STRUCTURAL AND FUNCTIONAL CHANGES IN THE TESTIS AND EPIDIDYMIS OF CROSS BRED BULLS WITH IMPAIRED FERTILITY

By

T. SREEKUMARAN

THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy

Faculty of Veterinary and Animal Sciences

Kerala Agricultural University

Department of Animal Reproduction

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

MANNUTHY, THRISSUR - 680651

KERALA, INDIA

2000

DECLARATION

I hereby declare that the thesis entitled **"STRUCTURAL AND FUNCTIONAL CHANGES IN THE TESTIS AND EPIDIDYMIS OF CROSSBRED BULLS WITH IMPAIRED FERTILITY"** 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.

Sreekumaran

T. SREEKUMARAN

Mannuthy,
18 .05.2000.

CERTIFICATE

Certified that the thesis, entitled **"STRUCTURAL AND FUNCTIONAL CHANGES IN THE TESTIS AND EPIDIDYMIS OF CROSSBRED BULLS WITH IMPAIRED FERTILITY"** is a record of research work done independently by Dr. T. Sreekumaran, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Dr. P. P. Balakrishnan
(Chairman, Advisory Committee)
Professor and Head
Department of Animal Reproduction
College of Veterinary and
Animal Sciences, Mannuthy

Mannuthy,
18-05.2000.

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Dr. T. Sreekumaran,** a candidate for the degree of Doctor of Philosophy in Animal Reproduction, agree that the thesis entitled **"STRUCTURAL AND FUNCTIONAL CHANGES IN THE TESTIS AND EPIDIDYMIS OF CROSSBRED BULLS WITH IMPAIRED FERTILITY"** may be submitted by Dr. T. Sreekumaran, in partial fulfilment of the requirement for the degree.

Dr. P.P. Balakrishnan
(Chairman, Advisory Committee)
Professor and Head
Department of Animal Reproduction
College of Veterinary and Animal Sciences, Mannuthy

Dr. E. Mathai
Professor (Retd)
Department of Animal Reproduction
(Member)

Dr. V. Vijayakumaran
Associate Professor
Department of Animal Reproduction
(Member)

Dr. T. Sreekumaran
Professor
Centre of Excellence in
Veterinary Pathology,
(Member)

Dr. K.V. Raghunandanan
Associate Professor
Centre for Advanced Studies
in Animal Breeding and Genetics
(Member)

External Examiner 26/12/2000
(DR. K. VENUGOPAL NAIDU M.V.Sc, Ph.D)

ACKNOWLEDGEMENT

I am deeply indebted and grateful to Dr. P.P. Balakrishnan, Professor and Head and Chairman, Advisory Committee for the valuable guidance, support and constant encouragement during the period of investigation.

I express my sincere gratitude and indebtedness to Late Dr. C.P. Neelakanta Iyer, former Head, Department of Animal Reproduction and former Chairman of Advisory Committee for his inspiring guidance, scholastic advice, perpetual support and enthusiasm.

I am very much grateful and obliged to Dr. E. Mathai, Professor (Retired) Department of Animal Reproduction, Dr. T. Sreekumaran, Professor, Centre of Excellence in Pathology, Dr. K.V. Raghunanadanan, Associate Professor, Centre for Advanced Studies in Animal Breeding and Genetics and Dr. V. Vijayakumaran, Associate Professor and Head, Cattle Breeding Farm, Thumburmuzhi for their constant help, guidance and encouragement as members of the Advisory committee.

I express my sincere appreciation and gratitude to Dr. K. Prabhakaran Nair, Former Head, Department of Animal Reproduction, Dr. G. Mukundan, Former Director, Centre for Advanced Studies in Animal Breeding and Genetics and Late Dr. G. Nirmalan, Former Dean-in-charge of College of Veterinary and Animal Sciences for the scholastic advice and consistent help rendered as members of advisory committee.

Grateful acknowledgement is made to Dr. M. Nagasundharam, General Manager, Dr. R. Rajeev, Deputy General Manager, Dr. B.N. Kishan and Dr. (Mrs). K.V. Kunjammini, Former Deputy Managers, Dr. Roy Mathew and Dr. Rajeev Shetty, Assistant Managers and Dr. A.M. Sajith and Dr. Biju Raj, Former Assistant Managers and all Scientific and Technical staff of Dhoni Farm, Palghat for affording all facilities to conduct the research programme.

I am deeply indebted to Dr. E. Madhavan, Former Professor and Head, Veterinary Hospital, Kokkalai for his ever willing help, perpetual support and consistent encouragement throughout the period of study.

The invaluable help and assistance rendered by Dr. K.V. Athman, Dr. K.N. Aravinda Ghosh, Dr. S.P. Suresh Nair, Associate Professors, Dr. K. Ramachandran, Dr. Metilda Joseph, Dr. A.M. Vahida, Dr. G. Ajitkumar, Dr. C. Ibrahimkutty, Assistant Professors, Dr. C. Jayakumar, Dr. K. Pramod, Dr. Manju Soman, Teaching Assistants, Dr. R. Geetha, Dr. B. Bibin becha and other Post-graduate students and all other staff of the Department of Animal Reproduction are gratefully acknowledged.

I express my profound gratitude to Dr. K.M. Ramachandran, Director, Centre of Excellence in Veterinary Pathology, Dr. N. Divakaran Nair and staff of the Department of Pathology and Sri. A.P. Peter, Farm Assistant, Department of Animal Reproduction for their valuable help and assistance for the histopathologic and ultrastructural studies.

My thanks are also due to the staff of Meat Technology Unit for help rendered in the slaughter studies.

The help rendered by Smt. U. Narayanikutty, Assistant Professor and Head and K.P. Santha Bai and K.V. Prasad, Programmers and other staff of the Department of Statistics for the statistical analysis of the data is hereby gratefully acknowledged. I am also equally thankful to all my colleagues in the faculty for their help and encouragement.

Thanks are due to Professor, Research Co-ordinator and Director of P.G. Studies for their valuable co-operation and guidance for undertaking this investigation.

I sincerely acknowledge the help rendered by the Managing Director, Kerala Livestock Development Board for giving permission to undertake the studies at Dhoni Farm, Palghat.

I am grateful to Dr. S. Sulochana, Dean, College of Veterinary and Animal Sciences, and Dr. A Rajan, former Dean for their valuable guidance and encouragement at the various stages of this study.

The whole hearted appreciation to Peagles, Mannuthy is hereby acknowledged for the excellent typing of the thesis.

I have no words to express my gratitude to my beloved mother, wife and children who have been a constant source of inspiration to me.

T. Sreekumaran

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

1. INTRODUCTION

Genetic progress in a population of livestock can be achieved by using superior males and females to produce the next generation. The transfer of genetic material from one generation to the next is mainly taking place through bulls to bulls and cows to bulls. In developed countries, semen of proven bulls are employed for large scale artificial insemination. It is generally felt that importance should be given to bull mother farm to isolate superior genotype from among the lot. A proper selection from among the bull mothers is possible, only if they are kept in a standard plain of reproduction. In India, massive crossbreeding programme of local non descript stocks with exotic dairy breeds such as Jersey, Holstein Friesian and Brown Swiss was launched five decades back. This ambitious programme necessarily warrants extreme care in the selection of crossbred breeding bulls with respect to their reproductive ability. Galina and Arthur (1991) opined that delayed maturity, testicular hypoplasia and sperm abnormalities appear to be the most common problems in cross bred bulls and stressed the necessity for a suitable test to screen bulls for adequate reproductive performance. To fulfil the above demands, high fertile bulls are essentially needed for frozen semen bull stations. But often it is difficult to identify the high fertile bulls, unless they are put to use for sometime in the breeding operations. Therefore investigations have to be carried out to predetermine the fertility of breeding bulls before it is extensively used.

Evaluation of bull for its fertility has therefore been a demanding task which necessitates standardization of criteria and techniques. Progressive modification is always desirable but variation in evaluation can only reflect unfavourably in this programme. The necessity for evaluation of bull for fertility is important because a significant number of prospective breeding bulls with impaired fertility are subfertile and breeding of these bulls is time consuming, expensive and fruitless.

In order to exploit the full potential of breeding bulls, it is needless to say that sires of high pedigree should be selected and used to the maximum extent. The process of selection of breeding bulls is therefore a laborious process and various parameters have to be taken into account from birth to adult stage to exploit the maximum out of these breeding bulls. It is therefore important to study the structural and functional changes in the reproductive organs of bulls with normal and impaired fertility, so that only those with assured reprductive ability will be selected for breeding purpose. Consideration should also be given on the desirability of semen for freezing and factors which would affect the freezability of semen. It is also necessary to get an insight into the probable infertility conditions that might be encountered which would affect the rate of culling of bulls. The above factors have paramount importance in the success of breeding programme and the present work was therefore undertaken to investigate the structural and functional changes in testis and epididymis of different crosses with impaired fertility with the ultimate object of suggesting ways for better and efficient utilization of available crossbred breeding bulls in the breeding programme.

Review of Literature

2. REVIEW OF LITERATURE

Assessment of breeding performance of bulls in general and early detection of infertility are the main stay of any breeding programme since presence of questionable or unsatisfactory bulls would render the whole cattle development programme ineffective, wasteful and unpopular. Investigation of breeding bull depends on meticulously kept breeding records which have become all the more important, with wide scale application of frozen semen technology. Emphasis should be not only on elimination of bulls with questionable or unsatisfactory potential but also on identification of bulls with potential to produce good quality semen. It is generally accepted that 5 to 10 per cent of bull calves and young bulls have to be eliminated as sterile or subfertile at the very early stage (Annual Report of KLD Board, 1985-86). From the remaining 90 to 95 per cent, despite rigorous selection in respect of fertility the conception rate varies from 60 to 70 per cent.

In order to detect abnormalities in growing bulls breeding soundness evaluation has been used as a quick and cost effective method of predicting fertility of bulls (Rao and Rao, 1991). This includes general physical examination focussing on the parts of the bull's body most likely to be involved in cases of decreased reproductive efficiency. This has to be followed by examination of reproductive system including examination of external genitals and per rectal examination. Scrotal circumference measurements have been accepted as a part of fertility examination of young, growing and adult bulls (Gipson *et al.*, 1985; Das and Tomer, 1995).

2.1 Bull calves upto 12 months of age

2.1.1 Palpation of testicles – weight of testis

Spitzer and Hopkins (1997) reported that sperm production and testes weight of bull calves are closely related. They further remarked that the rate of sperm production was remarkably similar among bulls with similar testes weight because each gram of functional tissue had the same amount of tubular epithelium.

2.1.2 Shape of testis

Abdel Raouf (1960) reported that the shape of testes was cylindrical at birth and resembled date fruit and became more oval as the age advanced. Rao and Rao (1988) described different types of scroti in crossbred bulls as square (23.53 per cent), oblong (23.53 per cent), elongated (14.71 per cent), oval (14.71 per cent), round (7.35 per cent) and bifid (4.41 per cent). Veerapandian (1992) reported different shapes of scrotum in Jersey bulls ie., oval, elongated, rounded, rectangular and pear shaped as 62.50, 18.75, 8.33, 2.08 and 8.3 per cent respectively.

2.1.3 Scrotal circumference

Singh *et al.* (1967) observed a correlation of age and body weight with different testicular biometrics. A definite progressive growth of testis was observed from birth to 16 months of age. It was further observed that the growth was slow in the beginning, became rapid between 5 to 8 months, more rapid upto 12 months and most rapid after 12 months of age. They also noted that different testicular measurements showed consistent rise indicating constant shape of testis during

prepubertal period. The average testicular circumference at birth was 3.37 cm which increased to 4.33, 4.86, 5.75, 7.48, 9.23, 11.05 and 17.42 cm at 2, 4, 6, 8, 10, 12 and 16 months of age respectively. Hahn *et al.* (1969) reported that scrotal circumference averaged 28.4 cm at 7 to 12 months of age and age of bulls showed a curvilinear relationship.

It has been reported that, eventhough, there was tremendous variation in age at puberty among and within breeds, scrotal circumference of bull calves appeared to be more accurate predictor of fertility in prepubertal and adult bulls. According to Lunstra *et al.* (1979) individual bulls representing variety of genotypes reached puberty at a fairly consistent scrotal circumference regardless of breed, although, age and weight at puberty varied greatly among breeds. They also reported mean scrotal circumference as 27.9 ± 0.2 cm at puberty and reported the scrotal circumference at puberty in bulls somewhere as 29 cm irrespective of the breeds.

According to Hueston *et al.* (1988) the mean scrotal circumference of Jersey bull calves was 18.9 cm at 5 months of age. Rao *et al.* (1992) found a correlation of scrotal circumference with age and body weight in Indian breeds. Das and Tomer (1995) found positive correlation of scrotal circumference with initial motility, percentage of live sperm and serum testosterone level.

2.1.4 Infertility

The incidence of cryptorchidism has been reported to be less in bull calves compared to other species (Roberts, 1986 and Laing *et al.*, 1988). Although unilateral cryptorchids are partially sterile, bilateral cryptorchids are sterile owing to thermal suppression of spermatogenesis (Hafez, 1993). The time of descent of testes in bull was reported to be 3.5 to 4 months of gestation (Arthur *et al.*, 1996). Although abnormal gubernacular development and testicular descent are poorly understood, gonadotrophin deficiency has been attributed for failure of testicular descent.

The hypoplastic testis is detected only at puberty because of reduced fertility or sterility, the condition was suspected in bull calves by scrotal and testicular measurements (Almquist *et al.*, 1975). Nagasundaram (1986) noticed testicular problems in bull calves usually bilateral and reported high incidence of small eliptical testis indicative of Testicular hypoplasia. Testicular hypoplasia, has been detected in young bull calves of all breeds (Roberts, 1986).

2.2 Growing bulls from 12 to 24 months of age

In growing bulls, the factors responsible for reducing reproductive efficiency are many and varied. It has been reported that nearly 50 per cent of young bulls are being culled for poor semen quality during the growing stage. The number of growing bulls rejected on account of poor semen freezability was found to be alarmingly high in crossbreds (Annual Report of KLD Board, 1985-1986).

2.2.1 Scrotal circumference

Gipson *et al.* (1985) reported that scrotal circumference measurements in young bulls gave a relatively accurate estimation of their ability to produce semen in terms of quality and quantity. According to Hueston *et al.* (1988) the mean scrotal circumference of growing Jersey bulls was found to be 36.4 cm at 18 months of age. Spitzer and Hopkins (1997) reported that the mean scrotal circumference at puberty for bulls was somewhere close to 28 cm and most bulls should have attained puberty when a scrotal circumference reached 30 cm.

Blockey (1979) and Rao *et al.* (1993) reported a positive correlation between age and scrotal circumference. A positive correlation between body weight and scrotal circumference was also found in young bulls of 6 to 16 months of age (Pimentel *et al.*, 1984) in Holstein yearling bulls (Coulter and Foote, 1976) and Jersey bulls (Rao and Rao 1990).

Kuferschmied *et al.* (1985) found significant correlation between scrotal circumference and total number of spermatozoa per ejaculate in 51 to 55 and 77 to 80 weeks of age. On the contrary, Thompson *et al.* (1992) reported that total circumference and semen quality was unrelated. However, the reports on the interrelationship between scrotal circumference and semen characteristics of growing bulls are scanty.

Hopkins and Spitzer (1997) observed total circumference was positively related to sperm output and highly heritable. The minimum recommended scrotal circumference for various ages of bulls have been reported to be 30 cm in bulls in

the age of 12 to 15 months, 31 cm in 15 to 18 months, 32 cm in 18 to 21 months and 33 cm in 21 to 24 months.

Van Camp (1997) reported that alterations in the normal scrotal confirmation might indicate pathological condition. A short scrotum was reported to be an indication of testicular hypoplasia (Van Camp, 1997), while a flat or slab sided scrotum might indicate a unilateral testicular hypoplasia or atrophy. He also reported that distinct distorsion of scrotal silhoutte of the head or tail of epididymis was indicative of epididymitis and swelling of the neck of the scrotum indicated varicosities of the pampiniform plexus.

2.3 Adult bulls of above 24 months of age

2.3.1 Libido

Bane (1954) believed that libido was controlled by genetic factors, Smith (1961) did not find any such influence on sex libido.

Frazer (1974) showed that lack of libido was one of the most common problems of bulls leading to frequent culling. Iyer (1984) reported poor sex desire of 3.63 per cent and 4.44 per cent in Jersey crossbreds and purebreds respectively. Veerapandian (1992) found that 3 out of 11 Jersey bulls refused ejaculation and classified them with questionable libido. Smith *et al.* (1981) reported a significant correlation between sex libido and scrotal circumference. But Chenoweth *et al.* (1988) did not find any correlation in scrotal circumference between bulls of high and medium libido. This was also confirmed by Niwaklor and Ezinma (1989).

Verma and Singh (1992) remarked that lack of libido or weak sexual reflexus might be due to low testosterone levels.

2.3.2 Scrotal circumference (SC)

Hahn *et al.* (1969) found that SC was a better measure of testis size than various linear measurements. The variable was easy to measure, highly repeatable and was highly correlated with testis weight. Similar associations between testicular measurements and testis weight (Ansari *et al.*, 1972) and between SC and testis weight (Nema and Kodagali, 1983) were observed in buffalo bulls.

Coulter *et al.* (1975) reported that SC ranged from 30.0 to 43.9 cm in exotic breeds. Raju (1981) noted a mean of 37.33 cm in Jersey bulls. According to Larson *et al.* (1990) SC ranged from 34 to 42 cm in beef breeds.

Veerapandian (1992) reported that SC was similar in Jersey bulls with satisfactory and questionable semen quality and that SC was significantly lower (29 cm) in Jersey with unsatisfactory semen. SC increased upto 84 to 107 months of age and then declined. The bulls in higher SC groups produced three fold more concentrated semen (1142 vs 380 millions per ml) and four fold more total sperm per ejaculate (4993 vs 1239 millions/ml) than the least SC group in Jersey bulls. Higher age combined with lower motility, live sperm, sperm concentration and higher incidence of sperm abnormalities in the semen of Jersey bulls with SC <32 cm indicated age related degenerative changes in these bulls. SC was positively correlated with quantity and quality of semen. Rao and Rao (1995) remarked that scrotal circumference is a simple repeatable method of measuring testicular size

which reflects spermatozoan producing ability and they also reported that greater testicular circumference might result in good quality of semen which might be due to high spermatogenic activity in larger area.

Makarechian *et al.* (1985) have observed that scrotal circumference was not a reliable predictor of sperm output of bulls whose testis circumference was within normal range.

Tierney *et al.* (1982) found a definite correlation of scrotal circumference with sperm concentration and abnormal sperms. Scrotal circumference and semen volume were similar in bulls classified as satisfactory questionable, or unsatisfactory on the basis of semen characteristics and serum testosterene level. Mohanty *et al.* (1983) reported a highly positive correlation of SC with individual motility, sperm concentration, live sperm count and sperm abnormalities. Makarechian *et al.* (1985) noted a positive correlation between SC and semen quality and quantity. According to Veeramachaneni *et al.* (1986) decreased sperm motility, spermatozoan concentration and increased sperm abnormalities were seen in bulls with SC less than 30 cm.

Coulter and Foote (1976) reported that SC increased with age similar to that of body weight, but testes size tended to reach mature more rapidly by 30 months when compared to body size. They also reported that larger bulls tended to have larger testes. Makarechian *et al.* (1985) did not find any effect of age on scrotal circumference within each age group.

2.3.3 Testicular measurements

Abdel Raouf (1960) reported that bovine testicle became more oval as age advanced due to greater increase in breadth and thickness. Coulter *et al.* (1975) reported a high rate of testicular growth upto 36 months with limited growth in mature bulls and slightly decreased growth in old bulls after 144 months. Abdel Raouf (1960) and Sakala (1964) reported asymmetry in growth of right and left testis in different breeds. The right testis was found heavier than left.

Kozumplik (1983) observed mean testicular length and width as 11.7 to 12.4 and 8.2 to 8.8 cm respectively in different age groups. In Jersey bulls, the length and width of testes were reported to be 14.33 and 7.67 (Raju, 1981) and 12.93 and 7.45 cm (Singh and Pangawkar, 1989) respectively.

Kohli (1985) reported a case of bifid scrotum in a young Rathi bull with well developed testicles freely movable in the scrotum and without any rotation of scrotum on its longitudinal axis.

Krishna and Rao (1987) observed that oblong shape of scrotum was prominent in Jersey bulls. Rao and Rao (1988) studied the different types of scroti in crossbred bulls and observed oblong 23.53 per cent, square 23.53 per cent, elongated 14.71 per cent, oval 14.71 per cent, round 6.35 per cent and bifid 4.41 per cent and described the abnormal types of scroti as monorchid 8.32 per cent, asymmetrical 2.94 per cent and scrotum close to the abdomen 1.47 per cent. Veerapandian (1992) reported that oval shape of scrotum was predominant in Jersey bulls.

2.3.4 Semen characteristics

Extensive studies have been carried out on semen characteristics, freezability and sperm abnormalities of exotic bulls (Salisbury, 1944; Anderson, 1945 and Almquist *et al.*, 1963). However, the performance of crossbred bulls with particular reference to their semen quality and fertility under the varying environmental conditions prevailing in our country has not been adequately reviewed.

Attempts made to assess the potential fertility of a bull on the basis of ejaculate volume, initial motility, concentration, livability, freezability and magnitude of incidence of spermatozoan abnormalities would serve the purpose of a single sure test to select bulls of high fertility.

2.3.4.1 Ejaculate volume

The relationship between ejaculate volume and fertility of the bull appears conflicting. Bishop *et al.* (1954) found evidence of decline in the rate of fertility with increase in the volume of ejaculate. Hafez (1962) on the other hand, observed that ejaculate volume was found to vary between breeds, age, level of nutrition and climatic conditions. A genetic basis for the ejaculate volume was recognized by Tomar *et al.* (1965). Salisbury *et al.* (1978) observed variation in ejaculate volume in between and within bull and expressed doubts whether real breed differences in semen volume have been established. Roberts (1986) opined that fertility of the male was not influenced by an increase or decrease of the volume of semen.

The mean ejaculate volume was reported to be 6.4 ml (Mathew, 1974) and 4.34 ml (Raju and Rao, 1982) in Brown Swiss and 4.02 ± 1.61 ml (Rao and Rao, 1975), 4.34 ml (Raju and Rao, 1982), 3.67 ± 0.04 ml (Sharma *et al.*, 1982), 4.08 ± 0.34 ml (Khan and Kharche, 1983), 4.56 ml (Iyer, 1984), 3.79 ml (Sagdeo *et al.*, 1990), 5.75 ml (Singh and Pangaw kar, 1990), 7.6 ml (Reddy *et al.*, 1991) and 4.02, 3.65 and 4.63 ml in satisfactory, questionable and unsatisfactory bulls respectively (Veerapandian, 1992), in Jersey bulls. The average ejaculate volume for the various Indian breeds was 3.51 ml for Tharparkar, 4.71 ml for Red Sindhi, 5.70 ml for Gir (Bhattacharya and Prabhu, 1954) 4.37 ml for Sahiwal (Tomar, 1964), 4.19 ml (Rao and Rao, 1980) and 5.77 ml (Raju and Rao, 1982) for Ongole.

In Holstein Friesian x Hariana and Brown Swiss x Hariana crossbred bulls, Kaker and Arora (1973) reported an ejaculate volume of 3.84 ml and 5.56 ml, respectively. In Brown Swiss crosses with 75, 62.5 and 50 per cent exotic inheritance Mathew (1974) recorded 5.5 ml, 4.0 ml and 4.8 ml respectively as the mean ejaculate volume. In other crossbred bulls, the mean ejaculate volume was 3.65 ml in Jersey x Sindhi (Rao and Kotayya, 1977), 5.16 ml in Jersey x Sahiwal (Saxena and Tripathi, 1978), 4.83 ml (Rao and Rao, 1978) and 5.17 ml (Raju and Rao, 1982) in Brown Swiss x Ongole and 4.17 ml in Holstein Friesian x Ongole bulls (Rao and Rao, 1978). Raja (1981) found that the volume of semen per ejaculate in Brown Swiss crossbreds varied from 0.5 to 6.0 ml with a mean of 2.65 ± 0.084 ml. The ejaculate volume of semen of adult 50 per cent crossbred bulls ranged between 3.64 ml to 4.42 ml with a mean of 3.99 ml.

Iyer (1984) reported that ejaculate volume of young bulls at 18 to 19 months of age was 2.05 ml and that the ejaculate volume showed a gradual increase as age advanced from 18 to 24 months and reached a mean of 2.73 ml at 24 months of age in growing crossbred bulls.

According to Hultnas (1959) the volume of semen was significantly influenced by the age of the animal. Povliconko (1964) also observed an increase on ejaculate volume with advance in age in *Bos taurus* bulls. Abdel Raouf (1965) also observed a similar trend in Swedish Red and White cattle. In Hariana bulls, Singh *et al.* (1967) reported increase in semen volume upto six years of age. A significant correlation between ejaculate volume and age has been reported in Angus x Hereford bulls (Almquist and Cunningham, 1967) and in Brown Swiss x Ongole crossbred bulls (Rao and Rao, 1978). In contrast Kaker and Arora (1973) did not observe any difference in volume due to increase in age in Hariana crossbred bulls.

In crossbred bulls, Mathew (1974) observed increase in semen volume with increase in exotic inheritance. Pathak (1979) found no influence of nutritional treatments on the volume of semen in crossbred bulls. According to Raju and Rao (1982) the volume of semen per ejaculate significantly varied between various genetic groups.

2.3.4.2 Motility

Blom (1950) found that low rate of motility of spermatozoa was invariably associated with infertility. Significant relation between motility and fertility was

observed by Stewart *et al.* (1972). Rao and Rao (1979) observed positive correlation between fertility and initial motility in crossbred bulls. Mohanty *et al.* (1983) also reported significant correlation between sperm motility and fertility of bulls.

Average initial sperm motility in *Bos taurus* bulls was reported as 51.6 per cent (Lasley, 1951), 83 per cent (Bishop *et al.*, 1954), 63 per cent (Brown, 1959) and 65 per cent (Almquist *et al.*, 1963). Initial motility ranged from 52 to 73 per cent in Holstein Friesian x Hariana crossbreds and 35 to 57 per cent in Brown Swiss x Hariana (Kaker and Arora, 1973). Rao and Rao (1975) observed 81.62 per cent motility in Tharparkar bulls. In Jersey bulls the motility was recorded as 79.20 per cent (Rao and Rao, 1975), 73.92 per cent (Raju and Rao, 1982), 80.61 per cent (Sharma *et al.*, 1982), 80 per cent (Bhosrekar *et al.*, 1982 and Khan and Karche, 1983), 63.19 per cent (Iyer, 1984), 72.14 per cent (Sagdeo *et al.*, 1990), 75.00 per cent (Reddy *et al.*, 1991) and 56.54 ± 1.86 per cent (Veerapandian, 1992).

In Jersey x Sahiwal crosses, Saxena and Tripathi (1978) observed 71.17 per cent and for Brown Swiss x Ongole and Holstein Friesian x Ongole crosses Rao and Rao (1978) reported 84.13 and 80.00 per cent motility respectively. Raja (1981) recorded 66.24 per cent motility for Brown Swiss cross breds. Raju and Rao (1982) recorded 59.50, 62.25, 73.92 and 67.75 per cent initial motility for Brown Swiss x Ongole, Brown Swiss, Jersey and Ongole bulls respectively.

Initial motility ranged from 30.24 to 79.24 in crossbreds (Sagdeo *et al.*, 1990 and Singh and Pangawkar, 1990). In Holstein Friesian bulls the initial motility

ranged from 70.44 to 79.41 per cent (Bhosrekar *et al.*, 1982; Mohanty and Dugwekar, 1987; Pangawkar and Sharma, 1989 and Singh and Pangawkar, 1990). Iyer (1984) observed that the initial motility of adult crossbred bulls varied from 54.44 per cent to 62.61 per cent with a mean of 59.03 per cent. Several authors have reported that initial motility was influenced by various factors. Lindley *et al.* (1959) observed highly significant correlation between initial motility and age of the bull Dimitriev (1964), Malmberg (1965) and Morstin (1970) also concurred this. Abdel Raouf (1960) observed an increase in motility as age of bulls advanced. Almquist and Cunningham (1967) reported that the sperm motility showed a highly significant increase during the first 20 weeks after puberty in Jersey bulls. Almquist *et al.* (1975) found that the initial motility in Jersey bulls increased with age during the first 60 weeks after puberty. The rapid increase in initial motility from 34 per cent at 1 to 4 weeks to 56 per cent at 9 to 12 weeks after puberty accounted for most of the improvement in sperm motility as the bull was aged. Raju and Rao (1982) recorded a gradual increase in initial motility in the ejaculate with advancing age from 64.84 per cent in bulls below 24 months to 67.73 per cent in bulls above 36 months.

Iyer (1984) observed that the initial motility was poor (39.64 per cent) at 18 to 19 months of age which gradually increased and reached 53.47 per cent at 24 months of age in Jersey crossbred bulls.

Hultnas (1959), Maslov (1960) and Rüttle *et al.* (1975) did not find any significant correlation between motility and age of bull. This indicated that as bulls

get older they tended to produce semen of lower initial motility. Singh *et al.* (1968) and Morstin (1970) found positive correlation between motility and percentage of live sperms.

Veerapandian (1992) reported that sperm motility was highest and abnormalities were lowest in age group of 36-59 months. He found that the semen quality in terms of motility and abnormality declined as the age advanced and suggested that it would be prudent to utilize exotic breeds like Jersey to the maximum extent possible before they attained 6-7 years of age.

2.3.4.3 Sperm concentration

Lagerlof (1934) opined that concentration of spermatozoa in fertile bulls varied from 300 to 2000 millions per ml with an average of 800 millions per ml of semen. The concentration of first and second ejaculates in taurus bulls was 1.259 and 1.281 million per cmm respectively (Salisbury, 1944). In the same species the values were further reported as 873 million (Anderson, 1948), 1108 million (Blom, 1950), 1388 million (Stone *et al.*, 1950) and 1296 million (Rao and Rao, 1975) per ml.

In Hariana, Sahiwal, Tharparkar, Red Sindhi and Gir bulls, Paul *et al.* (1966) recorded 1.284, 1.426, 1.269, 1.729 and 1.674 million sperms per cmm respectively. The mean sperm concentration for other bulls was 1.396 million per c.mm (Mathew, 1974) and 1118:58 millions per ml (Raju and Rao, 1982) in Brown Swiss. The values were 1296 million per ml (Rao and Rao, 1975) 1300.33 million per ml (Raju and Rao, 1982), 1274 millions per ml (Sharma *et al.* 1982)

1590 millions per ml (Bhosrekar *et al.* 1982), 935 millions per ml (Khan and Kharche, 1983) 1692.71 millions per ml (Iyer, 1984), 1157.75 million per ml (Singh and Pangawakar, 1990) 1052 millions per ml (Reddy *et al.* 1991) and 1110.24 millions per ml in satisfactory, 616.25 millions per ml in questionable and 90.00 millions per ml in unsatisfactory classes. (Veerapandian, 1992) in Jersey bulls, 1332 million per ml (Rao and Rao, 1975) in Tharparkar, 1133 million for Red Dane (Porwal *et al.* 1977) and 765.90 million per ml (Rao and Rao, 1980), 1203.67 million 1070.00 to 1482 .85 millions per ml for Holstein Friesian (Mohanty *et al.* 1983).

In crossbred Brown Swiss bulls with 75,62.5 and 50 per cent level of exotic inheritance, Mathew (1974) recorded concentration as 1320 million, 1502 million and 1408 million sperms per ml respectively. Rao and Kotayya (1977) recorded 1472 millions per ml for Jersey x Sindhi crosses. Saxena and Tripathi (1978) recorded 912.33 million per ml for Jersey x Sahiwal crosses. Values for other crosses were 790 million per ml for Holstein Friesian x Hariana and 697 million per ml for Brown Swiss x Hariana (Kaker and Arora, 1973); 984.93 millions per ml for Brown Swiss x Ongole and 611.84 millions per ml for Holstein Friesian x Ongole crosses (Rao and Rao , 1978). In 62.5 per cent Brown Swiss crossbred bulls, Raja (1981) recorded 1599.64 ± 55.371 million sperms per ml of semen. Iyer (1984) reported that the sperm concentration increased from 500 millions per ml at 18 to 19 months of age to 907.78 million per ml at 24 months of age in cross bred Jersey bulls. He observed that the sperm concentration of bulls above 48 months was significantly higher than that in bulls between 36 and 48 months of age.

Lepard *et al.* (1941), Maslov (1960) and Singh *et al.* (1967) did not observe any significance of age on sperm concentration. Contrary to this, Laurans and Negriere (1964) reported that the total output of sperm was significantly high in adult bulls than in young ones. Morstin (1970) reported that concentration of spermatoza increased with advancement of age. Kaker and Arora (1973), Rao and Rao (1978) and Rao *et al.* (1979) also reported similarly increase in cross bred bulls.

Roberts (1986) observed that sperm cell concentration might be reduced to one third to one half of normal values or severe oligospermia in testicular degeneration. Veeramachaneni. *et al.* (1986) observed a decreased sperm concentration in bull with testicular lesions and regressed epididymal epithelium.

In partial testicular hypoplasia, the concentration was found to be 478 million/ml (Deshpande *et al.*1976). In cases of unilateral hypoplasia the sperm concentration was reported to be 115 (Kodagali and Kerur,1965) 150 to 300 (Rao *et al.* 1966) and 45 to 350 millions per ml (Akusu and Akpododje, 1983).

2.3.4.4 Livability

Roberts (1986) opined that a high incidence of necrospermia is usually associated with poor motility resulting in subfertility in bulls. The mean percentage of live spermatozoa in *Bos taurus* bulls was reported to be 77.9 per cent (Bishop *et al.* 1954) and 70.1 per cent (Bratton *et al.* 1956). In purebred bulls the live sperm concentration was 85.15 per cent (Rao and Rao, 1975), 82.20 per cent (Raju and Rao, 1982), 88.00 per cent (Bhosrekar *et al.*, 1982); 77.55 per cent (Bujarbaruah *et*

al., 1982); 75.38 per cent (Iyer 1984); 82.97 per cent (Singh and Pangawrkar, 1990) and 82.40 per cent (Veerapandian 1992) in Jersey, 64.7 per cent in Red Dane (Porwal *et al.*1977); 75.95 per cent in Brown Swiss bulls (Raju and Rao, 1982) and 76.95 ± 1.55 to 81.20 ± 1.54 per cent in Holstein Friesian bulls (Mohanty *et al.* 1983). The percentage of live sperms was 76.00 per cent (Tomor *et al.* 1966) and 80.60 per cent (Singh *et al.*1967) in Hariana bulls. The live sperm percentage in Tharparkar bulls was 88.22 per cent (Rao and Rao, 1980) and 72.52 per cent (Raju and Rao, 1982) in Ongole bulls respectively. The percentage of live sperms in crossbred bulls are also reported to be varied, the values being 86.34 per cent for Hariana x Holstein Friesian, 87.34 per cent for Hariana x Brown Swiss and 87.28 per cent for Hariana x Jersey (Biswas *et al.* 1976). In Holstein Friesian x Ongole and Brown swiss x Ongole bulls the same was reported to be 85.20 per cent and 87.22 per cent respectively (Rao and Rao 1978). Raju and Rao (1982) found live sperm concentration of 66.93 per cent in Brown swiss x Ongole crossbred bulls. According to Saxena and Tripathi (1978), Jersey x Sahiwal crossbred bulls recorded 83.07 per cent live sperms in the ejaculate. In 62.5 per cent Brown Swiss bulls , Raja (1981) recorded 72.55 per cent of live sperms which ranged from 45 to 90 per cent. Iyer (1984) reported that in growing crossbred bulls, the live sperm percentage steadily increased from 64.49 per cent at the age of 18 to 19 months to 74.01 per cent at 24 months of age. In adult cross breds the initial motility varied from 54.44 to 62.61 per cent with a mean of 59.03 per cent. Although, Tomar (1970) did not find any influence of age of bull on the live sperm concentration of the ejaculate, Singh *et al.* (1967) reported that age influenced the percentage of live

sperms. Mohanty *et al.* (1983) observed a significant correlation between scrotal circumference and sperm concentration in Holstein Friesian bulls.

2.3.4.5 Sperm Abnormalities

Conflicting views have been expressed with regard to the number of abnormal sperms in the semen sample in relation to fertility. Lagerlof (1934), Davis *et al.* (1940) and Anderson (1941) found lowered fertility when abnormal spermatozoa exceeded 17 per cent and opined that importance should be given to the head abnormalities like detached head and abnormal shape. However, Herman and Swanson (1941) remarked that presence of 30 per cent abnormal sperms is compatible to poor or good fertility. On the other hand Blom (1948, 1950) observed impaired fertility in bulls containing 15 percent abnormal sperms. However, Cupps *et al.* (1954); Bratton *et al.* (1956) and Campbell *et al.* (1960) found a high correlation between abnormal sperm count and fertility of bulls. This was also supported by Shaw (1972). A negative correlation between abnormal sperm count and fertility was also observed in Holstein Friesian x Ongole and Brown Swiss x Ongole crossbred bulls by Rao and Rao (1979). The percentage of abnormal sperms was 4.6 per cent (Blom, 1950) in normal bulls Rollinson (1951) opined that normal fertile bulls should not have more than 3 to 4 per cent abnormal heads, 4 to 10 per cent abnormal tails and 0.5 to 6 per cent loose heads. According to Hancock (1959), in normal bulls, the incidence of any single abnormality should not exceed more than 10 per cent. In Jersey, Guernsey, Jersey x Ongole and Brown swiss x Sahiwal bulls, Rao and Kotayya (1977) recorded an incidence of 10.60,

11.46, 38.95 and 13.26 per cent of total abnormalities of sperms respectively. The frequency of occurrence of head abnormalities, free loose heads, proximal protoplasmic droplets, midpiece and tail abnormalities in Jersey bulls was noted as 11.74, 3.75, 2.20, 1.20 and 15.4 per cent and in Tharparkar bulls 10.58, 2.11, 1.10, 1.15 and 2.38 per cent respectively by (Rao and Rao 1975). The percentage of head abnormalities in Hariana x Holstein Friesian, Hariana x Brown Swiss and Hariana x Jersey was reported to be as 14.56, 13.09, 16.34 per cent respectively (Biswas *et al.* 1976). Saxena and Tripathi (1978) found 18.93 per cent of abnormal sperms in Jersey x Sahiwal cross bred bulls. The head abnormalities, free loose heads, midpiece abnormalities and proximal and distal protoplasmic droplets in Brown Swiss x Ongole crosses were reported to be 9.46, 2.97, 0.74, 3.02 and 2.86 per cent whereas the corresponding values in Holstein Friesian x Ongole crosses were 15.86, 6.13, 0.92, 3.03 and 5.04 per cent (Rao and Rao, 1979). The frequency of occurrence of head abnormalities, free loose heads, midpiece and tail abnormalities in Ongole bulls was 7.13, 2.76, 0.75, 2.87 and 1.74 per cent respectively (Rao and Rao, 1980). In 62.50 per cent Brown Swiss bulls, Raja (1981) recorded 3.43, 4.91, 0.65, 6.44 and 1.20 per cent of head abnormalities, free loose heads, mid piece abnormalities, tail abnormalities and proximal protoplasmic droplets respectively. Sperm abnormalities in Jersey bulls was reported as an average of 14.03 per cent (Khan and Karche, 1983); 12.55 per cent (Talukdor *et al.* 1986); 10.45 per cent (Singh and Pangawkar, 1990); 10.25 per cent (Reddy *et al.* 1991) and 16.81 per cent (Veerapandian , 1992). Age was not found to influence the production of abnormal sperms in *Bos taurus* bulls (Bane, 1954; Hultnas, 1959 and Laurans and Negriere,

1964). On the other hand, Malmberg (1965); Abdel Raouf (1965); Singh *et al.* (1967); Morstin (1970) and Rao and Rao (1979) observed a decrease in the percentage of abnormal sperms as age advanced. But Raja (1981) observed an increase of free loose heads and a decrease of head and tail abnormalities with increase in age in 62.5 per cent crossbred bulls. In Jersey crossbred bulls Iyer (1984) recorded head abnormalities, free loose heads, middle piece abnormalities, tail abnormalities and proximal protoplasmic droplets as 2.85 per cent, 4.51 per cent, 1.63 per cent, 7.30 per cent and 1.19 per cent respectively. The corresponding values for pure bred Jersey were 2.79, 2.68, 0.97, 6.25 and 1.16 per cent respectively. Tail abnormalities and proximal protoplasmic droplets showed significant variation between age groups in purebred bulls while in crossbred bulls age influenced the incidence of all abnormalities other than middle piece abnormalities.

Singh and Pangawkar (1990) studied characteristics of exotic and crossbred bull spermatozoa and found that the mean values for ejaculate volume, mass activity, initial motility, live sperm count and sperm concentration in Holstein-Friesian, Jersey, Holstein-Friesian Sahiwal, Karan Friesian and Holstein Red Sindhi were within range stipulated for normal fertile bulls. The total abnormalities of sperm head, midpiece and tail including prototoplasmic droplets were 10.01 ± 0.14 per cent in HF, 10.45 ± 0.20 per cent in Jersey, 8.48 ± 0.15 per cent in HFS, 8.82 ± 0.16 per cent in KF and 15.80 ± 0.23 per cent in HRS bulls. A high significant breed difference in volume, initial motility, live sperm count and sperm abnormalities was also observed.

Veerapandian (1992) reported 4.33 ± 0.42, 8.12 ± 1.65 and 38.10 ± 5.30 per cent primary sperm abnormalities and 12.48 ± 1.07, 54.53 ± 4.35 and 38.39 ± 5.10 per cent secondary sperm abnormalities in the semen of bulls with satisfactory, questionable and unsatisfactory fertility respectively. There was an absolute positive correlation between secondary and total sperm abnormalities.

2.3.5 Freezability

Considerable attention is being paid in recent years on the freezability and post thaw livability of bull semen, since conception depends to a great deal on these factors. Variations were found in the ability of spermatozoa of different samples of different bulls to withstand freezing. This was confirmed by more extensive studies by Dunn *et al.* (1954) who found more significant difference between bulls than within bulls. According to them, density of semen samples and maturity of spermatozoa influenced the freezability. Ohms and Willet (1955) observed better freezability with the second ejaculate than the first, which they attributed to the sperm cell and not to seminal plasma. However, O" Dell *et al.* (1959) found no such difference with five successive ejaculates in the freezing ability. Maule (1962) also made similar observations.

Freezability expressed as post thaw motility was between 32 to 36.9 per cent in Brown Swiss bulls and 32.7 to 33.5 per cent in 62.5 per cent Brown Swiss crosses (Mathew, 1974). Rate of freezability increased with higher exotic inheritance in these bulls. Roy *et al.* (1975) recorded 54.6 per cent freezability in Brown Swiss x Hariana crosses and 48.60 and 47.80 per cent in crosses of Hariana

with Jersey and Holstein Friesian respectively. However, no significant difference was noticed between bulls of the same genetic group. Reddy *et al.* (1980) recorded 57.08 per cent post thaw motility in exotic bulls. In Jersey bulls, Sattar *et al.* (1980) recorded 59.10 to 63.45 per cent post thaw motility when different extenders were used. Raja (1981) observed 36.86 per cent post thaw motility in 62.5 per cent Brown Swiss bulls. He observed no seasonal variation in the freezability, which is in agreement with Mathew (1974). Iyer (1984) reported mean freezability of sperms of purebred Jersey and Jersey crossbred bulls to be 43.60 per cent and 42.32 per cent respectively. Age of bulls had no influence on the freezability in both the groups and seasonal influence of freezability was evident only in crossbred bulls. However, Raja (1981) observed that age of bulls significantly influenced freezability.

According to the report of the Milk Marketing Board, Scotland (1973-74), the pre-freezing and post-freezing rejection rates of ejaculates were 23.4 and 4.2 per cent respectively. Mathew (1974) found a higher rate of pre-freezing rejection in Brown Swiss crossbred bulls. He further observed that both pre-freezing and post-freezing discard rates decreased with increase in exotic inheritance. The corresponding rates for Jersey bulls were 11.9 and 5.36 per cent respectively as against 13.5 and 5.19 per cent respectively in Holstein bulls (Sattar *et al.* 1978). In Brown Swiss crosses, Raja (1981) reported pre-freezing rejection rate of 28.55 per cent and post-freezing rejection rate of 15.74 per cent. Month of collection and age of the bull had significant influence on the rejection rates. Iyer (1984) observed that rate of discard before freezing in cross bred bulls was 34.31 per cent and the post

freezing discard was 8.61 per cent. The corresponding discard rates for purebred Jersey bulls were 30.02 and 6.60 per cent. The percentage of total rejection rates in crossbred and purebred Jerseys was 38.98 and 34.95 respectively. Sajdeo *et al.* (1990) observed that in breeding bulls, exotic indigenous combination played a major role in the freezability of bovine semen. However they recommended further cytogenetic studies to confirm this. Singh *et al.* (1993) studied the effect of spermatozoan concentration on freezability and found that the post thaw motility and livability in samples with 15 x 10^6 per ml spermatozoan concentration differed significantly and proved to be the best in comparison to 20 x 10^6 and 30 x 10^6 per ml. Singh *et al.* (1993) observed the effect of spermatozoan concentration on freezability of purebred and crossbred bull semen and found highly significant difference in samples with 15 x 10^6 spermatozoan concentration when compared to 20 x 10^6 and 30 x 10^6 concentration per ml.

2.3.6 Infertility

Lagerlof (1934) reported an incidence of 23 per cent testicular hypoplasia in Swedish Highland bulls. In other countries, a low incidence of this condition was reported and its hereditary nature was not been established. Lagerlof (1936) and Eriksson (1943) attributed testicular hypoplasia a single recessive autosomal gene. Possibility of exogenous factors such as hormonal and vitamin deficiencies, toxic factors operating intrauterine or postnatal life were also attributed by Cohrs (1966). David Steffen (1997) observed hypoplasia more common in highly inbred cattle population.

Settergren (1978), Rao *et al.* (1986) and Patel *et al.* (1986) observed testicular hypoplasia due to low germ cell, resistance with poor freezablity of semen. Hypoplasia with arrested spermatogenesis was reported by Knudson (1958). Kodagali (1964) described unilateral gonadal hypoplasia in a Gir bull based on gross and histological changes. Rao *et al.* (1966) reported a similar case in a cross bred bull with conspicuous reduction in size and weight of the testicles. Based on clinical examination, Kodagali and Kerur (1968) observed six cases (4.5 per cent) unilateral gonadal hypoplasia in a herd of 133 Gir bull calves. Kodagali *et al.* (1971) reported nine cases of hypoplasia in Gir bulls of which six were left sided, two right sided and one bilateral. Deshpande *et al.* (1976) observed left sided partial hypoplasia and Kaikini *et al.* (1978) reported complete testicular hypoplasia in a Holstein Friesian bull. Bilateral hypoplasia was recorded in Red Dane cross bred bull by Narasimhan *et al.* (1981). Iyer (1984) reported unilateral testicular hypoplasia (3.63 per cent) bilateral hypoplasia (10.90 per cent) and disturbed spermatogenesis associated with scrotal defects (10.90 per cent) in Jersey cross bred bulls.

Patel and Kodagali (1985) reported 22.22 per cent of bulls having testicular hypoplasia and recorded clinical findings in normal vs problem bulls with respect to scrotal circumference (34.93 ± 0.72 vs 30.75 ± 0.51 cm), testicular volume 1037.59 ± 47.65 vs837.50 ± 23.72 ml); R-test (1154.81 ± 100.81 vs 425.00 ± 17.68) seconds and sperm survivability test in cervical mucus (1.96 ± 0.20 vs 84 ± 0.21) minutes.

Patel *et al.* (1986) observed complete bilateral testicular hypoplasia and low germ cell resistance type of hypoplasia with subsequent testicular degeneration in cross bred bulls. Rao *et al.* (1986) reported that the mean percentage of head, middle piece and tail abnormality and proximal droplets was 30.25, 0.425 and 32.58 respectively in low germ cell type of testicular hypoplasia.

Patel *et al.* (1988) reported 22.22 per cent of testicular hypoplasia in cross bred bulls. Normal bulls had significantly higher values of SC, testicular volume, R-test, sperm survivability in homologous cervical mucous, cold shock resistant sperm % and hot shock resistant sperm % than the problem bulls.

Veeramachaneni *et al.* (1986) found severe testicular hypoplasia with scrotal circumference below 30 cms with seminiferous tubules having only sertoli cells. Settergren and Mckentee (1992) reported sporadic cases of testicular hypoplasia in bulls of Swedish Red and White breed.

Settergren and Mckentee (1992) reported that bulls with typical case of hypoplasia often mature late. Veerapandian (1992) observed one per cent of unilateral hypoplasia of testis in Jersey bulls.

The incidence of cryptorchidism was low in bulls (Blom and Christensen, 1947, Carrol *et al.* 1963, Ladds *et al.* 1973). Raja and Nair (1961) Kodagali and Kerur (1968) reported unilateral cryptochidism in a Sindhi and Gir bull respectively. Rebhun (1976) reported bilateral crytorchidism in a Holstein Friesian bull.

The sensitivity of testicular epithelium to adverse conditions like excessive heat or cold, trauma, physical strain, irradiation, toxaemia and age has been well recognized (McEntee, 1970). Infectious diseases like Foot and Mouth disease and other viruses are known to produce degeneration of testis and also as a sequale to vaccination (Sharma, 1969; Radhakrishnan *et al.* (1975).

Iyer (1984) reported 7.27 per cent and 8.88 per cent of testicular degeneration in 50% Jersey cross breds and pure bred Jersey bulls respectively. Rao and Bane (1985) reported that increased abnormal speratozoan heads with pear shape, narrow at the base, lack of development, abnormal contour and microhead will increase in severity of testicular degeneration. The incidence of proximal cytoplasmic droplets increased with degeneration but that of tail abnormalities was not affected.

Rao and Rao (1991) reported reduced fertility with testicular degeneration with poor semen picture in three bulls with degenerative changes in the germinal epithelium and pronounced increase in interstitial connective tissue in all cases.

Epididymitis has been occasionally observed as an acquired lesion in all species of animals. In bulls the incidence varied from 0.1 to 2 per cent (Blom and Christensen, 1947; Carrol *et al.* 1963). Konig (1964) opined that epididymitis arises chiefly by spread of infection in genitourinary passage less frequently by haematogenous route and seldom by trauma. According to Blom and Christenson (1972) affections of epididymis and vas deferens occur in bulls. According to Roberts (1986) Brucella infection was the most important cause of epididymitis in

bulls. Parkinson *et al.* (1993) reported atypical granulomatous epididymitis in a Devon bull due to undetected traumatic rupture of an epididymal tubule resulting in cell mediated response and spermatozoan damage due to antisperm antibodies.

Epididymal dysfunction associated with sperm tail abnormalities has been reported by Gustafsson (1965) and Gustafsson *et al.* (1972). Cupps and Briggs (1965) opined that impaired epididymal function caused changes in the morphology of sperms. Twinbull (1977) recorded 7.9 per cent of epididymal affections while Iyer (1984) found epididymal dysfunction to the extent of 5.45 per cent in cross bred bulls and 13.33 per cent in pure bred Jersey bulls. The incidence of tail abnormalities decreased gradually if semen was collected frequently in a short time to exhaust epididymal reserve (Krishna and Rao, 1987). Veerapandian (1992) furnished bulls with testicular degeneration and epididymal dysfunction had lower incidence of proximal and distal droplets when compared to normal bulls. Incidence of head abnormalities was higher. Rao (1997) remarked that pathological sperms above 17 to 18 per cent indicate disturbances in spermatogenesis or epididymal dysfunction resulting in lowered fertility and recommended repeated examination for prognosis.

Van Camp (1997) opined that epididymitis and seminal vesiculitis are two most common diseases of secondary sex organs of bulls and are associated with each other.

Information on the incidence of seminal vesiculitis in bulls is scanty. Incidence was reported to be 0.8 per cent (Blom and Christensen, 1947), 4.2 per cent (Vandersluis, 1953), and 1.3 per cent (Bishop *et al.* 1954).

Ball *et al.* (1968) observed idiopathic testicular epithelial abnormality of mild nature in 89% of bulls and 84% of testicles in a herd of bulls with high incidence of seminal vesiculitis. They observed that primary sperm abnormalities increased as the severity of seminiferous testicular damage increased and no correlation was observed between secondary abnormalities and tubular changes. They also reported orchitis in 52%, epididymitis in 63%, ampullitis in 58%, seminal vesiculitis in 49%, prostatitis in 43% and bulbo urethral adenitis in 15% of bulls.

Zaitsev and Cherepakin (1988) reported autoantibodies in bulls with inflammation by an agglutination reaction with seminal plasma as cases of immunological infertility in bulls with diseased accessory sex glands. Kaveleri and Van Camp (1997) reported age as a predisposing factor with more common incidence in young, against peripubertal bulls over nine years. Chronic cases showed abscessation, fibrosis, adhesions with fistulations to the rectum, bladder, peritoneal cavity etc.

Reports from India on the incidence of this condition are few. Johari (1957) Sane *et al.* (1965) Pathak (1967) Kaikini *et al.* (1968) and Kerur and Kodagali (1970) reported incidence in individual bulls of different breeds. Rao and Rao (1981) recorded unilateral seminal vesiculitis in a Holstein Friesian bull.

Todorovic (1984) reported an incidence of 18 per cent primary and secondary prostatitis in 2 to 6 year old bulls and classified prostatitis as primary, secondary, focal, difuse, acute, subacute and chronic types. Zaitsev (1988)

observed immunological infertility in bulls with inflammation of prostate and vesicular glands by an agglutination reaction with seminal plasma.

Moorthy (1985) reported 23.8% cases of an outbreak of balanoposthitis in Boran bulls caused by herpes virus demonstrated in preputial mucosa of bulls. Infertility due to specific sperm abnormalities not associated with testicular pathology like decapitated sperms (Hancock and Rollinson, 1949), Knobbed sperms (Donald and Hancock, 1953), pouch formation in the nucleus of sperm head (Bane and Nicander, 1965).

Bane and Nicander (1965) described Diadem defect in bulls characterized by pouch formation appearing like a dark necklaces along the anterior edge of the post nuclear cap. Aughey and Renton (1968) observed small lateral appendages at the posterior end of sperm head inserted into separate implantation fossa in a sterile Ayreshire bull. Fawcett and Philips (1970) and Barth (1986) observed knobbed acrosomes characterized by localised swelling or bead formation on the apical ridge in bulls. A refractile bead at the apex of sperm head was seen less commonly in the infertile bulls. Saacke (1970). reported that Knobbed acrosomal defect with normal fertility in bulls was less frequent and found ruffled. acrosome characterised by wrinkled appearance causing subfertility in bulls. Acrosomal cap abnormalities of sperm like knobbed, ruffled and incomplete acrosomes from subfertile Jersey and Holstein Friesian bulls and their sons were reported by Saacke *et al.* (1968).

Anderson *et al.* (1990) reported that a swollen apical segment of acrosome resulted in subfertility in Ayreshire bulls. Singh *et al.* (1992) reported that the

acrosomal abnormalities decreased with increase in percentage of live spermatozoa and the highest level of defects noted in samples with less than 60 per cent live sperms and indicated the usefulness of such studies as an aid to indirect assessment of fertility.

Barth *et al.* (1992) observed that a moderate degree of sperm head narrowness in the absence of other seminal signs of disturbances of spermatogenesis was not detrimental to fertility. However extreme narrowness of post acrosomal region of the head of spermatozoa without other signs of disturbance in spermatogenesis resulted in significant reduced fertility. Thundathil *et al.* (1999) reported that pyriform head had reduced capability to bind and to penetrate zona pellucida and (zygote resulting from this fertilization of oocyte by pyriform sperm) appeared to have reduced ability for cleavage.

Blom (1950) reported corkscrew sperm defect of the midpiece in Danish Red Jersey and Friesian bulls. Munro *et al.* (1961) observed an incidence of 28 to 46 per cent of abnormal middle piece in a Jersey bull. Incidence of abaxial attachment of mid piece in a Norwegian Red and White bull was also reported by Onstad (1963). Peet *et al.* (1988) observed infertility in bulls with semen containing 60-80 per cent abaxial middle piece and 40-50 per cent swollen middle piece.

Barth (1989) reported that among attachment of bovine spermatozoa in 10.5 per cent of bulls only 0.48 per cent bulls produced sperms with greater than 50 per cent abaxial tails and 0.86 per cent produced sperms with accessory and double

tails. He found that spermatozoa with abaxial tail attachment fertilize ova at a normal rate and are not associated with any increase in embryonic death.

Coubrough and Barker (1966) reported various mid piece abnormalities in sterile bulls. Blom (1968) observed in five related Friesian bulls rounded or elongated thickened areas in the middle piece and described it as pseudodroplet defect . A hereditary defect called "Dag defect" where 40 to 50 per cent sperm tails were defective with very low motility was described in a Jersey bull by Blom (1968). Similar tail defects were also described in Jersey bulls by Swanson and Boyd (1962), Gustafsson (1965); Gustafsson *et al.* (1972); Koefoed-Johnson and Friis (1965). Ogasa *et al.* (1967) observed a high incidence of looped tail in a Holstein Friesian bull. Arriola *et al.* (1985) reported sterilizing Oligoteratozoospermia known as sperm tail stump defect in a bull. Williams (1987) also reported such defects in Charolais bulls with watery semen, low concentration, sperm akinesia and with cent per cent abnormal sperms. Partial development was the most striking abnormality with a short mid piece like remnant of cytoplasmic droplet replacing midpiece and tail, with high percentage of spermatogenesis. There was no normal elongation of spermatids or tail development.

Reports on sperm abnormalities from India are scanty. Raja and Nambiar (1962) reported a high incidence of abnormal sperms with abaxial attachment in a sterile Sindhi bull. Raja *et al.* (1974) reported complete absence of spermatozoan motility associated with large number of coiled sperm tails with apparently no

pathological changes in the sexual organs of a Jersey bull. Similar defects were reported in a Hallikar (Rao, 1976) and Jersey bull (Rao and Subbaih, 1982); Haranath *et al.* (1983) and Veerapandian (1992). Veerapandian *et al.* (1992) reported "Dag defect" in two Jersey bulls characterized by 40 to 50 per cent of sperm tail strongly coiled and folded together or split up into fibres in the ejaculates and also found that bulls born to this sire with different mothers had same abnormalities. Gopakumar (1994) reported a case of knobbed spermatozoa in a cross bred bull.

2.3.7 Seminal Enzymes

Enzymatic evaluation of semen quality and fertility has been recognised as one of the useful means to predict fertility of bulls (Crabo *et al.* 1971; Breeuwsma, 1973; Pandit and Garg, 1983; and Belorkar 1986). Semen transminases and phosphatases play important role in transamination and phosphorylation of sperm metabolism, sperm survival and fertilization (Mann, 1964). Phosphatases in semen reflects functional state of accessory sex glands and are helpful in differentiating the reproductive biology of different breeds and species. Belorkar (1986) reported the overall mean values for Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT), GOT GPT ratio, Alkaline phosphatase (AKP), Acid phosphatase (ACP), AKP:ACP ratio, freezability and fertility as 90.75 ± 11.65 micro mol/litre, 13.08 ± 4.98 micromol/litre 6.94:1. 152.80 ± 79.22 KAU/100 ml, 262.04 ± 90.09 KAU/100 ml, 0.59:1.1, 54.27 ± 1.89 per cent and 43.29 per cent respectively. Significant higher values were observed for freezability, GPT and

AKP during hot, wet and cold season respectively while GOT and ACP levels were lower during cold seasons. They also reported that semen of bulls with good freezability showed higher GPT, AKP and ACP values.

Pangawkar *et al.* (1988) studied the acid and alkaline phosphatase (ACP & AKP) and GOT, GPT activities of seminal plasma of Holstein Friesian bulls and found a significant low level of acid phosphatase and alkaline phosphatase activity and higher level of GOT and GPT in semen of bulls with low freezability. GOT in seminal plasma was found to be negatively correlated with fertility indicating higher release of spermatozoan GOT into the plasma.

Verma and Singh (1992) observed a low succinic dehydrogenase activity in a bull having low conception rate with normal libido and serving behaviour. According to Dhami *et al.* (1994) the mean enzymic activity for Friesian bulls was GOT 80.02 ± 3.73 micro mol /litre, GPT 12.88 ± 0.69 micro mol/litre, GOT GPT ratio 6.21, AKP 226.98 ± 15.88 KAU/100 ml. ACP 234.94 ± 11.10 KAU/100 ml, AKP ACP ratio 0.97 and LDH 391.24 ± 11.06 IU/l respectively Effect of bull was found to be significant for Friesians for AKP. A significant negative correlation for AKP activity with ejaculate volume and a positive correlation with GOT and ACP were also noticed. LDH had significant positive correlation with sperm motility and GOT and negative correlation with pH. Other correlations of enzymes with seminal attributes were not significant.

Rao *et al.* (1999) reported the mean activities of AKP, ACP, and LDH enzymes in neat semen of Punganellur bulls were 584.87 ± 11.22 KAU/10 ml,

247.47 ± 2.31 KAU/100 ml and 356.22 ± 9.45 IU/L respectively. No significant difference was noticed between different bulls and between collection in all the three enzymes, but a higher leakage of these enzymes when preserved in EYC extender compared to tris extender

2.4 Rate of culling

Culling rates of bulls of different ages at KLD and MM Board was 28.3 per cent, 63.9 per cent, 73.8 per cent and 76.9 per cent in 100 per cent, 75 per cent, 62.5 per cent and 50 per cent Brown Swiss bulls respectively. The reason for culling was poor individual motility of sperm, poor or absence of libido, azoospermia, oligospermia and other minor conditions like poor body confirmation etc (Annual Report of KLD Board, 1985-86).

It was also reported that the percentage of culling due to poor semen production was higher in crossbred bulls than in exotic bulls. Mathew *et al.* (1982) reported earlier that poor semen quality in bulls was the cause for culling of 15 per cent of bulls.

Nagasundaram (1986) reported at KLD Board, a high percentage of culling during the period from 1982 to 1984 and found poor initial motility and loose head abnormality of sperm was the major reason for culling.

2.5 Scanning Electron Microscopy (SEM)

Reports of scanning electron microscopic studies of testis and epididymis of bull are scanty. However, morphological alteration of these tissues on SEM reported by Jubb *et al.* (1993).

Materials and Methods

3. MATERIALS AND METHODS

The present study was carried out at Dhoni Farm, Palghat attached to the Kerala Livestock Development Board during the period from 1993 to 1998. The object of the farm is to meet the requirements of frozen semen of different crossbred bulls for the state. With the object of investigating the incidence, nature and magnitude of prevalence of infertility conditions among crossbred bulls, the records maintained at the Dhoni Farm was scrutinized for a period of five years. In addition to this, 153 crossbred males of different crosses of Jersey (CBJ), Brown Swiss (CBBS) and Holstein-Friesian (CBHF) were divided into the following three age groups and subjected to detailed andrological investigations.

Group I - Bull calves from birth to 12 months of age

Group II - Growing bulls from 13 months to 24 months of age

Group III - Adult bulls above 24 months of age

3.1 Group I - Bull calves

3.1.1 Palpation and measurement of Testis and Epididymis

Forty six bull calves from birth to 12 months of age were subjected to detailed andrological examinations for presence of any infertility or pathological conditions. Testis and epididymis of both sides were examined, measurements were recorded and inspected for any asymmetry, enlargement, lesions or scars. Scrotum was palpated to assess mobility of testis inside the scrotal sac. Both testes were

examined separately for size, shape, consistency and normal descent. Epididymis was then palpated and the head, body and tail of epididymis were examined for any inflammatory lesion, spermatocoele, hypoplasia or aplasia and their size and consistency were noted.

3.1.2 Scrotal circumference

The testes were pulled firmly into the lower part of scrotum by encircling its base with hand and pulling down both the testes. The thumb and fingers were positioned on the side of the scrotum to prevent separation of testis. The circumference of the scrotum was measured at its maximum diameter using flexible tape formed into a loop.

The testis length, thickness and width were measured at the point of maximum dimensions with the help of vernier calipers as described by Hahn *et al.* (1969) and recorded in cm. Any deviation and symmetry was evaluated.

3.1.3 Infertility conditions observed

Number of various infertility conditions observed during the period of study was ascertained and detailed investigation was carried out.

3.1.4 Histopathology

Those having gross pathological conditions were preserved for further histopathological and scanning electron microscopic studies after slaughter of these bull calves.

3.1.5 Rate of culling

The rate of culling of bull calves was ascertained from the records maintained at Dhoni farm and various infertility conditions detected were recorded.

3.2 Group II - Growing bull

3.2.1 Palpation and measurement of testis and epididymis

Sixty seven growing bulls between 13 and 24 months of age were subjected to detailed andrological examination for detecting any gross pathological changes. The scrotum was visually inspected for asymmetry, enlargement of scrotal sac or any lesions. Scrotum was palpated and the testicles were examined for mobility. Depending upon the shape, scroti were classified as oval, elongated, rounded, rectangular, pear shaped, square, oblong and bifid.

Testicles were examined for its size, shape and consistency. The length, thickness and width were measured at the point of maximum dimension with the help of vernier calipers as described by Hahn *et al.* (1969) and recorded. Any deviation from the balance and symmetry was noted. Shape of the testis was noted and deviation if any due to the presence of abscess, tumour etc. was recorded. The consistency was noted as turgid, elastic, firm or soft and flabby. Head, body and tail of epididymis were examined for any inflammatory changes, sperm granuloma, spermatocoele, hypoplasia or aplasia and their size and consistency were noted.

3.2.2 Scrotal circumference

(As in 3.1.2)

3.2.3 Libido

The mating behaviour of bulls was observed and recorded. The bulls which failed to mount or serve artificial vagina beyond a reaction time of 15 mts. or more were recorded.

3.2.4 Semen evaluation

Semen was collected from all other bulls after allowing sufficient reaction time and one or two false mounts. Two ejaculates were collected from each bull using an artificial vagina between 6 AM and 7 AM at weekly intervals for a period of 6 weeks. Detailed evaluation was carried out for each of the ejaculate immediately after collection and the vials were placed in water bath at 37°C.

3.2.4.1 Volume

Volume of each ejaculate was recorded directly from the graduated collection vials.

3.2.4.2 Initial motility

A small drop of fresh semen was then examined under the high power (1x400) of the phase contrast microscope at 37°C for assessing the initial motility and this was expressed in terms of percentage.

3.2.4.3 concentration of spermatozoa

Sperm concentration was estimated using a calibrated photoelectric colorimeter. Five ml of 2.9 per cent sodium citrate solution was taken in a colorimeter tube and placed in the photometer. The transmission percentage was then adjusted to 100. By using Eppendorf microlitre pipette 0.1 ml of undiluted semen was dropped into the photometer tube. The transmission percentage of the mixture was then observed after mixing the contents thoroughly. The sperm concentration was determined by plotting the value against a reference standard and expressed as numbers per ml of semen.

3.2.4.4 Live sperm

The live sperm percentage was estimated by differential staining using eosin-nigrosin stain as described by Hancock (1959).

3.2.4.5 Sperm morphology

The head and mid-piece abnormalities were assessed by using eosin-nigrosin staining technique. Semen samples fixed in the buffered formol saline were used for estimating the tail abnormalities, proximal and distal photoplasmic droplets using phase contrast microscope.

Smear of the semen sample from infertile bulls were made on cover glass and fixed in 2.5% glutaraldehyde, in phosphate buffer and dehydrated in different grades of acetone and coated with gold in a sputter coater and examined under Scanning Electron Microscope (Hitachi) at 50 KV.

3.2.4.6 Freezability

The semen samples after initial evaluation were diluted in Tris egg yolk extender. These were then filled in 0.25 ml French straws after adjusting the number of sperms to 30 million per dose. Using the rapid horizontal vapour freezing method, the straws were frozen in liquid nitrogen vapour in LNR 250 container. After thawing, in water at room temperature the motility was assessed at 37°C. Freezability was expressed in per cent post thaw motility. The number of ejaculates discarded before and after freezing and the total rejections were also recorded separately.

3.2.5 Infertility conditions observed

Total number of infertility conditions observed among growing bulls were recorded.

3.2.5.1 Histopathological and ultrastructural studies

Nineteen growing bulls suspected to have testicular involvement were slaughtered periodically. The organs were collected at slaughter and subjected to detailed examination. Slice of tissue of testis and epididymis of about 3 to 5 mm thickness were then removed, fixed in Bouins fluid, dehydrated and embedded in paraffin as per the method described by Humason(1967). Sections were then cut at 5μ thickness and stained by Haemotoxylin-eosin and examined for histopathological lesions.

Tissue samples from testis and epididymis fixed in 2.5 per cent glutaraldehyde in phosphate buffer were post fixed in 1% osmium tetroxide. After dehydration in ascending grades of acetone, the specimens were coated with gold in sputter coater and examined in a Hitachi scanning electron microscope at 50 KV.

3.2.6 Culling rate

The rate of culling of growing bulls of three different crosses for a period of five years from 1993 to 1998 at the Dhoni farm was gathered, tabulated and analysed.

3.3 Group III - Adult bulls

3.3.1 Palpation and measurement of testis and epididymis

Forty adult bulls of three different crosses were subjected to detailed clinico-andrological examination as done in the case of group II bulls. Data were tabulated and analysed.

3.3.2 Scrotal circumference

The scrotal circumference was measured as in group II bulls.

3.3.3 Libido

The mating behaviour and libido were assessed and semen was collected from these bulls for evaluation of semen characteristics as in group II bulls.

3.3.4 Semen evaluation

3.3.4.1 Volume

The volume of semen of adult bulls was observed as in group II.

3.3.4.2 Initial motility

Initial motility of semen of adult bull was assessed as in group II bulls.

3.3.4.3 Concentration of spermatozoa

Sperm concentration was measured as in group II bulls.

3.3.4.4 Live sperms

The live sperm percentage was estimated by differential staining technique using eosin and nigrosin.

3.3.4.5 Sperm morphology

The morphology of spermatozoa was assessed as in group II bulls.

3.3.4.6 Freezability

Freezability and rate of rejection were recorded from 241 ejaculates as in group II bulls.

3.3.5 Infertility conditions observed

Total number of infertility conditions observed among adult bulls was recorded.

3.3.5.1 Histopathological and ultrastructural studies

Fourteen adult bulls suspected to have testicular involvement were slaughtered periodically. The organs were collected at slaughter and subjected to histopathological and scanning electronmicroscopic studies as in group I.

3.3.5.2 Seminal enzymes

Seminal enzymes as acid phosphatase (ACP), alkaline phosphatase (AKP), glutamic oxaloacetic transaminase (GOT) or aspartate transaminase, glutamic pyruvic transaminase (GPT) or alanine transaminase and lactic dehydrogenase (LDH) were estimated. Semen samples were centrifuged to separate seminal plasma and was stored in 0.25 ml French straws frozen in liquid nitrogen. The seminal plasma enzymes as Transaminases (Reitman and Frankel, 1957), phosphatases (King and Armstrong, 1934) and lactic dehydrogenase (Wooton, 1964) were estimated by colorimetry to assess the variation in the different seminal enzymes in 31 bulls with a freezability of 50 per cent and above and in nine bulls with freezability less than 50 per cent.

3.3.6 Rate of culling

The rate of culling of adult bulls of the three different crosses for the period from 1993 to 1998 at Dhoni farm was also gathered and assessed.

The results obtained in the investigation were statistically analysed using methods described by Snedecor and Cochran (1967).

Results

4. RESULTS

Results of investigation of andrological examination of bull calves from birth to 12 months of age are presented in Table 1 and Fig.1 to 7.

4.1.1 Palpation and measurement of testis and epididymis

The texture of testis on palpation was turgid and resilient. The normal texture was found to vary in abnormal bulls.

The length, breadth and thickness of testis and length of cauda of the right and left side are presented in Table 1. On analysis significant variation in the measurement of testis and epididymis was observed between different crosses and between right and left side organs.

4.1.2 Scrotal circumference

The mean scrotal circumference of the three crosses CBJ, CBBS and CBHF was 20.07 ± 1.49 cm, 19.47 ± 1.86 cm and 18.83 ± 1.77 cm respectively. The overall mean scrotal circumference of all the three breeds was 19.42 ± 1.03 cm. Significant variation was observed between different crosses in scrotal circumference (Table 1).

4.1.3 Infertility conditions observed

Out of 46 bull calves studied, one case of bilateral cryptorchidism was detected.

On palpation of scrotum both testicles could not be traced. The scrotal pouch could only be felt which measured 10 cm in circumference. The scrotum appeared shrunken and the scrotal skin was wrinkled (Fig.1).

The bull calf was culled and testis and epididymis were recovered from the inguinal canal at slaughter (Fig.2).

4.1.4 Histopathology

On histopathological examination, the following lesions were observed.

4.1.4.1 Testis

Testicular tissue showed extensive fibrocollagenous proliferation and presence of occasional tubules (Fig.3). Tubular lining cells were inconspicuous and the lumen contained cellular debri (Fig.4). Thickening of the intertubular connective tissue was observed. Variation in the size of the tubules, irregularity of the lining cells, loss of germinal epithelium, wide separation of the tubules and presence of hyalinised homogeneous mass within the lumen were characteristic (Fig.5). Hyaline thickening of the tubular basement membrane and atrophy of germinal epithelium were prominent. A few spermatogenic cells along with sertoli cells were seen in few tubules. Interstitial cells appeared relatively numerous at certain parts of the testicular parenchyma.

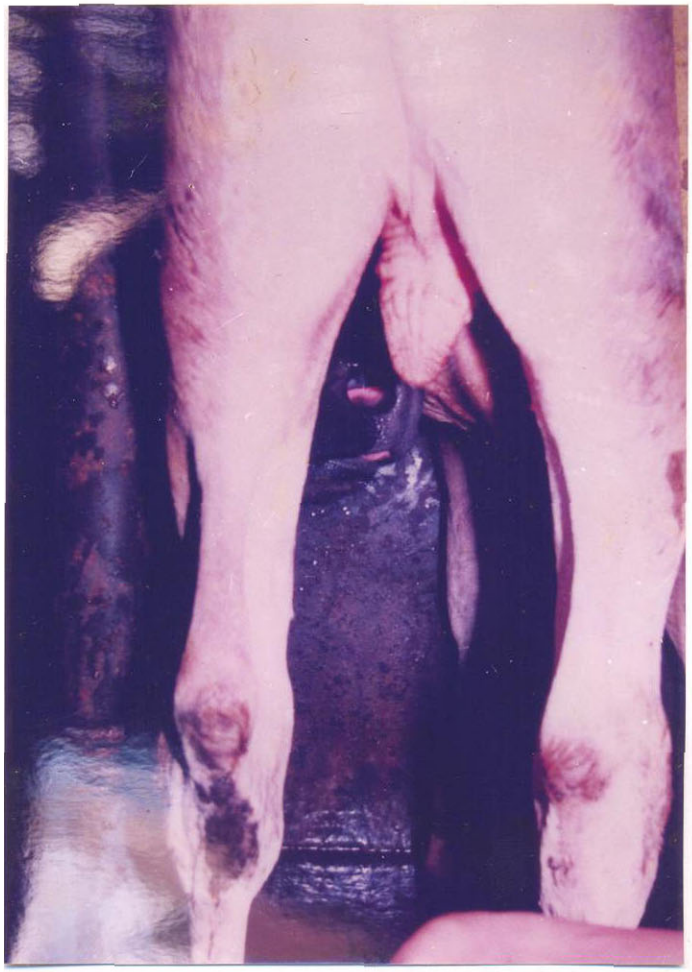
Table 1. Testicular measurements of calves below 12 months of age

Cross bred type	No. of calves observed	Scrotal circum-ference (cm)	Left side				Right side				
			Length (cm)	Breadth (cm)	Thickness (cm)	Length of cauda (cm)	Length (cm)	Breadth (cm)	Thickness (cm)	Length of cauda (cm)	Age at observation (months)
CBJ mean	13	20.07 ± 1.49	7.91 ± 0.68	4.53 ± 0.41	3.48 ± 0.32	1.73 ± 0.22	7.52 ± 0.72	4.72 ± 0.44	3.49 ± 0.20	1.76 ± 0.20	8.75 ± 0.97
Range		9.0 – 29.5	4.0 – 11.5	1.5 – 6.6	2.0 – 6.0	0.5 – 3.0	4.0 – 11.6	2.0 – 7.0	2.0 – 4.7	0.5 – 2.8	3 – 12
CBBS mean	18	19.47 ± 1.86	7.53 ± 0.78	4.48 ± 0.42	3.06 ± 0.28	1.82 ± 0.17	7.77 ± 0.82	4.81 ± 0.46	3.01 ± 0.28	1.71 ± 0.81	7.22 ± 0.95
Range		7.5 – 34.5	2.0 – 12.0	2.0 – 8.4	1.3 – 6.0	1.0 – 3.7	2.5 – 13.5	2.0 – 9.1	1.3 – 6.1	0.5 – 3.0	1.0 – 12.0
CBHF mean	15	18.83 ± 1.77	6.97 ± 0.73	4.10 ± 0.45	3.04 ± 0.26	1.69 ± 0.13	7.51 ± 0.78	4.41 ± 0.40	3.14 ± 0.29	1.57 ± 0.17	5.17 ± 0.93
Range		8.9 – 30.0	3.0 – 12.0	2.0 – 7.0	1.4 – 5.5	0.4 – 2.5	3.5 – 13.0	2.1 – 7.6	1.3 – 5.5	0.5 – 3.0	1.0 – 12.0
Total Mean	46	19.42 ± 1.03	7.44 ± 0.43	4.36 ± 0.24	3.16 ± 0.17	1.75 ± 0.10	7.61 ± 0.45	4.65 ± 0.25	3.18 ± 0.16	1.68 ± 0.10	7.69 ± 0.55
Range		7.5 – 34.5	2-12	1.5 – 8.4	1.3 – 6.0	0.4 – 3.7	2.5 – 13.5	2 –9.1	1.3 - 6.1	0.5 - 3.0	1 – 12
F value		20.052**	16.691**	11.475**	6.135**	9.23**	19.914**	15.855**	6.297**	3.080**	23.674**

** Highly significant (P<0.01)

Fig.1 Bilateral cryptorchidism

Fig.2 Bilateral cryptorchidism – Testicles and epididymis exposed



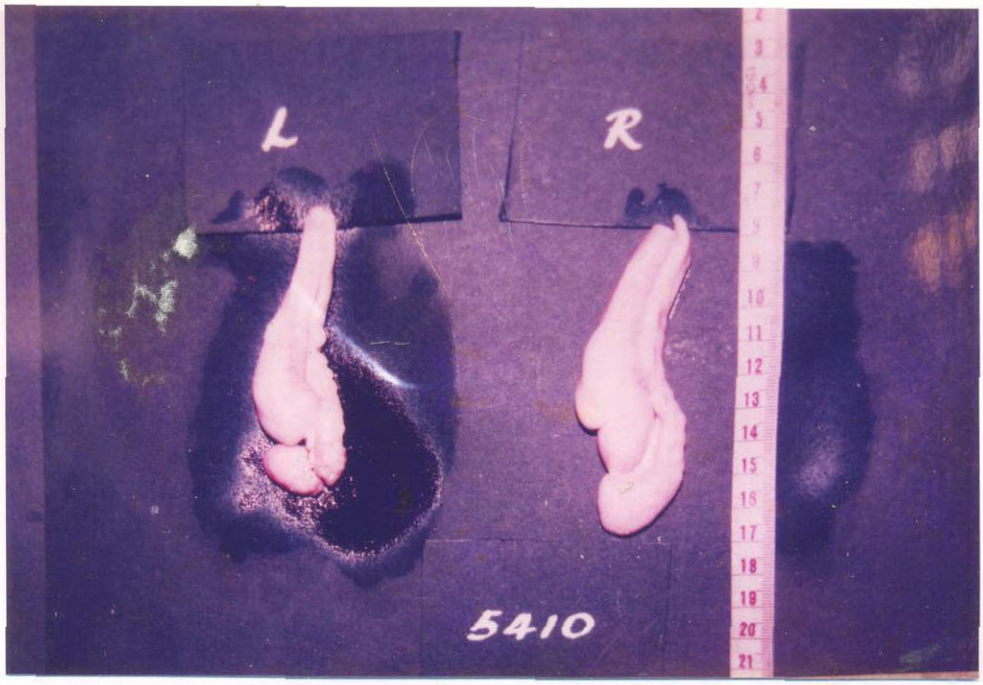
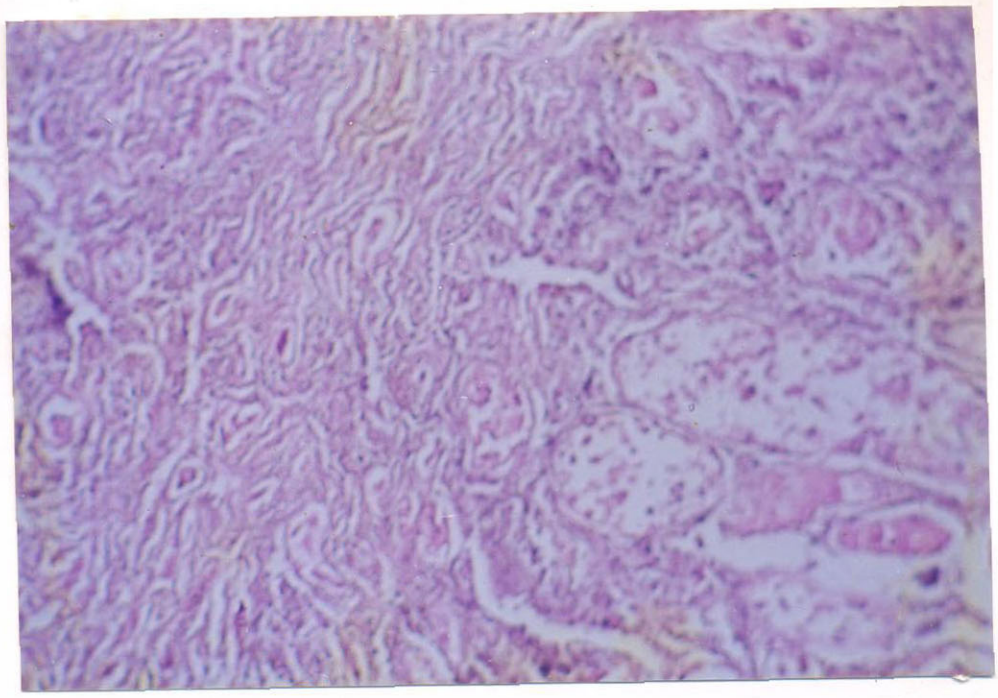

L
R
5410

Fig.3 Cryptorchid testis

Fibrocollagenous proliferation, hypoplastic testicles with inconspicous lining cells
H&E x 165

Fig.4 Cryptorchid testis

Intertubular connective tissue proliferation, hypoplastic tubules containing cellular debris x 250



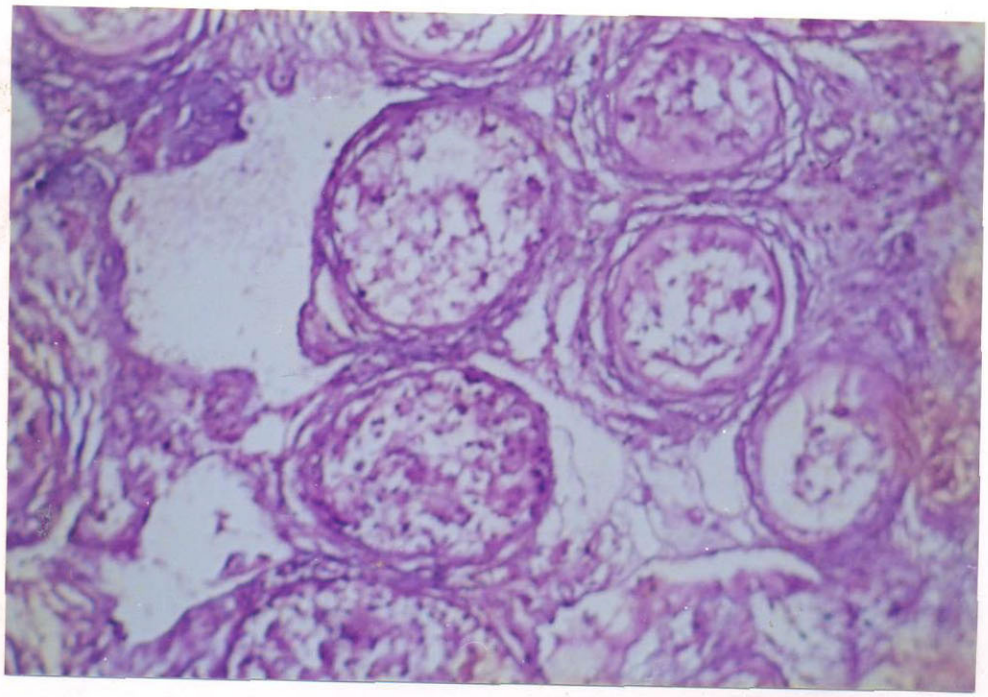
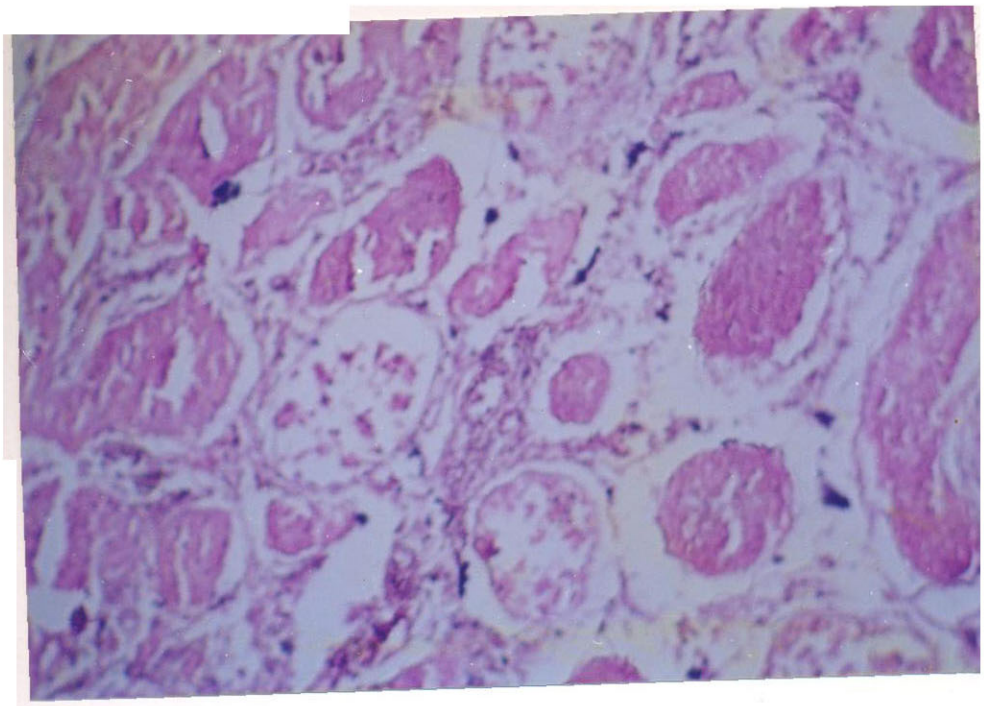


Fig.5 Cryptorchid tesis

Hyalinised homogenous mass within the lumen
H&E x 165

Fig.6 Cryptorchid testis

Epididymis : Peritubular fibrosis – H&E x 250



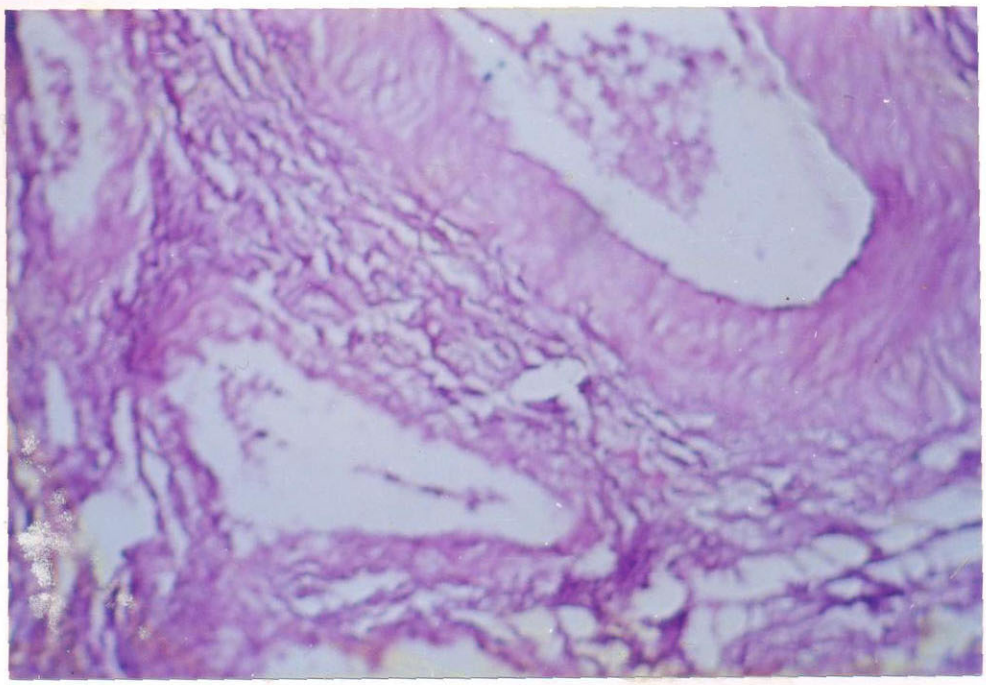
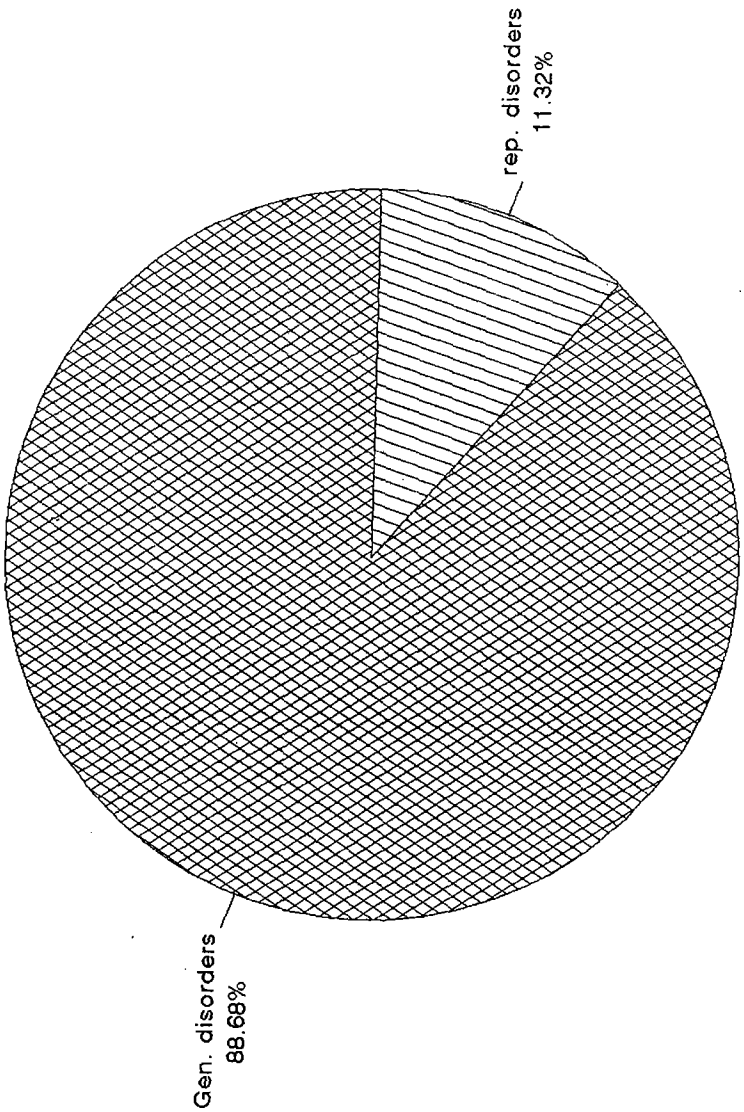


Fig.7 RATE OF CULLING IN CALVES



4.1.4.2 Epididymis

Epididymis showed detachment of tubules and loss of lining cells. Many of the tubules were found dilated. Many dilated tubules contained necrosed germinal epithelial cells. Peritubular fibrosis was prominent (Fig.6).

4.1.5 Rate of culling

The rate of culling of bull calves ascertained from records maintained at Dhoni Farm revealed that out of 222 bull calves 53 were culled (23.87 per cent) due to various reasons (Fig.7). Out of these, 20.75 per cent was CBJ, 16.98 per cent CBBS and 13.20 per cent CBHF. Among this, six bull calves (11.32 per cent) were culled due to various reproductive disorders such as hypoplasia, cryptorchidism, umbilical hernia and inguinal hernia.

4.2 Group II - Growing bulls

4.2.1 Palpation and measurement of testis and epididymis

The texture of testis on palpation of normal bulls were turgid and resilient. The testicles of hypoplasic bulls were harder and those with testicular degeneration were soft and plum like.

The length, breadth, thickness and length of cauda epididymis of right and left side are presented in Table 2. Statistical analysis did not show any significant variation between different crosses and between the right and left organ within crosses.

The shape of the scrotum of 67 growing bulls were found to be oval in 35 (52.24 per cent), elongated in 13 (19.40 per cent) rounded in 5 (7.46 per cent) rectangular in 3 (4.48 per cent) pear shaped in 4 (5.97 per cent) square in 3 (2.99 per cent), oblong in 3 (4.48 per cent) and bifid in 2 (2.99 per cent).

4.2.2 Scrotal circumference

The mean scrotal circumference of three crosses CBJ, CBBS and CBHF was 29.84 ± 0.54, 30.21 ± 0.61 cm and 29.14 ± 0.11 cm respectively. The overall mean scrotal circumference of all the three breeds was 29.86 ± 0.38 cm. On analysis, there was no significant variation between breeds in scrotal circumference.

4.2.3 Libido

Out of 67 bulls, semen could be collected only from 52 bulls since the rest 15 bulls failed to mount during the period of study. Out of these, 4 animals were slaughtered and histopathological studies revealed scanty interstitial cells.Sertoli cells appeared less and spermatids were seen in few of the tubules with degeneration and necrosis of many of the tubules (Fig.8). In some bulls the testis and epididymis appeared normal.

4.2.4 Semen evaluation

The physical characteristics of semen of the three different crossbreds CBJ, CBBS and CBHF are presented in Table 3.

4.2.4.1 Volume

The mean ejaculate volume of CBJ, CBBS and CBHF was 2.58 ± 0.21 ml, 3.27 ± 0.32 ml and 3.09 ± 0.35 ml respectively. the overall mean was 2.91 ± 0.16 ml and ranged from 1.12 to 5.92 ml.

4.2.4.2 Initial motility

The mean initial motility of spermatozoa of CBJ, CBBS and CBHF was 47.36 ± 3.05 per cent, 51.85 ± 3.38 per cent and 52.29 ± 5.89 per cent respectively. The overall mean was 49.77 ± 2.12 per cent and ranged from 13.30 to 66.83 per cent.

4.2.4.3 Concentration of spermatozoa

The sperm concentration of CBJ, CBBS and CBHF was 1244.60 ± 86.43 million per ml, 1366.11 ± 88.95 million per ml and 1320.11 ± 135.84 million per ml respectively. The overall mean was 1299.73 ± 56.25 million per ml and ranged 563.00 to 2075.00 millions per ml.

The length and breadth of testis was significantly correlated with motility, volume and live sperm count ($P<0.01$).

4.2.4.4 Live sperm

The mean live sperm count of CBJ, CBBS and CBHF was 56.28 ± 2.80 per cent, 60.64 ± 3.50 per cent and 61.90 ± 6.90 per cent respectively. The overall mean was 58.76 ± 2.15 per cent and ranged from 12.24 to 80.51 per cent (Table 4).

4.2.4.5 Sperm morphology

The morphological characters of the three different breeds CBJ, CBBS and CBHF are presented in Table 4 and Fig.9 to 11. There was no significant difference in the morphological characters of different breeds. The overall mean of head, mid-piece and tail abnormalities were 6.57 ± 0.79 per cent, 1.94 ± 0.20 per cent and 12.97 ± 0.73 per cent and the mean proximal and distal protoplasmic droplets were 4.88 ± 0.83 per cent and 2.08 ± 0.19 per cent respectively.

4.2.4.6 Freezability

The pre-freezing post-freezing and total rejection of 291 ejaculates frozen was 39.50 per cent, 12.70 per cent and 52.27 per cent respectively (Table 5 and Fig.12).

Scrotal circumference revealed highly significant correlation ($P<0.01$) with volume, initial motility, live sperm count and freezability.

4.2.5 Infertility conditions

The incidence of various infertility conditions observed among growing bulls are presented in Table 6 and Fig.13.

4.2.5.1 Unilateral testicular hypoplasia

Three out of 67 crossbred bulls (4.48%) detected had unilateral hypoplasia. These bulls were fully grown up and evinced excellent libido. The difference in size between the normal and affected testicles was apparent (Fig.14 and 15).

Semen picture revealed the following:

Ejaculate volume	1 to 2.5 ml
Sperm concentration	520 to 830 million per ml
Motility	20 to 40 per cent
Free abnormal heads	10 to 15 per cent
Other head abnormalities	11 to 18 per cent
Proximal protoplasmic droplets	16 to 20 per cent
Distal protoplasmic droplets	3 to 4 per cent
Tail abnormalities	4 to 8 per cent

Total abnormalities	44 to 65 per cent

Histological studies of the affected testicles revealed that the seminiferous tubules were underdeveloped and lined by a single layer of spermatogonial cells (Fig.16). Complete vacuolation, thinning of the basement membrane and irregularity of cellular lining were observed in few of the tubules (Fig.16 and 17). Cytoplasmic vacuolation and occasional detachment of tubules from the basement

membrane were also observed. Mild fibrosis of the interstitial connective tissue and moderate increase in the Leydig cells were seen.

4.2.5.2 Bilateral partial testicular hypoplasia

Six crossbred bulls showed bilateral partial hypoplasia (8.96 per cent). General health and libido of the bulls were normal. Clinical examination of the bulls did not reveal any apparent abnormality. The size and shape of the testicles were normal.

Physical characteristics of semen examination revealed:

Mean ejaculate volume	1.5 to 2.5 ml
Motility	20 to 40 per cent
Sperm concentration	410 to 1210 million per ml
Head abnormalities	16 to 18 per cent
Mid-piece abnormalities	6 to 8 per cent
Detached heads	15 to 18 per cent
Proximal protoplasmic droplets	17 to 32 per cent
Tail abnormalities	4 to 6 per cent
Total abnormalities	56 to 82 per cent

Testes revealed hyperchromatosis of the nucleus of epithelial cells lining the seminiferous tubules. Mild degree of cytoplasmic vacuolation was seen. No

marked changes in the intertubular connective tissue and Leydig cells could be observed. A few of the tubules appeared cystic (Fig.18) without any lining cells. Spermatozoa were absent in many of the tubules. Normally appearing tubules were seen amidst the hypoplastic ones. Some of the seminiferous tubules were devoid of germinal cells and varying degrees of spermatogenesis were present in the rest of the tubules.

4.2.5.3 Testicular degeneration

Eleven bulls (16.41 per cent) showed testicular degeneration. Clinical examination did not reveal any apparent abnormalities except loss of firmness in the testis of the four bulls. The mean ejaculate volume of the bulls ranged from 3 to 5 ml, sperm concentration 600 to 1000 million per ml and initial motility 20 to 35 per cent. Semen generally appeared milky. Morphological examination of spermatozoa revealed detached heads (20 to 24 per cent), head abnormalities (15-20 per cent), proximal protoplasmic droplets (15 to 20 per cent) and tail abnormalities (10 to 16 per cent).

Histopathological studies of testes revealed varying degrees of changes in testicular degeneration from early mild to moderate or more advanced and even resulting in necrosis. Lesions appeared either focal or diffuse.

Mild to moderate testicular degeneration characterized by hyperchromatic nucleus, vacuolation of the cytoplasm of seminiferous epithelial cells and loss of scattered primordial germ cells were seen (Fig.19). In two of the animals much of

the epithelium lining the tubules has been found disappeared and the remnants were vacuolated (Fig.20). In advanced degenerative changes complete loss of germinal epithelial cells, cystic dilatation of the most of the tubules thickening of the basement membrane and hyalinisation were seen (Fig.21 and 22). Degenerated, necrosed and calcified sperm mass was present in many tubules.

4.2.5.4 Epididymal dysfunction

One bull was identified to have epididymal dysfunction (1.49 per cent).

Clinical examination revealed no abnormality in the genital organs. The spermatozoal motility was poor in this case with a mean of 18 per cent, the mean sperm concentration was 1980.83 millions per ml of semen, and the live sperm percentage averaged 14.20 per cent. Morphological examination of sperms revealed a mean tail abnormality of 29 per cent (Fig.11). The tail abnormalities noted were sample bent, bent at right angle and coiled tail. Head abnormalities did not exceed more than 15 per cent. Exhaustion tests revealed an increase in motility and a decrease in the tail abnormalities in subsequent ejaculates.

4.2.6 Rate of culling

Out of the 248 growing bulls observed 122 were culled (49.12 per cent) due to various reasons. Among this 35.59 per cent was with poor semen quality, 26.27 per cent with poor libido, 8.47 per cent with poor freezability, 16.94 per cent with poor libido and poor semen quality,10.16 per cent with poor libido, poor

Table 2. Testicular measurements of growing bulls between 13 and 24 months of age

Cross bred type	No. growing bulls observed	Scrotal circumference (cm)	Left side				Right side				Age at observation (months)
			Length (cm)	Breadth (cm)	Thickness (cm)	Length of cauda (cm)	Length (cm)	Breadth (cm)	Thickness (cm)	Length of cauda (cm)	
CBJ mean	32	29.84 ± 0.54	11.14 ± 0.24	6.0 ± 0.12	4.84 ± 0.14	1.69 ± 0.11	11.18 ± 0.31	5.80 ± 0.15	5.11 ± 0.15	1.82 ± 0.10	18.84 ± 0.62
Range		24.0 – 34.0	9.0 – 14.0	4.5 – 8.0	4.0 – 7.0	0.8 – 3.0	5.5 – 14.5	2.5 – 8.0	25 - 7.0	0.8 - 3.0	13.0 – 24.0
CBBS mean	24	30.21 ± 0.61	11.67 ± 0.37	6.08 ± 0.19	5.18 ± 0.18	1.79 ± 0.09	11.57 ± 0.41	6.24 ± 0.16	5.49 ± 0.21	1.79 ± 0.12	18.59 ± 0.97
Range		24.0 – 35.0	8.0 – 14.0	4.5 – 8.0	3.0 - 6.5	0.5 - 3.5	6.5 – 15.5	3.5 - 7.5	3.5 - 6.0	0.5 - 2.0	12.0- 29.0
CBHF Mean	11	29.14 ± 0.11	11.77 ± 0.38	5.96 ± 0.33	4.91 ± 0.31	1.46 ± 0.25	10.86 ± 0.64	5.91 ± 0.37	4.91 ± 0.29	1.55 ± 0.17	17.82 ± 1.66
Range		22.0 – 35.0	10.0 - 14.0	4.5 – 8.0	3.0 - 6.5	0.5 - 3.5	6.5- 15.5	3.5 - 7.5	3.5 - 6.0	0.5 - 2.0	12.0 - 29.0
Total Mean	67	29.86 ± 0.38	11.43 ± 0.19	6.02 ± 0.10	4.97 ± 0.10	1.69 ± 0.07	11.27 ± 0.23	5.97 ± 0.11	5.21 ± 0.11	1.76 ± 0.07	18.59 ± 0.52
Range		22.00 – 37.00	8.00 - 14.00	4.5 - 8.0	3 00 - 7.00	5.0 - 3.5	5.50 – 15.00	2.5 - 8.6	2.5 - 7.0	0.5 - 3.0	0.17 - 29.00
F value		1.050^{NS}	1.118^{NS}	0.093^{NS}	0.948^{NS}	0.495^{NS}	1.029^{NS}	1.943^{NS}	1.699^{NS}	1.034^{NS}	0.131^{NS}

NS – Non significant

Table 3. Physical characteristics of semen of growing bulls

Cross bred type	No. of bulls observed	Number of ejaculates	Volume (ml)	Initial motility (%)	Concentration million/ml
CBJ Mean	25	150	2.58 ± 0.21	47.36 ± 3.05	1244.60 ± 86.43
Range			1.12 – 5.50	17.10 – 66.66	567.00 – 2075.00
CBBS Mean	18	108	3.27 ± 0.32	51.85 ± 3.38	1366.11 ± 88.95
Range			1.25 – 5.92	25.00 – 65.00	587.00 – 1813.00
CBHF Mean	9	54	3.09 ± 0.35	52.29 ± 5.89	1320.11 ± 135.84
Range			1.40 – 4.50	13.30 – 66.83	563.00 – 1813.00
Total Mean	52	312	2.91 ± 0.16	49.77 ± 2.12	1299.73 ± 56.25
Range			1.12 – 5.92	13.30 – 66.83	563.00 – 2075.00
F value			2.002^{NS}	0.594^{NS}	0.473^{NS}

NS – Non significant

Table 4. Morphological characteristics of spermatozoa of growing bulls

Cross bred type	No. of bulls observed	No. of smears	Live sperms	Abnormalities of			Protoplasmic droplets	
				Head %	Mid piece %	Tail %	Proximal %	Distal %
CBJ Mean	25	150	56.28 ± 2.80	7.14 ± 1.26	1.73 ± 0.18	13.68 ± 1.19	4.72 ± 1.18	1.82 ± 0.23
Range			23.82-75.81	1.89-24.12	0.69 - 3.95	6.10 - 27.79	0.98 - 21.74	0.89-6.10
CBBS Mean	18	108	60.64 ± 3.50	6.88 ± 1.37	2.15 ± 0.46	12.79 ± 1.12	4.51 ± 1.13	2.11 ± 0.29
Range			29.61 - 80.51	2.25 - 26.15	0.91 - 8.87	4.57-21.55	1.03-18.10	0.80-5.88
CBHF Mean	9	54	61.90 - 6.90	4.35 ± 1.12	2.10 ± 0.49	11.39 ± 1.42	6.06 ± 2.90	2.74 ± 0.73
Range			12.24 - 79.14	1.36 - 12.75	1.08 - 5.01	3.32 - 18.30	1.16-22.22	1.13 - 8.20
Total Mean	52	312	58.76 ± 2.15	6.57 ± 0.79	1.94 ± 0.20	12.97 ± 0.73	4.88 ± 0.83	2.08 ± 0.19
Range			12.24 - 80.51	1.36 - 26.15	0.69 - 8.90	3.30 - 27.79	0.98 - 22.22	0.80 - 8.20
F value			0.630^{NS}	0.934^{NS}	0.440^{NS}	0.628^{NS}	0.065^{NS}	1.609^{NS}

NS – Non significant

Table 5. Pre freezing, post freezing and total rejection of ejaculates of growing crossbred bulls

Freezing	Number of ejaculates	Number of rejected	Percentage
Pre freezing	291	115	39.50
Post freezing	291	37	12.70
Total	291	152	52.27

Table 6. Infertility conditions investigated in 67 growing bulls between 12 and 24 months of age

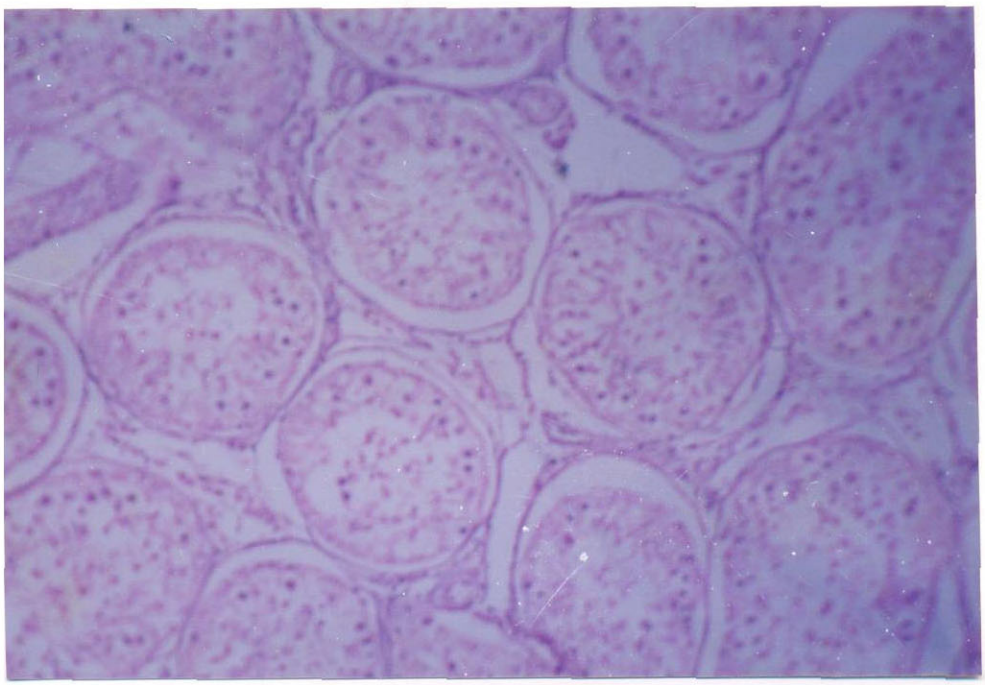
Sl. No.	Infertility conditions observed	Number of conditions observed	Percentage of infertility
1	Unilateral hypoplasia	3	4.48
2	Bilateral partial hypoplasia	6	8.96
3	Testicular degeneration	11	16.41
4	Epididymal dysfunction	1	1.49

Fig.8 Testis of bull with lack of libido

Moderate degeneration of the tubules scanty interstitial cells. Occasional sertoli cells and few spermatids in some of the tubules – H&E x 250

Fig.9 Sperm abnormalities

Spermatozoa showing loose abnormal heads E&N x 500



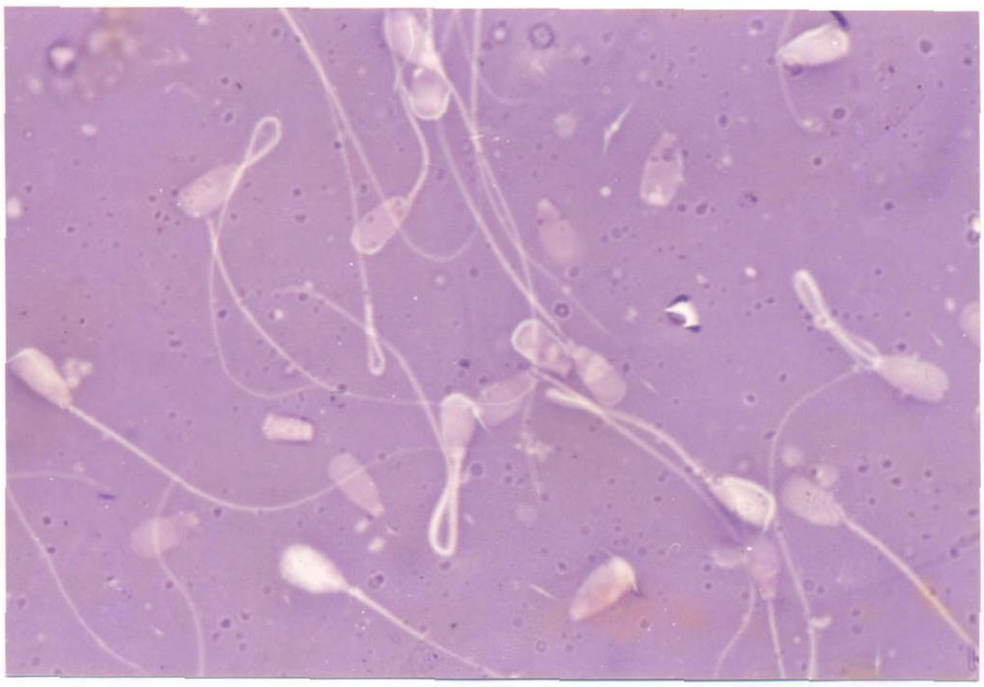
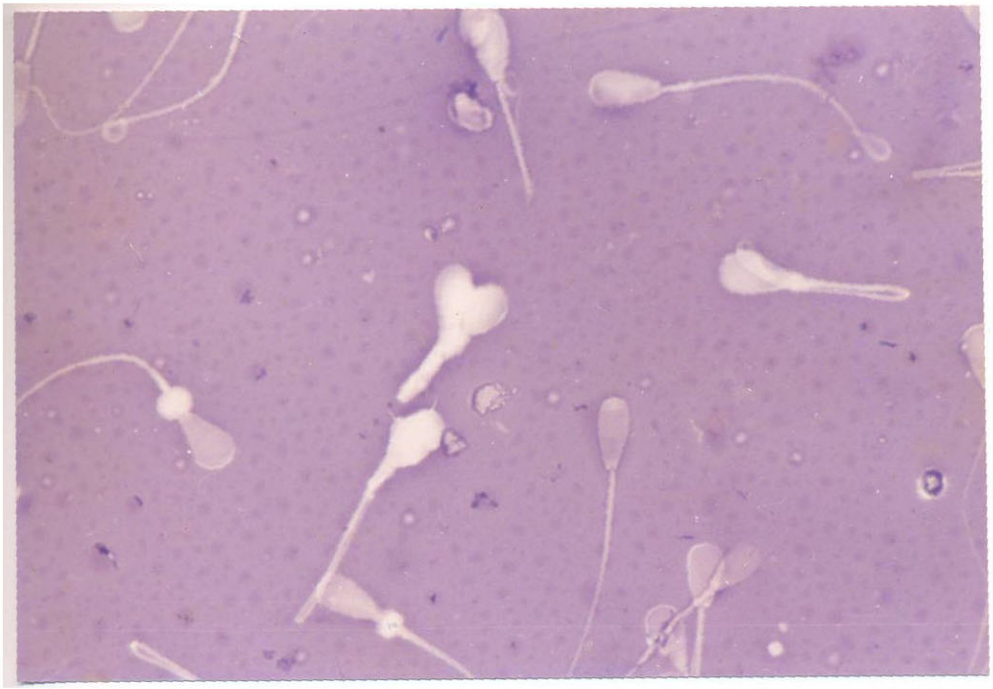


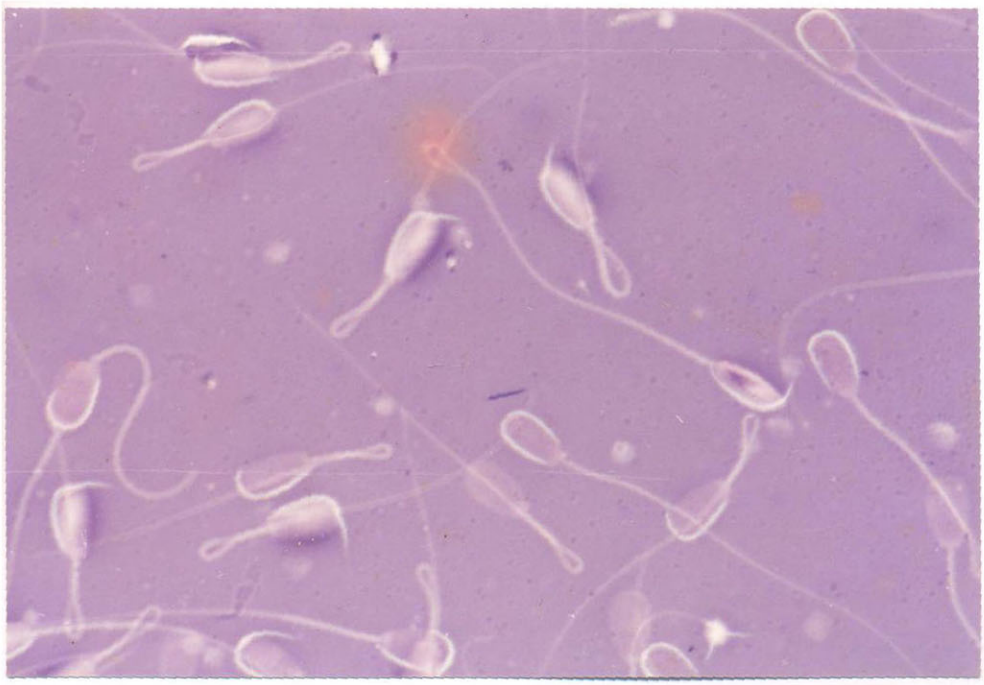
Fig.10 Sperm abnormalities

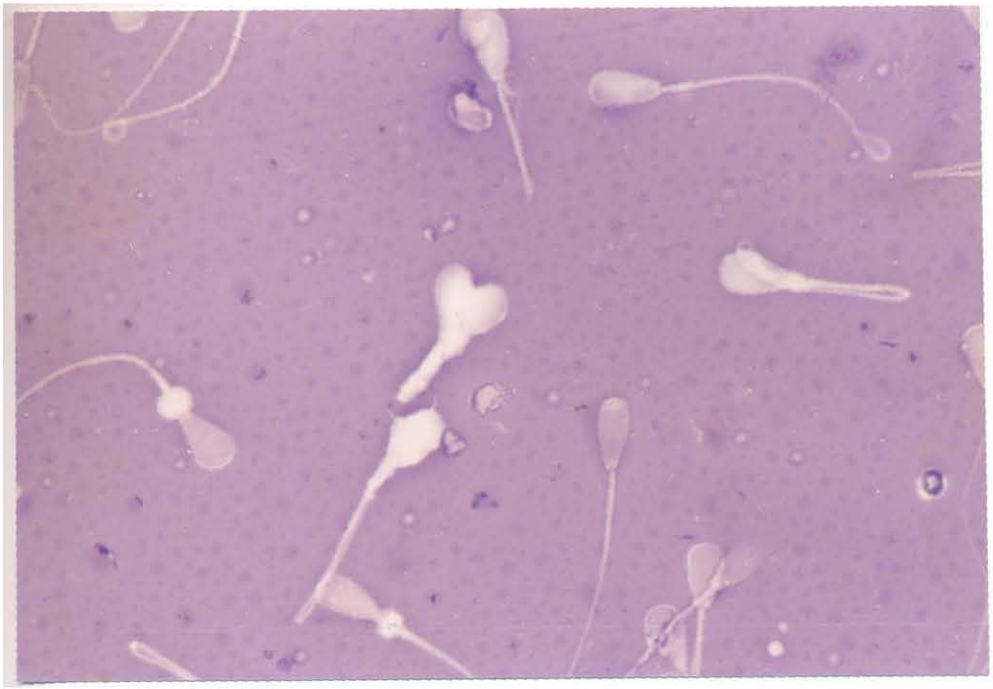
Spermatozoa with double heads and heads narrow at the base E& N x 500

Fig.11 Epididymal dysfunction

Spermatozoa with large number of bent tails E& N x 500







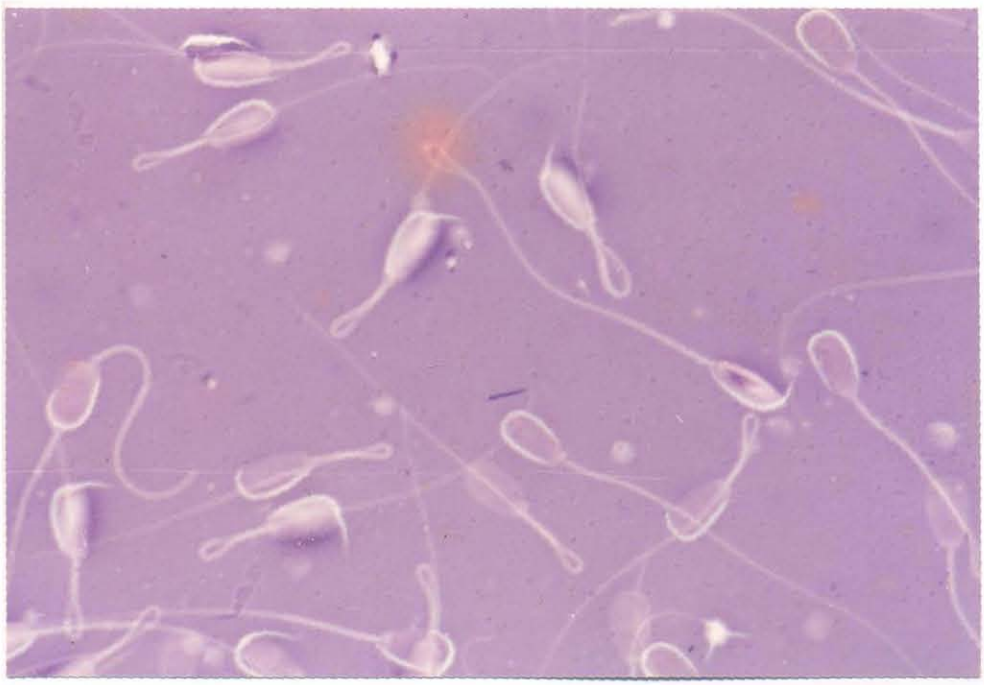


Fig. 12. PREFREEZING, POSTFREEZING AND TOTAL REJECTION OF EJACULATES OF GROWING CROSS BRED BULLS

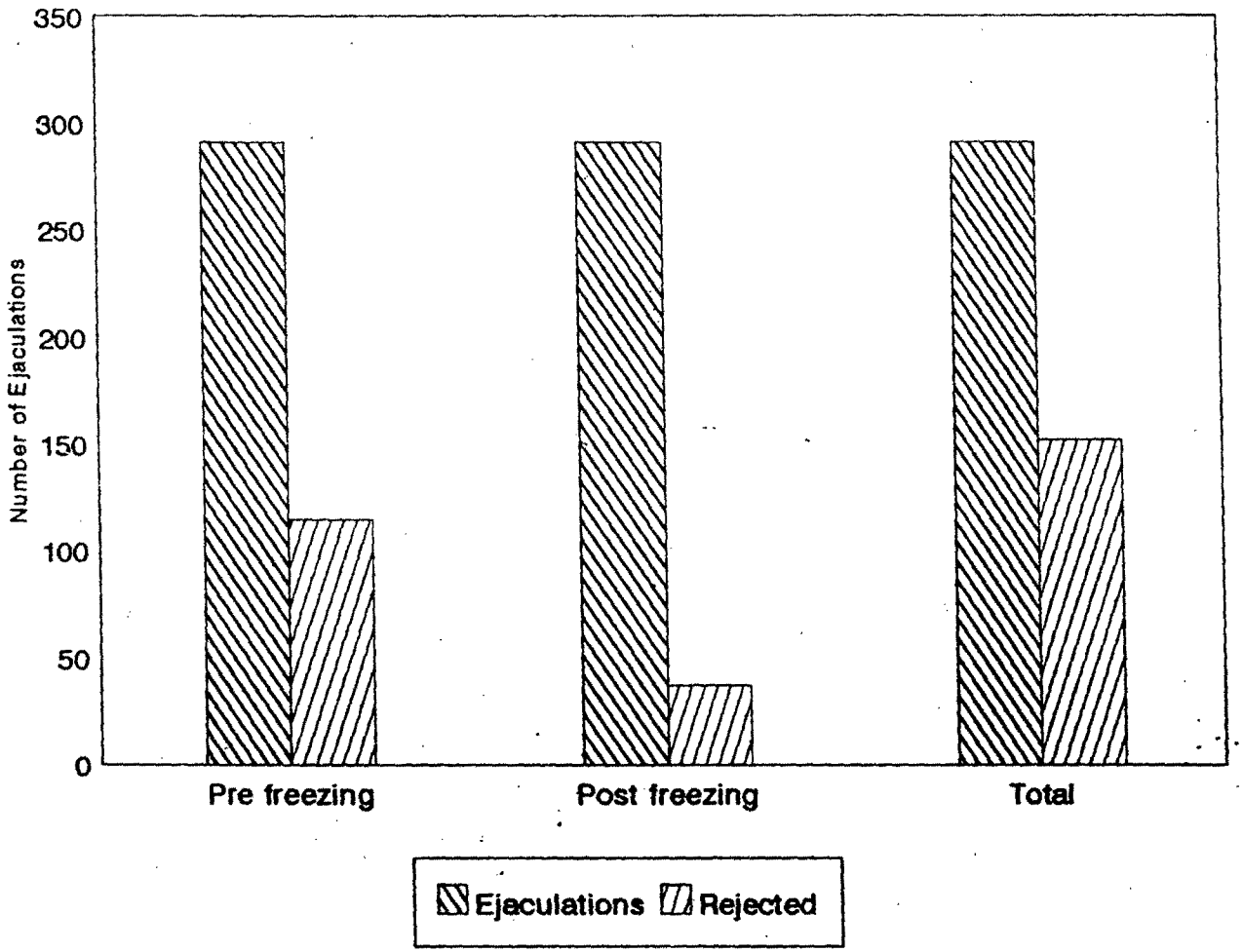


Fig.13 PERCENTAGE OF INFERTILITY CONDITIONS INVESTIGATED IN GROWING CROSS BRED BULLS

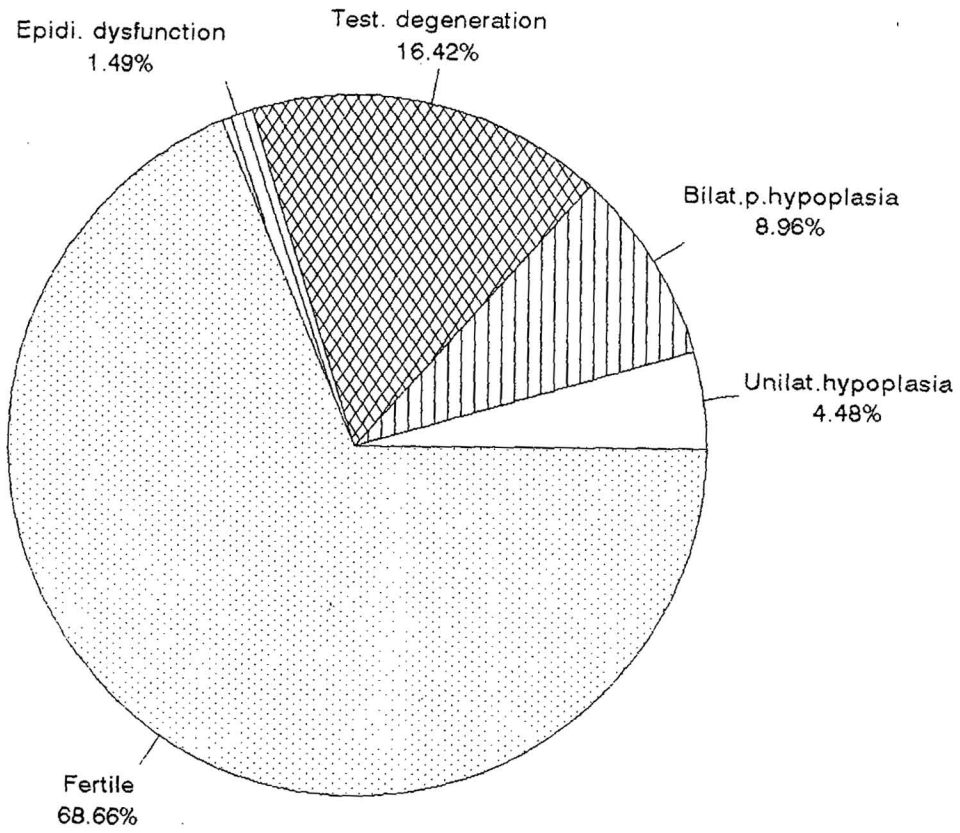
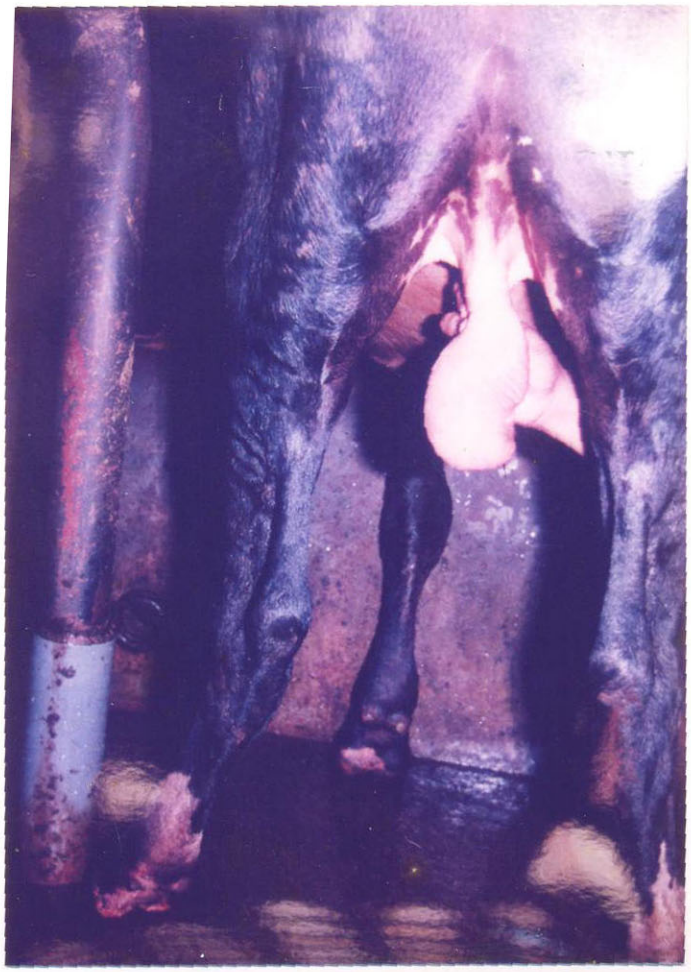


Fig.14 Unilateral testicular hypoplasia

Apparently small and shrunken right testicle

Fig.15 Unilateral testicular hypoplasia – testis and epididymis recovered



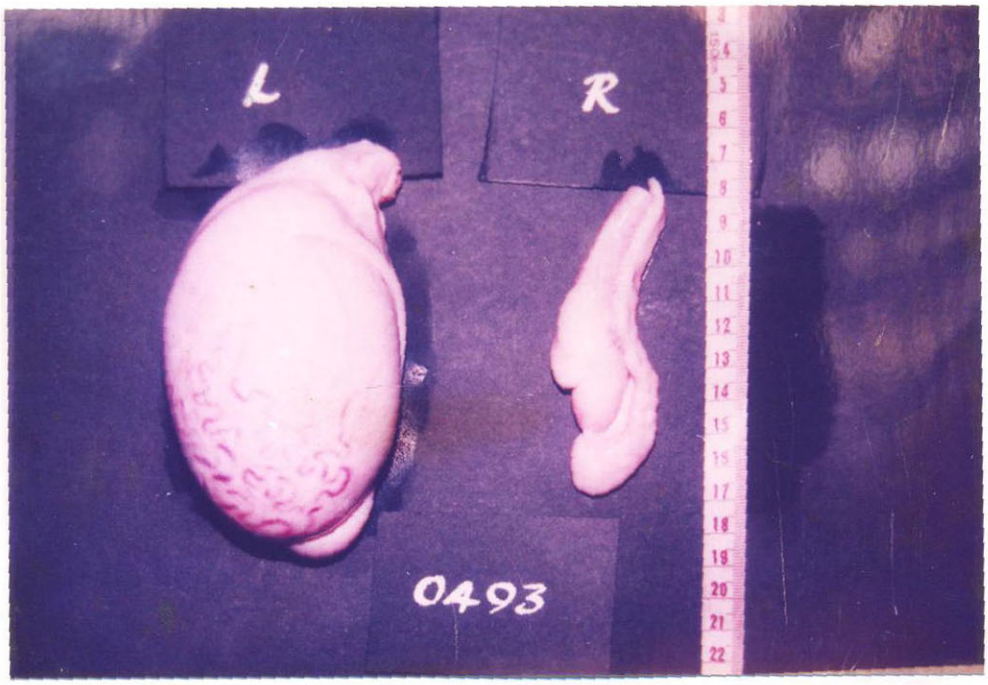
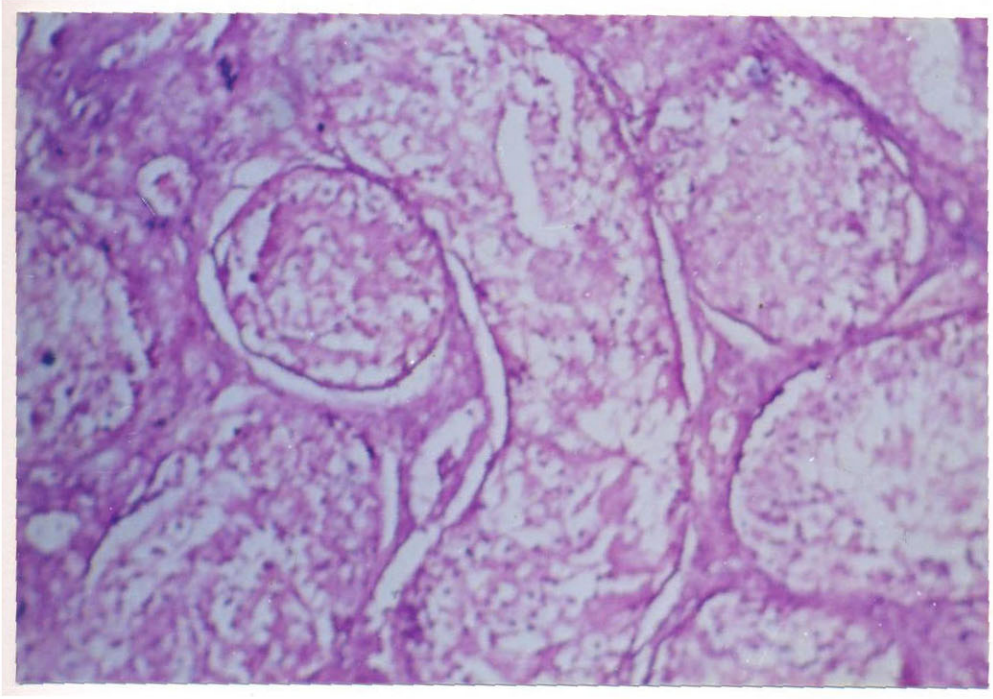

L
R
0493

Fig.16 Unilateral testicular hypoplasia

Irregular seminiferous tubules, vacuolation of the cytoplasm of the lining cells – H&E x 250

Fig.17 Unilateral testicular hypoplasia

Irregular cellular lining, vacuolation and thining of the basement membrane of the tubules – H&E x 250



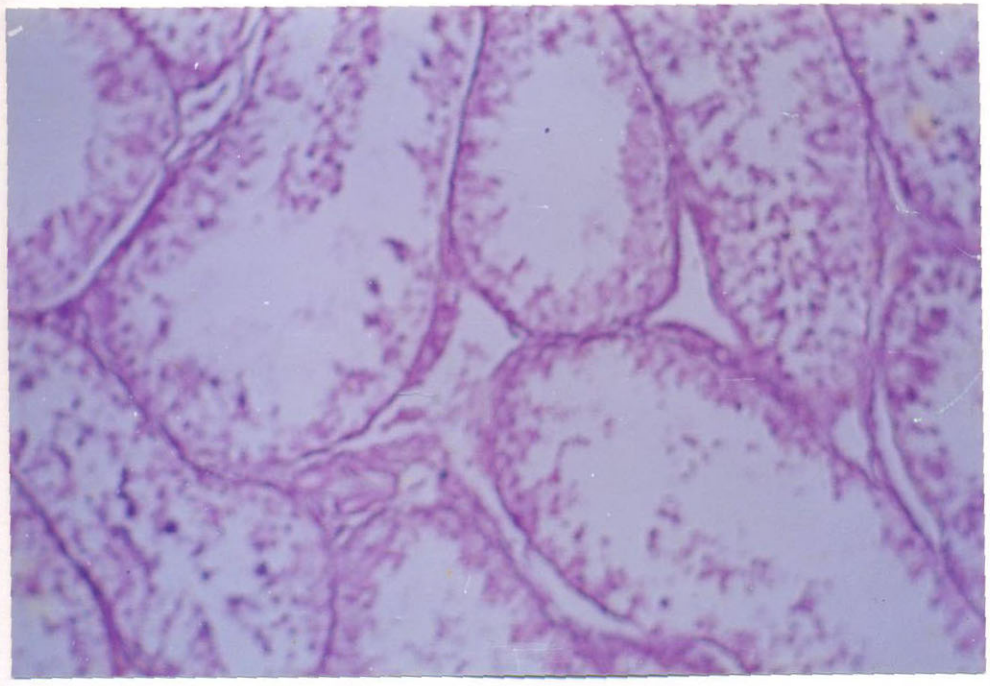
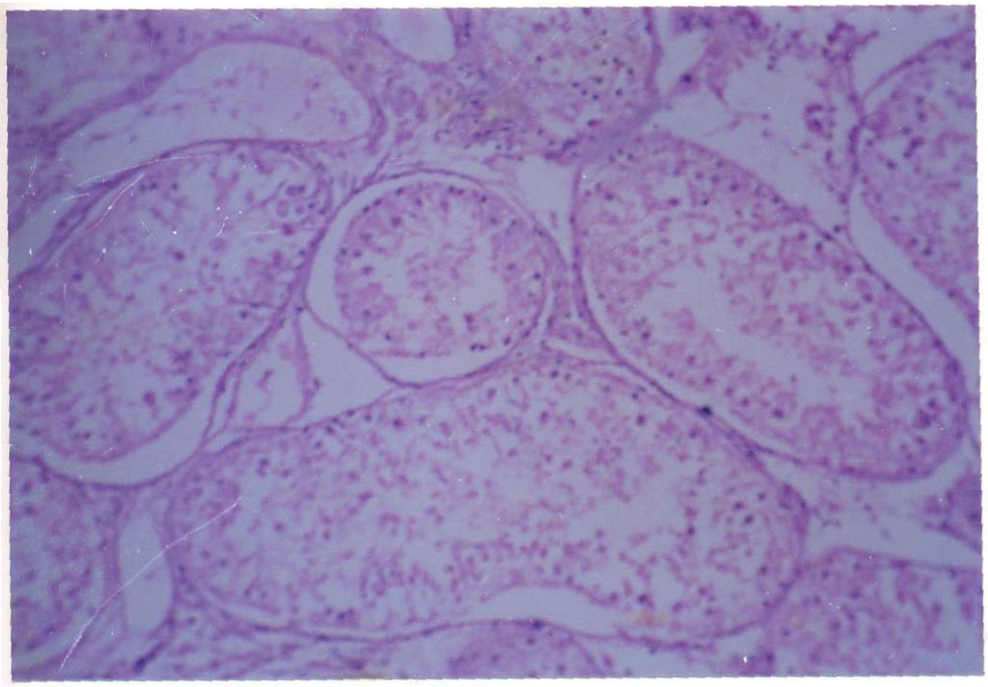


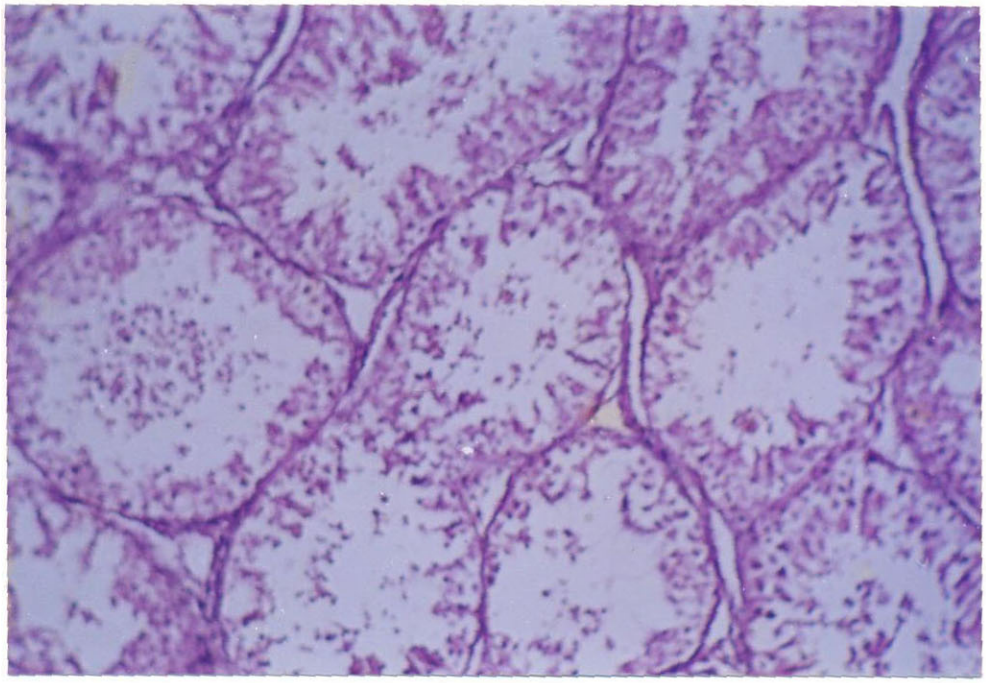
Fig.18 Bilateral partial testicular hypoplasia

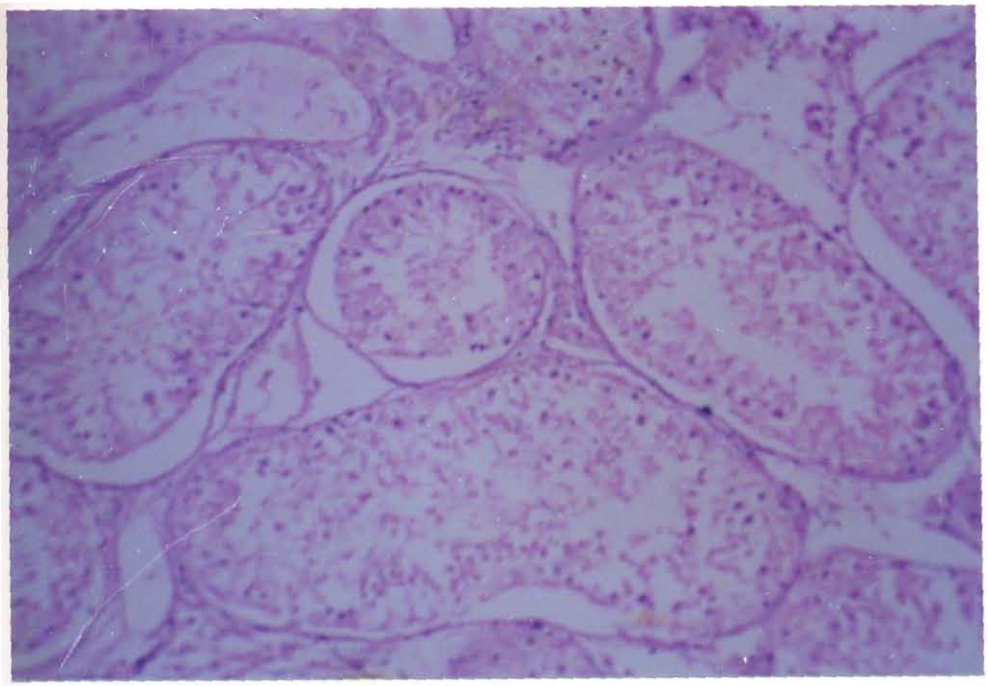
Variation in size of the tubules – Few of the tubules appeared cystic. Absence of spermatozoa in many of the tubules - H&E x 250

Fig.19 Testicular degeneration – mild to moderate

Vacuolation of cytoplasm of seminiferous epithelial cells, loss of primordial germ cells - H&E x 250







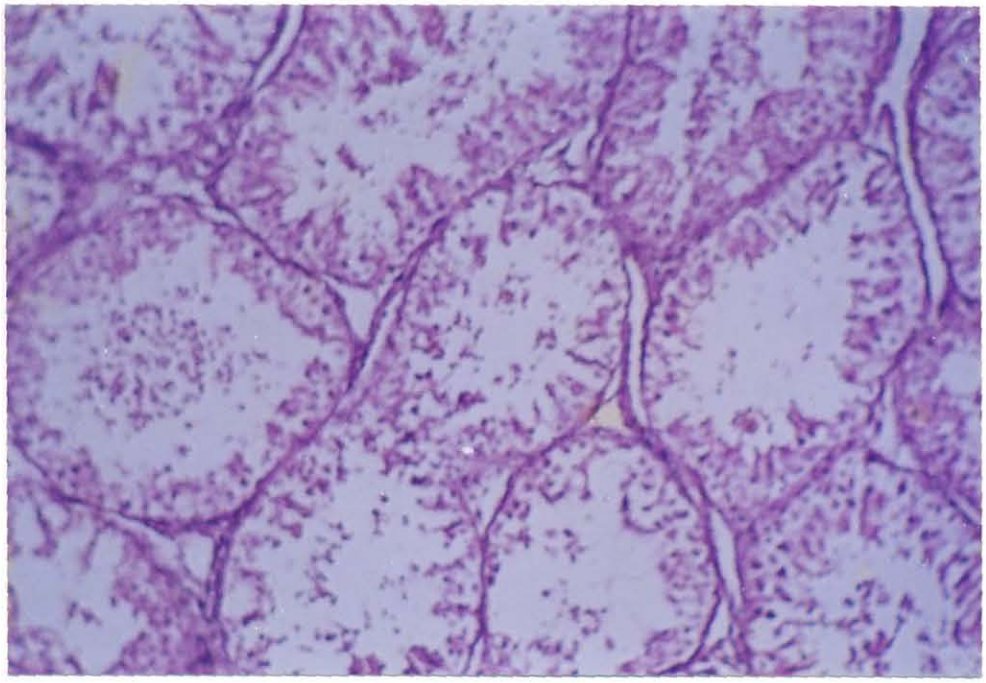
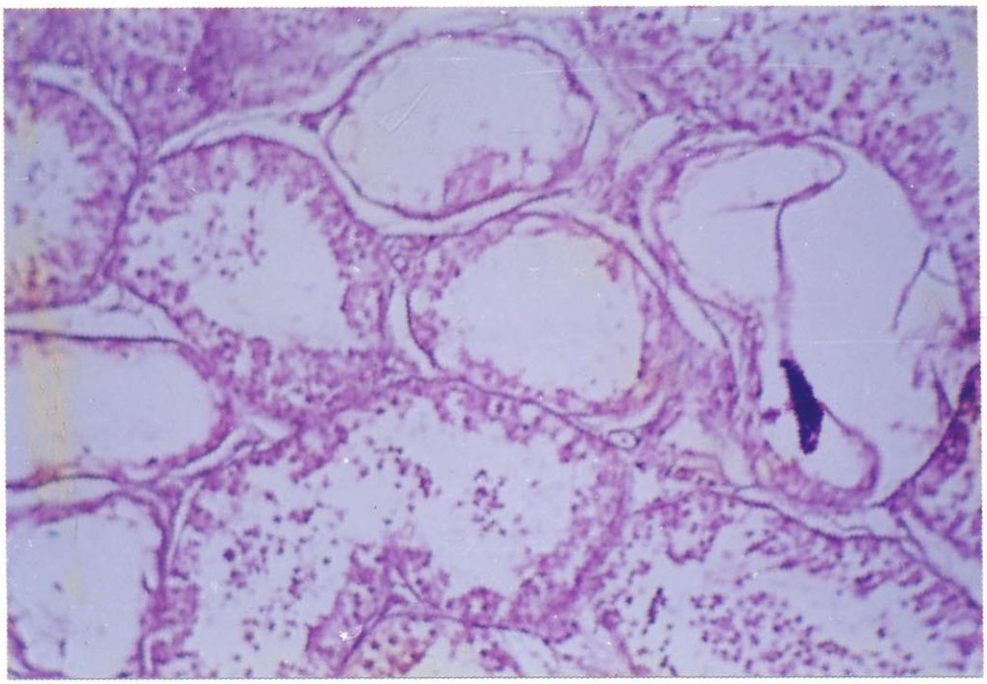


Fig.20 Severe testicular degeneration

Loss of germinal epithelial cells dilatation of tubules and cytoplasmic vacuolation of lining cells H&E x 250

Fig.21 Advanced testicular degeneration

Dilatation of tubules, thickening of basement membrane with degenerated necrosed and calcified sperm mass in the tubules - H&E x 250



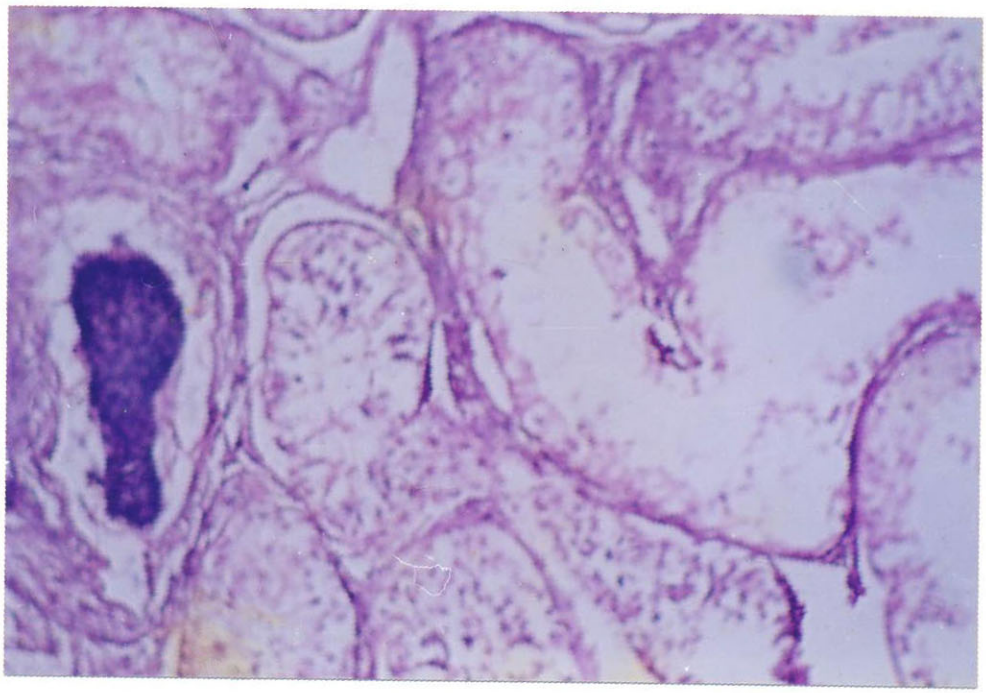


Fig.22 Testicular degeneration – Advanced

Presence of necrosed, calcified sperm mass within the tubule - H&E x 250

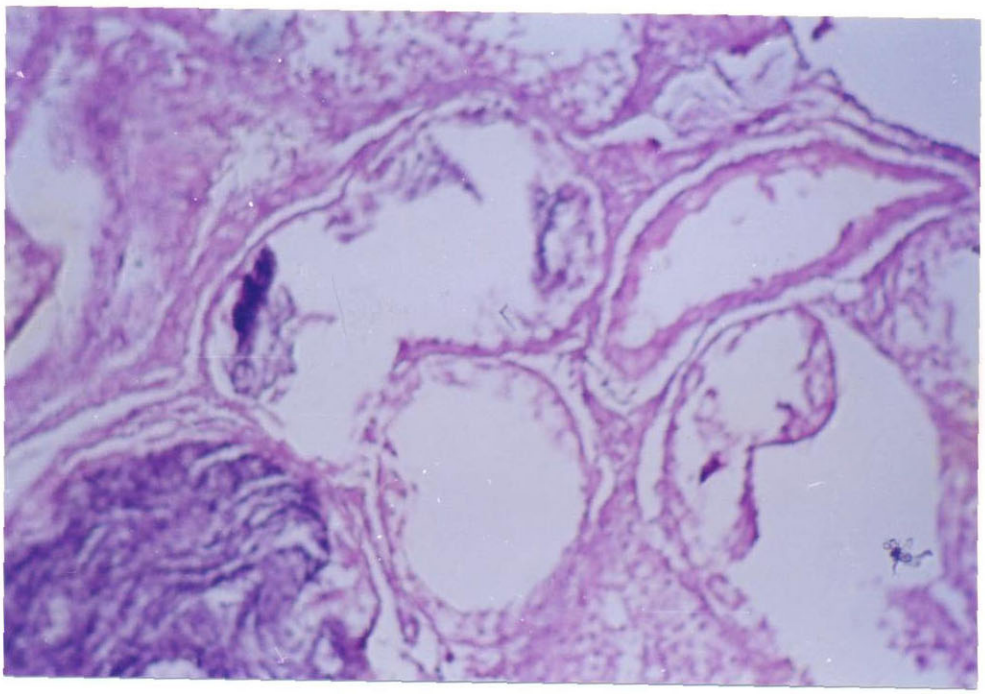
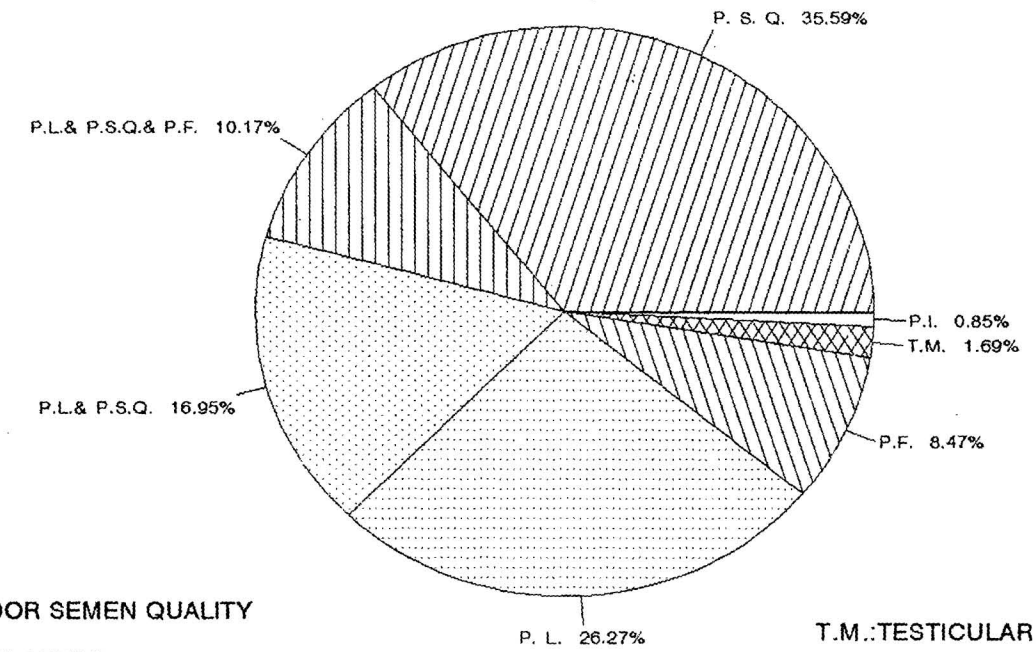


Fig.23 RATE OF CULLING OF GROWING CROSS BRED BULLS



P.S.Q.:POOR SEMEN QUALITY

P.L.:POOR LIBIDO

P.F.: POOR FREEZABILITY

T.M.:TESTICULAR MALFORMATION

P.I.:PENILE INFECTION

semen quality and poor freezability, 1.69 per cent with testicular malformation and 0.84 per cent with penile infection (Fig.23).

4.3. Group III - Adult bulls

4.3.1 Palpation and measurement of testis and epididymis

The texture of testis on palpation of normal bulls were turgid and resilient. The testis of hypoplastic bulls were more hard to feel and these with mild to moderate degenerative degeneration were more soft and flabby and hard in advanced degeneration.

The length, breadth, thickness of testis and length of cauda epididymis of right and left side are presented in Table 7. Statistical analysis did not reveal any significant variation between crosses in testicular measurements.

4.3.2 Scrotal shape and circumference

Out of 40 adult bulls, the shape of the scrotum was observed as oval in 19 (47.50 per cent), elongated in 8 (20.00 per cent), rounded in 3(7.50 per cent), rectangular in 2 (5.00 per cent), pear shaped in 3 (7.50 per cent), square in 2 (5.00 per cent), oblong in 2 (5.00 per cent) and bifid in 1 (2.50 per cent) (Fig. 24 to 35).

The mean scrotal circumference of CBJ, CBBS and CBHF bulls was 35.98 ± 1.25 cm, 33.36 ± 0.99 cm and 36.13 ± 0.73 cm. The overall mean of all the three bulls was 35.29 ± 0.74 cm. On analysis of data there was no significant variation between crosses in scrotal circumference.

4.3.3 Libido

All the adult bulls were having good libido and semen could be collected from all the bulls.

4.3.4 Semen evaluation

The physical and morphological characters of semen from 40 adult bulls are presented in Table 8 and 9.

4.3.4.1 Volume

The mean ejaculate volume of CBJ, CBBS and CBHF was 5.28 ± 0.26 ml, 4.11 ± 0.41 ml and 4.86 ± 0.34 ml respectively. The overall mean was 4.87 ± 0.20 with a range of 1.92 to 8.75 ml.

The volume of semen of CBJ and CBBS show significant variation ($P<0.05$).

4.3.4.2 Initial motility

The initial motility of spermatozoa of CBJ, CBBS and CBHF was 59.01 ± 1.46 per cent, 58.65 ± 2.41 per cent and 63.65 ± 0.70 per cent respectively. Overall mean for the three breeds was 59.84 ± 1.05 per cent and ranged from 40.00 to 70.00 per cent.

4.3.4.3 Concentration of spermatozoa

The sperm concentration of CBJ, CBBS and CBHF was 1444.57 ± 45.54 million per ml, 1498.27 ± 80.14 million per ml and 1551.75 ± 70.50 millions per

ml respectively. Overall mean for the three breeds was 1480.78 ± 35.15 million per ml and ranged from 971 to 2015 millions per ml.

4.3.4.4 Live sperms

Mean live sperm count of the CBJ, CBBS and CBHF was 71.94 ± 1.49 per cent, 66.02 ± 2.90 per cent and 65.77 ± 6.20 per cent respectively. Overall mean for three breeds was 69.08 ± 1.68 per cent and ranged from 23.20 to 81.89 per cent. (Table 9).

4.3.4.5 Sperm morphology

Morphological characters of the three different breeds of CBJ, CBBS and CBHF are presented in Table 9 and Fig.36 to 39. The overall mean of head, mid-piece and tail abnormalities was 2.67 ± 0.26 per cent, 1.07 ± 0.05 per cent and 11.18 ± 0.71 per cent respectively. The mean proximal and distal protoplasmic droplets were 1.70 ± 0.28 per cent and 1.56 ± 0.21 per cent respectively. There was no significant difference in the morphological characters of the three different crossbreds.

4.3.4.6 Freezability

The prefreezing, post freezing and total rejection of 241 ejaculates, were 12.00 per cent, 11.20 per cent and 23.20 per cent respectively (Table 10 and Fig.40).

4.3.5 Infertility conditions

The various infertility conditions observed among adult bulls are presented in Table 11.

4.3.5.1 Bilateral partial hypoplasia

Two crossbred bulls showed bilateral partial hypoplasia (5%). These bulls were normal clinically. Semen picture showed high incidence of abnormalities. The seminogram revealed:

Mean ejaculate volume	2 to 4 ml
Motility	30 to 40 per cent
Sperm concentration	620 to 1251 million per ml
Head abnormalities	10 to 15 per cent
Mid piece abnormalities	7 to 8 per cent
Detached heads	10 to 15 per cent
Proximal protoplasmic droplets	15 to 30 per cent
Tail abnormalities	4 to 5 per cent
Total abnormalities	46 to 73 per cent

There was variation in the size of the seminiferous tubules. Most of the tubules were irregularly lined and the nucleus of the lining cells appeared hyper chromatic. Mild degree of vacuolation of cytoplasm was observed. The intertubular connective tissue and Leydig cells did not reveal any marked changes. Pyknotic nuclei were evenly distributed throughout the seminiferous epithelium. Primary spermatocytes had chromosomes in various stages of development with most appearing as single chromatin mass. Secondary spermatocytes and spermatids in few of the tubules formed groups. Karyolysis and karyopyknosis were observed in a few.

4.3.5.2 Testicular degeneration

Testicular degeneration of various degrees was detected in six adult crossbred bulls. The testicles showed loss of firmness with the following seminal characters.

Mean ejaculate volume	4 to 5 ml
Sperm concentration	680 to 1280 million per ml
Initial motility	20 to 40 per cent
Sperm head abnormalities	15 to 25 per cent
Detached heads	10 to 20 per cent
Mid piece abnormalities	3 to 4 per cent
Proximal protoplasmic droplets	15 to 20 per cent
Tail abnormalities	20 to 25 per cent
Total abnormalities	63 to 94 per cent

Mild to moderate testicular degeneration characterised by single layer of lining cells, cytoplasmic vacuolation, loss of germinal epithelium and desquamation were seen as in (Fig.19). Irregular dilatation of tubules with degeneration and necrosis of lining cells was observed in three cases (Fig.41). Remnants of cells were seen filling the lumen of the tubules. Irregular and dilated tubules with complete loss of lining cells leaving only the thickened basement membrane was a feauture in few of the cases. Some of the tubules have become cystic and the lumen contained homogeneous pink staining material (Fig.42). Advanced degenerative changes were observed in two cases. The basement membrane was highly thickened in all the cases. There was an increase in the proliferation of the intertubular connective tissue (Fig.43). Only a few pyknotic germinal cell remained in some of the tubules. Loss of all the epithelial cells from the seminiferous tubules which consisted merely of thickened, wrinkled fibrous tissue was seen in one case (Fig.44) sperm accumulated in seminiferous tubules near the rete testis in more advanced lesions.

4.3.5.3 Scanning Electron Microscopy (SEM)

SEM of tissues from cryptorchid hypoplasia and degenerative conditions revealed mild to moderate morphological alterations. In cryptorchid testis only few segments of seminiferous epithelium was observed. Outer connective tissue appeared highly thickened and pitted. Differentiation of germinal epithelial cells was absent in most of the seminiferous tubules. Within tubules spermatozoa could

not be detected. Lumen of epididymis showed no evidence of spermatozoa. Steriocila were not seen at the free margins (Fig. 45).

In hypoplastic testis coiled tubules of very small diameter surrounded by connective tissue were noted. Seminiferous epithelial lining revealed scarcely populated epithelial cells of varying size and shape (Fig.46).

In degenerated testicular tissue only few spermatogenic cells were seen. Within the lumen, scanty coiled sperm tails could be observed (Fig.47). Compactness of the epithelium was absent.

SEM of spermatozoa was made (Fig.48 to 51). The result was in conformation with the morphological changes observed under the present study.

4.3.5.4 Seminal enzymes

The enzyme profile of ACP, AKP, GOT, GPT and LDH of bulls with freezability of fifty per cent and above was 556.34 ± 2.93 KAU per 100 ml, 804.03 ± 3.59 KAU per 100 ml, 753.14 ± 1.59 units/ml, 33.33 ± 2.21 units per ml and 355.88 ± 1.28 IU per l and those of bulls having freezability less than 50 per cent was 600.42 ± 7.73 KAU units/100 ml, 1003.50 ± 9.38 KAU units per 100 ml, 815.76 ± 2.21 units per ml, 36.33 ± 2.21 units per ml and 397 ± 84.0 ± 2.58 IU per L respectively (Table 12). The seminal plasma enzymes were significantly higher in group II than those in group I ($P<0.01$).

4.3.6 Rate of culling

Out of 280 adult bulls observed 87 adult bulls (31.10 per cent) were culled due to various problems (Fig.52). Among this, 63 bulls (72.40 per cent) were culled due to various reproductive disorders as poor semen quality (49.20 per cent), poor libido (14.28 per cent), poor freezability (12.69 per cent), poor libido and poor semen quality (6.34 per cent), poor semen quality and poor freezability (12.69 per cent), testicular degeneration (1.58 per cent), testicular malformation (1.58 per cent) and hard testis (1.58 per cent).

Table 7. Testicular measurements of adults cross bred bulls above 24 months of age

Cross bred type	No. Adults bulls observed	Scrotal circum-ference (cm)	Left side				Right side				Age at obser-vation (months)
			Length (cm)	Breadth (cm)	Thickness (cm)	Length of cauda (cm)	Length (cm)	Breadth (cm)	Thickness (cm)	Length of cauda (cm)	
CBJ mean	21	35.98 ± 1.25	12.48 ± 0.59	6.74 ± 0.19	5.29 ± 0.18	2.12 ± 0.15	13.24 ± 0.43	7.02 ± 0.18	5.78 ± 0.22	2.02 ± 0.22	51.00 ± 3.16
Range		29-55	2.5 – 16.0	5.0 – 8.5	4.0 – 7.5	1.0 – 4.0	9.0 – 17.0	5.5 – 9.0	4.0 – 7.7	1.0 - 6.0	30.0 – 86.0
CBBS mean	11	33.36 ± 0.99	12.27 ± 0.45	6.64 ± 0.15	5.50 ± 0.35	1.86 ± 0.14	12.77 ± 0.58	6.73 ± 0.26	5.46 ± 0.25	2.20 ± 0.21	42.64 ± 4.03
Range		29.0 – 41.0	11.0 – 16.0	6.0 – 7.5	3.5 - 7.5	1.0 – 2.5	10.5 – 17.5	5.5 – 8.0	4.0 – 7.0	1.0 – 3.0	27.0 – 67.0
CBHF Mean	8	36.13 ± 0.73	13.31 ± 0.38	6.63 ± 0.34	4.55 ± 0.15	1.75 ± 0.09	13.75 ± 0.47	7.00 ± 0.19	5.56 ± 0.38	1.50 ± 0.13	65.63 ± 4.61
Range		36.0-38.5	11.5-15.0	5.5-8.5	4.0-5.0	1.5-2.0	12.0-16.5	6.5-8.0	4.5-8.0	1.0-2.0	42.0-79.0
Total Mean	40	35.29 ± 0.74	12.59 ± 0.34	6.69 ± 0.12	5.20 ± 0.14	1.98 ± 0.09	13.21 ± 0.29	6.94 ± 0.12	2.01 ± 0.16	2.01 ± 0.16	51.63 ± 2.48
Range		29.0-55.0	2.5-16.0	5.0-8.5	3.5-7.5	1.0-4.0	9.0-17.5	5.5-9.0	4.0-8.0	1.0-6.0	27.0-86.0
F value		1.312^{NS}	0.586^{NS}	0.091^{NS}	2.986^{NS}	1.446^{NS}	0.649^{NS}	0.544^{NS}	0.445^{NS}	1.448^{NS}	6.370**

NS – Non significant

** Highly significant (P<0.01)

Table 8. Physical characteristics of semen of adult bulls

Crossbred type	No. of adult bulls observed	No. of ejaculates	Volume (ml)	Initial motility (%)	Concentration (million per ml)
CBJ mean	21	126	5.28 ± 0.26 [a]	59.01 ± 1.46	1444.57 ± 45.54
Range			3.17 - 8.75	41.67 - 70.00	1092.00 - 2008.00
CBBS mean	11	66	4.11 ± 0.41 [b]	58.65 ± 2.41	1498.27 ± 80.14
Range			1.92 - 6.17	40.0 - 66.67	971-00 - 2015.00
CBHF mean	8	48	4.86 ± 0.34	63.65 ± 0.70	1551.75 ± 70.50
Range			3.67 - 6.67	60.00 ± 66.67	1302.00 – 1815.00
Total mean	40	240	4.87 ± 0.20 [ab]	59.84 ± 1.05	1480.78 ± 35.15
Range			1.92 - 8.75	40 - 70	971 – 2015
F value			3.434*	1.730^{NS}	0.709^{NS}

Figures having different superscript in column varies significantly (P<0.05)

* Significant (P<0.05)

NS – Non significant

Table 9. Morphological characters of spermatozoa of adult cross bred bulls

Cross bred type	No. of bulls observed	No. of smears	Live sperm (%)	Abnormalities of			Protoplasmic droplets	
				Head (%)	Mid-piece (%)	Tail (%)	Proximal (%)	Distal (%)
CBJ mean	21	126	71.94 ± 1.49	2.42 ± 0.34	1.14 ± 0.08	9.93 ± 0.76	1.98 ± 0.51	1.64 ± 0.34
Range			55.21 - 81.89	1.16 - 7.89	0.55 - 1.88	3.81 - 18.57	0.38 - 9.36	0.63 - 8.18
CBBS mean	11	66	66.02 ± 2.9	2.70 ± 0.43	1.01 ± 0.08	12.91 ± 1.89	1.56 ± 0.16	1.67 ± 0.40
Range			48.04 - 800	0.96 - 5.36	0.40 - 1.34	4.09 - 25.53	0.95 - 2.52	0.83 - 4.90
CBHF mean	8	48	65.77 ± 6.2	3.29 ± 0.76	0.96 ± 0.05	12.11 ± 1.21	1.15 ± 0.46	1.19 ± 0.12
Range			23.2 - 76.05	1.75 - 8.37	0.67 - 1.16	4.33 - 15.17	0.66 - 2.11	0.63 - 1.71
Total mean	40	240	69.08 ± 1.68	2.67 ± 0.26	1.07 ± 0.05	11.18 ± 0.71	1.70 ± 0.28	1.56 ± 0.21
Range			23.20 - 81.89	0.96 - 8.37	0.40 - 1.88	3.81 - 25.53	0.38 - 9.36	0.63 - 8.18
F value			1.673^{NS}	0.558^{NS}	0.932^{NS}	1.604^{NS}	0.558^{NS}	0.389^{NS}

NS – Non significant

Table 10. Pre freezing, post freezing and total rejection of ejaculates of adult crossbred bulls

Freezing	Number of ejaculates	Number rejected	Percentage
Pre freezing	241	29	12.00
Post freezing	241	27	11.20
Total	241	56	23.20

Table 11. Infertility conditions investigated in 40 adult crossbred bulls

Sl. No.	Infertility conditions observed	Number of observed	Percentage
1	Bilateral partial testicular hypoplasia	2	5
2	Testicular degeneration	6	15

Table 12. Mean enzyme profile of seminal plasma in freezability groups of adult crossbred bulls

Freezability groups	No. of bulls examined	ACP KAU/100 ml	AKP KAU/100 ml	GOT/AST Units/ml	GPT/ALT Units/ml	LDH IU/L
Group I 50% and above	31	556.34 ± 2.93	804.03 ± 3.59	753.14 ± 1.59	33.33 ± 2.21	355.88 ± 1.28
Group II less than 50%	9	600.42 ± 7.73	1003.50 ± 9.38	815.76 ± 2.21	36.33 ± 2.21	397.84 ± 2.58
T value		6.7035**	24.855**	18.7645**	9.9335**	15.1047**

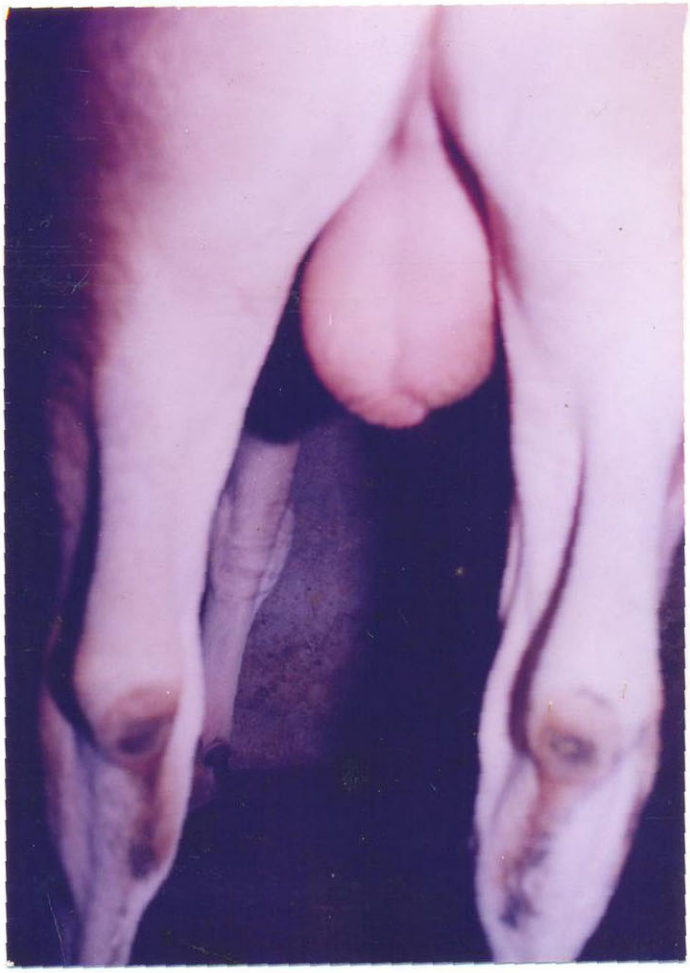
**($P < 0.01$)

Fig.24 Shapes of scroti

Oval

Fig.25

Elongated



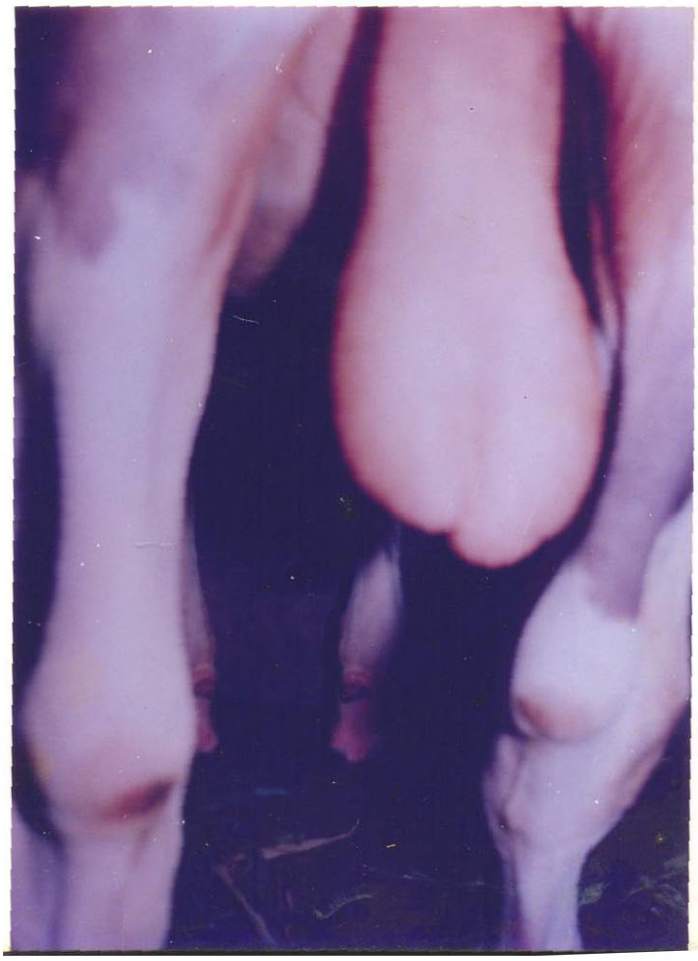
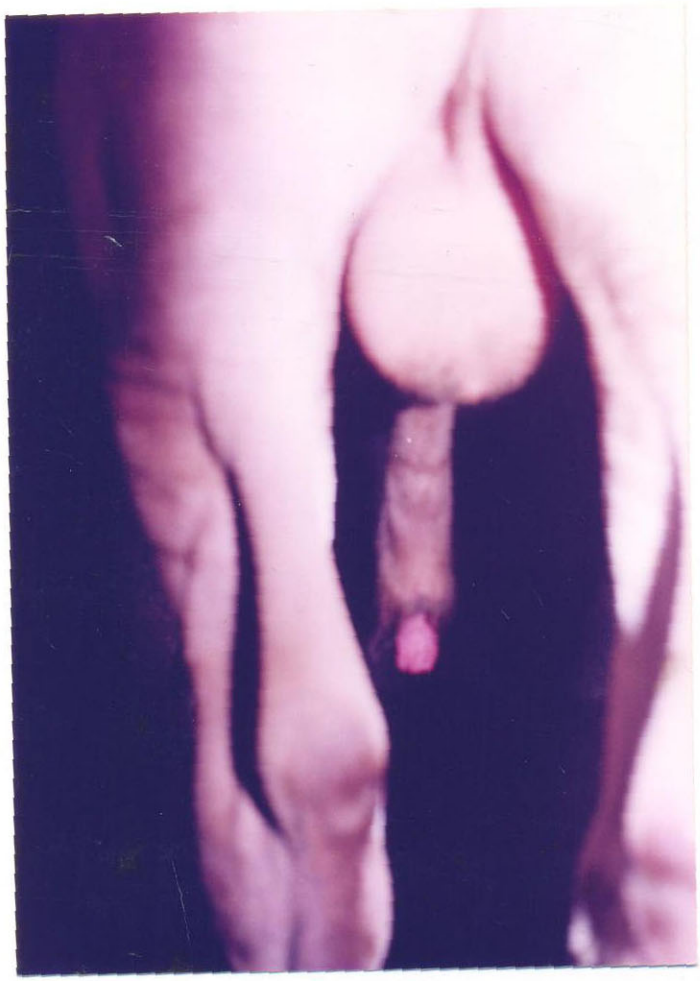


Fig.26 Shapes of scroti

Rounded

Fig.27

Rectangular



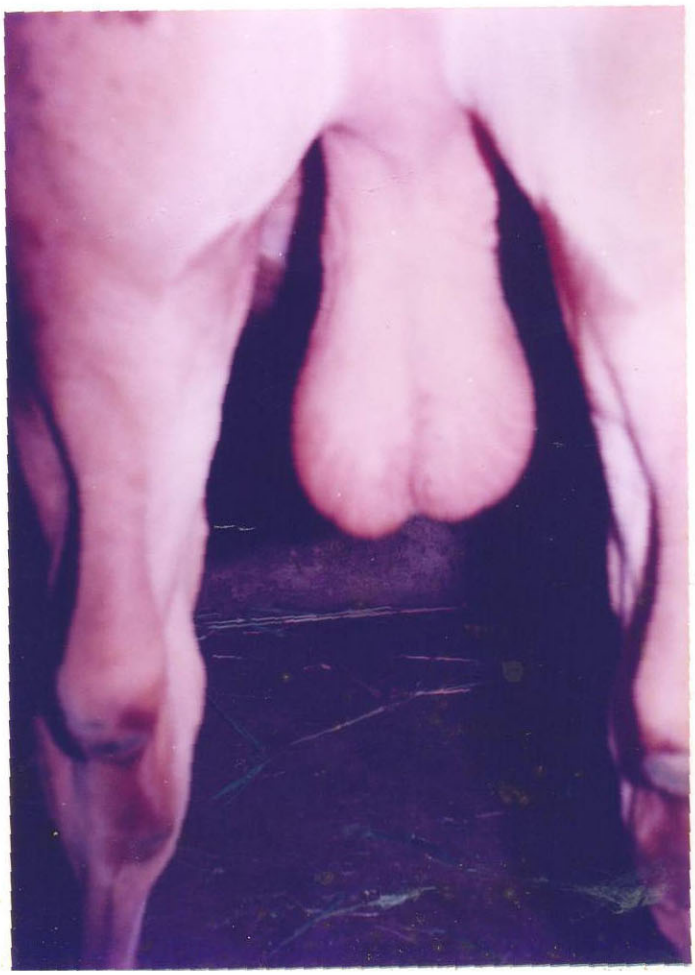
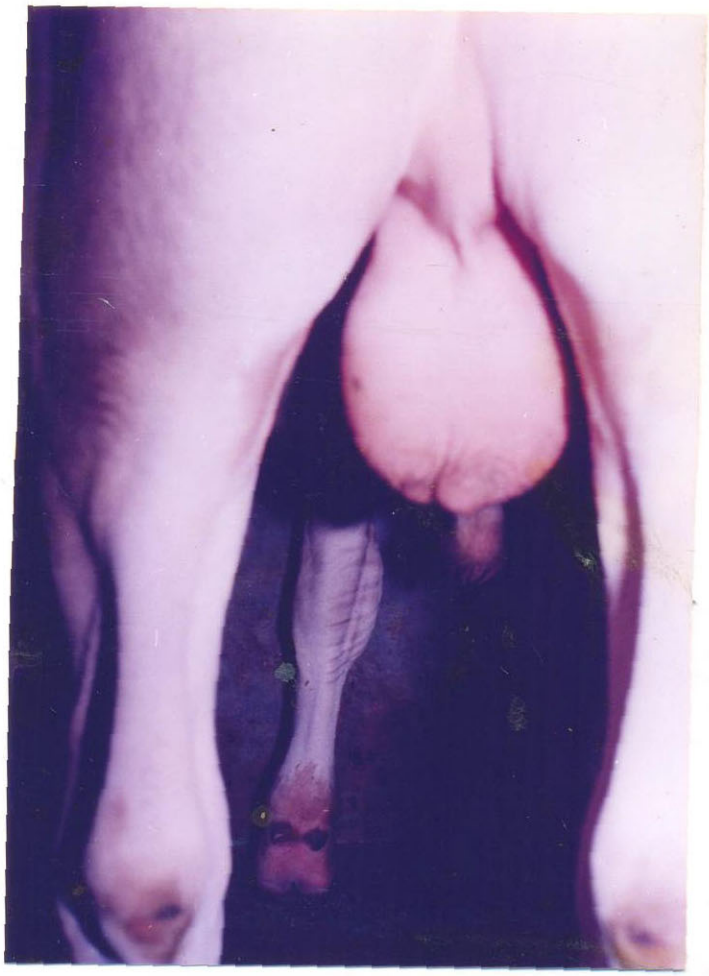


Fig.28 Shapes of scroti

Pear shaped

Fig.29

Square



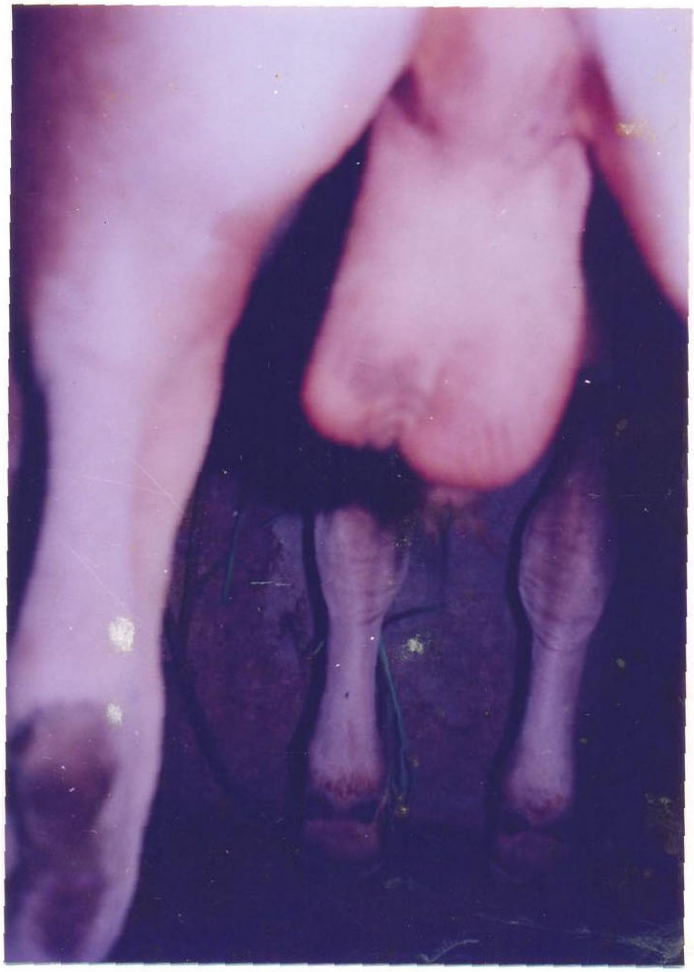
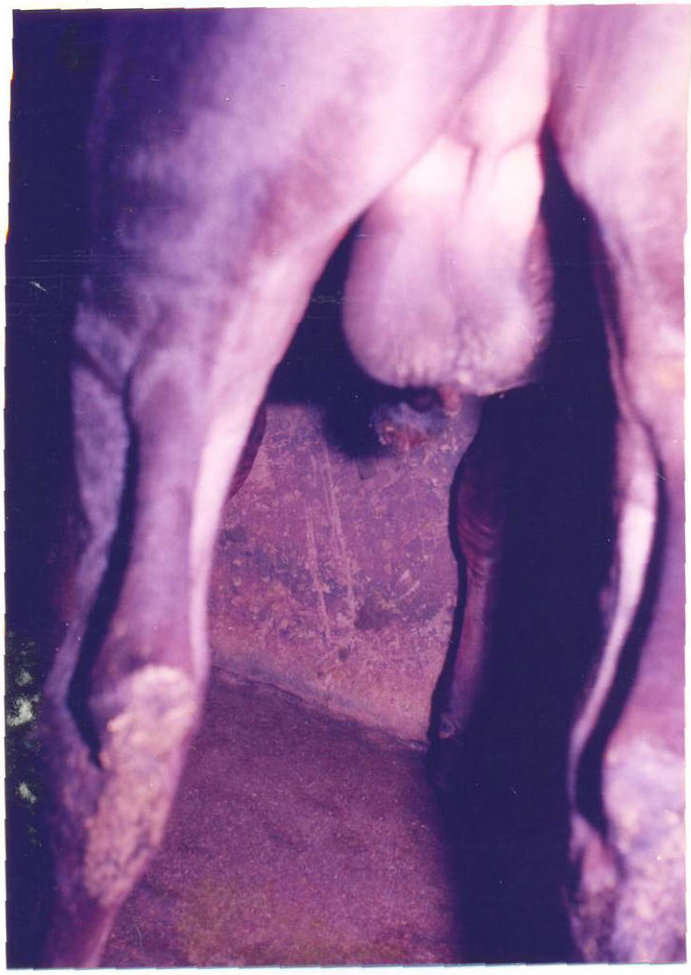


Fig.30 Shapes of scroti

Oblong

Fig.31

Bifid



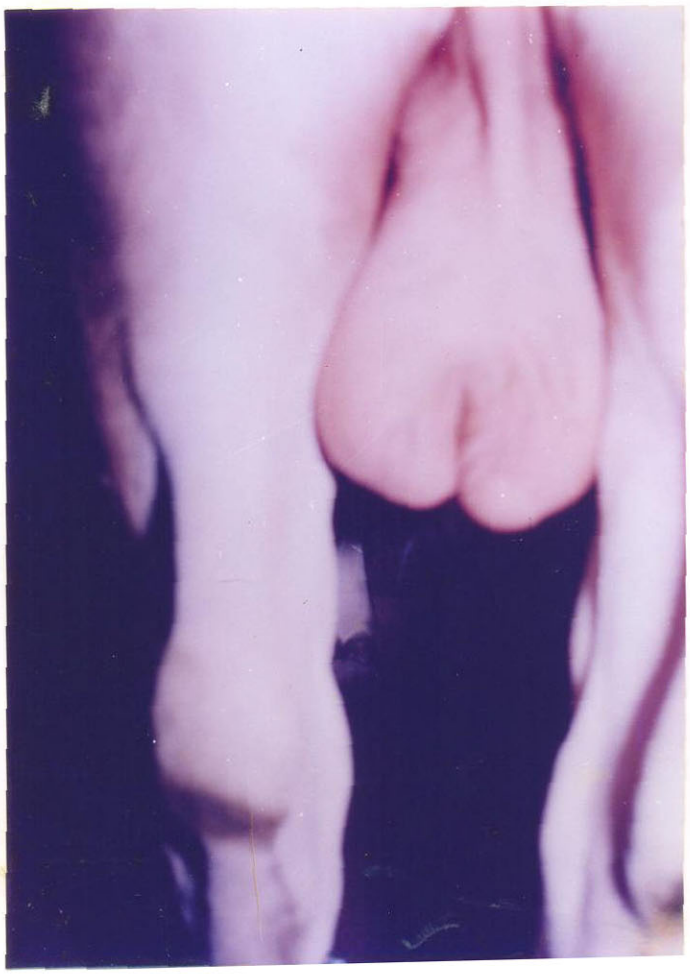
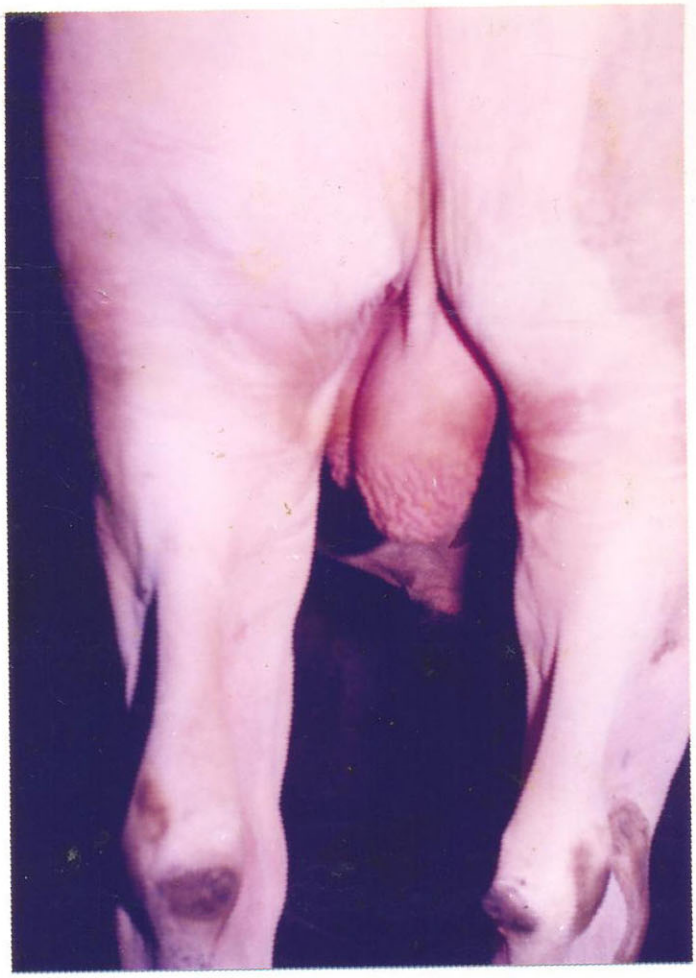


Fig.32 Shapes of scroti

Testicles twisted side ways with wrinkles

Fig.33

Asymmetric



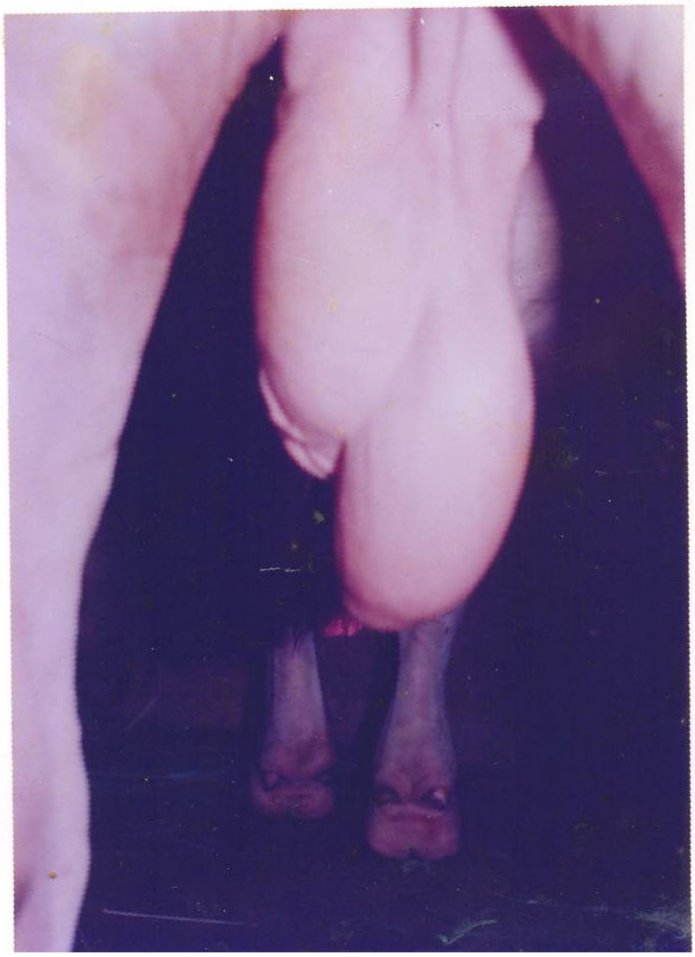


Fig.34 Shapes of scroti

Testicles held close to abdomen

Fig.35

Testicles twisted backwards with wrinkles

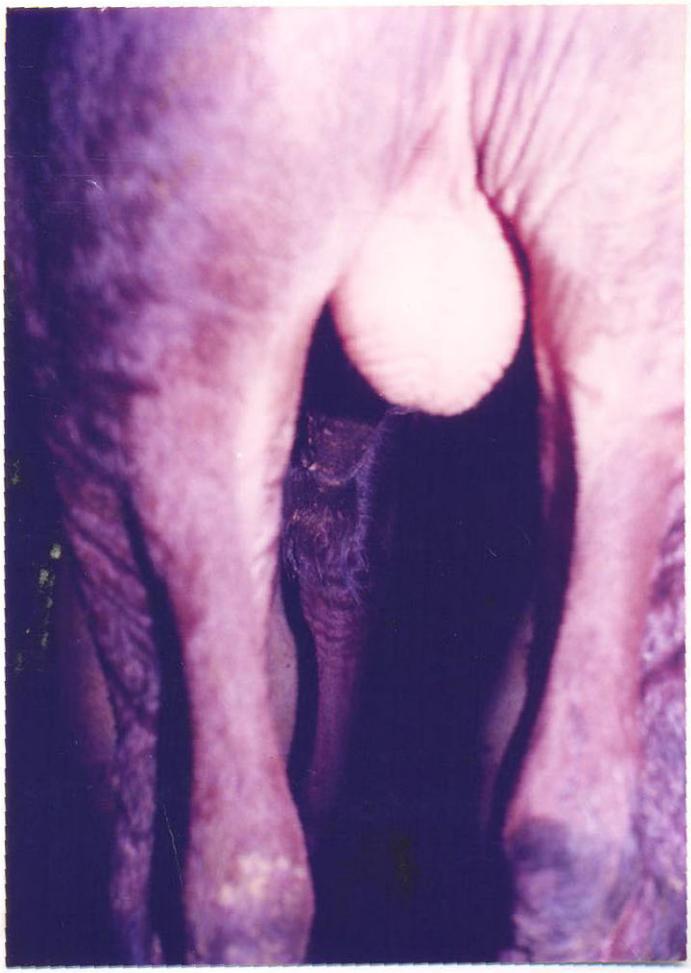
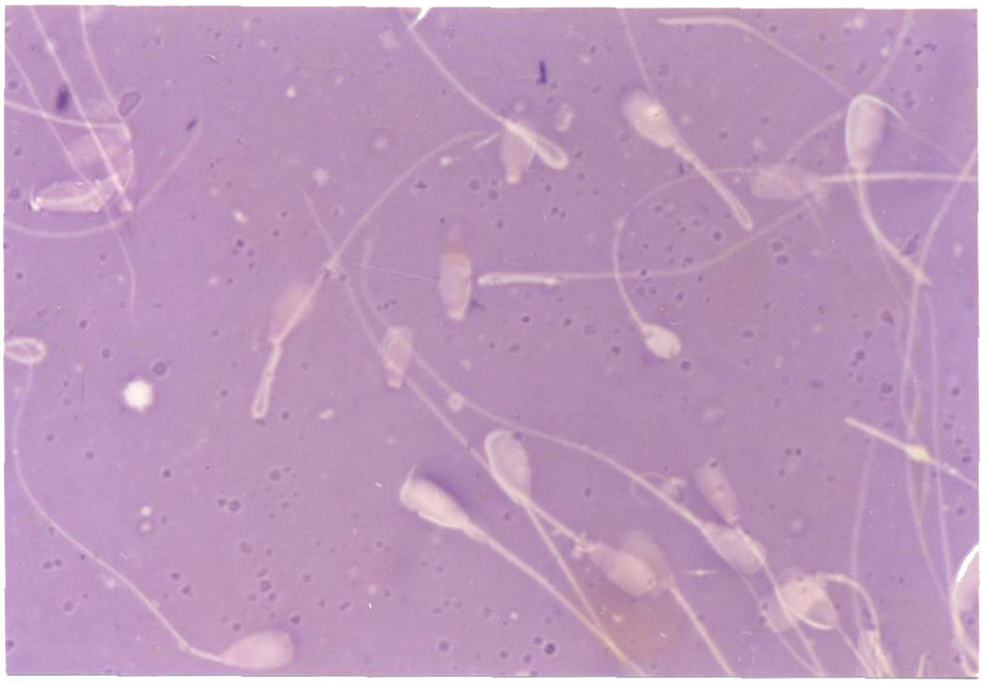


Fig.36 Sperm abnormalities

Spermatozoa with large number of abnormal and loose heads. E&N x 500

Fig.37 Sperm abnormalities

Spermatozoa with proximal protoplasmic droplets and abaxial attachment. E&N x 500



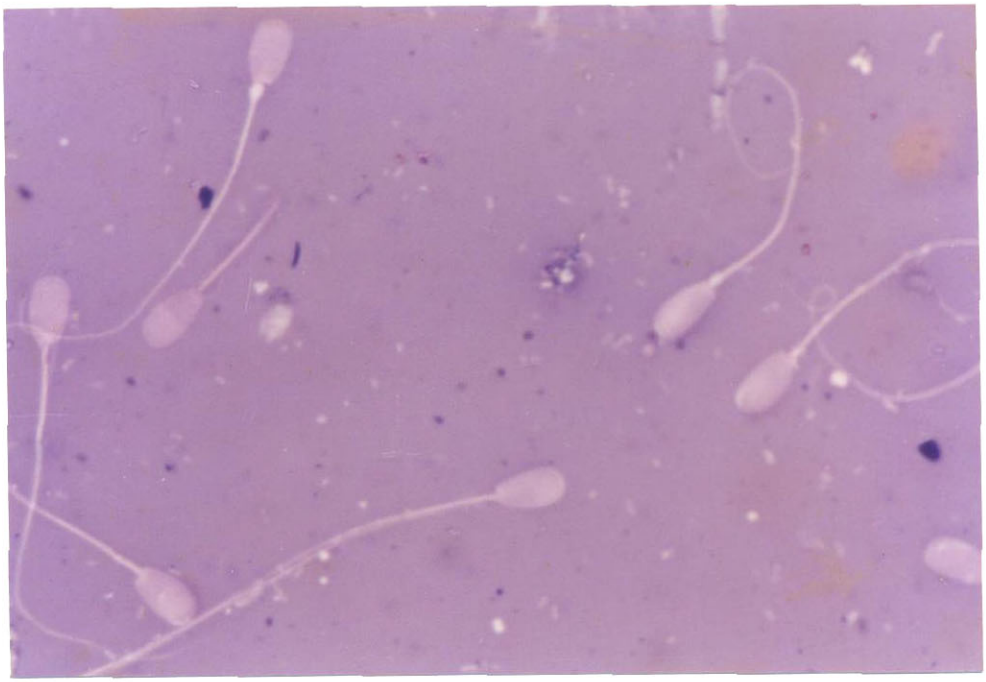
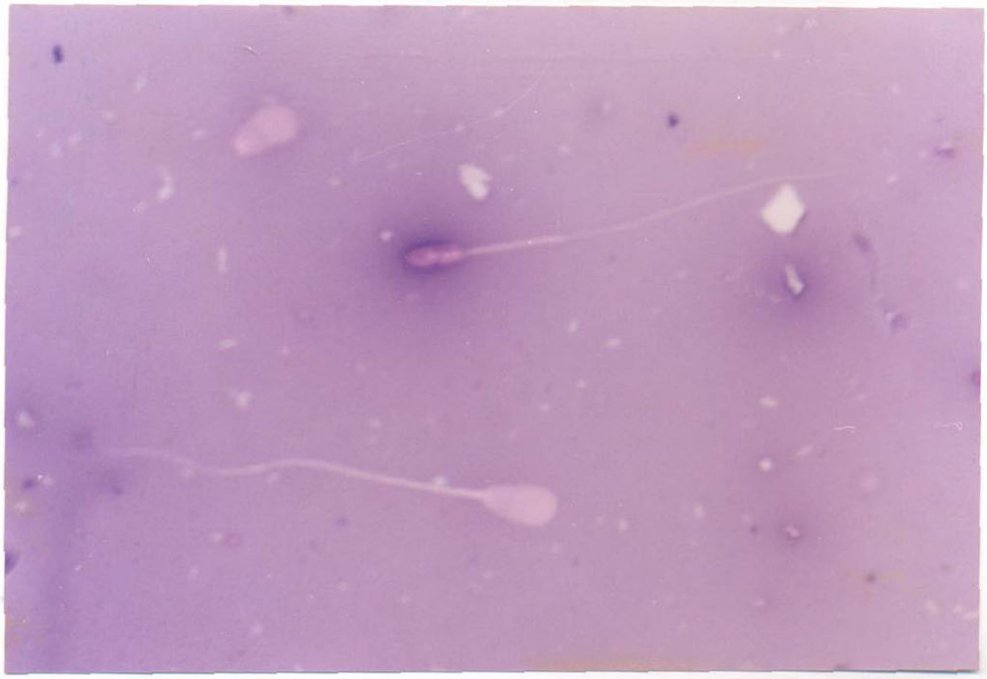


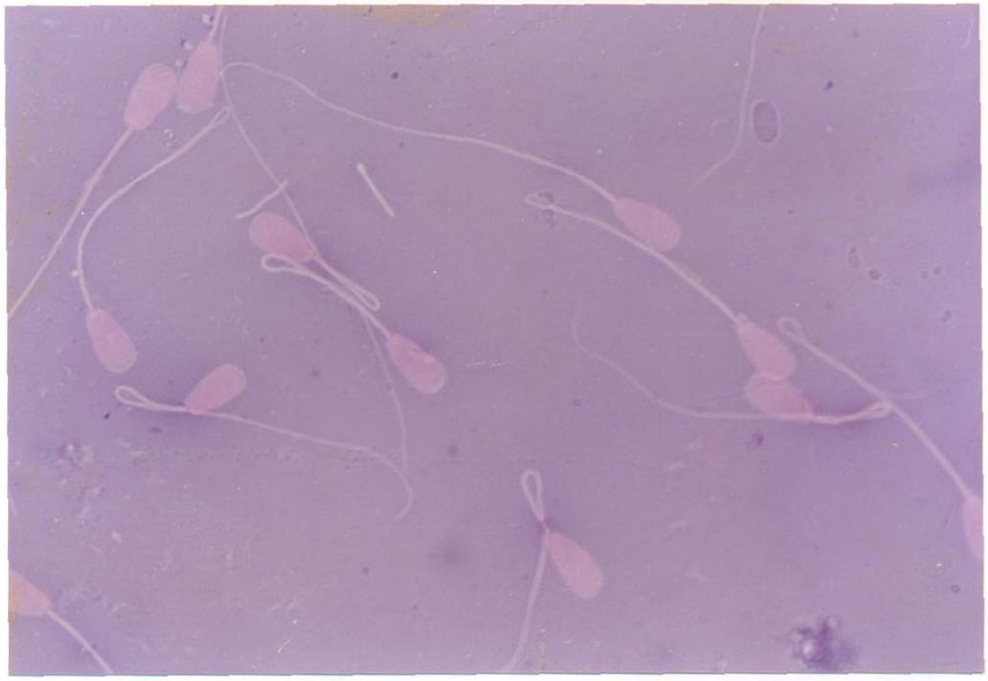
Fig.38 Sperm abnormalities

Loose head and microcephalous sperms
E&N x 500

Fig.39 Sperm abnormalities

Spermatozoa with bent tails and terminally coiled
Tails. E&N x 500





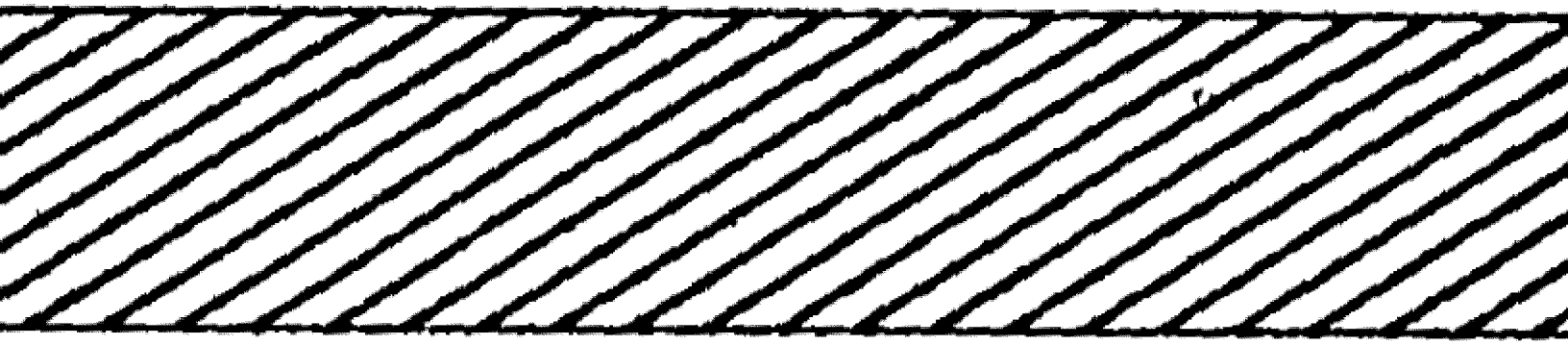
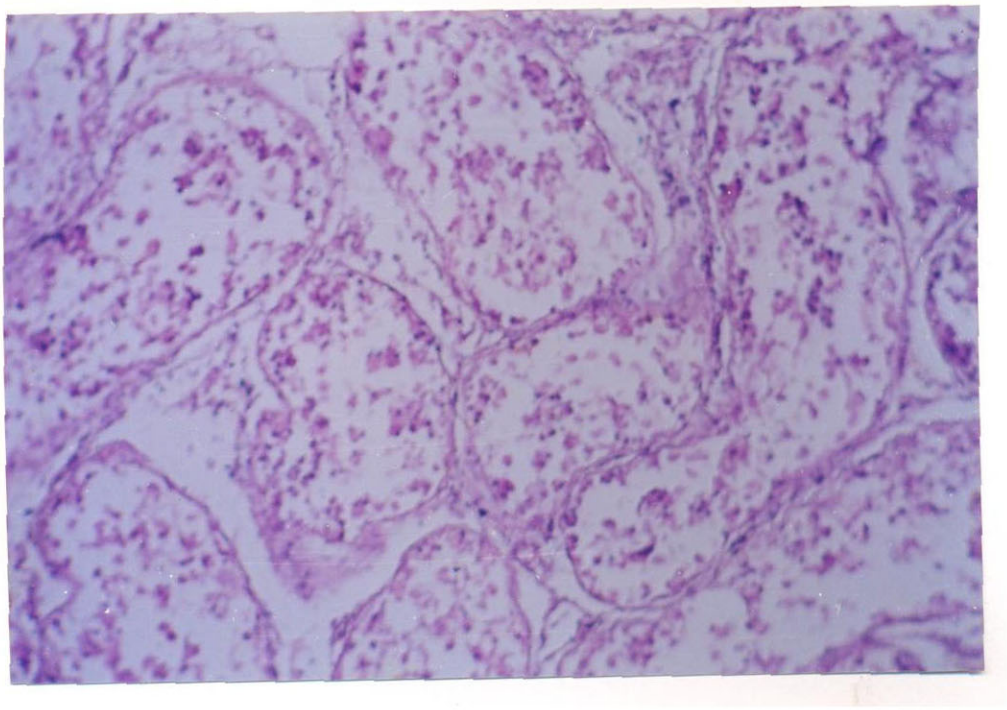


Fig.41 Testicular degeneration with necrosis of lining cells

Irregular dilatation of tubules with degeneration and necrosis of lining cells - H&E x 250

Fig.42 Testicular degeneration with cystic changes

Cystic tubules with lumen containing pink staining material. The basement membrane is seen highly thickened - H&E x 250



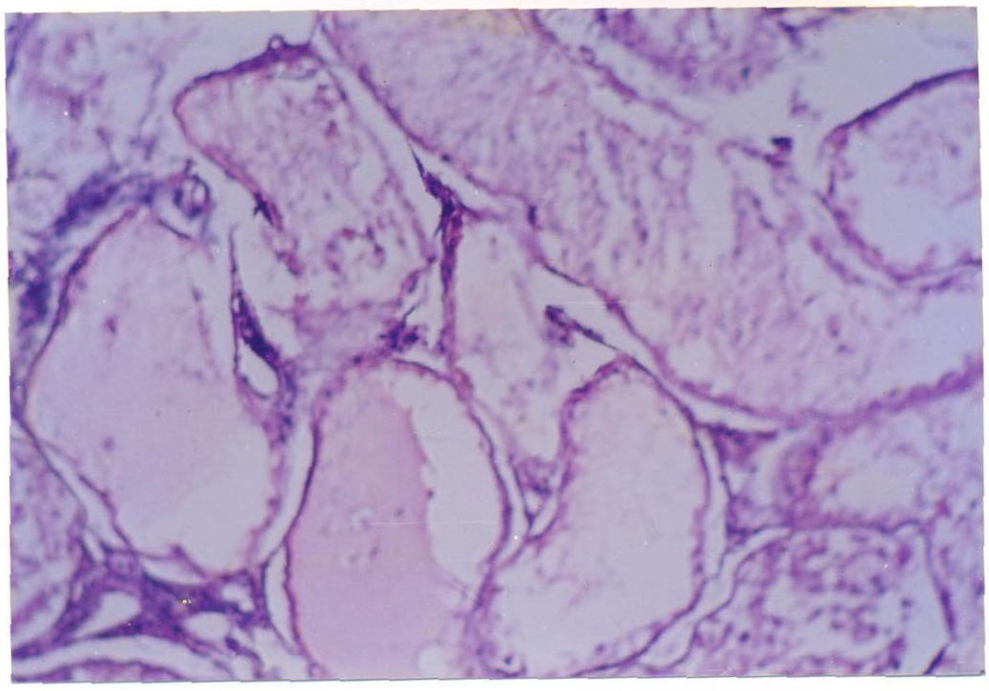
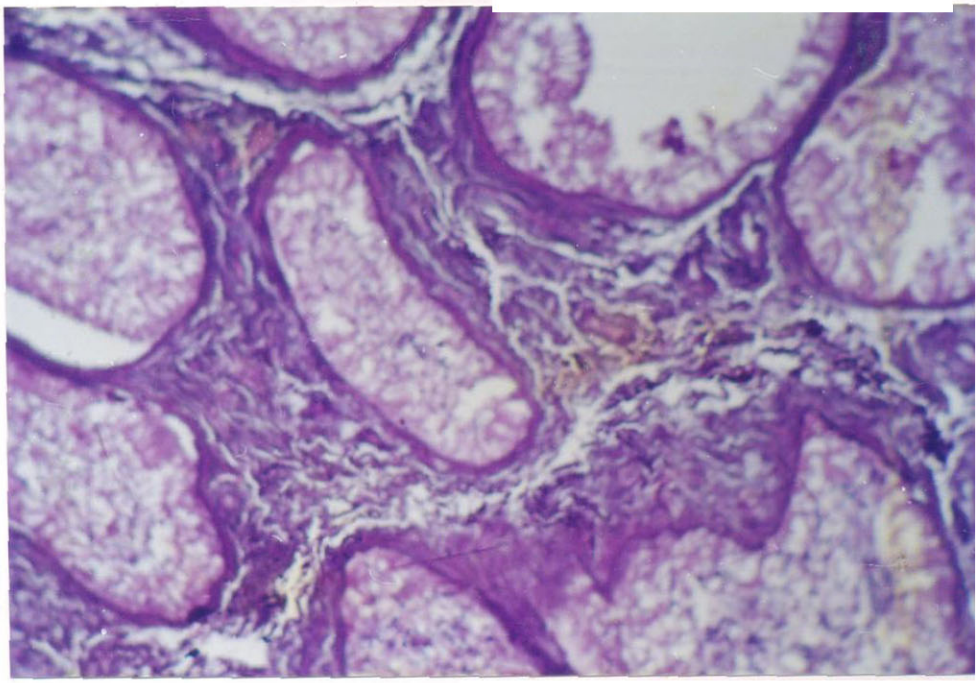


Fig.43 Advanced degenerative changes in testis

Proliferation of intertubular connective tissue and loss of all epithelial cells - H&E x 250

Fig.44 Testicular degeneration with fibrosis

Seminiferous tubules consisting merely thickened and wrinkled fibrous tissue - H&E x 250



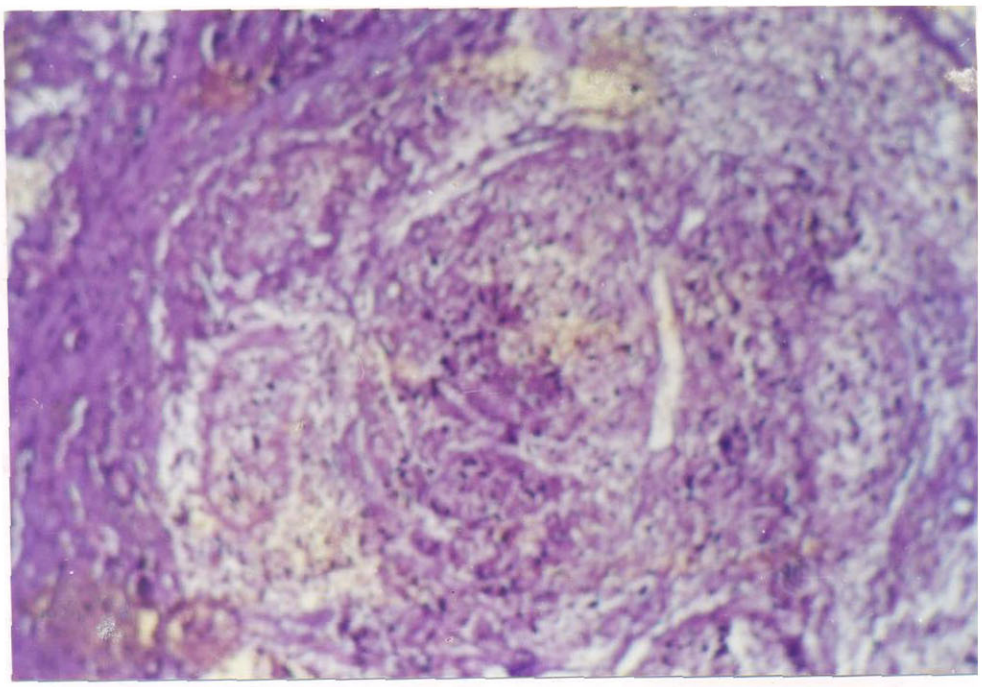
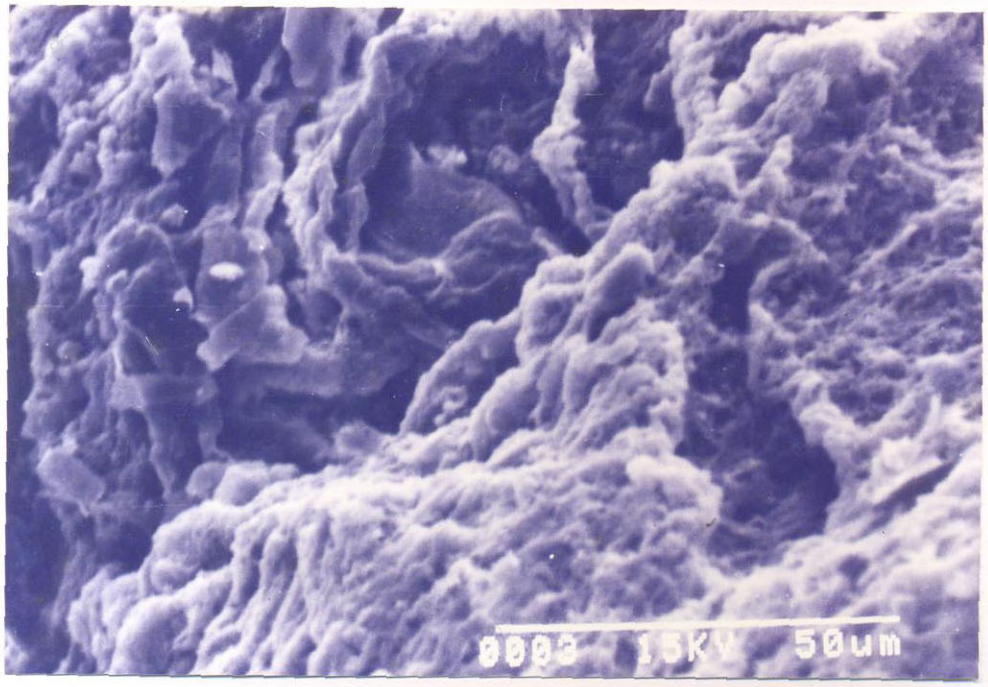


Fig.45 SEM – Cryptorchid testis

Highly thickened and pitted outer connective tissue layer of the walls of the seminiferous tubules

Fig.46 SEM Hypoplastic testis

Tightly packed mass of coiled tubules surrounded by connective tissue


0003 15KV 50um

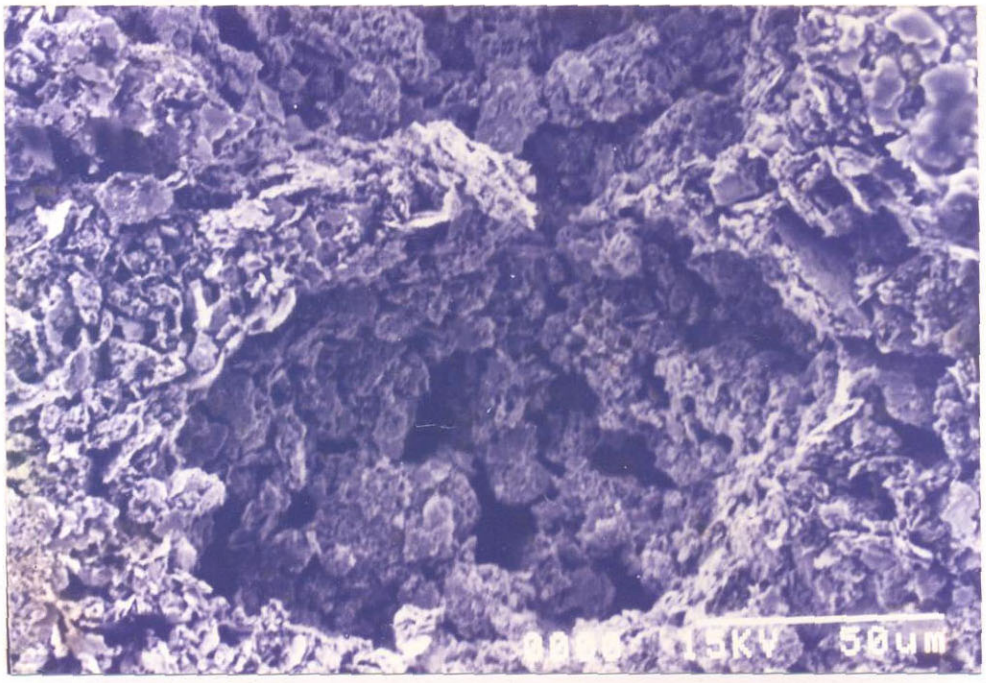
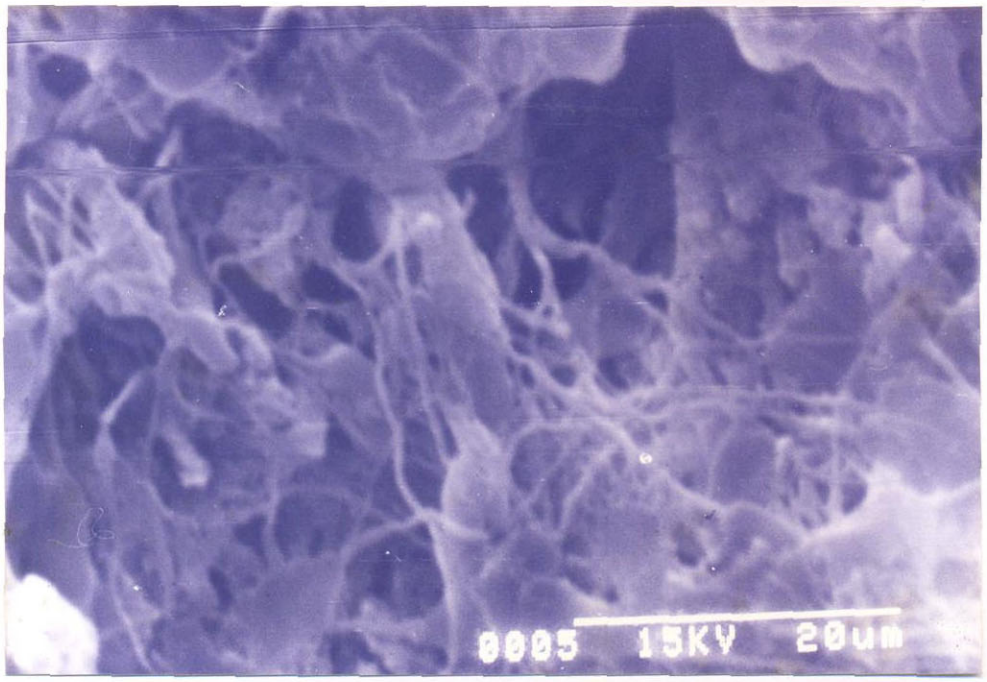

15KV 50um

Fig.47 SEM – Testicular degeneration

Shows few spermatogenic cells – Scanty spermatozoa – Coiled spermatic tails could also be seen

Fig.48 SEM – Sperm abnormalities

Spermatozoa with abnormal contour of head, thick mid piece, protoplasmic droplet and tightly coiled tail.


0005 15KV 20um

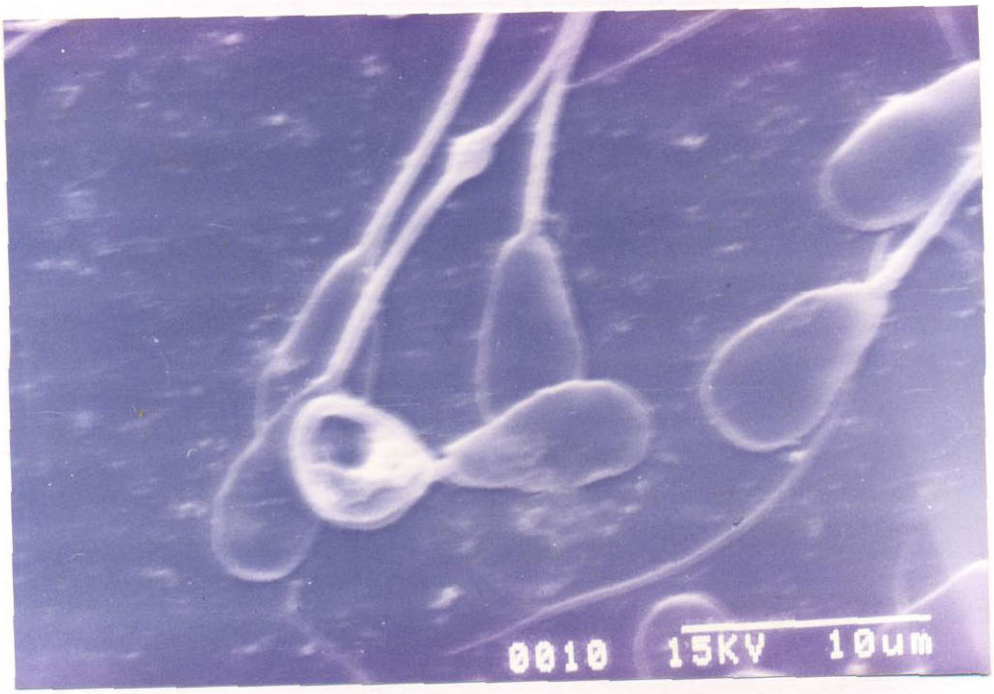
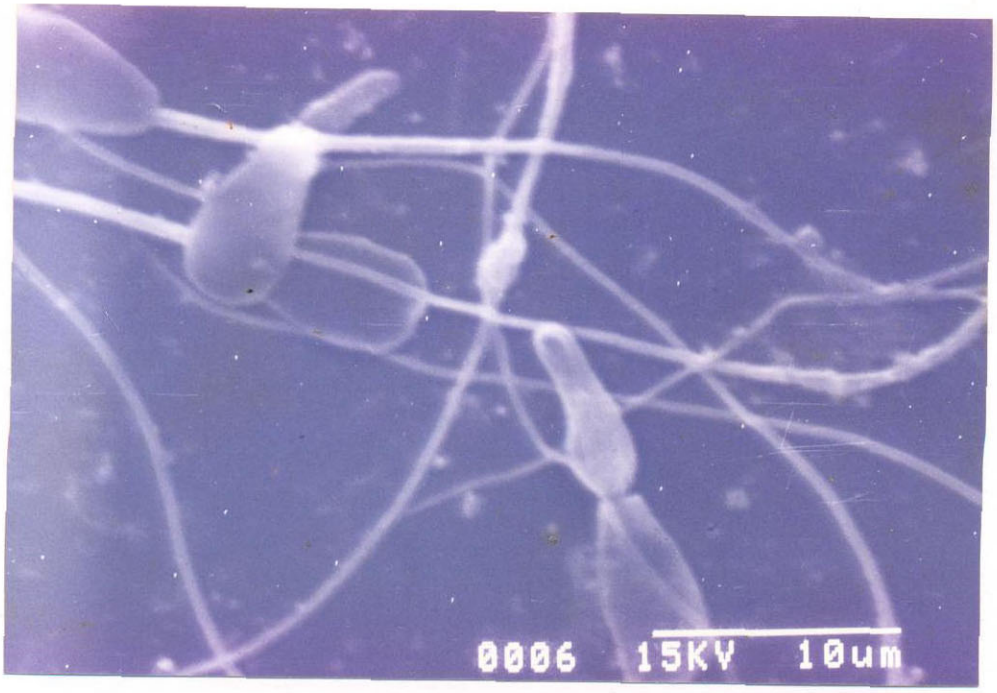

0010 15KV 10um

Fig.49 SEM Sperm abnormalities

Sperms with thick middle piece coiling at mid piece with pseudo droplets

Fig.50 SEM – Sperm abnormalities

Abnormal loose heads, bent tails and a split middle piece are seen


0006 15KV 10um

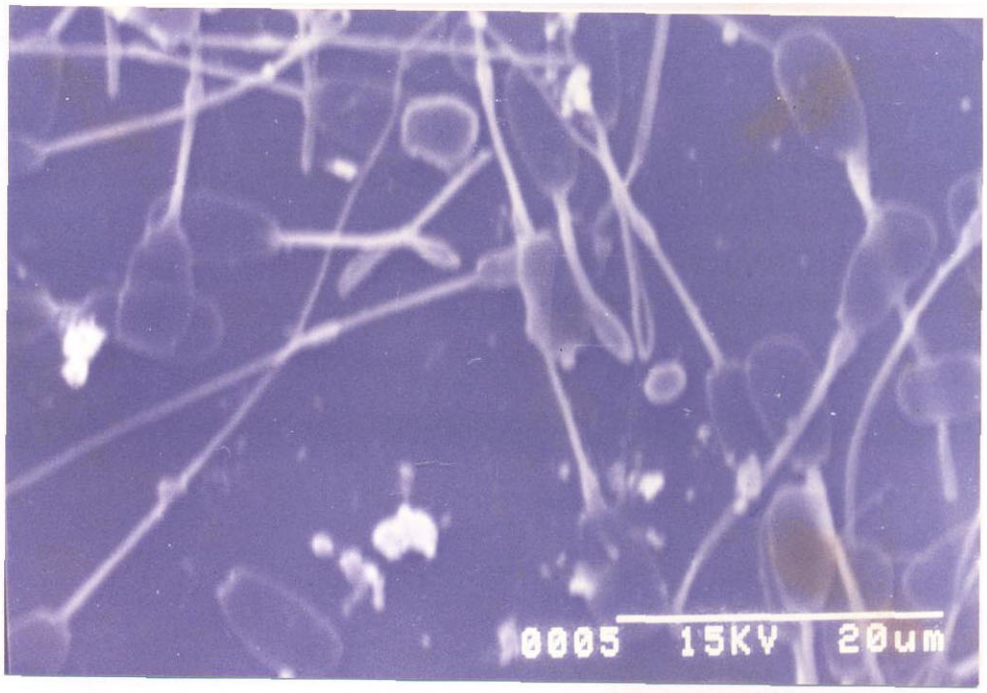

0005 15KV 20um

Fig.51 SEM – Sperm morphology

Sperms with thick mid piece, abaxial attachment of mid piece and pseudo droplets

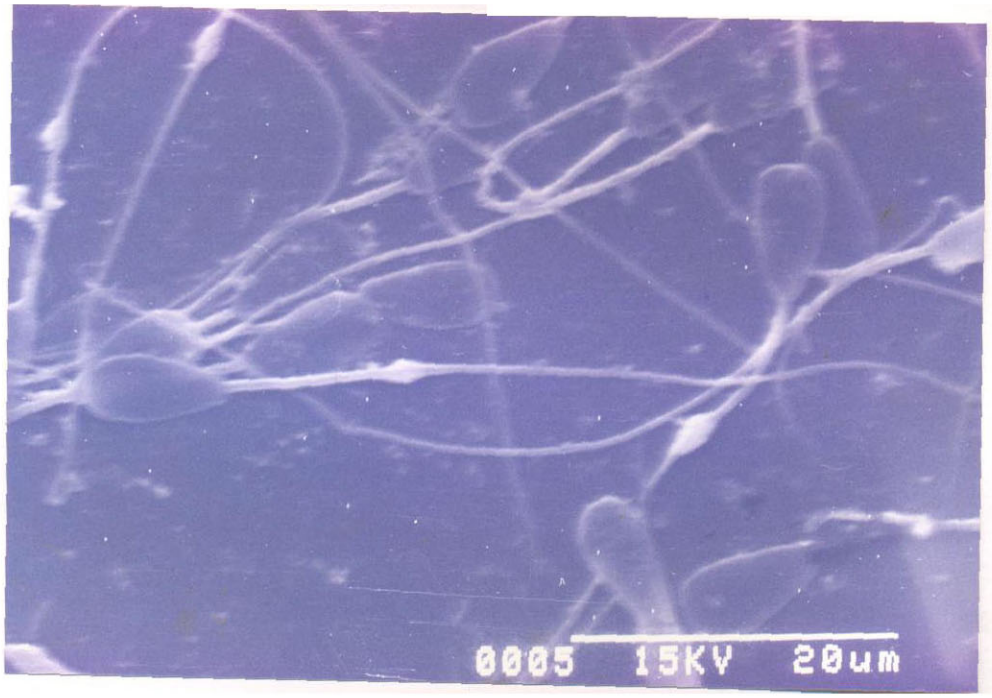
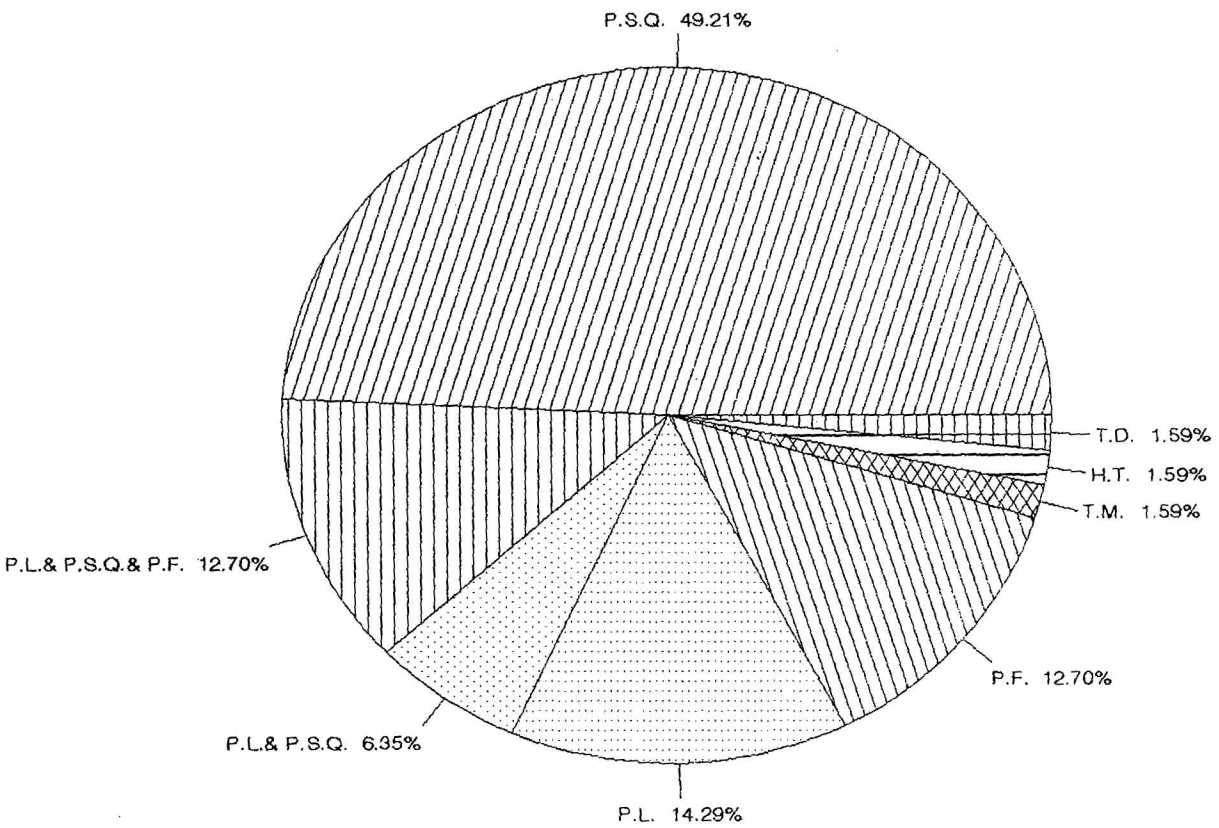

0005 15KV 20um

Fig.52 RATE OF CULLING OF ADULT CROSS BRED BULLS



P.S.Q.- POOR SEMEN QUALITY

P.L.- POOR LIBIDO

P.F.- POOR FREEZABILITY

H.T.- HARD TESTIS

T.D.- TESTICULAR DEGENERATION

T.M.- TESTICULAR MALFORMATION

Discussion

5. DISCUSSION

5.1 Group I - Bull calves

5.1.1 Palpation and measurement of testis and epididymis

Palpation and measurement of testis and epididymis (Table 1) revealed that there was significant difference between testis and epididymis of bull calves in different crosses. Singh *et al.* (1967) observed that the growth of testis and epididymis was slow in the beginning and became rapid from 5 to 8 months and more rapid upto 12 months and most rapid after 12 months of age. They also reported that different testicular measurements showed consistent rise indicating constant shape of testis during prepubertal period. The present study revealed that the testicular measurements were influenced by genetic make up of animals upto 12 months of age.

5.1.2 Scrotal measurements

The mean scrotal measurement of the different crosses (Table 1) showed significant variation and averaged 19.42 ± 1.03 cm. Hahn *et al.* (1969) reported scrotal circumference of 28.40 cm at seven months of age. Singh *et al.* (1967) also reported testicular circumference at birth as 3.37 cm which increased to 4.33, 4.86, 5.75, 7.48, 9.23 and 11.05 cm at 2, 4, 6, 8, 10 and 12 months of age respectively. According to Lunstra *et al.* (1979) the scrotal circumference averaged 27.90 ± 0.20 cm and it reached 29 cm at puberty irrespective of the bred. Hueston *et al.* (1988) reported scrotal circumference of Jersey bulls as 18.9 cm at five months of age.

Spitzer and Hopkins (1997) opined that scrotal circumference was closely related to sperm production. The variation in the scrotal circumference in the present study with the early report could be attributed to the different genetic make up of the bull and management factors.

5.1.3 Infertility

Out of 46 bull calves only one case of bilateral cryptorchidism was detected during the course of investigation (Fig.7). The incidence of cryptorchidism in bull calves has already been reported (Roberts, 1986; Hafez, 1993 and Arthur, 1996).

5.1.4 Histopathology

5.1.4.1 Testis

The histopathological lesions of testis revealed extensive fibrocollagenous proliferation with necrosis of seminiferous tubular lining are consistent with McKentee (1990). There was hyperplasia of interstitial cells as reported by Jubb *et al.* (1993).

5.1.4.2 Epididymis

Epididymis showed detachment of tubules and loss of lining cells, peritubular fibrosis and tissue proliferation as described by Jubb *et al.* (1993).

5.1.5 Rate of culling

Screening of the records of the last five years revealed that out of 222 bull calves, six were culled due to various reproductive disorders. This is in accordance with the findings of Roberts (1986) who suggested that 5 to 10 per cent of bull calves have to be eliminated as sterile or subfertile at the very early stage of selection. The necessity of screening from birth in order to obtain an acceptable conception of adult bull was stressed by Settergren (1978) and Laing *et al.* (1988).

5.2 Group II - Growing bulls

5.2.1 Palpation and measurement of testis and epididymis

Out of 67 growing bulls 52.24 per cent had oval scrotum, 19.40 per cent elongated, 7.46 per cent, rounded, 4.48 per cent rectangular 5.97 per cent pear shaped, 2.99 per cent square, 4.48 per cent oblong and 2.99 per cent were bifid. The testicular measurements did not show any variation between different crosses. Rao and Rao (1988) described different types of scroti in cross bred bull as oval, elongated, square, oblong, round and pear shaped. Krishna and Rao (1987) observed oblong type of scrotum as predominant in cross bred bull. However, Veerapandian (1992) reported oval shape of scrotum as predominant. Although, literature on the relationship between the pathological conditions of testis, shape and confirmation of scrotum are scanty. Van Camp (1997) opined that alterations in the normal scrotal confirmation might indicate pathological condition. He further remarked that short scrotum as an indication of testicular hypoplasia while

flat or slab sided scrotum indicated unilateral testicular hypoplasia or atrophy. Although such deviations of scrotum was not detected during the study. Stray cases of asymmetry and scrotum held close to abdomen (Fig.34) was also detected without any pathological significance.

5.2.2 Scrotal circumference

The mean scrotal circumference of the three crosses did not vary significantly and the overall mean was 29.86 ± 0.38 cm. According to Hahn (1969) and Gipson *et al.* (1985), the scrotal circumference in young bulls gave a relatively accurate estimation of ability to produce semen in quality and quantity. In Jersey bull Roberts (1986) and Hueston *et al.* (1988) recorded a mean scrotal circumference of 36.40 at 18 months of ages. Spitzer and Hopkins (1997) remarked that scrotal circumference at puberty should be close to 28 cm which is almost in accordance with the present finding Hopkins and Spitzer (1997) recommended minimum circumference for various age of bulls, as 30 cm between 12 to 15 months, 31 cm between 15 to 18 months, 32 cm between 18 to 21 months and 33 cm between 21 to 24 months of ages.

5.2.3 Libido

Out of the 67 growing bulls semen could be collected from only 52 bulls since the rest 15 bulls failed to mount (22.38 per cent). Frazer (1974) showed that lack of libido was one of the common problem of bulls leading to culling. Iyer (1984) reported that poor sex drive or libido of 33.63 and 4.40 per cent in Jersey

cross breds and pure breds respectively. Veerapandian (1992) found that three out of eleven bulls refused ejaculation and classified them with questionable libido. Bane (1954) believed that libido was genetic. Smith (1961) did not find any such influence. Although, Verma and Singh (1992) remarked that lack or weak libido might be due to low testosterone levels it may be presumed that the different percentage of lack of libido in different studies might also be due to adverse management and climatic factors existed in different studies.

5.2.4 Semen evaluation

The results of evaluation of semen is presented in Table 3 and Table 4.

5.2.1 Volume

The mean ejaculate volume of different crosses was 2.91 ± 0.16 ml. This is in accordance with Abdel Raouf (1965) who reported that young bulls when they reach puberty would produce 0.5 to 2.5 ml of semen. Rao *et al.* (1979) have also reported that the average ejaculate volume was 2.42 ± 0.71 ml of semen in crossbred bulls at this age. The ejaculate volume showed a gradual increase as age advanced from 18 to 24 months of age. Hultnas (1959), Abdel Raouf (1965), Morstin (1970) and Rao *et al.* (1979) reported that ejaculate volume increased with increase in age during the period of growth. A similar trend was also reported by Raja (1981) and Iyer (1984) in Brown Swiss cross bred and Jersey cross bred bulls respectively.

5.2.4.2 Initial motility

The mean initial motility of spermatozoa of CBJ, CBBS and CBHF was 47.36 ± 0.35 per cent, 51.85 ± 3.38 per cent and 52.29 ± 5.89 per cent respectively. The overall mean of the three crosses was poor (49.77 ± 2.12 per cent) in this age group which gradually increased and reached 59.84 ± 1.05 per cent at above 24 months of age. Similar trend was also reported by Rao *et al.* (1979), Raja (1981) and Iyer (1984).

5.2.4.3 Concentration of spermatozoa

The sperm concentration of CBJ, CBBS and CBHF was 1244.60 ± 86.43 millions per ml, 1366.11 ± 88.95 millions per ml and 1320.11 ± 135.84 million per ml respectively. The overall mean was 1299.73 ± 56.25 millions per ml at the growing age which reached 1480.78 ± 35.15 millions per ml in adult group. Similar trend was reported by Rao *et al.* (1979), Raja (1981) and Iyer (1984). In general the concentration of spermatozoa increased with increase in age which concurs with the earlier findings of Hultnas (1959), Abdel Raouf (1965) and Rao *et al.* (1979).

5.2.4.4 Live sperm

The overall mean live sperm count of the three different crosses was 58.76 ± 2.15 per cent. The live sperm percentage steadily increased to 69.08 ± 1.68 per cent in the age group above 24 months. The influence of age on the improvement of live sperm percentage is also in accordance with Raja (1981) and Iyer (1984) in

Brown Swiss and Jersey cross bred bulls respectively. Although statistically not significant, all the characters showed a steady increase with increase in age (Table 3 and Table 4). The low motility observed initially could be correlated with low concentration and live sperm count. The increase in live sperm is related to the improved functioning of testis during growth phase of the animal (Roberts, 1986).

5.2.4.5 Sperm morphology

The morphological characters of the three different crosses CBJ, CBBS and CBHF are presented in Table 4. The overall mean of head, middle piece and tail abnormalities were 2.57 ± 0.79 per cent, 1.94 ± 0.20 per cent and 12.97 ± 0.73 per cent respectively. the mean proximal and distal protoplasmic droplets were 4.88 ± 0.83 and 2.08 ± 0.19 respectively for the three crosses. The values showed a gradual decrease as age advanced and reached 2.67, 1.07, 11.8 per cent for head, midpiece and tail defects respectively and 1.70, 1.56 per cent for proximal and distal droplets respectively in bulls above 24 months of age. Although not statistically significant except in the case of proximal protoplasmic droplets, the reduction was gradual and convincing. This phenomenon in the reduction of abnormal sperms with increase in age was also reported earlier (Hultnas, 1959; Abdel Raouf, 1965; Rao and Rao, 1979; Raja, 1981 and Iyer 1984). Hultnas (1959) and Abdel Raouf (1965) opined that the improvement in the physical characteristic of semen with corresponding decrease in abnormal sperm is related to normal growth of reproductive organs. Rao *et al.* (1979), Raja (1981) and Iyer (1984) observed this phenomenon in cross bred bulls. They further remarked that

semen with good quality can be obtained when cross bred bull attain 23 to 24 months of age. The present observation of gradual improvement in quality of semen with increase in age and satisfactory semen quality at 24 months of age in three different crosses of Jersey, Brown Swiss and Holstein Friesian supports the above view.

Correlation of scrotal circumference with physical and morphological characters revealed highly significant correlation with volume, initial motility, live sperms and freezability. The length and breadth of testis was significantly correlated ($P<0.01$) with volume, motility and live sperm count. Blocky (1979) and Rao and Rao (1990) reported a positive correlation with age and circumference. A significant correlation between scrotal circumference and total number of sperm per ejaculate was observed by Kuferschmied *et al.* (1985). On the other hand, Thomson *et al.* (1992) found no relationship between the quality of semen and scrotal circumference. However, Hopkins and Spitzer (1997) observed that the circumference was positively related to sperm out put. However, reports on the interrelation between scrotal circumference and semen characteristics of growing bulls are scanty.

5.2.5 Infertility conditions

Three cases of unilateral hypoplasia eleven cases of testicular degeneration and a case of epididymal dysfunction were observed (Table 6). Perusal of literature did not give detailed investigation or reports on the incidence of various infertility conditions of growing bull.

Taking into consideration the object of the present investigation the incidence of gonadal hypoplasia currently observed warrant detailed investigation on a larger scale.

Out of 67 crossbred growing bulls culled for gonadal hypoplasia three had right unilateral hypoplasia and the remaining six had bilateral partial hypoplasia. Kodagali and Kerur (1968) reported 4.50 per cent incidence of unilateral hypoplasia in Gir bull calves. Kodagali *et al.* (1971) reported nine cases of hypoplasia in Gir bulls of which six were left sided, two were right sided and one bilateral. Iyer (1984) reported that out of 23 crossbred bulls culled 8 had gonadal hypoplasia (14.54 per cent).

5.2.5.3 Testicular degeneration

Degeneration of testis was observed in 11 bulls (16.41 per cent). Poor quality semen along with high incidence of morphological abnormalities led to the evidence of testicular degeneration. Histological studies as described by Jubb and Kennedy (1993) also revealed evidence of degenerative changes. Isolated instances (Venkataswamy and Pattabiraman, 1970; Rao and Rao, 1979) have been reported in various breeds of bulls. A variety of causes could be attributed to the high incidence of testicular degeneration. However, adverse effect of hot and humid environment might have predisposed to the rate of testicular degeneration in young bulls.

5.2.5.4 Epididymal dysfunction

One bull was identified to have epididymal dysfunction (1.49 per cent). The condition was characterised by presence of large number of abnormal sperm tails as reported by Gustafsson (1965), Blom (1968) and Gustafsson *et al.* (1972). In the present study the tail abnormalities were simple, bent at right angle and coiled tails. Raja *et al.* (1974) and Rao and Subbhaiah (1982) have also reported similar instances in Jersey bulls. Iyer (1984) and Veerapandian (1992) also detected similar instances in Jersey and Jersey crossbred bulls. The present observation therefore supports their views on the incidence of this condition in Jersey and their crosses. Though not established the involvement of a hereditary nature of this defect in pure and crossbred Jersey cannot be ruled out as reported by Blom (1968).

5.2.6 Rate of culling

The rate of culling of growing bulls observed in the present study agrees with the earlier report of Mathew *et al.* (1982). It was also found that the percentage of culling due to poor semen quality was higher in crossbred bulls which also agrees with the earlier report (Mathew *et al.*, 1982). Nagasundaram (1986) reported a high percentage of culling and found that poor initial motility and spermatozoan abnormalities were major reasons for culling.

5.3. Group III - Adult bull

5.3.1 Palpation and measurement of testes and epididymis

Different types of scrotum observed was oval (47.50 per cent) elongated (20.00 per cent), round (7.50 per cent), rectangular (5.00 per cent), pear shaped (7.50 per cent), square (5.00 per cent), oblong (5.00 per cent) and bifid (2.50) per cent.

Although different shape of scrotum (oblong, elongated, oval, round and bifid) have been described by Rao and Rao (1988) oval was found to be predominant by Veerapandian (1992). Krishna and Rao (1987) however observed oblong type as predominant. Although no disturbed spermatogenesis due to shape of scrotum have been described, Iyer (1984) observed disturbed spermatogenesis due to torsion with wrinkles of scrotum in one bull, backward tilting of scrotum in another bull and bifid scrotum with wrinkles in yet another bull.

5.3.2 Scrotal circumference

The scrotal circumference of different crosses did not show significant variation. The overall mean was 35.29 ± 0.75 cm. Coulter *et al.* (1975) recorded similar scrotal circumference in Jersey, Holstein and Brown Swiss crosses. Raju (1981) observed a mean scrotal circumference of 37.33 cm in Jersey bulls. Veerapandian (1992) observed that scrotal circumference was lower in Jersey with unsatisfactory semen.

5.3.3 Libido

Semen could be collected from all adult bulls under study. This could be attributed to the fact that bulls having poor libido were eliminated or culled during the process of selection to adult stock.

5.3.4 Semen evaluation

5.3.4.1 Volume

The mean ejaculate volume of the three crosses was 4.87 ± 0.20 and ranged from 1.92 to 8.75 ml. This is in accordance with the findings of Mathew (1974) for similar crosses. the mean ejaculate volume was reported to be 3.65 ml, 5.16 ml and 4.83 ml in Jersey (Rao and Kotaya, 1977), Brown Swiss (Saxena and Tripati, 1978) and Holstein, Friesian crosses (Raju and Rao, 1982). Raja (1981) found that the volume of semen per ejaculate in Brown Swiss cross varied from 0.5 to 6.0 ml. Iyer (1984) reported ejaculated volume 3.64 to 4.42 ml with a mean of 3.99 ml in Jersey crossbred bulls. Raju and Rao (1982) reported higher ejaculate volume in bull of different groups. The variation in the ejaculated volume observed currently from earlier reports could be attributed to the genetic constitution of the animals as contemplated by Raju and Rao (1982) and Roberts (1986).

5.3.4.2 Initial motility

The initial motility of CBJ, CBBS and CBHF was 69.10 ± 0.46, 58.65 ± 2.41 and 63.65 ± 0.70 per cent respectively with an overall mean of 59.84 ± 1.05

per cent. The mean observed is comparable to the reports in crossbred bulls by Rao and Rao (1975), Raju and Rao (1982) and Iyer (1984) and slightly lower than that observed by Almquist *et al.* (1963), Bhosrekar *et al.* (1982), Sagdeo *et al.* (1990); Singh and Pangawkar (1990) and Reddy *et al.* (1991). The initial motility was reported to range from 30.24 to 79.24 per cent in crossbreds (Sagdeo *et al.*, 1990 and Singh and Pangawkar, 1990). However, a lower motility rating than the present observation was reported by Lasley (1951).

5.3.4.3 Sperm concentration

The sperm concentration was found to be 1480.78 ± 3515 millions per ml within a range of 971 to 2015 millions per ml. The present value appear to be lower than that reported by Iyer (1984) in crossbred Jersey bulls. In Brown Swiss crosses, Rao and Rao (1975) reported lower values. However, Bhosrekar *et al.* (1982) reported similar concentration in cross bred bulls. Mathew (1974) recorded a concentration of 1320 million, 1502 million and 1408 million sperms per ml respectively in bulls with 75, 62.5 and 50 per cent of exotic inheritance.

5.3.4.4 Live sperm

The mean live sperm count of three crosses was 79.94 ± 1.149, 66.02 ± 2.90 and 65.77 ± 6.20 respectively with an overall mean of 69.08 ± 1.68 per cent. The percentage of live sperms in crossbred bulls are reported to be varied, the values being 86.34 per cent for Haryana x Holstein Friesian, 87.34 for Haryana x Brown Swiss and 87.28 for Haryana x Jersey (Biswas *et al.*, 1976). Rao and Rao

(1978) found live sperm percentage as 85.02 per cent in Hosltein Friesian crosses. In Brown Swiss crosses, Raja (1981) recorded 72.55 per cent live sperms which ranged from 45 to 90 per cent.

5.3.4.5 Sperm morphology

The overall mean of head, middle piece and tail abnormalities was 2.67 ± 0.26 per cent, 1.07 ± 0.05 per cent and 11.18 ± 0.71 per cent respectively. The mean proximal and distal protoplasmic droplet was 1.70 ± 0.28 and 1.56 ± 0.21 per cent respectively. The mean obtained is in near agreement with that of Raja (1981) in Brown Swiss crossbreeds and Iyer (1984) in Jersey crossbreds. The mid-piece abnormalities observed in the present studies are comparable to Rao (1976), Rao and Rao (1978), Raja (1981) and Iyer (1984). The tail abnormalities was comparable to the values reported by Rao and Rao (1980) but higher than that of Rao and Rao (1978), Raja (1981) and Iyer (1984). The protoplasmic droplets is similar to the values reported by Raja (1981), but lower to that reported by Rao and Rao (1980) and higher than the values reported by Iyer (1984).

5.3.4.6 Freezability

The pre-freezing, post freezing and total rejection of ejaculates were 12.00 per cent, 11.20 per cent and 23.20 per cent respectively. The prefreezing, post freezing and rejection rates of ejaculates was reported to be 23.4 and 4.2 respectively (Milk Marketing Board, Scotland 1973-74). Mathew (1974) reported a higher rate of pre-freezing rejection in Brown Swiss crossbred bulls and opined that pre-freezing and post freezing discard rate decreased with increase in exotic

inheritance. In Jersey bulls, the pre freezing and post freezing rejection rates were reported to be 11.9 and 5.36 per cent as against 13.5 and 5.19 per cent in Holstein Friesian bull (Sattar *et al.*, 1978). In Brown Swiss crosses, Raja (1981) reported pre freezing and post freezing rejection rate as 28.55 and 15.74 per cent. In Jersey crosses, Iyer (1984) found the corresponding values as 34.31 and 8.61 per cent. The percentage of total rejection in Jersey crosses was reported to be 38.98 per cent (Iyer, 1984). There are conflicting reports regarding the various factors influencing rejection rates during freezing. Mathew (1974) attributed this variation in exotic inheritance. Sagdeo *et al.* (1990) also concurred with this. However Singh *et al.* (1993) opined that spermatozoa concentration influenced the freezability of semen.

5.3.5 Infertility

The various infertility conditions in adult bulls were presented in Table 11. Among 11 bulls culled, 2 bulls were discarded due to testicular hypoplasia, incidence being 18.18 per cent. Perusal of literature does not reveal evidence of systematic investigation on gonadal hypoplasia in crossbred bulls in India. Most of the reports are isolated instances in different breeds Kodagali (1964), Rao *et al.* (1966), Deshpande *et al.* (1976). However, Iyer (1984) on a similar investigation reported 14.54 per cent as testicular hypoplasia. Taking into consideration the object of the present study to identify various forms of infertility in crossbred bulls, the incidence of testicular hypoplasia currently observed need further detailed screening with larger number of herds.

5.3.5.1 Bilateral partial hypoplasia

The incidence of bilateral,partial hypoplasia was higher than that reported by Iyer (1984). The difference in size of the testicles and the histologic changes in testis led support to the above diagnosis. These bulls continued to produce semen of poor quality. The morphological characters of spermatozoa also suggest disturbance in spermatogenic activity. Histological studies of the affected testis point to a lack or disturbance in spermatogenic activity in certain tubules amidst tubules with normal activity. These tubules were apparently small with hyperchromatosis of the nuclei of epithelial cells lining the seminiferous tubules with mild degree of vacuolation of cytoplasm.

5.3.5.2 Degeneration of testis

The degeneration of testis was detected in 3 bulls (15.00 per cent). The incidence is higher than that reported by Iyer (1984) in crossbred Jersey bulls (8.88 per cent). Lagerlof (1936) reported testicular degeneration account to 50 to 60 per cent of all testicular disorders. Isolated instances have been reported by Venkataswamy and Pattabiraman (1970) and Rao and Rao (1979). Poor quality semen with high evidence of morphological abnormalities led to the evidence of testicular degeneration. Histological studies showed varying degrees of degenerative changes. The higher incidence of testicular degeneration would be attributed to the hot and humid environment with advancement of age in the bulls currently studied.

5.3.5.3 Seminal enzymes

The enzyme profile of seminal plasma of the adult bulls with varying degrees of exotic inheritance with above 50 per cent freezability and below 50 per cent freezability of ACP, AKP, GOT and LDH was presented in Table 12. Significant variation was found in the seminal enzymes between the freezability groups revealing that bulls with higher freezability were with lower enzyme profile. Similar observation was reported by Belorkar (1986). Semen of bulls with good freezability had higher values of seminal enzymes. Pangawakar *et al.* (1988) also reported the relationship of the freezability and enzyme profile of Holstein Friesian bull and found a significant low level of acid phosphatase and alkaline activity and higher level of GOT and GPT with low freezability. However Pangawkar *et al.* (1988) suggested that freezability of semen was affected with increased level of phosphatase activity. The higher percentage of seminal enzymes in sample with less than 50 per cent freezability may be attributed to the leakage of seminal enzymes.

5.3.6 Rate of culling

Out of 280 adult bulls observed 87 were culled due to various reasons. Among these 72.41 per cent were culled due to various reproductive disorders. Poor semen quality (49.20 per cent) was the main reason for culling of adult bulls. The present finding agrees with the earlier reports of Mathew *et al.* (1982) and Nagasundaram (1986).

5.3.7 Scanning Electron Microscopy (SEM)

Testicular tissue from cryptorchid, hypoplasia and degenerative conditions examined revealed mild to moderate surface morphological alterations lesser scanning electron microscopy.

Only a few segments of the seminiferous epithelium were observed in the cryptorchid testes. The outer connective tissue layer of the walls of the seminiferous tubules appeared highly thickened and pitted (Fig.45). Seminiferous epithelium was either absent or without germinal cells differentiation in most of the areas. The seminiferous epithelium contained only sparsely populated cells which were undifferentiated in certain regions. No spermatozoa could be detected within the tubules. The epithelial cells at times were found to be detached from the basement membrane.

Epididymis showed absene of cytoplasmic processes or stereocilia at their free margins. Spermatozoa was absent.

Tightly packed mass of coiled tubules of very small diameter surrounded by connective tissue was seen in the hypoplastic testes. The seminiferous epithelium lining the wall showed sparsely populated cells of varying shape and size (Fig.46).

A few spermatogenic cells could be seen and coiled within the lumen. Scanty coiled spermatic tails could be seen in the degenerated testicular tissue

(Fig.47). In some of the degenerated testes, few spermatid tails were seen attaching to the epididymal duct. Compactness of the epithelium was not present.

The scanning electron microscopic study of the semen sample confirmed the morphological changes observed under light microscopy (Fig.48 to 51).

Summary

6. SUMMARY

With the object of investigation of various infertility conditions among crossbred bulls, records maintained at Dhoni Farm for the past five years were screened and 153crossbred males of different crosses of Jersey (CBJ), Brown Swiss (CBBS) and Holstein Friesian (CBHF) were investigated by dividing them into three age groups viz. birth to 12 months (Group I), 13 months to 24 months (Group II) and 24 months and above (Group III).

6.1 Group I (Bull calves)

6.1.1 Palpation and measurement of testis and epididymis

The measurement of epididymis and testis revealed (Table 1) significant variation between different crosses.

6.1.2 Scrotal circumference

The mean scrotal circumference was 19.42 ± 1.03 cm which did show significant variation between different crosses.

6.1.3 Infertility

One case of bilateral cryptorchidism was detected. The scrotum could not be traced on palpation. The scrotal pouch measured only 10 cm which appeared shrunken with wrinkled skin.

6.1.4 Histopathology

6.1.4.1 Testis

Testicular tissue showed extensive fibrocollagenous proliferation and presence of occasional tubules. Necrosis of tubular lining with cellular debris within the lumen and separation from the basement membrane was detected.

6.1.4.2 Epididymis

Detached tubules and peritubular fibrosis were observed.

6.1.5 Rate of culling

Out of 222 bull calves 53 were culled due to various reasons. 11.32 per cent of bull calves were culled due to various reproductive disorders.

6.2 Group II - Growing bulls

6.2.1 Palpation and measurement of testis and epididymis

The various shape of scroti were oval (52.24 per cent), elongated (19.40 per cent), rounded (7.46 per cent), rectangular (4.48 per cent), pear-shaped (5.97 per cent), square (2.99 per cent), oblong (4.48 per cent) and bifid (2.99 per cent).

6.2.2 Scrotal circumference

The scrotal circumference did not show any variation between breeds. the mean value was 29.86 ± 0.38 cm.

6.2.3 Libido

Out of 67 bulls, 15 showed lack of libido and histopathological studies of the testis showed scanty interstitial cells with thinning and widening of intertubular septum.

6.2.4 Semen evaluation

6.2.4.1 Volume

The mean ejaculate volume was 2.91 ± 0.16 ml and ranged from 1.12 to 5.92 ml.

6.2.4.2 Initial motility

The overall mean was 49.77 ± 2.12 per cent and ranged from 13.30 to 66.83 per cent.

6.2.4.3 Concentration

6.2.4.3.1 The mean concentration was 1299.73 ± 56.25 millions per ml and ranged from 563.00 to 2075.00 million per ml.

6.2.4.4 Live sperm

The mean live sperm count was 58.76 ± 2.15 per cent and ranged from 12.24 to 80.51 per cent.

6.2.4.5 Sperm morphology

The overall mean of head, middle piece and tail abnormalities were 6.57 ± 0.79 per cent, 1.94 ± 0.20 per cent and 12.97 ± 0.73 per cent and the mean proximal and distal protoplasmic droplets were 4.88 ± 0.83 and 2.08 ± 0.19 respectively.

6.2.4.6 Freezability

The prefreezing, postfreezing and total rejection of 291 ejaculates frozen was 39.50 per cent, 12.70 per cent and 52.27 per cent respectively. Highly significant correlation with volume, initial motility and freezability was observed.

6.2.5 Infertility

Three crossbred bulls (4.48 per cent) had unilateral hypoplasia and six (8.96 per cent) bilateral hypoplasia. The mean ejaculate volume of these bulls varied from 1.0 to 2.5 ml and sperm concentration varied from 420 to 1200 million. There was high incidence of head abnormalities and presence of protoplasmic droplets. Eleven bulls (16.41 per cent) had testicular degeneration. Semen of these bulls appeared watery with high percentage of detached heads and head abnormalities. Various degrees of degenerative changes of tubules were examined on histopathological examination. One bull (1.49 per cent) had epididymal dysfunction.

6.2.6 Rate of culling

The number of bulls culled was 122 (49.12 per cent). 118 bulls (96.72 per cent) were culled due to reproductive disorders.

6.3 Group III - Adult bulls

6.3.1 Palpation and measurement of testis and epididymis

Out of 40 bulls, the shape of scrotum was observed as oval 47.50 per cent, elongated 20.00 per cent, rounded 7.50 per cent, rectangular 5.00 per cent, pear shaped 7.50 per cent, square 5.00 per cent, oblong 5.00 per cent and bifid 2.50 per cent.

6.3.2 Scrotal circumference

The mean scrotal circumference was 35.29 ± 0.74 cm.

6.3.3 Libido - all bulls showed good libido.

6.3.4 Semen evaluation

6.3.4.1 Volume

The overall mean ejaculate volume was 4.87 ± 0.20 and ranged from 1.92 to 8.75 ml.

6.3.4.2 Initial motility

The mean initial motility was 59.84 ± 1.05 per cent and ranged from 40.00 to 70.00 per cent.

6.3.4.3 Concentration of spermatozoa

The overall mean for the three breeds was 1480.78 ± 35.15 million per ml and ranged from 971 to 2015 millions per ml.

6.3.4.4 Live sperms

Mean live sperm count was 69.08 ± 1.68 per cent and ranged from 23.20 to 81.89 per cent.

6.3.4.5 Sperm morphology

The overall mean of head, mid-piece and tail abnormalities was 2.67 ± 0.26 per cent, 1.07 ± 0.05 per cent and 11.18 ± 0.71 per cent respectively. The mean proximal and distal protoplasmic droplets were 1.70 ± 0.28 per cent and 1.56 ± 0.21 per cent respectively.

6.3.4.6 Freezability

The prefreezing, post freezing and total rejection of 241 ejaculates were 12.00 per cent, 11.20 per cent and 23.20 per cent respectively.

6.3.5.1 Infertility

Two crossbred bulls had bilateral partial hypoplasia. The mean ejaculate volume of these bulls was 2 to 4 ml. Sperm concentration varied from 620 to 1251 million per ml. There was high incidence of head abnormalities with proximal protoplasmic droplets. Histologic studies showed small tubules with mild degree of vacuolation of cytoplasm. Six bulls showed testicular degeneration, the mean ejaculate volume of these bulls was 4. to 5 ml and sperm concentration 680 to 1280 millions per ml. High degree of head and tail abnormalities were detected. Severe degenerative changes of seminiferous epithelium was detected as indicated by presence of single layer of epithelium with thickening of the basement membrane.

6.3.5.2 Scanning Electron Microscopy (SEM)

Samples of tissues from testis affected with cryptorchudism, hypoplasia and degeneration were subjected to SEM.

6.3.5.3 Seminal enzymes

The enzyme profile of ACP, AKP, GOT, GPT and LDH of bulls revealed that those which had above 50% freezability had lower levels of the above enzymes than those which showed freezability less than 50 per cent.

6.3.6 Rate of culling

Out of the adult bulls observed 87 adult bulls were (31.10 per cent) were culled due to various problems. Among this bulls, 63 (72.40 per cent) were culled due to various reproductive disorders.

It could thus be summarized that selection of crossbred bulls for breeding should be done from birth itself. Measurement of scrotal circumference should be done systematically and growth of testis and epididymis should be watched to pubertal stage. Incidence of hereditary conditions like crytorchidism should eb viewed seriously before selection of bulls. Although shape of scrotum has no influence on the reproductive performance of bull, oval shape should be considered as normal and acceptable. Marginal variation in scrotal circumference need not be considered abnormal because of its variation due to level of exotic inheritance. Lack of libido of growing bulls should not be neglected because of its inheritance. High incidence of testicular hypoplasia and testicular degeneration needs further investigation. Usefulness of enzyme profile in seminal plasma in adult bulls for selection of semen samples for freezing needs due consideration.

References

REFERENCES

Abdel-Raouf, M. (1960). The post natal development of the reproductive organs in bulls with special reference to puberty. *Acta Endocrinol.* **34**: 49.

Abdel - Raouf, M. (1965). Sexual behavior and semen picture of bulls of the Swedish Red and White breed between the ages of 9 and 15 months *Nord. Vet. Med.* **17**: 318.

Akusu, M.O. and Akpododje, J.V. (1983). Unilateral testicular hypoplasia in a white Fulami bull: A case report *Br. Vet. J.*, **139**: 204-207.

Almquist, J.O., Amann, R.P. and Hale, E.B. (1963). Sperm output and sexual behaviour of Holstein bulls from 2 to 3 years of age when collected from puberty at high frequency continuously or when given prolonged sexual rest. *J. Dairy Sci.* **46**: 1176.

Almquist, J.O. and Cunningham, D.C. (1967). Reproductive capacity of beef bull I. Post pubertal changes in semen productions of different ejaculation frequencies. *J. Anim. Sci.* **26**: 174.

Almquist J.O., Branas, R.J. and K.A. Barber (1975). Post pubertal changes in semen production of charolais bulls ejaculated at high frequency and the relation between testicular measurements and sperm output. *J. Anim. Sci.* **42**: 670-676.

Anderson, J. (1941). Further investigations on the semen of bull. *Vet. Rec.* **53**: 197.

Anderson, J. (1945). The semen of animal and its use for artificial insemination. Imperial Bureau of Animal Breeding and Genetics (Edinburgh) No.6, pp. 151.

Anderson, J. (1948). Cited Maule (1962).

Anderson, M., Vierula, M. and Alanko, M. (1990). Three types of acrosomal aberrations of bull spermatozoa and their relation to fertility. *Acta. Vet. Scand* Vol. **31**(2): 175-179.

Annual Report of Kerala Livestock Development and Milk Marketing Board Ltd. 1985-86.

Ansari, M.M. Thomas, B.P., Shukla, R.K. and Memon, G.N. (1972). Association of testicular measurements with semen production in surti buffalo bulls. *Indian J. Anim. Sci.* **42**: 465-667.

Arriola J., Johnson L.A., Kaproth M. and Foote R.H. (1985). A specific oligoteratozoospermia in a bull. The sperm tail stump defect. *Theriogenology* **6**: 899.

Arthur, G.H., Noakes, D.E., Pearson, H. and Parkinson, T.J. (1996). Veterinary Reproduction and Obstetrics. 7th ELBS Edn. Bailliere tindall Ltd. London.

Aughey, E. and Renton, J.P. (1968). Abnormal spermatozoa in an Ayrshire bull, *Vet. Rec.* **82**: 129-131.

Ball, L., Young, S. and Carroll, E.J. (1968). Seminal vesiculitis syndrome: Lesions in genital organs of young bull. *Am. J. Vet. Res.* **29**: 1173-1184.

Bane, A. (1954). Sexual functions of bulls in relation to heredity, rearing intensity and somatic conditions. *Acta. Agric. Scand.***4**: 97.

Bane, A. and Nicander, N.L. (1965). Pouch formation by invagination of the nuclear envelope of bovine and porcine sperm as a sign of disturbed spermiogenesis. *Nord. Vet. Ned.* **36**: 328.

Barth, A.D. (1986). The knobbed acrosome defect in beef bulls. *Canadian Vet.* **27**(10): 379-384.

Barth. A.D. (1989). Abaxial tail attachment of bovine spermatozoa and its effect on fertility Canadian *Vet. Journal.* Vol. 30 **8**: 656-662.

Barth, A.D., Bowman, P.A., Mapletoft, R.J. (1992). Effect of narrow sperm shape on fertility in cattle. *Canadian vet. J.*. **1**: 31-39.

Belorkar, P.M. (1986). Studies on seminal characters freezability and fertility of crossbred (K x HF, K x J) bulls. Ph.D. thesis abst. *Indian J. Anim. Reprod.* **7**: 98-99.

Bhattacharya, P. and Prabhu, S.S. (1954). Field application of artificial insemination in cattle II. *Indian J. Vet. Sci.* **24**: 67.

Bhosrekar, M.R., Char, S.N., Waghmare, B.S., Thakur, Y.A., Badre, V.C. and Rangnekar, D.V. (1982). Note on the adpation mechanism of exotic bulls to water stress under seminarid conditions of tropical India. *Indian J. Dairy Sci.* **52**: 579-583.

Bishop, M.W.H., Campbell, R.C., Hancock, J.L. and Walton, A. (1954). Semen characteristics and fertility in the bull. *J. Agric. Sci.* **44**: 227.

Biswas, J.C., Raina, B.L., Kumar, R. and Bhat, P.N. (1976). Studies on live and abnormal spermatozoa in crossbred bulls. *Indian J. Anim. Health.* **15**: 129.

Blockey, MA. (1979). Observation on group mating of bulls at pasture. *Applied Animal Ethology* **5**: 15-34.

Blom, E. (1948). On the methods of evaluating bull semen. Medlemsble, Daurko, Dyrlaese frozen 31, 446. (A.B.A. 16: 1380).

Blom, E. (1950). On the evaluation of bull semen with special reference to its employment for artificial insemination. Ph.D. thesis, Copenhagen.

Blom, E. (1968). A new sperm defect pseudodroplet in the middle piece of the bull sperm. *Nord. Vet. Med.* **20**: 279.

Blom, E. and Christenson, N.O. (1947). Studies on pathological conditions in the testis, epididymis and accessory sex glands in the bull. *Scand. Vet.* **37**: 1-4.

Blom, E. and Christensen, N.O. (1972). A systematic research for abnormalities in testis, epididymis of pedigree bulls in Denmark Studies on pathological conditions in the genitals of the bull. VII royal Vet. and Agri. Uni. Yearbook. p. 1-36.

Bratton, R.W., Fotte, R.H., Henderson, C.R., Musgrave, S.D., Durber (Jr.), R.S., Dunn, H.O. and Beardsley, J.P. (1956). Relative influence of combination of laboratory tests for predicting the fertility of bovine semen. *J. Dairy Sci.* **39**: 1542.

Breeuwsma, A.J. (1973). The interrelationship between seminal glutamic oxaloacetic transaminase and fertility in bulls. VIIth Int. Cong. on Anim. Reprod. and AI Munich. *Theriogenology* **29**(6): 1393-1397.

Brown, M.A. (1959). Relationship of season to fertility of dairy bulls in Texas. *South West Vet.* **13**: 49.

Bujarbaruah, K.M., Rajkonwar, C.K., Bergolain, B.N. and Kakati, B.N. (1982). Note on semen characteristics in young Jersey and Red Dane bulls. *Indian J. Dairy Sci.* **52**: 589-593.

Campbell, R.C., Hancoch, J.L. and Shaw, I.G. (1960). Cytological characteristics and fertilizing capacity of bull spermatozoa. *J. Agri. Sci.* **55**: 91.

Carrol, E.J., Ball, L. and Sott, J.A. (1963). Breeding soundness in bulls - a summary of 10940 examinations. *J. Amer. Vet. Med. Ass.* **142**: 1105-1111.

Chenoweth, P.J., Farin, P.W., Mateos, E.R., Rupp, G.P. and Rexton, J.E. (1988). Relationship between breeding soundness and sex drive classification in beef bulls. *Theriogenology* **30**: 227-233.

Cohrs, P. (1966). Textbook of the special pathological anatomy of domestic animals. Ed. Nieberle Cohrs. Pergamon Press, Oxford, 1st English Edition. pp. 793-822.

Coubrough, R.I. and Barker, C.A.U. (1966). Spermatozoa : an unusual middle piece abnormality associated with sterility in bulls. *Vet. Bull.* **36**: 1629.

Coulter, G.H., Larson, L.L. and Foote, R.H. (1975). Effect of age on testicular growth and consistency of Holstein and Angus bulls. *J. Anim. Sci.* **41**: 1383-1389.

Coulter, G.H. and Foote, R.H. (1976). Relationship of testicular weight to age and scrotal circumference of Holstein bulls. *J. Dairy Sci.* **59**: 730-732.

Crabo, B., Gustefsson, B., Nicander, L. and Rao, A.R. (1971). Subnormal testicular function in a bull concealed by phagocytosis of abnormal spermatozoa in the efferent duct. *J. Reprod. Fert.* **26**: 393-396.

Cupps, P.T. and Briggs, T.R. (1965). Changes in the epididymics associated with morphological changes in the spermotozoa. *J. Dairy Sci.* **48**: 1241-1244.

Cupps, P.T. Laben, R.C. and Mead, S.W. (1954). The relation of semen quality tests to breeding efficiency and characteristics of semen from low fertility bulls before and after hormone infection. *J. Dairy Sci.* **36**: 442.

Das, S.K. and Tomer, O.S. (1995). Studies on testicular biometry and semen quality in cattle and buffalo. *Indian J. Dairy Sci.* **48**: 78-81.

David Steffen (1997). Genetic causes of bull infertility. *Vet. Clin. Nor. Am. Food Anim. Pract.* **13**: 243-353.

Davis, H.P., Trimberger, G.W. and Underbjerg, G.K.L. (1940). Fecundity and certain other characteristics of fresh and stored bovine semen. *J. Dairy Sci.* **23**: 532.

Deshpande, B.R., Hekeri, V.B. and Velhankar, D.P. (1976). Study of seminal attributes in a Holstein Friesian bull associated with partial testicular hypoplasia. *Indian Vet. J.* **53**: 408-413.

Dhami, A.J., Sahni, K.L., Greeshmohan and Tripathi, R.P. (1994). Comparative evaluation of initially static and motile semen ejaculates from Friesian and Murrah buffalo bulls for physio-morphological, biochemical enzymatic and mineral constituents of seminal plasma. *Indian J. Anim. Sci.* **64**: 926-932.

Dimitriev, N.G. (1964),. Semen production. In artificial insemination and reproduction in cattle and buffaloes, Tomar, N.S. (Ed.) Saroj Prakash 646, Katra, Allahabad, (1970) p. 70.

Donald, H.P. and Hancock, J.L. (1953). Evidence of a gene controlled sterility in bulls. *J. Agric. Sci.* **43**: 178.

Dunn, H.O., Buckner, P.J., Young, G.F., Conard, E.D., willet, E.L. and Larson, G.L. (1954). A comparative study of fertility of bovine spermatozoa stored at 5°C and -79°C. *J. Dairy Sci.* **37**: 1429.

Eriksson, K. (1943). Hereditory forms of sterility in cattle. *Lund.* p. 155.

Fawcett, D.W. and Philips, D.M. (1970). Recent observations on the ultra structure and development of the mammalian spermatozoan comparative spermatology. p. 13-27. B. Baceebti (Ed), Academic Press.

Frazer, A.F. (1974). Sexual behaviour, In Farm Animal Behaviour Balliere, Tindall, London. p. 101-110.

Galina, G.S. and Arthur, G.H. (1991). Review of Reproduction in the Tropics. Part 6. *The Male Anim. Breeding Astracts.* **59**. No.5: 412.

Gipson, T.A., Vogt D.W., Massey J.W. and Ellersieck M.R. (1985). Associations of scrotal circumference with semen traits in young beef bulls. *Theriogenology* **24**: 217-225.

Gopakumar, C.P. (1994). Chromasome profile of crossbred bulls in Kerala. M.V.Sc. thesis, Kerala Agricultural University, Mannuthy.

Gustafsson, B. (1965). A case of Akinesia of bull sperm associated with a functional disturbances in the epididymis. *Nord. Vet. Med.* **17**: 65-72.

Gustafasson, B., Crabo, B. and Rao, A.R. (1972). Two cases of bovine epididymal dysfunction. *Cornell Vet.* **62**: 392-402.

Hafez, E.S.E. (1962). Reproduction in farm animals. I Edn. Lea and Febiger, Philadelphia. pp. 152.

Hafez, E.S.E. (1993). Reproduction in farm animals. 6th edn. 6th Edn. Lea and Febiger, Philadelphia. pp. 165-170.

Hahn, J., Foote, R.H. and Seidel(Jr.) G.E. (1969). Testicular growth and related sperm output in dairy bulls. *J. Anim. Sci.* **29**: 41-47.

Hancock, J.L. (1959). The morphologic characteristics of spermatozoa and fertility. *Int. J. Fert.* **4**: 345-359.

Hancock, J.L. and Rollinsen, D.H.L. (1949). A seminal defect associated with sterility in Guernsy bulls. *Vet. Rec.* **61**: 742-743.

Haranath, G.B. Rao, A.V.N. and Duff, K.L. (1983). Knobbed defect of sperm head of possible viral origin in a Jersey bull. *Indian. J. Anim. Reprod.* **3**: 102.

Herman, H.A. and Swanson, E.W. (1941). Variation in dairy bull semen with respect to its use in artificial insemination. *Res. Bull. No. Agri. Exp. St.* No. 326: 82.

Hopkins, M.F. and Spitzer, C.J. (1997). The new society for theriogenology. Breeding soundness evaluation system. *Vet. Clin. North. Am. Food. Anim. Pract.* **13**: 283-293.

Hueston, W.D, Monke, D.R. and Milburn, R.J. (1988). Scortal circumference, measurements on young Holstein bulls. *J. Am. Vet. Med. Asso.* **192**: 766-768.

Hultnas, C.A. (1959). Studies on variation in mating behaviour and semen picture in young bulls of the Swedish Red and White breed and on causes of this variation. *Acta Agri. Scand. Suppl.* **61**: 1-82.

Humason, G.L. (1967). Animal Tissue Techniques. Edn. W.H. Freeman and company, San Francisco and London.

Iyer, C.P.N. (1984). Studies on the semen characteristics, freezability and fertility of pure bred and crossbred jersey bulls. Ph.D. thesis, Bombay Vet,. College Konkan Krishi Vidyapeeth, Dapoli.

Johari, M.P. (1957). Semino vesiculitis in a bull at an AI Centre. *Indian J. Vet. Sci.Anim. Husb.* **27**: 17.

Jubb, KV.F., Kennedy, P.C. and Palmer, N. (1993). Pathology of domestic animals. 4th edn. Vol. **3** pp. 486.

Kaikini, A.S., Bhandari, R.M., Gafoor, M.A., Pandit, A.V. and Khonde, P.A. (1968). Seminal vesiculitis in Gadao bull,. *Nagpur Vet. College Mag.* **4**: 124-127.

Kaikini, A.S., Dindorkar, S.V. and Pargaonkar, D.R. (1978). Impotentia generandi due to gonadal hypoplasia in exotic bull. Paper presented at FAO/SIDA follow up seminar on Animal Reproduction, Tirupathi.

Kaker, M.L. and Arora, K.L. (1973). Studies on biochemical components of semen of young Holstein Friesian x Hariana and brown Swiss x Hariana corssbred bulls as affected by post pubertal age. *Indian J. Anim. Sci.* **43**: 193-194.

Kavalieri, J. and Van Camp, S.D. (1997). Bovine seminal vesiculitis – a Review and update. *Vet. Clin. Nor. Am. Food. Anim. Pract.* **13**: 233.

Kerur, V.K. and Kodagali, S.B. (1970). Degeneration vesiculitis, and epidiymo orchitis in a Gir bull - A clinico path bacteriological report. *Guj. Vet. J.* **4**: 33-36.

Khan, S.M. and Kharche, K.G. (1983). Semen characteristics and fertility of Jersey bulls in tropcis. *Indian J. Anim. Reprod.* **3**: 88.

King, E.J. and Armstrong, A.R. (1934). In Clinical Biochemistry. Varley, H.C. (1963). 3rd Edn. Interscience Books. INC, New York.

Knudsen, O. (1958). Studies on spermato cytogenesis in the bull. *J. Fet.* **3**: 389-403.

Kodagali, S.B. (1964). Testicular hypoplasia in Gir bulls. *Indian Vet. J.* **41**: 394-396.

Kodagali, S.B. and Kerur, V.K. (1965). Gonodal hypoplasia in Gir cattle. *Indian Vet. J.* **42**: 114-118.

Kodagali, S.B. and Kerur, V.K. (1968). Gonadal hypoplasia in Gir cattle. *Indian Vet. J.* **45**: 602-603.

Kodagali, S.B., Vaishnava, T.N., Herajal, D.D. and Kerur, V.K. (1971). Studies on histopathological changes in testis associated with hypoplasia in Gir cattle. *Indian J. Anim. Sci.* **41**: 92-95.

Koefoed - Johnson, H.H. and Friis-N.F. (1965). A case of Dag defect in a charolais bull. *Anim. Breeding. Abstr.* **53**(4): 289.

Kohli, I.S. (1985). A case of bifid scrotum in a young Rathi bull. *Indian J. Anim. Reprod* **6**: 109.

Konig, H. (1964). Cited Raja and Mathew (1981).

Kozumplik, J. (1983). The concentration of plasma testosterone in relation to age, size of testes and libido., *Veterinari Medicina* **28**: 393-399. Abst in Anim. Breed Abst. **52**: 4584.

Krishna, K.M. and Rao, A.R. (1987). A note on rear leg conformation in Murrah breeding bulls. *Indian Vet. J.* **64**: 175.

Kuferschmied, H., Bachmann, F. andGaillard, C. (1985). Testis measurements on Simmental, Crossbred Red and White Holstein and black spotted cattle. *Mitteilungen-des-Schweizerischen verbandes-fur-Konstliche-Besamung-Und-der-Interessengemeinschaft- Schweizerischer - Besamungszuchter.* **23**(5): 194-195.

Ladds, P.W., Dannet, D.P. and Glazebrook, J.S. (1973). A survey of the genitalia of bulls in Northern Australia. *Aust. Vet. J.* **49**: 335-340.

Lagerlof, N. (1934). Morphological Studies on the changes in the sperm structure and in the testis of bulls with decrease or abolished fertility. Acta Pathol. Microbiol. Scand. Suppl. **19**: 254-267.

Lagerlof, N. (1936). Sterlity in bulls. *Vet. Rec.* **48**: 1159-1170.

Laing, J.A., Morgan, B. and Wagner, W.J. (1988). Fertility and Infertility in Veterinary Practice. 4th Edn. ELBS Baillieri Tindall Ltd. London.

Larsen, R.E., Littell, R., Rooks, E., Adams, E.L.,Falcon, C. and Warnick, A.C. (1990). Bull influences on conception percentage and calving date in Angus, Hereford, Brahman and Senepol single-sire herds. *Theriogenology* **34**: 549-568.

Lasley, J.F. (1951). Spermatozoan motility as a measure of semen quality. *J. Anim. Sci.* **10**: 211.

Laurans, R. and Negriere, M. (1964). Cited Raja (1981).

Lepard, O.L. and Shurat, C.E. and Foster, A. (1941). The effect of exercise on the amount and quality of dairy bulls semen. *J. Dairy Sci.* **25**: 509.

Lindley, C.E., Easley, G.T., Whatley, J.A. and Chambers, D. (1959). Certain semen characteristics and their relation to the reproductive performance of a purebred Hereford herd. *J. Anim. Sci.* **18**: 55-63.

Lunstra, D.D., Ford, J.J. and Echtemkamp, S.E. (1979). Puberty in beef bulls. Hormone concentrations, growth, testicular development, sperm production and sexual aggressiveness in bulls of different breeds. *J. Anim. Sci.* **46**: 1054-1062.

Makarechian, M., Farid, A. and Bery, R.T. (1985). Scrotal circumference, semen characteristics, growth parameters and their relationships in young beef bulls. *Can. J. Anim. Sci.* **65**: 789-798.

Malmberg, G. (1965). Semen characteristics in bulls at Brotsra, *Svensk Husdjursskots*, 46. (*Anim. Breed. Abstr.* **33**: 2358).

Mann, T. (1964). The biochemistry of semen and of the male reproductive tract. Ist Edn. Methuen & Co. Ltd. London. pp. 182-186.

Maslov, N. (1960). Evaluation of stud bulls by semen production. *Mol. Mjsn. Skotovod.* **5**: 47. *Anim. Breed. Abstr.* **29**: 218.

Mathew, A. (1974). Semen characteristics of crossbred and purebred bulls. Tech. paper presented at X International Dairy congress, New Delhi. 1974. pp. 57-58.

Mathew, A., Joseph, P.J. and Jose, T.K. (1982). Semen characteristics of purebred and corssbred bulls. *Indian Vet. J.* **59**: 364-367.

Maule, J.P. (1962). Semen of animals and artificial insemination. 1st Edn. Commonwealth Agricultural Bureau, England, pp. 133-145.

Mc Entee, K. (1970). Pathology of domestic animals. 2nd Ed. Vol. I. Edited by Jubb and Kennedy, P.C. Academic Press, New York.

Mc Entee, K (1990). Reproductive Pathology of Domestic Animals. Academic Press, Inc, Bosson. pp. 231-241, 252-257.

Milk Marketing Board, Scotland Report 1973-74.

Mohanty, D.N. and Dugwekar, Y.G. (1987). Spermiograms of Holstein-Friesian bulls and their relationship with fertility. *Indain J. Anim. Reprod.* **8**: 81-83.

Mohanty, D.N., Biswas, R.K., Singh G.B. and Dugwekar, Y.G. (1983a). Reproductive efficiency of Holstein Friesian bulls in Punjab. *Indian J. Anim. Reprod.* **3**: 97.

Moorthy, A.R.S. (1985). Outbreak of balanoposthitis in breeding bulls. *Vet. Rec.* **116**(4): 98.

Morstin, J. (1970). Observation on the incidence of morphological changes in the semen of young bulls. *Medycyna Vet.* **26**: 744-746.

Munro, I.B. , Stewart, D.L. and Melrose, D.R. (1961). Middle piece defect in bovine spermatozoa. *Vet. Rec.* **73**: 577-578.

Nagarsundaram, M (1986). Selection of breeding bulls for reproductive efficiency. A.I. News **1**(2): 8-9.

Narasimhan, K.S., Venkitaswamy, V., Pattabhiraman, S.R., John, D. and Perumalswamy, S. (1981). Bilateral testicular hypoplasia in a crossbred bull. *ndian J. Anim. Reprod.* **1**: 94-96.

Nema, S.P. and Kodagali (1983). Studies on biometry of testes and its correlation to sperm producing ability in Surti buffalo bulls. *Indian J. Anim. Reprod.* **4**: 89-91.

Nwakalor, L.N. and Ezinma, C.O. (1989). Libido serving capacity and breeding soundness in Mutura and N'Dama beef bulls. *Theriogenology* **32**: 901-911.

O'Dell, W.T., Almquist, J.O. and Amann, R.P. (1959). Freezing bovine semen Vs. Practicability of collecting and freezing a large number of successive ejaculates. *J. Dairy Sci.* **42**: 1209-1215.

Ogasa, A., Suguira, K., Iizuka, M., Yonumura, T., Yokoki, Y. and Ochi, T. (1967). Reduction of sperms with looped tail and improvement of sperm motility by frequent ejaculation in a bull. *Bull. Nath. Inst. Anim. Health.* **54**: 24-23. *Anim. Breed.Abstr.* **36**: 3603.

Ohms, J.I. and Willet, E.L. (1955). Study of possible factors related to freezability of spermatozoa. *J. Dairy Sci.* **38**: 604.

Onstad, O. (1963). Abaxial attachment of the middle piece of the spermatozoa in connection with sterility in a bull. *Nord. Vet. Med.* **15**: 92-96.

Pandit, R.K and Garg, U.G. (1983). Freezability, GOT release and fertility of crossbred bulls semen. *Indian J. Animal Reprod.* **4**:36.

Pangawkar, G.R. and Sharma, R.D. (1989). Spermiogram and cytomorphology of spermatozoa in relation to freezability of Holstein-Friesian semen. *Indian J. Anim. Reprod.* **10**: 6-63.

Pangawkar, G.R., Sharma, R.D. and Sharma, R. (1988). Phosphatase and transaminase activity in the seminal plasma of bulls in relation to freezability of semen. *theriogenology* **29**: 1393-1399.

Parkinson, J.J., Brown, P.J. and Crea P.R. (1993). A typical granulomatous epididymitis in a bull. *Vet. Rec.* **132**(20): 509-510.

Patel, K.V. and Kodagali, S.B. (1985). Diagnostic and investigative andrology in crossbred bulls. M.V.Sc. thesis abst. *Indian J. Anim. Reprod.* **6**(2): 107-110.

Pathak, N.N. (1979). A short note on same sexual behavior and semen atributes of crossbred bull calves raised on adlibitum feeding of urea molasses liquid diet with restricted cereal forage and intact proteins. *Indian Vet. J.* **56**: 69-70.

Pathak, R.C. (1967). A study of bulls with seminal vesiculitis. *Indian Vet. J.* **44**: 47-50.

Patel, K.V., Dhami, A.J. and Kodagali, S.B. (1986). Andrological investigations in crossbred bulls with testicular hypoplasia. *Indian J. Anim. Reprod.* **2**: 48-54.

Patel, K.V., Dhami, A.J. and Kodagali, S.B. (1988). Comparison of physico-biochemical semen characteristics in normal and problem bulls. *Indian J. Anim. Sci.* **58**: 1169-1172.

Paul, A.K., Acharya, A.K. and Bhattacharya, S. (1966). Studies on different seminal attributes of Indian Dairy breeds. *Indian J. Dairy Sci.* **19**: 79.

Peet, R.L., Kluck, P., and Mc carthy, M. (1988). Infertility in 2 Murray Grey bulls associated with abaxial and swollen midpiece sperm defect. *Aust. Vet. J.* **65**(11): 359-360.

Pimentel, C.A., Ferreira, J.M.M., Moraes, J.C.F, Chagas, P.R., Amoral, C.O., Medeiros, E.L. and Bento, C.L.R. (1984). Testicular and body development in beef bulls. *Revista-Brasileira-de-Reproduce - Animal* **8**(1): 27-33.

Porwal, M.L., Saxena, H.R. and Karaudikar, G.W. (1977). Effect of seasons on seminal attributes of Red Dane bulls. *Indian Vet. J.* **54**: 163-166.

Povliconko, I.N. (1964). The effect of age, body weight and condition of bulls on their semen production. *Zivotnorodstovo* **26**: 71. *Anim. Breed. Abstr.* **33**: 1257.

Radhakrishnan, R., Venkitaswami, V. and Pattabiraman, S.R. (1975). Further report on the effect of protective vaccination on the semen quality of breeding bulls. *Indian Vet. J.* **52**: 620-625.

Raja, C.K.S.V. (1981). Postnatal development of testis and epididymis, semen characteristics and fertility of Brown Swiss Crossbred bulls. Ph.D. thesis, APAU.

Raja, C.K.S.V. and Nair, D.V.P. (1961). A case of infertility in a bull. *Indian Vet. J.* **38**: 118-123.

Raja, C.K.S.V. and Nambiar, K.,G. (1962). Infertility in bulls due to sperm with a afaxial attachment of head and Middle price. *Indian Vet. J.* **39**: 541-544.

Raja, C.K.S.V., Namboothiripad, T.R.B. Mathai, E. and Nair, K.P. (1974). Testicular fibrosis in a Jersey bull. *Kerala J. Vet. Sci.* **2**: 71-74.

Raju, M.S. (1981). Semen characteristics and freezability of spermatozoa in crossbred exotic and Indian bulls. M.V.Sc. thesis submitted to APAU, Hydrabad.

Raju, M.S. and Rao, A.R. (1982). Note on the semen characteristics of crossbred and pure bred bulls. *Indian J. Anim. Sci.* **52**: 1230-1232.

Rao, A.R. (1976). Sperm Akinesia in a Hallikar bull. *Indian Vet. J.* **53**: 414-418.

Rao, A.R. (1997). Seminology as an indicator of epididymal dysfunction in bulls. *Indian J. Anim. Reprod.* **18**(2): 147-148.

Rao, A.R. and Bane, A. (1985). Pathological conditions of genital organs of normal fertile bulls. *Indian Vet. J.* **62**(3): 242-246.

Rao, A.R., Naidu, K.V. and Rao, K.B. (1986). A case of low germ cell resistance type of testicular hypoplasia in crossbred bull. *Indian Vet. J.* **63**: 745-746.

Rao, A.R., Rao, P.N. and Rajulu, P.S. (1966). Testicular hypoplasia in a crossbred bull. *Indian Vet. J.* **43**: 778.

Rao, A.R., and Rao, P.R. (1979). Testicular fibrosis in Young Red Dane bull. *Indian Vet. J.* **57**: 1041-1042.

Rao, A.R. and Rao V.J. (1981). Seminal Vesiculitis in a Holstein Friesian bull. *Indian Vet. J.* **58**: 497.

Rao, A.R., Reddy, M.N. Rao, T.L.N. (1979). Semen characteristics of young corssbred (F1) bulls. *Indian Vet. J.*. **56**: 1013.

Rao, A.R. and Subbaih, G (1982). Note on a case of sperm akinesia in a Jersey bulls. *Indian J. Anim. Sci.* **52**: 123-1237.

Rao, A.V.N. (1990). Treatment with gonadotrophins releasing hormone for correction of libido problems in Murrah bulls. *Indian Vet. J.* **67**: 760-762.

Rao, A.V.N., Harnath, G.B. and Somasekharan, G. (1993). Revivability and acrosomal integrity of buffalo spermatozoa deep frozen in straws using different equilibration and thaw rates. *Indian J. Anim. Reprod.* **14**(1): 44-47.

Rao, A.V.N. and Kotayya, K. (1977). Studies of ejaculate characteristics of Bos taurus and crossbred bulls under the tropical conditions of Andhra Pradesh. *Indian J. Anim. Health.* **16**: 45.

Rao, A.V.N., Rao, G. and Haranath, G.B. (1991). Effect of collection frequency, age and season on quantitative seminal characteristics in Murrah bulls. *Indian Vet. J.* **68**: 1084-1085.

Rao, C.V., Rao, A.V.N. and Venkatramaiah, P. (1992). Age and breed related changes in body weight and testis size in bulls. *Indian Vet. J.* **69**: 415-418.

Rao, K.B. and Rao, A.R. (1988). Different types of scroti and preputial sheaths in crossbred bulls. *Indian J. Anim. Reprod.* **9**(2): 114-117.

Rao, K.B. and Rao, A.R. (1988). A note on rearleg conformation in crossbred breeding bulls. *Indian J. Anim. Reprod.* **10**: 69-70.

Rao, K.B. and Rao, A.R. (1990). Body, scrotal, testicular measurements and semen characterestics in 50% and 75% exotic crossbred bulls. *Indian Vet. J.* 67: 238-241.

Rao, K.B. and Rao, A.R. (1991). Testicular degeneration in crossbred bulls. *Indian Vet. J.* **2**: 132-134.

Rao, K.B. and Rao, V.V.V.S.P. (1995). Relationship between suotal circumference and seminal characteristics in crossbred bulls. *Indian J. Anim. Reprod.* **16**(2): 129.

Rao, K.B., Venugopal Naidu, K., Vijaya Singh, and Seshagiri Rao, A. (1999). Studies on some enzyme levels and their extracellular leakage during preservation of Punganellur bulls semen. *Indian J. Anim. Reprod.* **20**(1): 47-50.

Rao, R.M. and Rao, A.R. (1975). Studies on the semen characteristics of Tharparkar and Jersey bulls. *Indian Vet. J.* **52**: 889-892.

Rao, T.L.N. and Rao, A.R. (1978). Semen characteristics of crossbred bulls. *Indian Vet. J.* **55**: 692-695.

Rao, T.L.N. and Rao, A.R. (1980). Studies on semen characteritics of Ongole bulls. *Indian Vet. J.* **57**: 316-321.

Rebhun, W.C. (1976). Bilateral cryptorchidism in a bull. *Cornell Vet.* **66**: 10-13.

Reddy, J.V., Chetty, A.V., Ramachandraiah, S.V. and Sreeraman, P.K. (1991). Effect of foot and mouth disease vaccination on semen quality in bulls. *Indian J. Anim. Reprod.* **12**: 13-14.

Reddy, N.C., Gupta, C.L.R. and Radhakrishna, T.G. (1980). Freezability and fertility results of bulls and buffalo semen. Paper presented at II All India symposium on Animal Reproduction, Bangalore.

Reitman, S. and Frankle, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and serum glutamic pyruvic transaminase. *Amer. J. Clin. Path.* 28: 56-60.

Roberts, S.J. (1986). Veterinary Obstetrics and Genital diseases 3rd Edn. David and Charles Inc. North Pomfret, Vermont 05053, USA.

Roy, J.J., Biswas, J.C., Bandyopadlyay and Bhatit, N. (1975). Cited Raja (1981).

Rollinson, D.H.L. (1951). Studies on the abnormal spermatozoa of bull semen. 1. *Br. Vet. J.* **107**: 203.

Ruttle, J.L., Ezaz, Z. and Sacery, E.J. (1975). Some factors influencing semen characteristics in Range bulls. *J. Anim. Sci.* **41**: 1069-1076.

Saacke, R.G. (1970). Morphology of the sperm and its relationship to fertility. Proc. 3rd Tech. Cnf. AI Reprod. Chic. III. NAAB 17-29.

Saacke, R.G., Amann, R.P. and Marshall, C.E. (1968). Acrosomal cap abnormalities of sperm from sub fertile bulls. *J. Anim. Sci.* **27**:1391-1400.

Sagdeo, L.R., Chitnis, A.B., Deshmukh, S.N. and Kaikini, A.S. (1990). Studies on semen freezability of pure Jersey and crossbred bulls with varying levels of exotic inheritance. *Indian J. Anim. Reprod.* **11**: 79-84.

Sakala, J. (1964). The relation between biometrical values of the testes and various physiological process in stud bulls. *Anim. Breed. Abst.* **33**: 260.

Salisbury, G.W. (1944). A controlled experiment in feeding wheat germ oil as a supplement to the normal ration of bulls used for artificial insemination. *J. Dairy Sci.* **27**: 551.

Salisbury, G.W. Vandemark, N.L. and Lodgi, J.R. (1978). Physiology of Reproduction and artificial insemination of cattle. 2nd Edn. W.H. Freesmann and Co., San Francisco. p. 649-650.

Sane, C.R. Deshpande, B.R. and Chinavavalleas, (1965). Record of seminal vesiculities in a khillar bull. *Bombay Vet. Coll. Magazine*. **12**: 27-32.

Sattar, A., Deka, B.C. and Baruah, J.N. (1980). Effect of extenders on freezability of bovine semen. Paper presented at 2nd All India Symposium on Animal Reproduction, Bangalore.

Satter, A., Deka, B.C. and Baruah, J.N. (1978). A comparative study on conception rate of liquid and frozen bovine semen paper presented at FAO/SIDA follow up seminar, Faisalabad.

Saxena, V.B. and Tripathi, S.S. (1978). Studies on the physico-chemical attributes and preservability of semen of crossbred bulls. *Indian J. Anim. Sci.* **48**: 865-869.

Settergren, I. (1978). Compendium – FAO/SIDA. Follow up seminar on Animal Reproduction Faisalabad and Tirupathi.

Settergren, I. and Mckentee, K. (1992). Germ cell weakness as a cause of testicular hypoplasia in bulls. *Acta Vet. Scand.* **33**: 273-282.

Sharma, N.C. (1969). Impact of foot and Mouth disease on sexual behavior and semen quality in cow and buffalo, bulls including study on testicular tissue, M.V.Sc. Dissertation - Maharastra Agricultural University, Bombay.

Sharma, R.K., Pareek, P.K. and Vyas, K.K. (1982). Climatic effect on sexual behaviour and semen quality of Jersey bulls in arid zone of Rajastan. *Indian J. Anim. Reprod.* **2**: 45.

Shaw, R.C. (1972). The problem of variation of abnormal sperm level between ejaculates from the same bull. Paper presented at VII *Int. Cong. Anim. Reprod.* **2**: 45.

Singh, B., Singh, B.P. and Tomar, N.S. (1967). Studies on the semen characteristics of Hariana and Murrah bulls 1 per ejaculate volume, initial pH, initial sperms concentration per cu.mm. *Indian J. Dairy Sci.* **20**: 80-85.

Singh, D.M. and Pangawkar, G.R. (1989). Biometry of genitalia of exotic and crossbred bulls. *Indian J. Anim. Reprod.* **10**(1): 65-67.

Singh, D.M., and Pangawkar, G.R. (1990). Studies on some characteristics of exotic and crossbred bull spermatozoa. *Indian. J. Anim. Reprod.* **11**(2): 92-95.

Singh, G., Bhatragar, M.K. and Pillai, S.K. (1968). Studies on the effect of higher intensities of collection and seasonal variation in semen quality of Hariana bulls. *J. Anim. Morp. Physiol.* **15**: 88.

Singh, K.T., Mohnty, B.N., Ray, S.K.H. and Mohanty, D.N. (1993). Effect of spermatozoan correlation on freezability of bull semen. *Indian J. Anim. Reprod.* **2**: 103-104.

Singh, R.S., Tomar, N.S. Sharma, K.C. Sharma K.B. (1992). Studies on acrosomal abnormalities of cattle and buffaloe spermatozoa in relation to other semen characteristics and fertility. *Indian Vet. J.* **69**: 267-268.

Smith, G.F. (1961). Behaviour of bulls at artificial insemination centres. *Proc. 13th Meet of Brit. Soc. of Anim. Prod.* pp: 25-35.

Smith, M.F., Morris, D.L., Amoss, M.S., Parish, N.R., Williams, J.D. and Wittbank, J.N. (1981). Relationships among fertility, scrotal circumference, seminal quality and libido in Santa Gertrudis bulls. *Theriogenology* **16**: 379-397.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. 6th edn. The Iowa State Univ. Press. USA.

Spitzer, J.C. and Hopkins, F.N. (1997). Breeding soundness evaluation of yearling bulls. Bull infertility *Vet. Clin. North. Am. food Anim. Pract.* **13**(2): 295-304.

Stewart, D.L., O' Hagen, C. and Glorer, F.A. (1972). The prediction of the fertility of bull semen from laboratory tests. VII Int. Congress on Animal Reproduction. Munich. Vol. II 12280-1283.

Stone, E., Johnstone, J.E. and Mixner, J.P. (1950). Live spermatozoa relationships and fertility of dairy bull semen. *J. Dairy Sci.* **33**: 442.

Swanson, E.W. and Boyd, J. (1962). Factors affecting coiled fowl spermatozoa in the bull. *Amer. J. Vet. Res.* **23**: 300.

Talukdar, H.K., Borgohain, B.N. and Deka, B.C. (1986). Semen characteristics of Jersey and crossbred bulls. Abst. of paper presented of Sixth national congress on Anim. Reprod. November, Gauhati.

Thompson, J.A. Buhr, M.M. and Johnson, W.H. (1992). Scrotal circumference does not accurately predict degree of germinal epithelial loss or semen quality in yearling hereford and simmental bulls. *Theriogenology* **38**: 1023-1032.

Thundathil, J., Palasz, A.T. Mapletoft., A.T. and Barth, A.D. (1999). An investigation of the fertilizing characteristics of pyriform shaped bovine spermatozoa. *Anim. Reprod. Sci.* **57**: 35-50.

Tierney, L.A., Hallford, D.M., Ruttle, J.L., Barlett, D.C. and Leigton, E.A. (1982). Serum testosterone, scrotal circumference and semen characteristics in Hereford and Brangus bulls under range conditions. Proc. Western Section, American Soc. Anim. Sci. 33: 114-117. Abst. in Anim. Breed. Abst. 51: 3625.

Todorovic, D. (1984). Primary and secondary prostatitis in bulls. *Veterinarski Glaswik* **38**(1): 45-49.

Tomar, N.S. (1964). Selection of bulls. In "Artificial Insemination and Reproduction of cattle and buffaloes". Tomar (Ed). Saroh prakash 646, Katra, Allahabad 1970. p. 16.

Toma;r, N.S., Sharma, S.C. and Desia, R.N. (1965). Repeatability estimates of volume and motility of semen of bulls kept at AI centres. *Indian Vet. J.* **42**: 360.

Tomer, N.S., Misra, B.S. and Johari, C.B. (1966). Seasonal variation in reaction time and semen production and predictionof some semen attributes on initial motility of spermatozoa in Hariana and Murrah bulls. *Indian J. Dairy Sci.* **19**: 87.

Tomar, N.S. (1970). Unpublished data "In Artificial Insemination and Reproduction of cattle and Buffaloes". Tomar (Ed). Saroj Prakash, 646. Ketra. Allahabad.

Twinbull, P.A. (1977). An abattoir survey of bull genitalia. *Aust. Vet. J.* **53**: 274-279.

Van Camp, S.D. (1997). Common causes of infertility in the bull. Bull infertility. *Vet. Clin. North Am. Food Anim. Pract.* **7, 13**(2): 203-231.

Vanderslius, L. (1953). Experiences with the examination into herd infertility. Proceedings I world congress, fertility and sterility **2**: 703.

Veeramachaneni, D.N.R., Ott, R.S., Health, E.H., McEntee, K., Boot D.J. and Hixon J.E. (1986). Pathophysiology of small testes in beef bulls. Relationship between scrotal circumference, histopathologic features of testes and epididymis, seminal characteristics and endocrime profiles. *Am. J. Vet. Res.* **47**: 1988-1999.

Veerapandian, C. (1992). Breeding Soundness Examination and Hormone profile in breeding bulls. Ph.D. thesis Madras Veterinary College Tamil Nadu Veterinary and Animal Sciences University, Madras.

Veerapandian, C., Edwin, J., Quayam, S.A., Rajasekaran, J. and Austian, A.J.S. (1992). Dag defect in two Jersey bulls (Half-sibs). *Indian J. Anim. Reprod.* **13**: 143-144.

Venkataswamy, V. and Pattabiraman, S.R. (1970). Calcareous testicular degeneration in a bull. *Ceylon Vet. J.* **18**: 15.

Verma, M.C. and Singh, N.P. (1992). Histopathological changes in relation to testosterone levels in bulls with complete loss of libido. *Indian J. Anim. Sci.* **62**(2): 1170-1171.

Williams, G. (1987). Tail stump defect affecting spermatozoa of two charolais bulls. *Vet. Record.* **121**(11): 248-250.

Wooton, I.D.P. (1964). Microanalysis in Medical Biochemistry. 4th Edn. J and A Chill Ltd. London pp.112.

Zaitsev, E.A. and Cherepakin, D.A. (1988). Immunological infertility in bulls with diseased accessory sex glands. *Sel'Skokhozjairkvennaya Biologiya* **3**: 136-137.

Zaitsev, E.A. (1988). Immunological infertility in bulls with diseased accessory sex glands. *Vet. Bull.* **58**(9): 5638.

STRUCTURAL AND FUNCTIONAL CHANGES IN THE TESTIS AND EPIDIDYMIS OF CROSS BRED BULLS WITH IMPAIRED FERTILITY

By

T. SREEKUMARAN

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy

Faculty of Veterinary and Animal Sciences

Kerala Agricultural University

Department of Animal Reproduction

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

MANNUTHY, THRISSUR - 680651

KERALA, INDIA

2000

ABSTRACT

With the object of suggesting suitable measures in the selection of breeding bulls the structural and functional changes in testis and epididymis of 153 crossbreed males of different crosses viz., Jersey (CBJ), Brown Swiss (CBBS) and Holstein Friesian (CBHF) were investigated during the period from 1993 to 1998. The records maintained at Dhoni farm for the past five years were also screened to assess the incidence of various infertility conditions and the rate of culling. The study was carried out by dividing the males into three age groups viz., birth to 12 months (Group I), 12 months to 24 months (Group II) and 24 months and above (Group III).

Group I – Bull calves

Measurement of testis and epididymis showed variation between different crosses. The mean scrotal circumference 19.42 ± 1.03 cm was found varied between different crosses. One case of bilateral cryptorchidism was detected. Among 53 bull calves 6 (11.32 per cent) were culled due to various reproductive disorders.

Group II – Growing bulls

Although different types of scroti were detected oval shape was predominant. The scrotal circumference was found to be 29.86 ± 0.38 cm. Lack of libido was predominant among growing bulls. The mean ejaculate volume, initial motility, sperm concentration and live sperm count were found to be 2.91 ± 0.16 ml, 49.77 ± 2.12 per cent, 1299.73 ± 56.25 million per ml and 58.76 ± 2.15 per cent

respectively. Freezability of semen was highly correlated with volume and initial motility. Unilateral hypoplasia (4.48 per cent) and bilateral partial hypoplasia (8.96 per cent) were the main pathological conditions detected. Testicular degeneration was found to be in the extent of 16.41 per cent. One case of epididymal dysfunction was also detected. The rate of culling among growing bull was 47.58 per cent due to reproductive disorders.

Group III – Adult bulls

Although different shapes of scrotum were detected, oval shape was predominant. The scrotal circumference was 35.29 ± 0.74 cm. The prefreezing, post-freezing and total rejection were 12.00, 11.20 and 23.20 per cent respectively. The main infertility conditions noted were bilateral partial hypoplasia (5 per cent) and testicular degeneration (15 per cent). Estimation of seminal plasma enzymes revealed that those having 50 per cent and above freezability had lower level of seminal enzymes than those showing less than 50 per cent freezability. The percentage of adult bulls culled due to various reproductive disorders were 22.50 per cent. Thus it could be concluded that screening of bulls for better breeding performance should be started from birth onwards. Measurement of testis and epididymis and scrotal circumference give good indication for future breeding performance. Incidence of cryptorchidism should be guarded. Shape of scrotum has negligible importance. Lack of libido in growing bulls should not be neglected. Incidence of testicular hypoplasia and testicular degeneration needs special

attention. Estimation of seminal plasma enzymes is a good indication for the suitability of semen for freezing.

Samples of tissues from testis affected with cryptorchidism, hypoplasia and degeneration were subjected to scanning electron microscopy.