution	EYC				Tris				Skim milk			
		0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
B_1	4.75	4.77	4.73	4.71	4.72	4.75	4.74	4.73	4.74	4.71	4.73	4.72	4.73
B_2	4.64	4.73	4.73	4.74	4.72	4.74	4.73	4.71	4.70	4.74	4.71	4.70	4.69
B_3	4.75	4.73	4.73	4.73	4.71	4.71	4.75	4.73	4.73	4.74	4.71	4.71	4.73
B_4	4.75	4.77	4.75	4.75	4.74	4.73	4.73	4.73	4.72	4.74	4.73	4.75	4.74
B_5	4.73	4.73	4.72	4.71	4.72	4.71	4.69	4.72	4.69	4.71	4.73	4.69	4.70
Mean	4.72	4.74	4.73	4.72	4.72	4.72	4.72	4.72	4.71	4.72	4.72	4.71	4.71

Table 35

Head breadth of bull spermatozoa

ANOVA

Source	DF	SS	MS	F
Animal	4	0.082	0.020	4.094**
Diluent	2	0.011	0.005	1.109
Period	4	0.056	0.014	2.924
Animal x Diluent	8	0.023	0.002	0.584
Animal x Period	16	0.017	0.001	0.219
Diluent x Period	8	0.007	0.0009	0.184
Animal x Diluent x Period	32	0.042	0.001	0.269
Error	675	3.380	0.005	

** significant at 1 per cent level

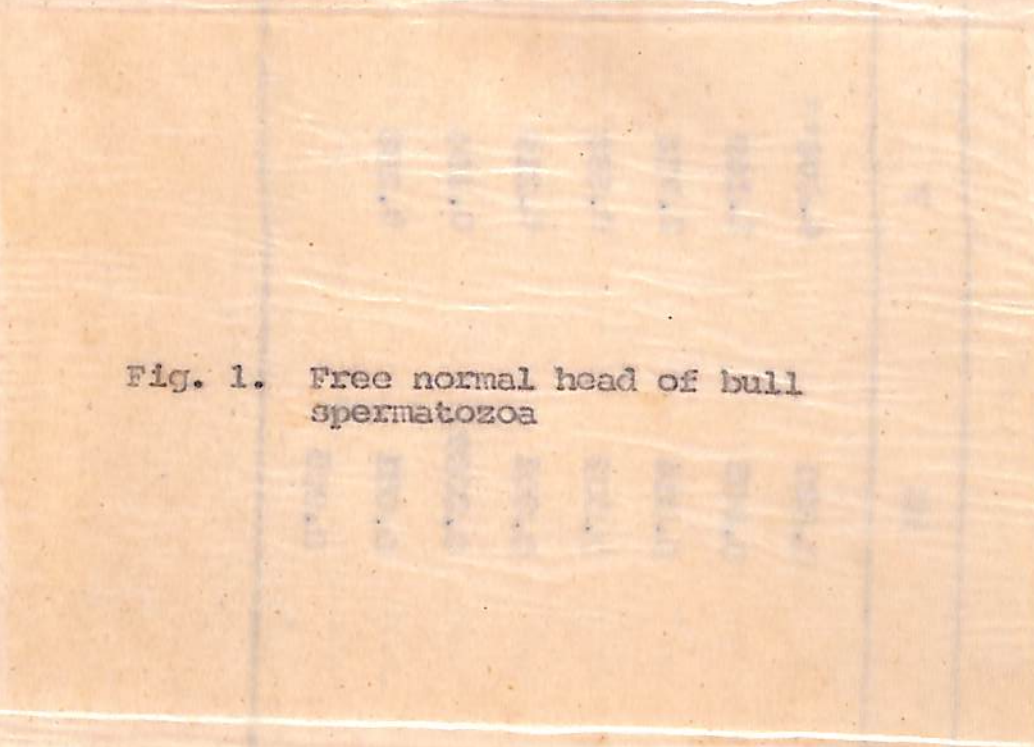


Fig. 1. Free normal head of bull spermatozoa

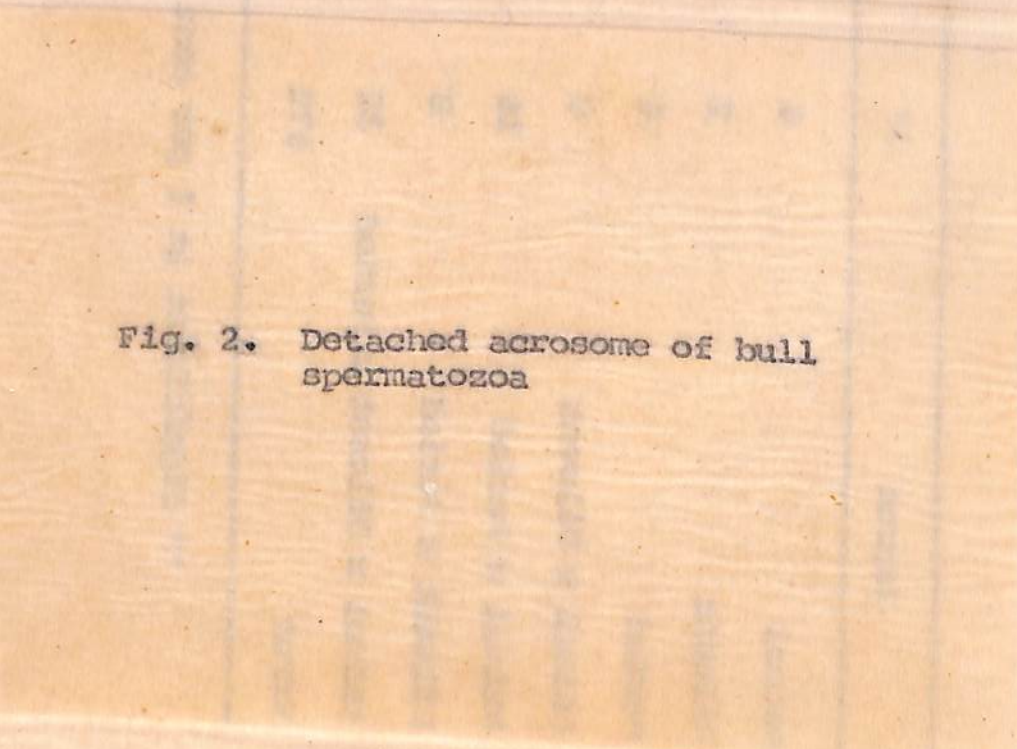
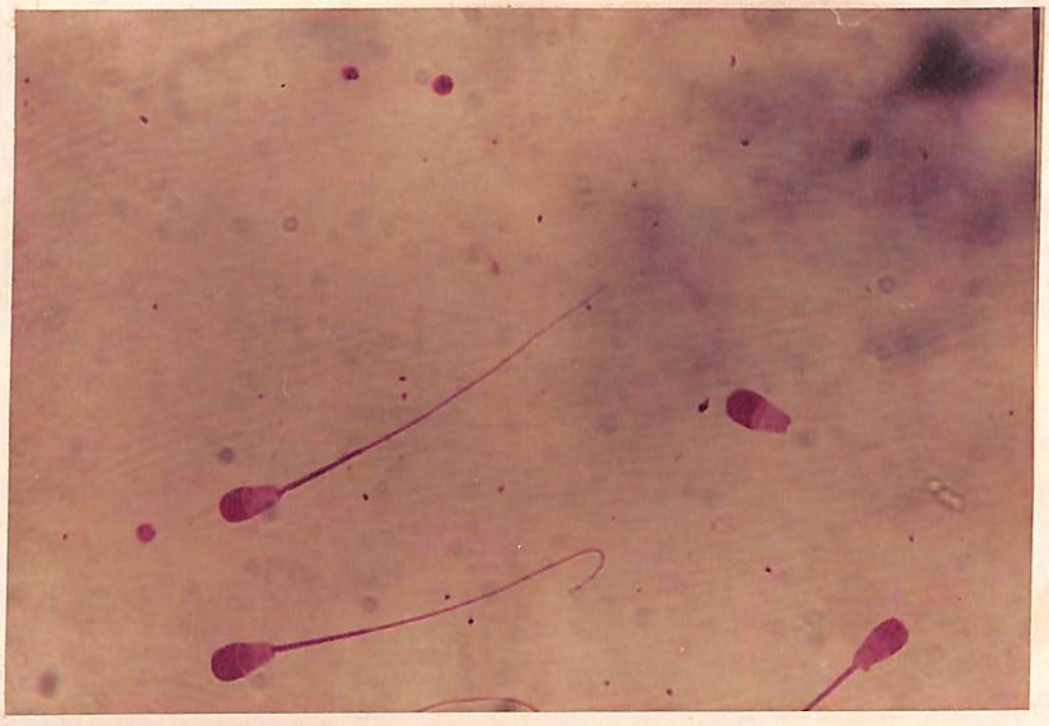
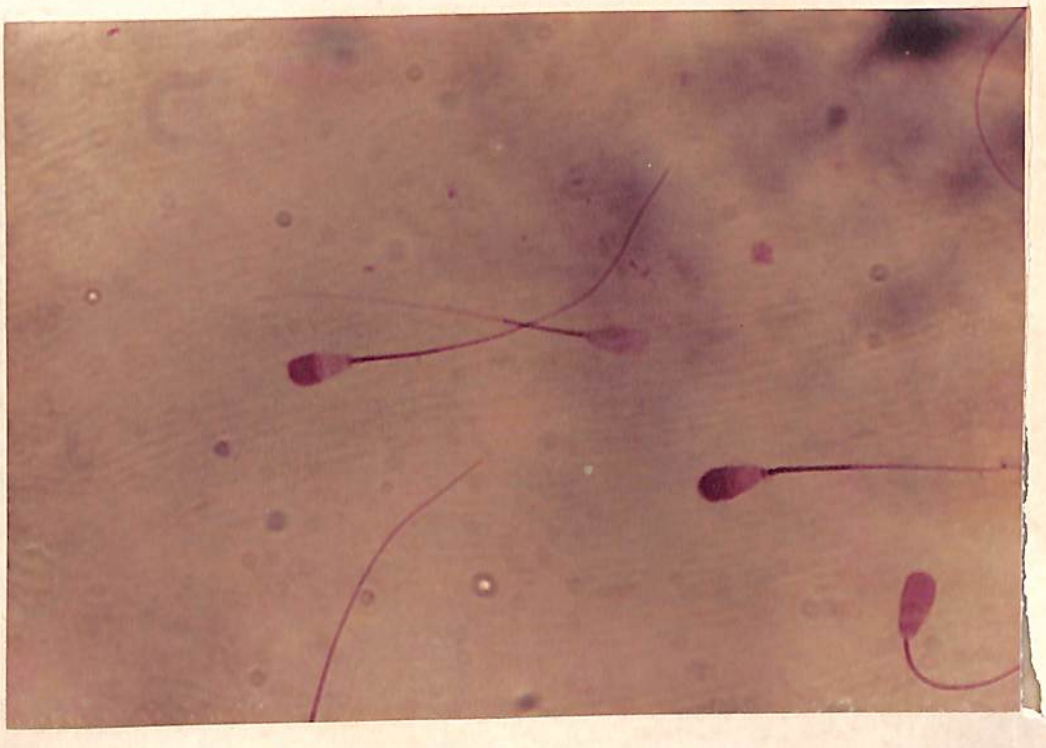


Fig. 2. Detached acrosome of bull spermatozoa





DISCUSSION

DISCUSSION

The average concentration of spermatozoa of crossbred bulls under investigation was found to be 1.59 ± 0.025 million per cmm which ranged from 1.290 to 1.910 million per cmm (Table 1). The mean value obtained in the present study is much higher than those recorded in exotic purebred bulls (Salisbury, 1944; Blom, 1950) and in Indian cattle (Rao and Rao, 1975; Rao and Rao, 1980). The values reported by Mathew (1974) and Rao and Kotayya (1977) in respect of crossbred bulls are also slightly lower than the present findings. These variations in sperm concentration may be attributed to the different genetic groups and to the age of the bulls used in different investigations. Contrary to the reports of Rao and Rao (1978) no significant variation in sperm concentration was observed between bulls (Table 2). Essentially the same observation was made by Rao and Rao (1980) in Ongole bulls and Raja (1981) in Brown Swiss crossbred bulls.

The average percentage of live spermatozoa was found to be 83.45 ± 0.522 within a range of 79.88 to 94.29 (Table 3). These values are comparable to that reported in bull semen by Tripathi (1978) and Rao and Rao (1978). However, this is higher than those reported by Bratton et al. (1956) and Tomar et al. (1966) and Singh et al. (1967) but lower than the values reported by Rao and Rao (1975). These variation

in the percentage of live sperm in various studies could be attributed to the different genetic make up and age of the bull employed and also to the different seasons in which the studies were carried out. There was no significant differences in the live sperm count of the ejaculates between bulls (Table 4). This observation is in accordance with the findings of Rao and Rao (1980) in Ongole bulls and Raja (1981) in Brown Swiss crossbred bulls, but, contrary to the findings of Rao and Rao (1975) in purebred bulls and Rao and Rao (1978) in crossbred bulls. The study also revealed no significant variation in percentage of live sperm between diluents (Table 4). However, Klein et al. (1975) found a higher live sperm percentage during preservation in Tris-yolk-glycerol extender compared to egg yolk-citrate and sodiumcarb-glucose extender.

It could be seen from table 5 that the percentage of motile sperm averaged 63.00 ± 1.00 immediately after dilution in EYC, Tris and skim milk diluent. The mean value presently observed is comparable with that reported by Bishop et al. (1954) but higher than those reported by Brown (1959) and Almquist et al. (1963), Saxena and Tripathi (1979) and Rao and Rao (1978) in crossbred bulls. However Rao and Rao (1979) have reported a higher value for Brown Swiss-Ongole crossbreds. It could also be seen that motility of the sperms gradually decreased on preservation in EYC, Tris and skim milk

diluent upto 72 hours of storage. Analysis of data revealed highly significant ($P < 0.01$) difference in the percentage of motile sperm between bulls, and different storage periods (Table 6). This is in contrast to the findings of Raja (1981) who observed no significant variation in the initial motility of sperm between bulls in Brown Swiss crossbreds. The gradual decline in motility during preservation in EYC and skim milk diluent is in accordance with the findings of Albright and Erb (1958) who observed a significant decline in motility of the sperms after extension till 72 hours of storage. Gradual reduction in sperm motility during preservation in diluent containing Tris has also been reported by Bartlett and Van Demark (1961) and Davis et al. (1963) in bulls and Balakrishnan (1979) in bucks. The study also revealed no significant variation in sperm motility between various diluents (Table 6). However, Edvin et al. (1975) noticed a higher percentage of motile sperms when preserved in Tris-yolk-glycerol extender for 24, 48, 72, 96 and 120 hours at 5°C compared with egg yolk-citrate and soda-bicarb-glucose extender.

The average percentage of free normal head was found to be 2.20 ± 0.151 per cent (Table 6). During preservation upto 72 hours in EYC, Tris and skim milk diluent, though there was no significant variation in the incidence of free normal head, highly significant ($P < 0.01$) difference was

observed between the bulls (Table 9) under investigation. Similarly the mean percentage of free abnormal head was noted to be 1.56 ± 0.132 (Table 10) which also showed a marginal increase during preservation at 0, 24, 48 and 72 hours, although these variation was not found to be statistically significant. Between bulls (Table 11) this was highly significant ($P < 0.01$). The above findings are in accordance with the findings of Rao and Rao (1978) and Raja (1981) in crossbred bulls. However in Angole bulls no such variation was observed by Rao and Rao (1980).

The detached acrosome was found to the tune of 1.26 ± 0.160 per cent. During preservation in EEC this abnormality increased to 1.38 ± 0.150, 1.32 ± 0.182, 1.36 ± 0.160 and 1.42 ± 0.177 per cent at 0, 24, 48 and 72 hours respectively (Table 12). In Tris diluent the above values were 1.26 ± 0.160, 1.34 ± 0.180, 1.44 ± 0.163, and 1.48 ± 0.159 per cent and in skim milk diluent, 1.24 ± 0.163, 1.36 ± 0.163, 1.40 ± 0.176 and 1.42 ± 0.169 per cent respectively (Table 12). From the above data it is evident that there is significant increase in the incidence of detached acrosome in all the three diluents during preservation. Statistical analysis also revealed highly significant ($P < 0.01$) variation in the detached acrosome between bulls and between storage periods. However, between diluent this variation was not significant (Table 13).

The incidence of pear shaped head was 1.80 ± 0.130 which also showed a gradual increase during preservation at 0, 24, 48 and 72 hours in all the three diluents studied (Table 14). Though, on analysis, this variation in the incidence of pear shaped head was not significant neither between storage periods nor between diluents, highly significant ($P < 0.01$) difference was seen between bulls (Table 15). An observation similar to this was made in the case of another sperm abnormality viz., narrow at the base. The percentage of this abnormality was 1.22 ± 0.088 (Table 16). This also showed an increasing trend during preservation in all the three diluents though the variation was not statistically significant. However a highly significant ($P < 0.01$) difference was noticed between bulls in the incidence of this abnormality. Between diluents also the variation was not significant (Table 17).

The knobbed head defect was found to range between 0.40 to 1.30 per cent with a mean of 0.80 ± 0.122 (Table 18). On preservation in the EYC, Tris and skim milk diluent there was a tendency for a slight increase of the above defect as the storage period increased. But this was not statistically significant (Table 19). However, this defect was found to vary significantly ($P < 0.01$) between bulls before and during preservation (Table 19). The incidence of abnormal contour of head ranged between 1.30 to 1.70 per cent with an overall

mean of 1.53 ± 0.08 per cent (Table 20). Similarly the underdeveloped head varied from 0.60 to 1.10 per cent with an overall mean of 0.82 ± 0.052 per cent (Table 22). These two defects did not vary significantly between diluents and also between different storage periods (Table 21, 23). Highly significant (P <0.01) variation was however noticed between bulls in respect of this defect (Table 21, 23).

The percentage of sperm head abnormality presently observed were slightly higher than those reported by Hollinsen (1951), Rao and Rao (1973) and Raja (1981) in different exotic and crossbred bulls. The variation is attributable to genetic differences of the bulls employed for various studies and also to the difference in the handling and staining of smears in different studies. The bulls employed for the present study belonged to different genetic groups and age groups and these could be the probable cause for significant variation observed in the various abnormalities between bulls. This is also in the findings of Rao and Rao (1973) and Raja (1981), who also observed highly significant variation in head abnormalities between crossbred bulls. However in the case of crossbred bulls no such variation was observed by Rao and Rao (1980).

The incidence of proximal protoplasmic droplet varied from 0.70 to 1.60 per cent with a mean of 1.18 ± 0.156 per cent (Table 24). This is in accordance with the findings

of Raja (1981) in Brown Swiss crossbred bulls but lower than those reported by Rao and Rao (1978) and those suggested for normal fertile bull by Blom (1950) and Rao (1971). The incidence of proximal protoplasmic droplet varied significantly ($P < 0.01$) between bulls before and after preservation in various diluents (Table 25). This observation is similar to that of Rao and Rao (1975) and Raja (1981) in crossbred bulls. However, Rao and Rao (1980) did not observe any significant variation in the occurrence of proximal protoplasmic droplet between Ongole bulls.

Middle piece abnormalities were noted to be in a range of 0.70 to 1.20 per cent with a mean of 0.90 ± 0.093 per cent (Table 26). These values are slightly higher than those reported in crossbred bulls by Rao and Rao (1978) and Raja (1981) and lower than those reported in the ejaculates of Tharparkar and Jersey bulls by Rao and Rao (1975). The incidence of mid piece abnormality was found to differ significantly ($P < 0.01$) between bulls (Table 27). An observation similar to this was made by Raja (1981) in Brown Swiss crossbred bulls. The mid piece abnormality did not show significant variation during preservation in different diluents upto 72 hours (Table 27).

The incidence of simple bent tail and coiled tail was 2.18 ± 0.106 and 1.24 ± 0.102 per cent respectively (Table 28, 30). The mean value obtained in respect of the

tail abnormalities is lower than those reported by Rao and Rao (1975) and Raja (1981). Highly significant ($P < 0.01$) variation in the percentage of simple bent tail was observed between EYC, Tris and skim milk diluent and also during preservation at 0, 24, 48 and 72 hours (Table 29). However, in the case of coiled tail the difference was found to be significant in respect of preservation period only (Table 31). Similar observation of marginal increase with tail abnormality during preservation in the similar diluent was also observed by Rao and Rao (1979). Contrary to the findings of Raja (1981) the incidence of tail abnormalities was found to vary significantly between bulls (Table 29, 31).

The total tail abnormalities during present observation before and after preservation was within the normal limit prescribed for fertile bulls as suggested by Hollinsen (1951). The values also did not go beyond the values reported by Rao and Rao (1978) in crossbred bulls.

There is divergence of opinion as to the importance of presence of varying number of abnormal sperms seen in stained semen preparation. According to Lagerlof (1934) more than 17% abnormal sperms in the ejaculate indicate lower fertility. Similar observation was also made by Davis (1940) and Andersen (1941). Haq (1949) reported an average of 15.1% of abnormal sperms within a range of 6 to 26% in the case of normal fertile bulls. According to Hancock (1959) the presence

of 10% or more of any single type of abnormality is often associated with reduced fertility. In the present study the total abnormal sperms before and after preservation in three different diluents was not more than the admissible limits (Table 7). The abnormality presently observed is comparable to those reported by Nirwan et al. (1976).

The average head length of bull spermatozoa before dilution was 9.51 micron (Table 32). On preservation in EYC, Tris and skim milk diluent there was no significant change in head length (Table 33). Similarly the average head breadth which was 4.72 micron (Table 34) also did not vary significantly during preservation in the above three diluents (Table 35). This is in accordance with the findings of Van Demark and Salisbury (1953). However, Tomar (1964) found significant decrease in the head length during preservation for 3 days in Hariana and Murrah bull spermatozoa. On the contrary Tomar et al. (1980) reported no significant changes in sperm head biometry as a result of storage at 5°C for a period of 48 hours in egg yolk-glucose-fructose-sodium bicarbonate and D_2-Co_2 medium. They also concluded that there was no significant relationship between fertility of bulls and sperm head biometry.

It could be seen from foregoing paragraphs that the morphological changes noticed in the present investigation were in the normal limit for crossbred bulls, and that no

deleterious effects were noticed during preservation upto 72 hours in EYC, Tris and skim milk diluents. Thus it could be concluded that the above three diluents are suitable for preservation of semen of crossbred bulls upto 72 hours at 5°C.

SUMMARY

summary

A systematic study was made on the semen characteristics of crossbred bulls and changes, if any, during preservation in egg yolk-citrate, Tris and skim milk diluent upto 72 hours.

A total of 72 ejaculates, from five crossbred bulls maintained at A.I. Centre attached to College of Veterinary and Animal Sciences, Mannuthy were utilised for the study.

Immediately after collection the semen was subjected to routine evaluation and the concentration, live and dead sperm and motility were assessed. Smears of the semen sample prepared and stained with Giemsa stain were examined for various types of sperm abnormalities. The stained smears so prepared were also utilised to study the sperm head biometry.

To study the effect of preservation on the motility and morphology of sperm, semen samples were diluted at the rate of 1:20 in egg yolk-citrate, Tris and skim milk diluent and stored at 5°C in the refrigerator. One drop of diluted semen was taken from each sample and motility assessment was carried out from zero hour to 72 hours of preservation at 24 hours interval of storage. At the same intervals, smears of diluted semen were also prepared and stained with Giemsa stain and examined for various types of sperm abnormalities

and sperm head biometry. The results obtained and inferences drawn are summarised below.

The overall average sperm concentration was 1.59 ± 0.025 million per cmm within a range of 1.29 to 1.91 million per cmm. No significant difference in sperm concentration was found between the bulls.

The overall mean livability was found to be 83.45 ± 0.532 per cent, within a range of 79.00 to 94.29 per cent. No significant difference in the livability of sperm could be observed between the bulls.

The overall mean initial motility was 83.00 ± 1.00 per cent ranging from 82.00 to 86.00 per cent. Highly significant ($p < 0.01$) variation in initial motility of sperms was found between the bulls. The mean motility percentage in egg diluent was 83.00 at zero hour and 57.10 at 72 hours of storage. The respective values in Tris diluent were 83.00 and 58.00 per cent and in skim milk diluent, 83.00 and 55.80 per cent. There was no significant difference between the three diluents in preserving motility at 5°C upto 72 hours. In all the three diluents the motility percentage of sperm declined significantly starting from zero hour of storage to 72 hours. Comparative evaluation of motility percentage during preservation showed that motility above 55 per cent could be maintained upto 72 hours of storage at 5°C in all the three diluents, viz. egg yolk-citrate, Tris and skim milk.

The average percentage of total sperm abnormality was 16.68 ± 0.59 before dilution. It increased slightly to 17.48 ± 0.661 immediately after dilution and to 19.24 ± 0.558 at 72 hours of storage in EYC diluent. Similarly in Tris diluent the mean percentage increased to 18.96 ± 0.620 at 72 hours of storage from 16.92 ± 0.595 at zero hour of preservation. In skim milk diluent the mean total sperm abnormality increased to 19.82 ± 0.572 per cent at 72 hours from 17.66 ± 0.720 per cent at zero hour of preservation.

The mean percentage of free normal head was 2.20 ± 0.151 before dilution. During preservation in EYC, Tris and skim milk diluent upto 72 hours the values were found to be 2.38 ± 0.139, 2.40 ± 0.109 and 2.44 ± 0.102 per cent respectively which did not vary significantly. The mean percentage of free abnormal head, detached acrosome, pear shaped head, narrow at the base, knobbed head, abnormal contour and underdeveloped head in the present study was 1.56 ± 0.132, 1.26 ± 0.100, 1.90 ± 0.130, 1.22 ± 0.086, 0.80 ± 0.122, 1.52 ± 0.08 and 0.82 ± 0.082 respectively before dilution. The respective values at 72 hours of preservation in EYC diluent were 1.72 ± 0.124, 1.42 ± 0.177, 1.94 ± 0.150, 1.38 ± 0.08, 0.98 ± 0.115, 1.70 ± 0.083 and 1.04 ± 0.097 per cent. Similarly in Tris and skim milk diluent the values were found to be 1.70 ± 0.114, 1.48 ± 0.159, 1.86 ± 0.120, 1.36 ± 0.074, 0.96 ± 0.14, 1.70 ± 0.104 and 0.98 ± 0.101 per cent and 1.78 ± 0.106,

1.42 ± 0.163, 1.90 ± 0.114, 1.42 ± 0.073, 1.00 ± 0.077, 1.68 ± 0.101 and 1.10 ± 0.089 per cent respectively. The present study revealed that among all the head abnormalities, the detached acrosome of the bull spermatozoa increased significantly with the advancement of storage period upto 72 hours in egg yolk-citrate, Tris and skim milk diluent. All types of head abnormality were found to vary significantly between the animals irrespective of the diluents used. The present study also showed that there was no difference between the above three diluents in regard to the occurrence of various types of head abnormalities.

The proximal protoplasmic droplet in the present study was found to be 1.10 ± 0.136 per cent before dilution. During preservation upto 72 hours in EYC, Tris and skim milk diluent the incidence was found to be 1.40 ± 0.173, 1.30 ± 0.219 and 1.48 ± 0.198 per cent respectively. From the results it could be seen that though there was significant variation between the bulls there was no variation between the diluents as well as between the storage periods for the incidence of proximal protoplasmic droplet. The middle piece defect of bull spermatozoa was also found to remain unchanged upto 72 hours of storage in egg yolk-citrate, Tris and skim milk diluent. The respective values in the above three diluents were 1.10 ± 0.089, 1.04 ± 0.050 and 1.00 ± 0.040 per cent.

The average percentage of the defect before dilution was 0.90 ± 0.093. Significant difference was found between the bulls for the defect but there was no difference between the diluents.

In the case of simple bent tail, significant difference between the bulls and between the diluents was observed. The incidence also increased significantly from 2.36 ± 0.120, 2.23 ± 0.096 and 2.38 ± 0.139 per cent during zero hour of preservation to 2.74 ± 0.150, 2.74 ± 0.090 and 3.06 ± 0.037 per cent at 72 hours of preservation in EYC, Tris and skim milk diluent, respectively. Similarly the coiled tail also was found to increase significantly as the storage period advanced, irrespective of the diluent used. The incidence of coiled tail was found to be 1.24 ± 0.102 per cent before dilution which increased to 1.44 ± 0.092, 1.44 ± 0.092 and 1.48 ± 0.213 per cent at 72 hours of preservation in EYC, Tris and skim milk diluent. Present study showed significant difference between bulls for the incidence of coiled tail but no difference was noticed between the diluents.

The average head length and head breadth of the bull spermatozoa was found to be 9.51 and 4.72 microns respectively before dilution. Present study showed that there was no change of length and breadth of spermatozoan head as a result of preservation upto 72 hours at 5°C in egg yolk-citrate, Tris and skim milk diluent. The study revealed significant

difference between the bulls regarding the spermatozoan head length and head breadth.

The present study revealed that among egg yolk-citrate, Tris and skim milk diluent, one did not possess any superiority over the other as a diluent for bull semen, stored at 5°C upto 72 hours and all the three diluents preserved the spermatozoan morphology and motility equally without exerting any major deleterious effect upon them.

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CHANGES IN SPERM MORPHOLOGY OF CROSSBRED BULLS DURING PRESERVATION

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ABSTRACT OF A THESIS

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ABSTRACT

A systematic study was made on the semen characteristics of crossbred bull and their changes, if any, during preservation in egg yolk-citrate, Tris and skim milk diluent upto 72 hours.

A total of 72 ejaculates, from five crossbred bulls maintained at A.I. Centre attached to College of Veterinary and Animal Sciences, Mannuthy were utilised for the study.

Immediately after collection semen was subjected to routine evaluation and smears were prepared, stained with Giemsa stain and examined for various types of sperm abnormalities and sperm head biometry. Semen samples were then diluted at the rate of 1:20 in 37°C, Tris and skim milk diluent and stored at 5°C. To study the effect of preservation in the above diluents at 0, 24, 48 and 72 hours, motility, various sperm abnormalities and sperm head biometry were observed during the above storage periods.

The overall average concentration and livability of sperm was 1.39 ± 0.025 million per cmm and 93.45 ± 0.522 per cent, respectively. No significant difference in concentration and livability of sperm was found between bulls.

The overall average initial motility was 82.00 ± 1.00 per cent before dilution. During preservation upto 72 hours

in EYC, Tris and skim milk diluent sperm motility declined significantly to 57.10, 58.00 and 55.80 per cent respectively. There was no significant variation between the diluent used in maintaining sperm motility but significant difference was noticed between the bulls.

The mean percentage of free normal head, free abnormal head, detached acrosome, pear shaped head, narrow at the base; knobbed head, abnormal contour and underdeveloped head was 2.20 ± 0.151, 1.56 ± 0.132, 1.26 ± 0.180, 1.80 ± 0.130, 1.22 ± 0.086, 0.80 ± 0.122, 1.52 ± 0.08 and 0.82 ± 0.082 respectively, before dilution. Among all the head abnormalities, detached acrosome increased significantly with the advancement of storage period upto 72 hours irrespective of the diluent used. However, no variation in sperm head abnormalities was noticed between the diluents. All types of head abnormalities varied significantly between bulls.

The average percentage of proximal protoplasmic droplets and middle piece defect was 1.18 ± 0.156 and 0.90 ± 0.083 per cent respectively, before dilution. They remain unchanged upto 72 hours of storage in all the three diluents. Though, significant difference was found between the bulls, no variation could be noticed between the diluent used.

Simple bent tail and coiled tail showed significant variation between bulls and between the storage periods; mean

values being 2.18 ± 0.106 and 1.34 ± 0.102 per cent, respectively, before dilution. They increased significantly at 72 hours of storage in MC, Tris and skim milk diluent, the mean values being 2.74 ± 0.150, 2.74 ± 0.050 and 3.00 ± 0.037 per cent, respectively, for simple bent tail and 1.44 ± 0.092, 1.44 ± 0.092 and 1.48 ± 0.215 per cent, respectively for coiled tail. Though, in the case of simple bent tail significant variation was found between the diluents, no such variation could be noticed in case of coiled tail.

The average length and breadth of sperm head was found to be 9.51 and 4.72 microns, respectively, before dilution. No significant variation in spermatozoan head length and breadth could be noticed as the storage period advanced to 72 hours. There was also no significant variation between the diluents. However, significant variation was found between the bulls. Thus it could be inferred that all the three diluents were found to be equally good for preservation of bull semen upto 72 hours at 5°C.