POSTNATAL DEVELOPMENT OF TESTIS AND EPIDIDYMIS, SEMEN CHARACTERISTICS AND FERTILITY OF BROWN-SWISS CROSSBRED BULLS

THESIS SUBMITTED TO THE
ANDHRA PRADESH AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN THE MAJOR SUBJECT OF
ANIMAL REPRODUCTION AND GYNAECOLOGY

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ANDHRA PRADESH AGRICULTURAL UNIVERSITY
1981

C E R T I F I C A T E

This is to certify that the Thesis entitled "POSTNATAL DEVELOPMENT OF TESTIS AND EPIDIDYMIS, SEMEN CHARACTERISTICS AND FERTILITY OF BROWN-SWISS CROSSBRED BULLS" submitted for the Degree of Doctor of Philosophy in the Major Subject of Animal Reproduction and Gynaecology to the Andhra Pradesh Agricultural University, is a result of bonafide research work carried out by SRI C.K. SHACHDRA VARMA RAJA, under our supervision and that the thesis has not formed in whole or in part the basis for the award of any other degree, diploma or distinction.

The assistance and help received during the course of investigation have been fully acknowledged.

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Acknowledgement

The author is highly indebted to:

Dr. A. Ramamohana Rao, B.V.Sc., M.S., Ph.D., F.A.V.C.S. (Sweden), Professor and Head of the Department of Animal Reproduction and Gynaecology, College of Veterinary Science, Tirupati for the inspiring guidance given during the course of investigation and hospitality and kindness shown during my stay,

Dr. P. Narasimha Rao, B.V.Sc., Ph.D., Associate Professor, Department of Animal Reproduction, Dr. P. Rama Rao, M.V.Sc., Ph.D., F.A.V.C.S. (Sweden), Professor and Head, Department of Pathology, Dr. V. Satyanarayana Rao, M.V.Sc., Ph.D., Assistant Professor, Department of Pharmacology, College of Veterinary Science, Tirupati, for their academic help and valuable advice given from time to time,

Dr. C.V. Reddy, B.V.Sc., M.S., Ph.D., Dean of Faculty of Veterinary Science, A.P. Agricultural University and Dr. K. Satyanarayana Rao, G.M.V.C., B.V.Sc., M.S., Principal, College of Veterinary Science, Tirupati, for permission given to conduct the study,

Dr. S.V. Ramachandraiah, B.V.Sc., M.Sc.(Voty), Veterinary Clinician, for fruitful discussion and the invaluable help with the manuscript, Dr. K. Keseva Reddy, M.V.Sc., Assistant Professor, Dr. T.L. Narasimha Rao, M.V.Sc., Inst-

ructor and Dr. P.V. Chelapathi, M.V.Sc., Veterinary Officer, Centralised Semen Collection Centre, Tirupati, for help and encouragement.

Dr. K. Pulla Reddy, B.A., B.V.Sc. and Dr. P. Nanda Kishore, B.V.Sc., Post-graduate students of the Department of Animal Reproduction and Gynaecology, College of Veterinary Science, Tirupati, for the assistance,

Dr. K.R. Harshan, M.V.Sc., Assistant Professor, Department of Anatomy, College of Veterinary & Animal Sciences, Mannuthy, Kerala, for help rendered in the histological studies,

Dr. E. Mathai, B.Sc., B.V.Sc., M.Sc., Associate Professor, Dr. K.P. Nair, B.V.Sc., M.Sc., Associate Professor, Dr.T.Sreekumaran, M.V.Sc. and Dr. Joseph Mathew, M.V.Sc., Department of Animal Reproduction, College of Veterinary & Animal Sciences, Mannuthy, Kerala, for the assistance and cooperation,

The Managing Director, Kerala Livestock Development & Milk Marketing Board, Kerala and Dr. Unnikrishnan Vallathan, B.V.Sc., Dr. Abraham Mathew, B.V.Sc., F.A.V.C.S. (Sweden), Dr. V.P. Thomas, M.V.Sc., Dr. Sukesan Achari, B.V.Sc., and Dr. Mohan, B.V.Sc., Indo-Swiss Project, Mattupatty, Kerala, for the facilities provided and willing cooperation extended during the course of the study,

Sri N. Kaleswaran, I.A.S., Vice-Chancellor, Kerala Agricultural University for giving sanction for deputation for higher studies,

Dr. P.G. Nair, B.Sc., G.M.V.C., B.V.Sc., M.S., Ph.D., former Dean, Faculty of Veterinary and Animal Sciences, Mannuthy, Kerala for the interest evinced in the study.

Dr. P.U. Surendran, Ph.D., Professor and Sri Balakrishnan Assan, M.Sc., Assistant Professor, Department of Statistics, College of Veterinary & Animal Sciences, Mannuthy, Trichur, for help rendered in the statistical analysis.

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C O N T E N T S

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Introduction

The commanding impact of a flourishing Dairy industry on the economic upheaval and social transformation of the weaker sections of the rural community in our country had long been recognised. It was obviously known too, that the Dairy industry can be expanded in all aspects by augmenting the production of milk and milk products. The increased emphasis placed on accelerated milk production consequently, warranted the urgent need for a better understanding of the chain of events in the reproductive processes of the animals including puberty and that stimulated research on these lines. Results of carefully conceived and meticulously executed investigations, both fundamental and applied, clearly indicated that milk production can be increased to an appreciable extent through sound and scientific feeding and management. The most important and predominant implicating factor involved in the poor production performances of our animal was found to be attributable to heredity. Successive trials of selective breeding and repeated attempts to grade up the local animals failed to bring about rapid and perceptible improvement in the genetic potential of the indigenous stock. Hence, this achievement was sought to be accomplished by a massive cross-breeding programme using recognised exotic temperate breeds such as Jersey, Holstein-Friesian, Brown Swiss etc. Through the implementation of extensive crossbreeding programmes, emerged artificial insemination as the most amenable and ideal biological tool to obtain maximum genetic make up for improved

reproductive efficiency and increased production. The technique of artificial insemination which is no longer a novelty is now being used more meaningfully as a means of genetic manipulation to exploit the potentialities of cross-breeding that has been launched throughout the country. However, the practice of selecting sires for the collection of semen solely on the basis of milk production potentialities with scant attention to the fertility rates impeded the anticipated progress of crossbreeding but nevertheless yielded apparent spectacular results. While innumerable reports are available assessing the breeding performances of crossbred cows, systematic efforts to evaluate the same of crossbred bulls of different exotic inheritance under various and varying environments and agro-climatic conditions of the country have rarely come to light.

In Kerala, where massive and intensive crossbreeding programmes have been in operation since early sixties, more than 50% of the breedable cattle population are now estimated to be crossbred cows. Besides, nearly a lakh of female crossbreds are added to this population every year with consistent regularity. Jersey and Brown Swiss are the two exotic breeds used for crossbreeding operations in the State. A crossbred called "Sugandhini" having 62.5% Brown Swiss inheritance has been evolved as a result and it is claimed that this new "breed" has inherited the characters of adapt-

ability to the local conditions and hardiness and disease resistence from the indigenous cattle and the high yield production traits from the exotic animals. For sustaining the level of exotic inheritance in the progeny, large scale interserv mating will have to be carried out for which adequate crossbred bulls with 62.5 of exotic inheritance have got to be made available. Further, for maximum genetic progress by artificial insemination, progeny testing will necessarily have to be resorted to. In order to exploit the full potential of artificial insemination, it is not only important to select sires of high pedigree but also to make use of them to the maximum extent possible. Using different wording in the relevant sense, it points up the imperative need to determine how early the crossbred bulls can be successfully used for the collection of semen for artificial insemination. In brief and in substance, it should be possible to predict in all its mundane significance the fertility potency of a known crossbred bull calf. It is well to remember that the onset of puberty is not dependent on a single factor but on interplay of several which intervene to produce the pattern inherent in the species in question (Asdell, 1946).

Many are aware of the usefulness of artificial insemination but only very few have comprehended that its certain success is essentially dependent on (1) an explicit knowledge of the process of post-natal growth and development of testis

and epididymis to determine the precise age at which a bull can be pressed into service, (2) evidence of proved fertility potency of the bull, and (3) a clear and authentic picture of the desirable characteristics of good quality semen ideally suited for insemination. The limited literature available on these aspects is mainly confined to foreign breeds. Comparatively, very little is known in respect of Indian breeds; much less so as applied to the crossbred animals. Apart from acquiring basic information on the physiological processes of reproduction involved in the aforesaid aspects of study, it was so much mandatory as was the purpose to subject these to detailed enquiry in the case of Brown Swiss crossbred animals with 62.5% exotic inheritance which have earned paramount importance in the implementation of the crossbreeding programmes designed towards intensive cattle development in the State. These were the animating forces of thought behind this principal object of the present investigation.

Review of Literature

From a perusal of literature, it is seen that information available on the postnatal development of testis and epididymidis is practically nil in respect of Indian breeds of farm animals. As regards studies designed to determine the semen characteristics and the age at which the bulls can be used for breeding, a great deal of work has been carried out in exotic breeds of cattle but the data available with reference to Indian breeds, particularly crossbred bulls are scattered and scanty. A brief account of the salient observations so far made on all these aspects is given below:

Testes: Postnatal Growth and Development:

The pattern of prepuberal growth and development of testes in the mammalian species in general is stated to be sigmoid in nature, these physiological responses being gradual in the beginning, accelerated at about the time of puberty and then again gradual until adulthood (Courot et al., 1970). The postnatal growth of the testes has been adequately described in the literature in respect of laboratory animals. (Sayles, 1939; Mixner et al., 1943; Bond, 1943; Webster and Young, 1951; Widdowson and McCance, 1960; Clermond and Hoekins, 1961; Ennesc and Lebland, 1962; Huckins, 1962; 1963; 1963; El Gohary, 1964; Ewing et al., 1966; Wildt et al.,1966; Skinner, 1967; Huminski, 1969). The pattern of testicular growth in guinea pigs (Kibler et al., 1943; Mixner et al.,

1943), mice and rats (Onuma and Nishikawa, 1955; Enesco and Leblond, 1962) and rabbits (Skinner, 1967) is of the sigmoid type with a median zone of rapid weight increase.

Michatsch (1933) observed ten fold increase in the weight of the bovine testis from birth to 3 months of age and two fold increase from 4 to 7 months of age. Lagerlof (1934) reported that the rate of increase in the size of the bovine testis is slow during the initial period of postnatal life and rapid after the onset of puberty. In the case of three adult Italian cattle breeds, Brigatti (1951) found that with increase in age there is a proportionate gain in weight of the testis as well. The growth of the testis of Holstein bulls was found to proceed at a rapid rate till the animals attained maturity (Musgrave, 1951; Dunn, 1955). In the case of the Swedish Red and White breed (SRB), the growth curve of the testis has been reported to be of sigmoid type, the growth being slow between birth and 16 weeks, rapid between 16 and 32 weeks and more rapid between 32 and 48 weeks (Abdel Raouf, 1960). Although the increase in the testicular weight is depressed between 48 and 64 weeks, it is still higher then the relative increase during 0-16 weeks. An observation similar to this has been reported in Friesian bulls by Jay et al. (1961).

According to Yao and Eaton (1954), the birth weight of the kid usually affects the development of the reproductive

organs and germ cells, some of the animals with low birth weight producing mature sperms very late in life. It has been shown by these authors (Yao and Eaton, 1954) that the growth of the testis in the case of Toggenburg and mixed breeds of goats is independent of birth weight from birth to 90 days, but after this the growth is well correlated with birth weight until 120 days of age. Beyond this, no correlation between testis weight and birth weight is observed. Apparently, studies on these lines have not been carried out in other species.

Positive correlation between testicular weight and age of the animal has been recorded by Courot (1971) in rams, by Thomas (1973) in boars and by Unnikrishnan (1975) in goats. All the authors observed that the testicular weight is more closely related with the body weight than with the age.

Testicular weight is reported to be closely correlated with the body weight in rams (Carmon and Green, 1952; Courot, 1971), boars (Green and Winters, 1944; Mauser et al., 1952; Thomas, 1973) and goats (Unnikrishnan, 1975). Yao and Eaton (1954) found that the increase in the weight of the testis in Toggenburg and mixed breeds of goats is correlated with body weight upto 114 days of age. A high correlation between the size of the testis and the body weight has been recorded in bulls of varying breeds by Mauser et al. (1952); Van Demark

and Mauger (1954); Abdel Raouf (1960) and Gourot (1971). It has been reported (Musgrave, 1951 and Dunn, 1955) that in Holstein bulls, growth of the testis proceeds at a faster rate as compared with body growth till the animals attain maturity, but since then the growth rate of the testes gets reduced and becomes very close to the rate of body growth. A positive correlation between live body weight and testicular weight has been observed in White Fulani bull calf (Bos indicus) by Aire and Akpokodje (1975). In marked contrast, Brigatte (1951) found no correlation between testis weight and body weight at any stage of development in three Italian breeds of cattle.

Phillips and Zeller (1943), Green and Winters (1944), Hauser et al. (1952) and Thomas (1973) reported that the left testis is bigger than the right, in boars. In the case of Swedish High Land bulls, the right testis was found to be larger than the left (Erikson, 1943). Abdel Raouf (1960) observed asymmetry in growth between the right and the left testis, the right testicle always being found to be heavier than the left and the difference in size however being relatively small in newborn and young animals.

Abdel Raouf (1960) found that the bovine testis is more thick than broad in all the age groups. The length of the gland is about two times its thickness and three times

its breadth. According to this author (Abdel Rmouf, 1960) the bovine testis at birth is cylindrical in shape resembling a date fruit, becoming more oval as age advances due to greater increase in breadth than that in thickness or length.

Histological changes of Testis:

The histologic pat[illegible] of the development of the testi during postnatal period h[illegible]en studied in rams (Carman and Green, 1952; Watson et al., 1956; Skinner et al., 1968; Court 1971), goats (Yao and Eaton, 1954; Unnikrishnan, 1975); boars (Phillips and Andrews, 1936; Mauser et al., 1952; Niwa and Misuno, 1954; Niwa, 1954; McFee and Eblen, 1967; Thomas, 19 and bulls (Fossland, 1954; Abdel Rmouf, 1960; May et al.,196 Aire and Akpokodje, 1975). Based on the quantitative and qualitative changes occurring within the bovine testis, Abde Rmouf (1960) divided the postnatal period into five stages, viz. infantile, proliferative, prepuberal, puberal and post-puberal or adult-hood. This classification as outlined by Courot et al. (1970) is inpuberal, prepuberal, puberty, firs postpuberal, second postpuberal, second postpuberal and adul hood.

Size of the Seminiferous Tubules:

In the opinion of Carman and Green (1952), May et al. (1961) and Skinner et al. (1968) the size of the seminferou tubules is an excellent parameter for assessing the reproduc

efficiency of farm animals. The pattern of increase in tubular diameter has been reported to be S-shaped in rats (Skinner et al., 1968); goats (Unnikrishnan, 1975) and boars (Thomas, 1973). In bulls, (Mickatsch (1953) observed a two-fold increase in the tubular diameter from birth to three months of age after which again between 3 and 7 months of age. In Guernsey, Ayrshire, Shorthorn and Hereford breeds of cattle the seminiferous tubules were seen to increase in size with advancing age (Phillips and Andrews, 1936). A four-fold increase in the diameter of tubules between birth and 18 months was recorded in Jersey and Holstein bulls by Fossland (1954). The general pattern of growth of the seminiferous tubules in SRB bulls has been described as S-shaped (Abdel Raouf, 1960) just as in the case of rams, goats and boars (Skinner, 1968; Unnikrishnan, 1975; Thomas, 1973). Abdel Raouf (1960) has divided the growth period of the tubules into three phases. During the first phase extending from birth to 20 weeks of age there occurs a two-fold increase in the tubular diameter. The second phase extending from 20-30 weeks of age is characterized by more rapid absolute and relative increase in the size of the tubules. During the third phase, from 36 weeks of age to adult-hood, there is a considerable increase in the tubular diameter but at a lower rate than that in the second phase. Ray et al. (1961) have recorded the tubular diameter in the case of Friesian bull calf at birth and at the time of establishment of spermato-

genesis as 40 microns, and 100-12 ...spectively. The authors (...t al., 1961) have ... view that the stabilizati... of the size of the ...niferous tubules takes place only along with th... attainment of sexual maturity. In White Fulani bulls (Bos ...dicus), marked differences in the size of the seminifero... tubules were not observed between 4 weeks and 16 weeks o... ...t from 20 weeks onwards an obvious increase in ...meter was noticed (Aire and Akpokodje, 1975).

It has been reported that the diameter of the tubules is closely correlated with the weight of the testis rather than with the age in goats (Yao and Eaton, 1954), rams (Phillips and Andrews, 1936; Watson et al., 1956 and Courot, 1971) and boars (Phillips and Andrews, 1936; McFee and Eblen, 1967 and Thomas, 1973). On the other hand, Caremon and Green (1952) found that both age and testicular weight in rams are highly correlated with the diameter of the seminiferous tubules. A positive correlation between the testicular weight and the tubular diameter was observed in White Fulani bulls also by Aire and Akpokodje (1975) who reported a clear parallelism between the two parameters.

Lumen Formation:

Although the seminiferous cord which is solid at birth undergoes a series of postnatal changes to form lumenated

seminiferous tubules (Courot et al., 1970) the different factors involved in the process of lumen formation are not clearly understood. Vacuolation of the cytoplasm has been considered as the first step in the formation of lumen in rams (Watson et al., 1936) and boars (Thomas, 1973). Abdel Raouf (1960) perceived that the first sign of lumen formation in the sex cords of bulls is cracking of the tubular cytoplasm, the cracked cytoplasm later radiating from the centre of cords and forming circular or star-shaped lumen.

Lumen formation in sex cords was noticed in bulls at 6 months of age (Michatsch, 1933). Phillips and Andrews(1936) have reported that complete lumen formation in the cord with the characteristic peripheral arrangement of epithelial cells, occurs at the age of 142 days in bulls. Hooker (1942) noticed initiation of lumen formation at the age of 5½ months in Holstein, Jersey, Ayrshire and Guernsey bulls, most of the tubules showing fairly large lumina at six months of age. In contrast, Santamarina and Reece (1957) reported initiation of lumen formation in sex cords at the age of 80 days and its completion at 127 days of age. Abdel Raouf(1960) adduced evidence that the formation of lumen in SRB bulls starts at 20 weeks and ends by 32 weeks of age. In Angoni bull calves lumina are formed at about 7 months of age (Igboeli and Rakha, 1971). Canalisation was observed at the earliest at 44 weeks of age in White Fulani calf (Aire and

Akpokodje, 1975). Abdel Raouf (1960) pointed out that the variation in the onset of lumen formation as reported by different authors is attributable to breed differences. The size of the lumina varies within the same testis, the tubules lying at the periphery exhibiting larger lumina than those lying near to the rete testis (Abdel Raouf, 1960).

Intratubular Cells:

The cellular elements within the seminiferous tubules during the prepubertal growth and development of the testes have been well documented (Charmy et al., 1952; Abdel Raouf, 1960; Nicander et al., 1961; Sapsford, 1962; Skinner et al., 1968; Ortavant et al., 1969; Courot et al., 1970; Courot, 1971). It has been shown (Santamarina and Reece, 1957; Abdel Raouf, 1960; Ortavant et al., 1969; Courot et al., 1970; Courot, 1971) that the testes of mammals at birth are composed of sex cords containing two easily distinguishable types of cells, viz. the gonocytes and supporting cells which transform themselves into spermatogonia and sertoli cells respectively, during the prepuberal period.

Michaloh (1933) observed that in the bovine testes miotic division of germinal epithelium occurs at about 3 to 3½ months of age, the sperms appearing in the lumen of the seminiferous tubules at the age of 7 months. Phillips and Andrews (1936) detected the presence of loosely arranged row

of spermatogonia with occasional spermatocytes even at the age of 63 days in Guernsey, Holstein, Short-horn and Hereford bulls. They observed abundance of spermatocytes in all the tubules between 224 and 226 days of age. Mann et al. (1949) recognised spermatogonia, and both primary and secondary spermatocytes in the case of bull calves aged 6 months. Spermatids and sperm were seen at 12 months. Knudsen (1954) has recorded evidence of the commencement of spermiogenesis by 7 months of age in SMB bulls. Fossland (1954) could identify spermatozoa in the lumina of Jersey and Holstein bulls at 9 and 10 months of age, respectively. Abdel Raouf (1960) contended that bovine testis shows solid sex cords without differentiation of intratubular cells upto the age of 8 weeks, proliferation of the cells commencing from 8th week onwards. By 20th week, spermatogonia can be identified. In certain tubules, primary spermatocytes can be seen as early as 20 weeks of age. The formation of spermatids and spermatozoa takes place at about 32nd week of age. May et al. (1961) spotted the presence of spermatozoa in the lumen of seminiferous tubules at 9 months of age in Friesian bulls. Gier and Marion (1970) found in one month old bovine testes supporting cells and gonocytes within the seminiferous cord. Further, primary and secondary spermatocytes were seen formed by 3rd and 5th month, respectively and spermatozoa at the age of 8 months. Godinho (1970) has reported the presence of spermatozoa

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in Brazilian bull calves after 60 weeks of age. Igboeli and Makhe (1971) noticed spermatozoa in the lumen of the tubules at 73 weeks in Angoni bull calves. Aire and Akpokodje (1975) identified in White Fulani bulls primary spermatocytes between 36 and 44 weeks of age and spermatozoa for the first time at 48 weeks. Mature spermatozoa could be seen in all the tubules at 60 weeks of age.

It has been shown by several authors (Kibler, 1943; Asdell, 1946; Watson et al., 1956; Abdel Raouf, 1960; McFee and Eblen, 1967; Skinner et al., 1968; Courot et al., 1970; Courot, 1971) that the initiation of spermatogenesis is more related to and better influenced by body weight than by the age of the animal.

Michatsch (1933) observed Sertoli cells in bovine testis at the age of 5-5½ months. The process of transformation of supporting cells to Sertoli cells was studied in detail in SRB bulls by Abdel Raouf (1960). He concluded that the maturation of indifferent supporting cells takes place at the age of 28 weeks and all the indifferent cells are transformed into Sertoli cells, by 40 weeks the number of supporting cells being found to increase prior to the transformation. No cell division occurs once the Sertoli cells are formed. Attal and Courot (1963) also have reported mitosis of the supporting cells only upto the period of their

transformation to Sertoli cells. Compiling the data from different sources, Gier and Marion (1970) assessed 5 months as the age at which supporting cells are transformed to Sertoli cells.

Intertubular Cells:

The presence of Leydig cells in the foetal testes has been identified in many vertebrates (Gillman, 1948; Roosen-Runge and Anderson, 1959; Gier and Marion, 1969; 1970; Hooker, 1944). Conflicting views have been put forth on the origin of Leydig cells. It is now generally accepted that the mesenchymal cells seen between the sex cords transform themselves into the cells of Leydig (Mahmoud, 1969; Hooker, 1944).

Leydig cells were detected in 30 mm bovine embryo by Basson (1923), who observed that the number of cells is decreased at the time of birth and then increased during the postnatal life. The process of metamorphosis of mesenchymal cells into the cells of Leydig has been described by Hooker (1944). Abdel Raouf (1960) has reported that the progressive metamorphosis of mesenchymal cells present within the intertubular space takes place by 6 weeks after birth in SAB bull calves, with gradual reduction in the intertubular space along with an increase in the number of fully metamorphosed Leydig cells with advancing age. Gier and Marion (1970) detected the presence of fully matured Leydig cells in 8 months old bovine testes.

Ductus Epididymidis:
Postnatal growth and development:

The pattern of prepuberal growth of the ductus epididymidis in bulls was reported to be sigmoid just as that of the testis (Abdel Raouf, 1960). Brigatti (1951) reported that in bulls epididymal growth is not related to either age or body weight of the animal. Abdel Raouf (1960) observed that epididymal weight was significantly correlated to testicular weight at different ages in SRB bulls. Positive correlation between the weight of the epididymis and testicular weight has been recorded in rams (Terrill, 1968), goats (Yao and Eaton, 1952; Marshan, 1975), and boars (Thomas, 1973). In SRB bulls, the right epididymis was found to be heavier than the left at all ages (Abdel Raouf, 1960).

Histological Changes:

It has been shown (Trautmann and Fiebiger, 1952; Sisson and Grossman, 1953; Blom, 1968; Gier and Marion, 1969, 1970) that the ductus epididymidis originating from the mesonephric duct gradually develops into a single tortuous tube with three grossly distinct regions, viz. the caput, the corpus and the cauda. The entire tube is lined by pseudostratified ciliated columnar epithelium and surrounded by varying layers of muscle fibers. Due to the extensive coiling, the duct in all the regions forms lobules separated by collagenous leaflets arising from the tunica albuginea (Trautmann and Fiebiger, 1952)

The prepuberal development of the ductus epididymidis has been studied in goats (Harshan, 1975), boars (Thomas,1973) and bulls (Abdel Raouf, 1960). It has been reported by Abdel Raouf (1960) that there is a gradual increase in the diameter of the duct and lumen at all the three regions with advancing age, distinct regional differences being noticed in respect of the diameter measurements. The diameter was found to be greatest in the caudo, lesser in the corpus and least in the caput. The regional differences in the size of the duct and lumen seen at birth were discernible throughout the growth period. Similar observations have been made in rams (Carmon and Green, 1952), goats (Harshan, 1975) and boars (Thomas, 1973).

Abdel Raouf (1960) has observed that as age advances there is a proportional increase in the height of epididymal epithelium. Also, there is a gradual change in the type of epithelium, from simple columnar to pseudostratified. Wide variations were noted by the author in the degree of transformation of epithelial cells not only between the regions but also between the different locations within the same region. It was further observed that the process of transformation was completed earliest in the caudo, then in the corpus and last in the caput. Regional differences in the height of epithelium were evident. At birth the epithelium had maximum height in the caudo, next in the caput and then in the corpus.

At adult-hood, the tallest epithelium was observed in the caput and the shortest in the cauda. An interesting observation that was made was the completion of differentiation of pseudostratified epithelium much before the attainment of adult height in a particular region. While the cell differentiation was found completed in all the regions at 32 weeks of age, the height of the epithelium continued to increase even upto the age of 48 to 52 weeks. Stratification of epididymal epithellum with regional variations has been reported in rams (Carmon and Green, 1952); goats (Yao and Eaton, 1954; Harshan, 1975) and boars (Thomas, 1973).

Abdel Raouf (1960) has reported the presence of spermatozoa in the epididymis at the age of 32 weeks in the case of SNB bulls. In White Fulani bulls, mature sperm could be seen in all the three regions of epididymis between 60 and 64 weeks of age (Aire and Akpokodje, 1975). Bialy and Smith (1958) reported that cauda epididymis contained 45.42 per cent, corpus 18.34 per cent and caput 36.24 per cent of the total sperms counted in the epididymis. The authors speculated that the number of sperms present in the caput reflects the spermatogenic rate and that in the cauda, the rate of sperm production as well as the rate of sperm elimination. Abdel Raouf (1960) has reported that sperm density is greater in the cauda than in the corpus or in the caput and this feature is attributable to the differences in the rate of passage of

the sperms through the three regions. Mason and Shaver (1952) in human and Niesander (1957) in rabbits had observed that the passage of testicular products through the ductuli efferents and the first coils of the ductus epididymidis was considerably more rapid than that through the more distal segments of the ductus system.

Semen Characteristics:

Attempts made to evolve an accurate and objective test for assessing the potential fertility of a bull on the basis of some specific characteristics of a given semen sample have not been successful. The consensus of opinion prevailed was that a combination of such characteristics as ejaculate volume, initial motility, concentration and livability of spermatozoa and the magnitude of incidence of spermatozoan abnormality will serve the purpose of a single sure test to select bulls of high fertility from a mixed herd (Maule, 1962). Work carried out and the various and varying observations made on each of these factors of semen quality are briefly indicated below:

Ejaculate volume: Anderson (1945) found no relationship between ejaculate volume and fertility of the bull. On the other hand, Bishop et al. (1954) recorded evidence of a decline in the rate of fertility with increasing volume of

semen per ejaculate. A genetic basis for the ejaculate volume was recognized by Tomer et al. (1965) who reported a repeatability estimate of 0.431 for this character in the case of Hariana bulls. The volume of semen per ejaculate has been reported to vary not only between breeds but also between bulls within the same breed (Roberts, 1971).

The average ejaculate volume in the case of bulls of exotic breeds appears to be 4.0 ml (Anderson, 1945). The mean volume of semen per ejaculate in different breeds of Bos taurus has been reported as 3.40 ml by Salisbury (1944) and as 8 ml (2-15 ml) by Almquist et al. (1963). In the case of Brown Swiss bulls, Mathew (1974) recorded 6.5 ml as the mean volume of semen per ejaculate. In respect of the Indian cattle, Bhattacharya and Prabhu (1954) reported 3.52 ml, 4.71 ml and 5.70 ml as the ejaculate volume in Tharparkar, Red Sindhi and Gir bulls, respectively. As regards Hariana and Sahiwal bulls, the per ejaculate volumes noted were 4.38 ml and 4.37 ml, respectively (Tomar et al., 1964). Rao and Rao (1980) found 4.19 ml as the mean ejaculate volume in Ongole bulls. In Brown Swiss crosses with 75%, 62.5% and 50% exotic inheritance, the volume of semen per ejaculate was observed as 5.5 ml, 4 ml and 4.8 ml, respectively (Mathew, 1974). The mean ejaculate volumes in the case of Jersey-Sindhi (Rao and Kotaiyya, 1977) and Jersey-Sahiwal crosses (Saxena and Tripati, 1978) were found to be 3.65 ml and 5.16 ml, respectively and

the same in Brown Swiss-Ongole and Holstein Friesian-Ongole crosses were 4.83 ml and 4.17 ml, respectively (Rao and Rao, 1978).

The ejaculate volume has been reported to vary with increasing age and body weight of the animal. According to Zaliani (1957), maximum semen volume is obtained at the age of six years in Brown Swiss and at eight years of age in Friesian bulls. The average volume of 2 successive ejaculates is stated to be 7.04 ml at two years of age and 12.31 ml at seven years of age in the case of Bos taurus bulls (Povlicenko, 1964). A significant increase in ejaculate volume with advancing age has been observed in Angus and Hereford bulls (Almquist and Cunningham, 1967), and also in Brown Swiss-Ongole and Holstein Friesian-Ongole crossbred bulls (Rao and Rao, 1978).

Reports on the effect of season on ejaculate volume have been conflicting. Seasonal variations in the ejaculate volume have been observed by several workers (Erb et al.,1942; Mercier and Salisbury, 1947; Brown, 1959; Horie and Ishikura, 1964; Sinha and Prasad, 1966; Rao and Rao, 1975). Milicevic (1963) noted reduced ejaculate volume in bulls due to high temperature. Amann et al. (1966) reported that in Holstein bulls, the ejaculate volume was smallest during winter and early spring and highest during summer. Tomer et al. (1966) obtained a significantly higher volume of semen in Hariana

bulls during summer as compared with winter. The ejaculate volume was found to be low during summer in Tharparkar (Rao and Rao, 1975) and Ongole bulls (Rao and Rao, 1980). Bhosrekar et al. (1978) recorded higher ejaculate volume in Jersey and Holstein bulls during rainy and winter seasons. Swanson and Herman (1944), Mukherjee and Bhattacharya (1952) and Kodagali (1962) failed to observe any seasonal variation in the ejaculate volume of bulls.

Initial Motility: Assessment of the initial motility of spermatozoa is commonly made to measure the fertilizing ability of a given semen sample. The heritability and repeatability of the initial motility were estimated as 0.501 (Zelfel, 1964) and 0.40 (Tomar et al., 1965), respectively. Studies carried out by Lasley and Bogart (1943), Chang et al. (1949) and Cupps et al. (1954) to correlate initial motility with fertility yielded negative or indefinite results. Blom (1950) indicated that the low rate of motility of spermatozoa is invariably associated with infertility. Rao et al. (1979) reported positive correlation between initial motility of sperms and fertility, in crossbred bulls. Swanson and Herman (1944) expressed the view that good initial motility alone is not an accurate indication of fertility in bulls, since they did not find any significant difference in the conception rates obtained with semen samples having 50% actively motile spermatozoa and with those containing comparatively higher

percent of motile sperm cells. Motility below 50% was however, found to be often associated with low conception rate or poor fertility.

Lasley (1951) has reported that the average percentage of initial motility of sperms in the ejaculate of Bos taurus bulls is 51.6. Bishop et al. (1954), Brown (1959) and Almquist et al. (1963) have reported values as high as 63%, 63% and 65%, respectively. Rao and Rao (1975) observed 79.20 per cent of the spermatozoa showing progressive motility in Jersey bulls. In the case of Ongole bulls, the mean initial motility of sperms was reported to be 84.04% (Rao and Rao, 1980). The initial motility of sperms in Jersey-Sahiwal was observed to be 71.17% (Saxena and Tripati, 1978) and in Brown Swiss-Ongole and Holstein Friesian-Ongole, 84.13% and 80.80%, respectively (Rao and Rao, 1978).

Initial motility tends to increase with increasing age in exotic bulls (Dimitriev, 1964; Malberg, 1965). Highly significant correlation was observed between initial motility and age of the bull by Lindley et al. (1959). Singh et al. (1967) found improvement in the initial motility of sperms in Hariana bulls when they attained 3 years of age. Maslov (1960) did not observe any difference in the initial motility of sperms between semen samples collected from bulls of different age groups.

Initial motility has been found to vary with season of semen collection both in exotic breeds of cattle (Swanson and Herman, 1944; [illegible] and Bhattacharya, 1952; Johnston and Branton, 1955; Vadrali, 1962; Tomar et al., 1966 and Rao and Rao, 1977). In almost all cases, higher initial motility was obtained during spring season. Humid hot season does not appear to be conducive for production of semen with high motility sperms. Roussel (1954) noticed a decline in initial motility, when the bulls were exposed to incandescent light. Rao and Rao (1980) found no significant variation in the initial motility of sperms between monthly collections in Ongole bulls.

Initial motility is positively correlated with sperm concentration (Tomar et al., 1966) and with live normal percentage of sperms (Stone et al., 1950; Bishop and Hancock, 1955; Stereo, 1956; Tomar et al., 1966; Singh et al., 1968). Similar correlations with cold shock resistent percentage of sperms (Tomar et al., 1966), impedance change frequency (Rothschild, 1950) and fructolysis rate (Bishop and Hancock, 1955) have also been worked out. Sperm motility has been found to be negatively correlated with pH (Anderson, 1942; Reid et al., 1948), methylene blue reduction time (Beck and Salisbury, 1943; Branton et al., 1952) and resazurin reduction time (Arb et al., 1950). Ing (1949) and Hollinson (1951) have reported that

initial motility is correlated with the percentage of abnorm
sperms, particularly those with defective mid piece and tai

Sperm Concentration: Writing in as early as 1925, Williamsand Savage (1935) expressed the view that the total sperm count would serve as a valuable index for the detectio of male infertility. According to Cupps et al. (1954) and Bishop et al. (1954) the measurement of sperm concentration alone is not so much of practical value in assessing the potential fertility of the semen samples used for routine insemination work. Cummings (1954) found no change in the conception rate with initial spermatozoal concentration var from 2.5 to 19X10^6 per ml.

The concentration of spermatozoa in the case of fert bulls has been found to vary from 300 to 2000 millions with an average of 800 millions per ml of semen (Legerlof, 1954). The mean concentration of sperms for the first and second ejaculate in the exotic breeds of cattle has been shown as 1.239 and 1.281 million per cmm, respectively (Salisbury,19 The sperm count in Brown Swiss bulls has been found to be around 1,396 millions per cmm (Mathew, 1974). The concentra tion of spermatozoa per ml of semen in the case of Jos taur bulls has been reported as 873 millions by Anderson (1948), 1108 millions by Blom (1950), 1388 millions by Stone et al. (1950) and 1296 millions by Rao and Rao (1975). The mean

sperm concentration in the ejaculate of Hariana, Sahiwal, Tharparkar, Red Sindhi and Gir bulls is found to be respectively 1.284, 1.426, 1.269, 1.729 and 1.674 millions per cmm of semen (Paul et al., 1966). Rao and Rao (1980) found the mean sperm concentration in the ejaculate of Ongole bull as 765.90 million per ml. The sperm count in Brown Swiss crossbred bulls with 75%, 62.5% and 50% exotic inheritance has been observed as 1320, 1502 and 1408 millions, respectiv per ml of semen (Mathew, 1974). A sperm count of 1472 milli per ml of ejaculate has been reported in Jersey-Sindhi cross bulls by Rao and Kottayya (1977) and 912-33 millions per ml Jersey-Sahiwal crosses by Saxena and Tripati (1978). Rao an Rao (1978) have recorded 984.93 and 611.84 millions sperms per ml of semen in the case of Brown Swiss-Ongole and Holst Friesian-Ongole crosses, respectively.

In the opinion of Lapard et al. (1941), Maslov (1960) and Singh et al. (1967), the age of the bull exerts no influence on the sperm density of semen. Laurens and Negiere, (1964) found that the total output of sperms in adult bulls was high. A gradual increase in the sperm concentration wit advancing age has been recorded in bulls by Almquist et al. (1963), Rao and Rao (1978) and Rao et al. (1979).

Significant seasonal variations in the sperm count of Bos taurus and Zebu bulls have been observed by several work (Erb et al., 1942; Mukherjee and Bhattacharya, 1958; Tomar

et al., 1966; Andreev, 1971; Holy, 1971; Igboeli and Makhe, 1971). The sperm concentration was found to be lowest in the hot season and highest in the cold season (Kodagali,1962). Morie and Ishikura (1964) found highest sperm count during June-July and lowest during September-October. Stone et al (1950) attributed the reduction in sperm concentration in bulls to sudden temperature variations associated with high relative humidity. Ames et al. (1966) observed that the sperm count in the case of Holstein bulls was lowest during late summer and early winter and highest during early summer and late spring. It was further observed that the production of sperm was maximum during the period of rapidly increasing day length. Nishikawa et al. (1966) reported negative correlation between sperm count and environmental temperature. Holy (1971), while working with Holstein-Friesian and Brown Swiss bulls in Cuba under subtropical conditions, reported adverse effect of hot season on sperm production. The effect was noted to be comperatively more severe in the case of Holstein Friesian bulls. Bhosreker et al. (1978) obtained higher sperm count in rainy and winter seasons as compared with summer. In marked contrast, Salisbury (1944), Johnston and Branton (1953), Rao and Rao (1975) and Rao and Rao (1980) did not notice any significant seasonal influence on the sperm count.

Livability of Spermatozoa: The livability of spermatozoa is generally used as a criterion for testing the fertility of semen. Lasley et al. (1942) and Lasley and Bogart (1943) have reported that the semen samples containing less than 50% live sperm are of doubtful fertility. Madden et al. (1947) could not find any significant difference in the percentage of live spermatozoa between semen samples which effected conception and which did not. Similar observations were made also by Stone et al. (1950). Erb et al. (1950) and Cupps et al. (1954), however, found a relation between live sperm count and percentage of fertility. Even though, Bishop et al. (1954) were unable to find any correlation between the number of live spermatozoa per insemination and fertility rate, they observed a significant relationship in this regard between the percentage of dead sperm in the ejaculate and fertility. Campbell et al. (1960) failed to demonstrate any correlation between the frequency of occurrence of dead sperm and fertility. According to Roberts (1971), a high incidence of necrospermia is usually associated with poor motility and low fertility.

The average percentage of live spermatozoa in the semen of Bos taurus bulls has been reported to be 77.9 by Bishop et al. (1954) and 70.1 by Bratton et al. (1956). Rao and Rao (1975) found the value to be 85.15 in Jersey bulls. The percentage of live sperm in the ejaculate of Hariana bulls

has been recorded as 76.0 by Tomar et al. (1966) and 80.6 by Singh et al. (1967). In the case of Ongole bulls, it was found to be 88.22% (Rao and Rao, 1980). The percentage of live sperm in the semen of Mariana X Holstein Friesian, Mariana X Brown Swiss and Mariana X Jersey was found to be 86.34, 87.28 and 87.28, respectively by Biswas et al. (1976). The live sperm count in the ejaculate of Holstein Friesian-Ongole and Brown Swiss-Ongole bulls was reported as 85.20% and 87.22%, respectively by Rao and Rao (1978). Saxena and Tripati (1978) recorded 83.07% live sperm in the semen of Jersey-Sahiwal crossbred bulls.

In the opinion of Singh et al. (1967), the bulls over 3 years of age are likely to produce semen with higher percentage of live spermatozoa. Tomar (1970) has stated that the age of the bull has no effect on the occurrence of the live sperm count in the ejaculate.

Significant seasonal variations in the percentage of live sperm per ejaculate have been observed in exotic and Zebu bulls by several workers (Lasley and Bogart, 1943; Tomar et al., 1966; and Rao and Rao, 1975). According to Tomar et al. (1966), the percentage of live sperm is low during hot humid season. Rao and Rao (1980) did not observe any seasonal effect on the frequency of occurrence of live sperm in the ejaculate of Ongole bulls.

Abnormal Spermatozoa: There is divergence of opinion as to the importance of the presence of varying number of abnormal sperms seen in stained semen preparations. Williams and Savage (1925) and Lagerlof (1934) observed lowered fertili with more than 17% of abnormal spermatozoa in the ejaculate. Similar observations were made by Davis et al. (1940) and Anderson (1941). Blom (1948) recorded impaired fertility with bull semen containing more than 15% of sperm with primary abnormality. Herman and Swanson (1941) mentioned that presence upto 30% abnormal sperm is compatible to good or poor fertilit Cupps et al. (1954) found a high correlation between abnormal sperm count and fertility. A negative correlation between abnormal sperm count and fertility was noted in Holstein Friesian-Ongole and Brown Swiss-Ongole crossbred bulls by Rao and Rao (1979). Laing (1945) was unable to demonstrate any relationship between the number of abnormal spermatozoa and the conception rate in natural mating. Rollinson (1951) found no clear correlation between the incidence of abnormal spermatozoa and fertility. Rottenstein and Anderson (1956) stated that a high level of abnormal sperms is not necessarily indicative of low potency for fertility.

Haq (1949) reported an average of 15.1% of abnormal sperm within a range of 6-26% in the case of normal fertile exotic bulls. Blom (1950) recorded 1.2 to 10.4% with a mean of 4.6% primary abnormality in fertile semen samples. Haq(194

and Rollinson (1951) are in near agreement that the normal fertile bulls should not have more than 3 to 4% abnormal head, 4 to 10% abnormal midpiece, 0.5 to 2% abnormal tail and 0.5 to 6% free loose head. Bishop et al. (1954) reported a mean incidence of 6 to 10% abnormal forms excluding bent tails in normal Bos taurus bulls. In the opinion of Hancock (1959), the presence of 10% or more of any single type of abnormality is often associated with reduced fertility. An average of 14.1% of abnormal sperms was recorded in Hariana cattle by Tomar et al. (1968). Rao and Kotfayya (1974) reported 10.6%, 11.46%, 38.95% and 13.26% as the total abnormality of sperm in the ejaculate of Jersey, Guernsey, Jersey X Ongole and Brown Swiss X Sahiwal bulls, respectively. The frequency of occurrence of head abnormalities, free loose head, proximal protoplasmic droplets, midpiece and tail defects in Jersey bulls was noted as 11.74%, 3.75%, 2.20%, 1.20% and 15.47%, respectively by Rao and Rao (1975). The percentage of sperm abnormalities in respect of Hariana X Holstein Friesian, Hariana X Brown Swiss and Hariana X Jersey was observed to be 14.56, 13.09 and 16.34, respectively by Biswas et al. (1976). The mean percentage of abnormal sperm in Jersey X Sahiwal crosses was reported to be 18.93 by Saxena and Tripati (1978). The head abnormalities, free loose head, midpiece abnormalities, tail abnormalities and proximal protoplasmic droplets were respectively 9.46%, 2.97%, 0.74%,

3.02% and 2.86% in the case of Brown Swiss X Ongole crosses and 15.86%, 6.13%, 0.92%, 3.03% and 5.04%, respectively in the case of Holstein Friesian X Ongole crosses (Rao and Rao, 1978).

In Bos taurus bulls, Laurans and Negrier (1964) reported that the age of the bull is of little consequence in producing abnormal sperms. Malmberg (1965) on the other hand, observed a decrease in the proportion of abnormal sperms with an increase in the age of the bulls. Similar findings have been reported in Hariana bulls by Singh et al. (1967) and in crossbred bulls by Rao and Rao (1978) and Rao et al. (1979).

Phillips et al. (1943) observed significant seasonal variations in the total sperm abnormalities, the incidence of malformation being higher in summer than in winter. Johnston and Branton (1953) reported highest percent of sperm abnormalities during fall in Jersey bulls. Brown (1959) recorded the highest and lowest number of sperm abnormalities during summer and winter, respectively. Schroder (1963) found highest incidence of abnormal sperm in the semen samples collected during winter in Jersey bulls. Igboeli and Rakha (1971) noted 22% more of total sperm abnormality in summer than in winter. Rao and Rao (1975) also observed comparatively more number of abnormal sperms in the semen samples collected during summer

in Jersey bulls. Seasonal changes in the presence of abnormal sperms in the ejaculate of Zebu bulls have been reported by Mukherjee and Bhattacharya (1952). Rao and Rao (1960) observed significant seasonal variations in the occurrence of spermatozoan abnormalities in the ejaculate of Ongole bulls. Tomar et al. (1966) did not find seasonal effect on the abnormal sperm count in the case of Hariana bulls.

Freezability of Spermatozoa:

In as much as the successful conception depends great deal on the freezability and post-thawing survivability of sperms in the frozen semen used for artificial insemination, considerable attention has been paid to these aspects in recent years. The limited studies carried out in this regard and the observations made are briefly outlined below:

Variations in the ability of spermatozoa of different semen samples from different bulls to withstand the deep freezing processes have long been recognized (Rowson, 1953; Swanney, 1953). Dunn et al. (1954) found significant differences between bulls in regard to the ability of the spermatozoa to survive freezing. Holt (1953) and Rowson (1953) reported that with dense semen the survival rate of spermatozoa after freezing and thawing is lower than that with less dense samples. Ohms and Willett (1955) found that the second ejaculate gives a significantly higher recovery rate than

does the first ejaculate. The superiority of the second ejaculate in this respect has been credit to the spermatozoal cell and not to the seminal plasma (Willett and Ohms, 1958).

As with semen stored at 5°C, the prediction of the potential fertilizing capacity of a given sample of frozen semen is not readily possible. The assessment of post-thawing motility is considered as a reasonably accurate method for evaluating the fertility of frozen semen. However, divergent views have been expressed regarding the optimum temperature for thawing the frozen semen. Rowson (1953) was unable to demonstrate any significant difference in live-dead sperm counts in stained smears prepared from semen samples thawed in water at 100°C, 90°C and 5°C. Brugman and Schmidt (1958) found no significant difference in motility as between thawing at 5°C, 38°C and 40°C. On the other hand, Van Demark and Kinney (1954), Snyder et al (1955) and Bratton et al. (1957) obtained better results when thawing was done at 5°C. The results of Miller and Van Demark (1954) indicated that the thawing of frozen semen at 5°C helped revive higher percentage of motile spermatozoa as compared to 38°C. Further, the semen samples thawed at 5°C were found to retain motility longer when subsequently stored at the same temperature.

The freezability expressed in terms of percent post-thawing motility was found to be in the range of 32.0 to 36.9

in pure bred Brown Swiss bulls and 32.7 to 33.5 in Brown Swiss crossbred bulls with 62.5% of exotic inheritance (Mathew, 1974). A general tendency for an increase in freezability with increased rate of exotic inheritance was observed by Mathew (1974). The author has further reported that season has little influence on the freezability rate of sperms. The overall post-thawing motility in the ejaculates of exotic bulls maintained at the Centralised Semen Collection Centre, Hebbal, Bangalore has been reported to be 57.08% (Reddy et al.,1980). Sattar et al. (1980) recorded 59.10% and 63.45% of post-thawing motility for bull semen diluted respectively in egg yolk citrate and milk extenders.

Pre and Post-freezing Discard of semen:

According to the report of the Milk Marketing Board England for 1973-1974, the pre-freezing and post-freezing discard of the ejaculates are 23.4% and 4.2%, respectively. Mathew (1974) has reported that both pre-and post-freezing rejection rates are considerably high in Brown Swiss crossbred bulls. The average pre-freezing rejection of the ejaculates was observed to be 75%, 28%, 32% and 14% respectively for 50%, 62.5%, 75% crosses and purebred Brown Swiss bulls (Mathew, 1974). The corresponding value for the post-freezing rejection in these animals was observed by the author as 61%, 44%, 41% and 31% of all the frozen samples. Mathew (1974) concluded

that substandard semen quality led to higher rate of elimination. The pre-freezing and post-freezing rejection rates of the ejaculate in Jersey bulls have been reported to be 11.90% and 5.36% respectively against 13.5% and 5.99% respectively in Holstein bulls (Sattar et al., 1978).

Fertility Rate:

The conception rates with extended frozen semen in general, have been found to be slightly lower than those obtained with extended liquid semen (Roberts, 1971). Higher fertility rates with liquid semen than with frozen semen have been reported by Dunn et al. (1954), Henderson et al. (1956), Williamsand Green (1956) and Anderson et al. (1963). Emmerson and Martin (1957), Madden (1956) and Bratton et al. (1957) did not find marked differences in the fertility rate between 24 hour old, fresh and deep frozen semen samples. The overall conception rate in cows inseminated with deep frozen semen from Brown Swiss bulls in Kerala has been reported to be 40.18% (Nair, 1975). The fertility rate with frozen semen at All India Coordinated Research Project at Lam Farm, Guntur, Andhra Pradesh, has been reported to be 28% in Holstein Friesian, 28.9% in Brown Swiss and 28.8% in Jersey bulls (Annual Report, 1976-77). Sattar et al. (1978) did not find significant variation in the conception rate between chilled liquid semen (52.46%) and frozen semen (51.63%). The overall

conception rates in Sahiwal cows inseminated with frozen semen samples of Holstein-Friesian, Brown Swiss and Jersey bulls were reported to range respectively from 19.1 to 59.1%, 21.4 to 66.7% and 18.2 to 50.2% (Koul et al., 1979).

Variations in the conception rate between bulls have been recorded by Mathai et al. (1970) and Sattar et al. (1978). Rao and Rao (1978) observed that the fertility of Brown Swiss X Ongole bull was significantly higher than that of Holstein Friesian X Ongole bulls. Koul et al. (1979) did not find any significant difference in the rate of conception attributable to the breed of the bull.

Significant effect of season of insemination with fresh semen on the conception rate in cattle has been reported by several workers (Salisbury and Van Demark, 1961; Maule, 1962; Singh et al., 1965; Singh and Prasad, 1966; Tomar, 1964; Tomar et al., 1966; Ghosreker, 1973 and Mukherjee, 1973). The observations of these authors are, however, at variance with the findings of Kohli and Suri (1960), Nair (1975) and Koul et al. (1979) who obtained no significant difference in this respect.

Materials and Methods

Animals:

The experimental calves varying in age from 0 to 360 days (Table 1) sacrificed for the study of postnatal development of the testis and epididymis, the bulls of 22 to 40 months of age from which semen samples were collected with artificial vagina for assessing the semen characteristics and those employed for the determination of freezability, rejection rate and fertility rate, were all Brown Swiss crosses with 62.5% exotic inheritance reared and maintained at Mattupatty, Kerala, under the Indo-Swiss Project as per the feeding schedule furnished in Table 2.

Materials:

Twenty four pairs of testis-epididymis formed the material for the study of the postnatal development.

Four hundred and five semen samples collected from 7 bulls over a period of one year from July, 1979 to June, 1980 were used to assess the semen characteristics.

Six thousand six hundred and seventy nine collection particulars gathered from the records maintained at Indo-Swiss Project, Mattupatty provided the data for inferring the freezability of sperms and the pre-freezing and post-freezing discards of the ejaculates.

For determining the fertility rate, 7586 insemination data obtained from the cross breeding research centre, Muvathipuzha, Kerala, were made use of.

Methods:

Testes: Testes and epididymes were removed as one part immediately after slaughter. Within an hour of procurement, each testis was stripped off the tunics and epididymis and weighed to the nearest 50 mg using a monopan balance (Mettler). The length, breadth (mediolateral diameter) and thickness (cranio-caudal diameter), of each testis were measured using a pair of calipers. The circumference of the testis at mid-region was determined by means of a wire thread and a scale. All measurements were recorded in centimeters. The body weights of the animals were always recorded just before slaughter using a platform balance.

For histological studies, either the right or the left testis or epididymis, as the case may be, was selected from each animal in such a way as to get a more or less equal representation. After dividing the testis into two longitudinal halves, a slice of tissue of about 3 to 4 mm thickness was removed from the middle portion near to the rete testis, fixed in Bouin's fluid for 36 hours, dehydrated, cleared and embedded in paraffin as per the method described by Humason (1967). Sections of 5 μ thickness were then cut and stained

by Haemotoxylin-Eosin (regression method) following Hunason (1967).

Twenty clearly cut circular tubules from each testis, selected at random were examined under the microscope for size, process of lumen formation and morphological characters of germ cells and supporting cells following the method described by Abdel Raouf (1960). The size of the seminiferous tubule was measured to the nearest tenth of a micron using an eye piece micrometer scale calibrated against a stage micrometer. The stage of lumen formation was ascertained by noting the earliest age at which the lumen was first formed as also by the degree of lumen formation at different ages. The morphological characters of germ cells and supporting cells in the tubules were determined following the method of Abdel Raouf (1960) as detailed below:

The gonocytes were distinguished by their large size, irregular location in the centre of the sex cords, large nuclei with filamentous chromation and thin but distinct cytoplasm. The spermatogonia were identified as transformed gonocytes distributed in the parietal layer of the seminiferous tubules, having finely diffused chromatin material in the nuclei. The germ cells located central to the spermatogonial layer and having large spherical nuclei that increase in size in proportion to the progress of mitosis were consi-

dered as the primary spermatocytes. The secondary spermatocytes were spoted on the basis of their being located central to the primary spermatocytes, and also by the sizes of the nuclei which are intermediary between those of the primary spermatocytes and round spermatids. The round cells located central to the primary spermatocytes in two or more layers and having spherical nuclei were accepted as round spermatids and the deeply stained cells clustering on sertoli cells and having elongated nuclei as elongated spermatids. The cells having homogenously blue-violet stained head and rose-red stained tail with haemotoxyline-eosin, arranged in a regular ring fashion with head directed perpendicular to the basement membrane or as free in the lumen were reckoned as mature sperms. The cells with indistinct cytoplasmic boundaries and round nuclei containing deeply stainable granular chromatin, arranged in a line on the basement membrane of the sex cords were recognised as supporting cells. The cells with indistinct boundaries and having highly stained pyramidal or oval shaped large nuclei were distinguished as Sertoli cells. The large polygonal cells with round or oval nuclei with abundance of cytoplasm were differentiated as immature Leydig cells and those with spherical nuclei with less abundance of cytoplasm as mature Leydig cells.

Epididymis: The weight of each epididymis was recorded to the nearest 50 mg after transecting the vas deference at

a level with the middle of the corpus. The thickness of the epididymis at each of the three regions viz., the caput, the corpus and the cauda was measured using a pair of calipers.

For histological studies of the epididymis, tissue pieces representing the cross sections of the caput, the corpus and the cauda were removed and processed in the same manner as was done in the case of the testis. The sections were stained with haemotoxyline-eosin (Humason, 1967).

The diameter of the ductus epididymis in each of the three regions was measured using a micrometer scale calibrated against a stage micrometer. The height of the epithelium at each of the three regions was also determined. The process of differentiation of the epithelium from simple columnar to pseudo-stratified type was followed at the different regions.

Semen Chracteristics:

The semen samples were collected with Artificial Vagina Two successive ejaculates were taken with an interval of 5 minutes in between. The ejaculate volume was noted directly from the graduated collection vial. The initial motility of sperms was assessed by examining a small drop of undiluted semen immediately after collection under high power (X400) of a phase contrast microscope at 37°C. The motility was expressed in percentage. Sperm concentration was measured using a

calibrated photoelectric colorimeter. Five ml of 2.9% sodium citrate solution were taken in the colorimeter tube and placed in the photometer. After adjusting the transmission percentage to 100, 0.1 ml of undiluted semen was dropped into the photometer tube by means of Eppendorf microliter pippete. After mixing the contents thoroughly, the transmission percentage of the mixture was noted. The sperm concentration expressed in numbers per ml was determined by plotting the value against a reference standard. The percentage of live sperm was estimated by differential staining method using Nigrosin-Eosin stain as described by Blom (1950). The smears used for differential counts of live-dead spermatozoa were also made use of for assessing the head and middle piece abnormalities of spermatozoa. For observing tail abnormalities and proximal protoplasmic droplets, semen samples fixed in buffered formal saline (Hancock, 1957) were used.

The data on the different characteristics of semen were statistically analysed to determine the variations, if any, between bulls, between months/seasons and between ages of the bulls (Snedecor and Cochran, 1967). Based on the meteriological data gathered by the Indo-Swiss Project, Mattupatty over a period of 8 years from 1971 to 1978 (Fig.9) the year was divided into the following seasons for gauging the seasonal effect on these characteristics.

Season I (Cool - medium) - December and January

Season II(Moderate-Medium)- February to April

Season III(Moderate - high)-May to November

To determine the effect of age of the bull on the different characteristics, the animals were divided into 4 age groups as: below 24 months, between 25-30 months, between 31-35 months and over 36 months.

Freezability of Sperms and Pre-freezing and Post-freezing discard of ejaculates.

The semen samples, after initial evaluation, were diluted in Tris egg-yolk dilutor, filled in medium sized (½ ml) French straws and Frozen in liquid nitrogen vapour (rapid horizontal vapour freezing) using LR 250 container. The number of sperms per dose of semen was adjusted to 30 millions. The post-thawing motility at 37°C was recorded using a phase contrast microscope. Freezability of sperms was expressed in terms of per cent post-thawing motility.

The pre-freezing, post-freezing and total rejection rates of the ejaculates were recorded separately.

The data on the freezability and on the rejection rate of the ejaculates were subjected to statistical analyses to assess the effect of months/seasons of collection and age of the bull (Snedecor and Cochran, 1967). The grouping of month

into seasons was done in the same way as for the semen characteristics. The bulls were grouped as those aged below 2 years, 2-3 years, 3-4 years, and over 4 years.

Fertility Rate:

The fertility rate of the bulls was calculated by the following formula:

$$\text{Fertility rate} = \frac{\text{No. of animals conceived}}{\text{No. of animals tested for pregnancy}} \times 100$$

The data on the fertility rate were analysed to assess variations between bulls, between months/seasons of insemination and between the age groups of bulls (Snedecor and Cochran, 1967). To evaluate the seasonal effect, the year was divided into 3 seasons as indicated below following Singh *et al*. (1965).

Summer : February to May

Rainy : June to October

Winter : November to January

The bulls were divided into 2 age groups viz., 2-4 years and above 4 years.

Results

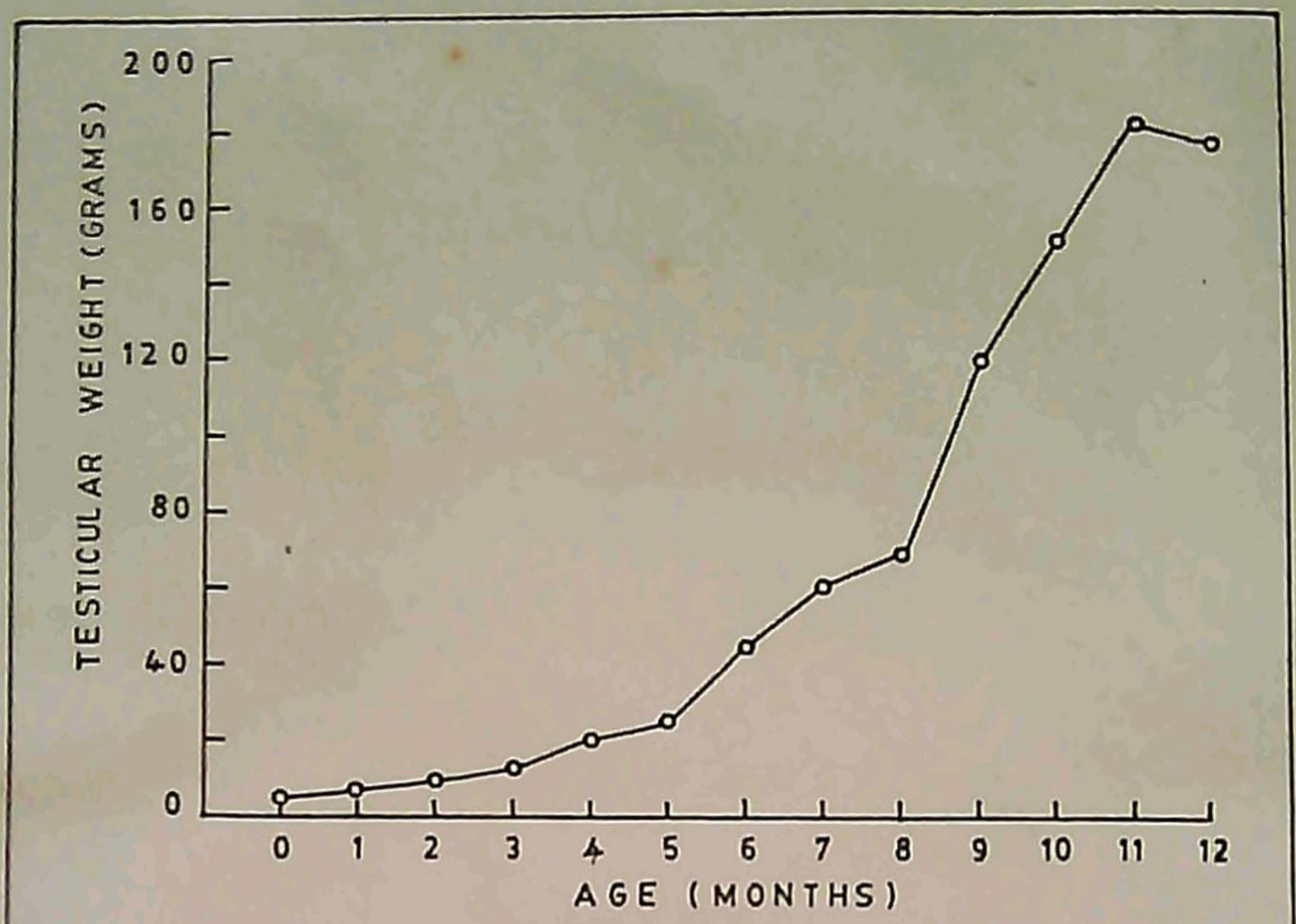

FIG.1. Testicular weight in relation to age

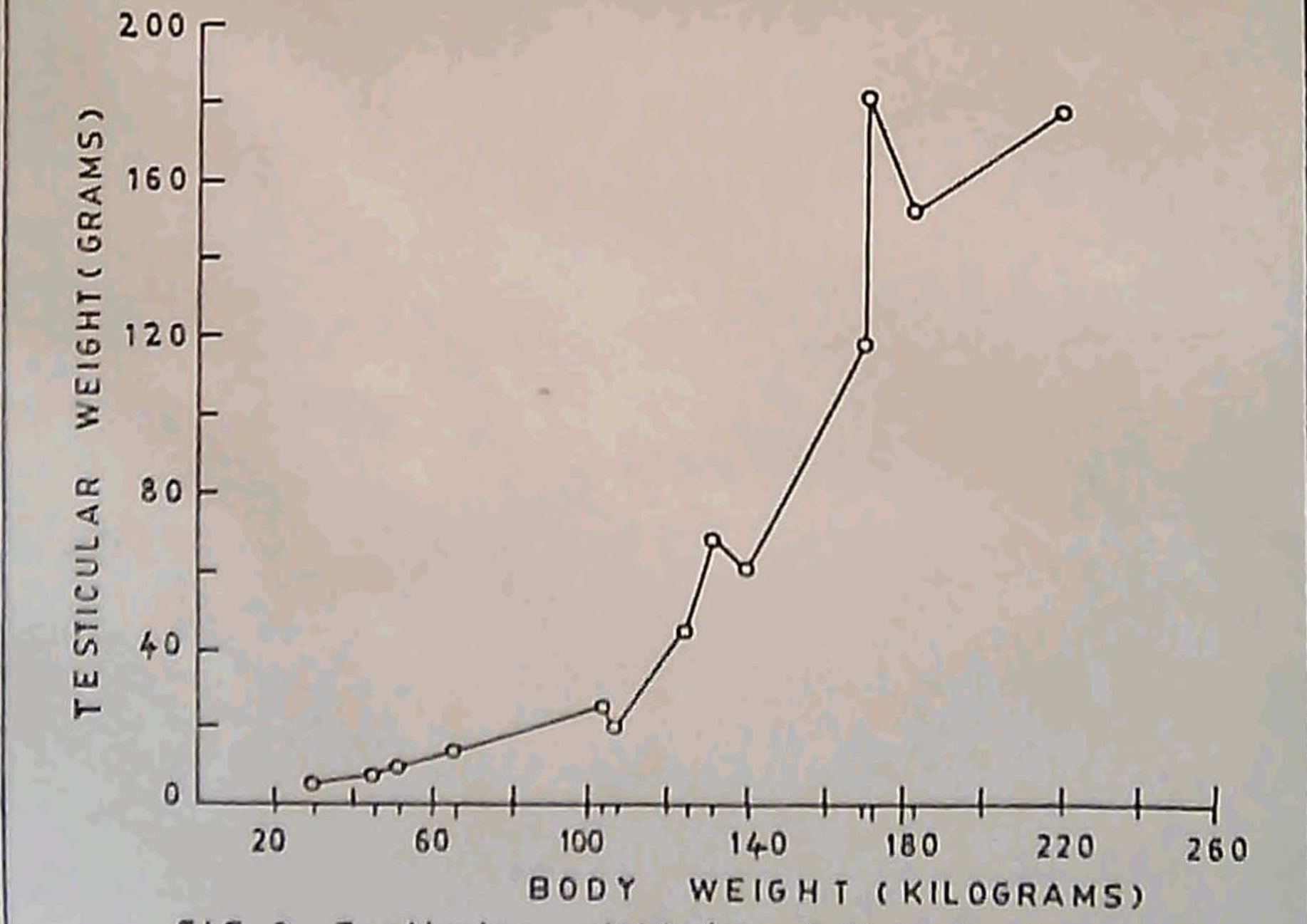

FIG.2. Testicular weight in relation to body weight

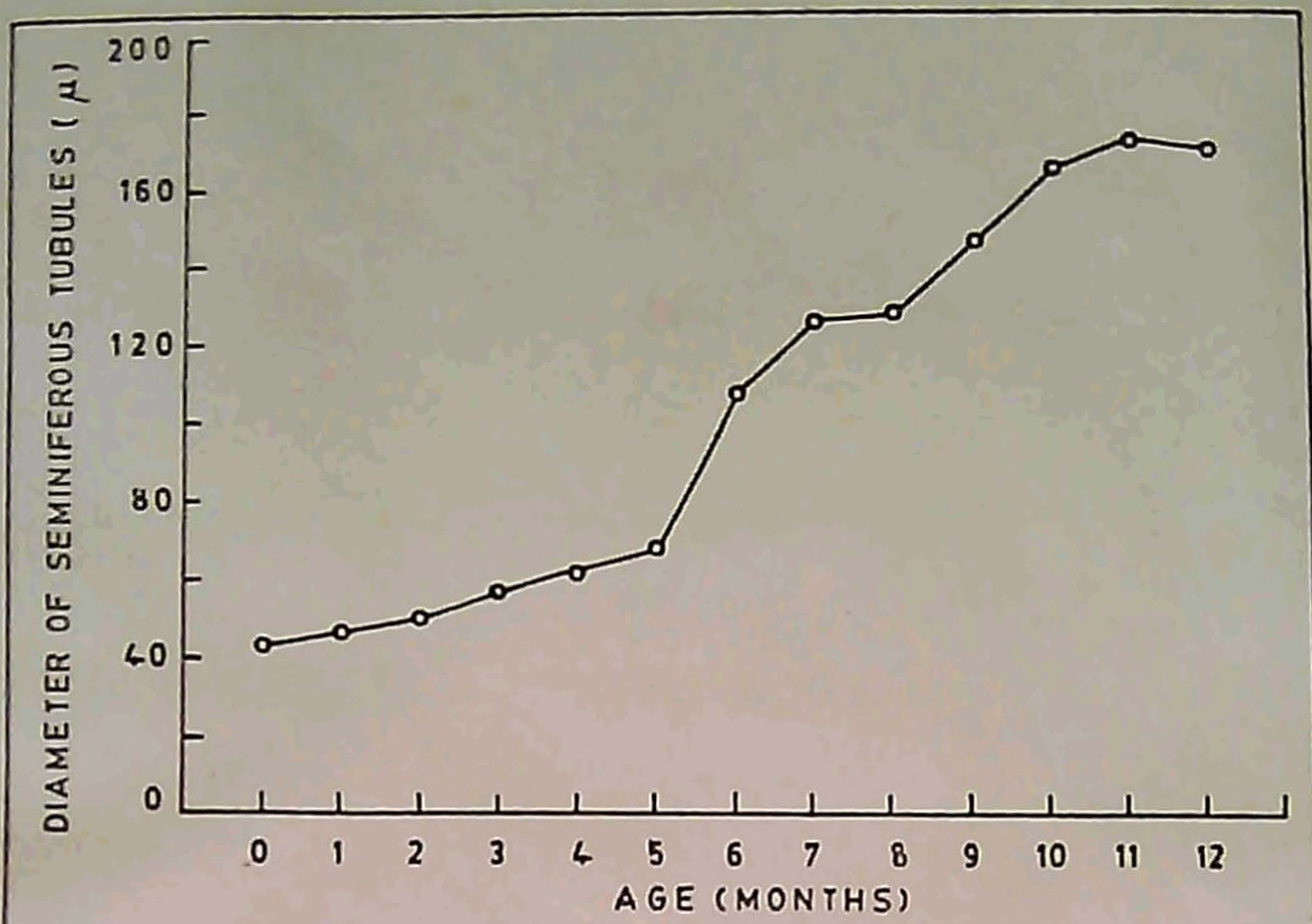

FIG. 3. Diameter of the seminiferous tubules in relation to age

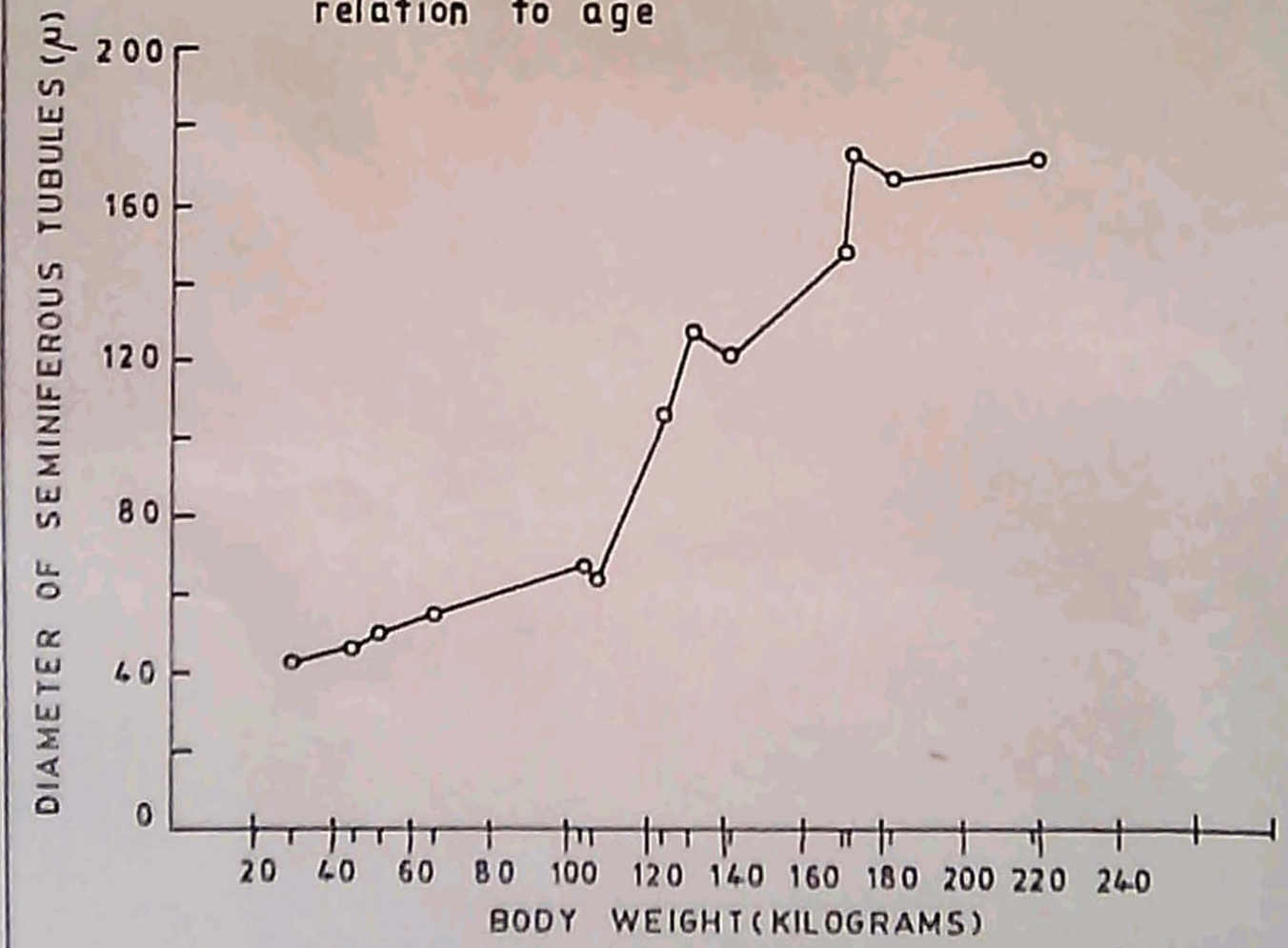

FIG. 4. Diameter of the seminiferous tubules in relation to body weight.

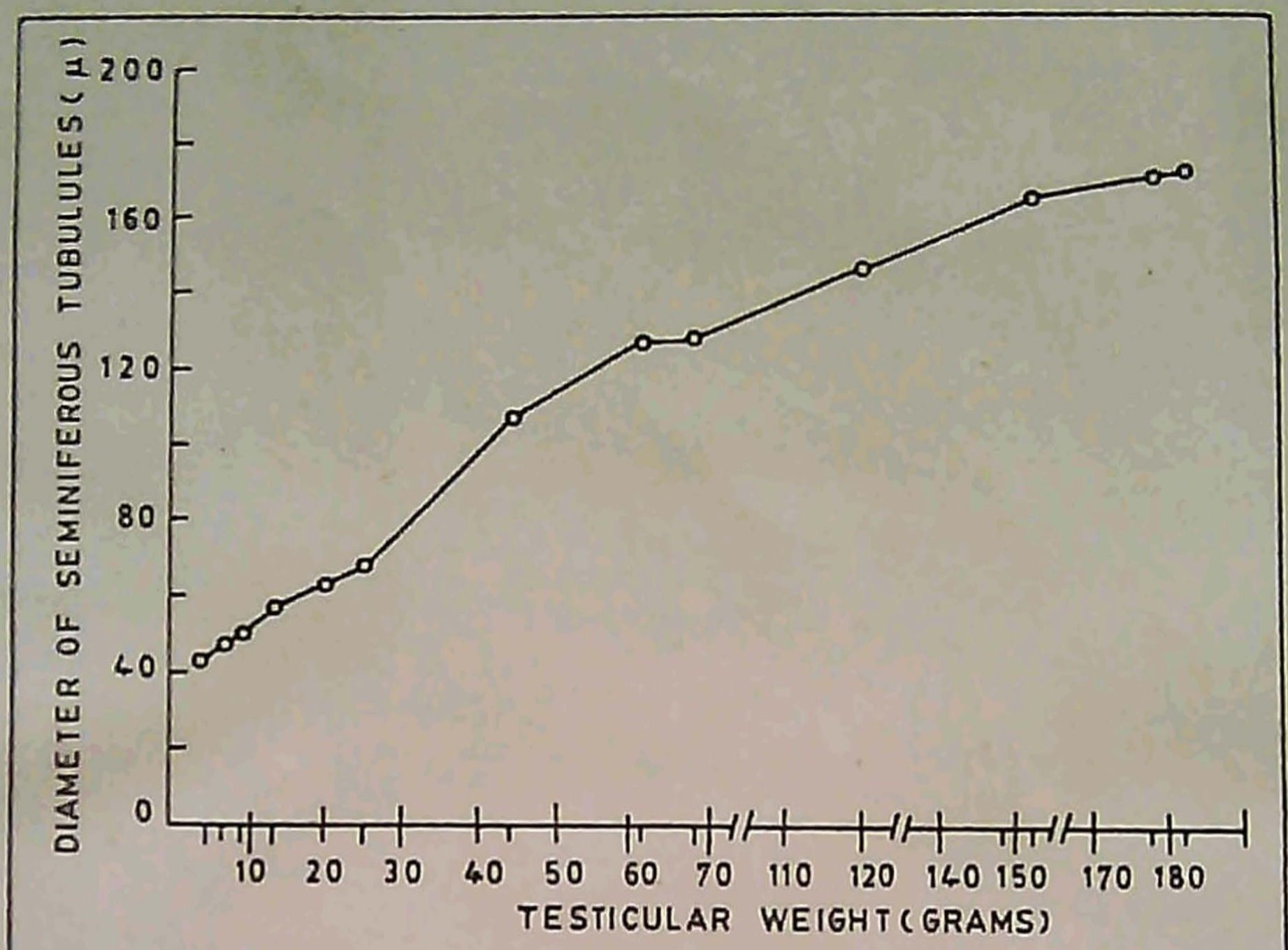

FIG.5. Diameter of the seminiferous tubules in relation to testicular weight.

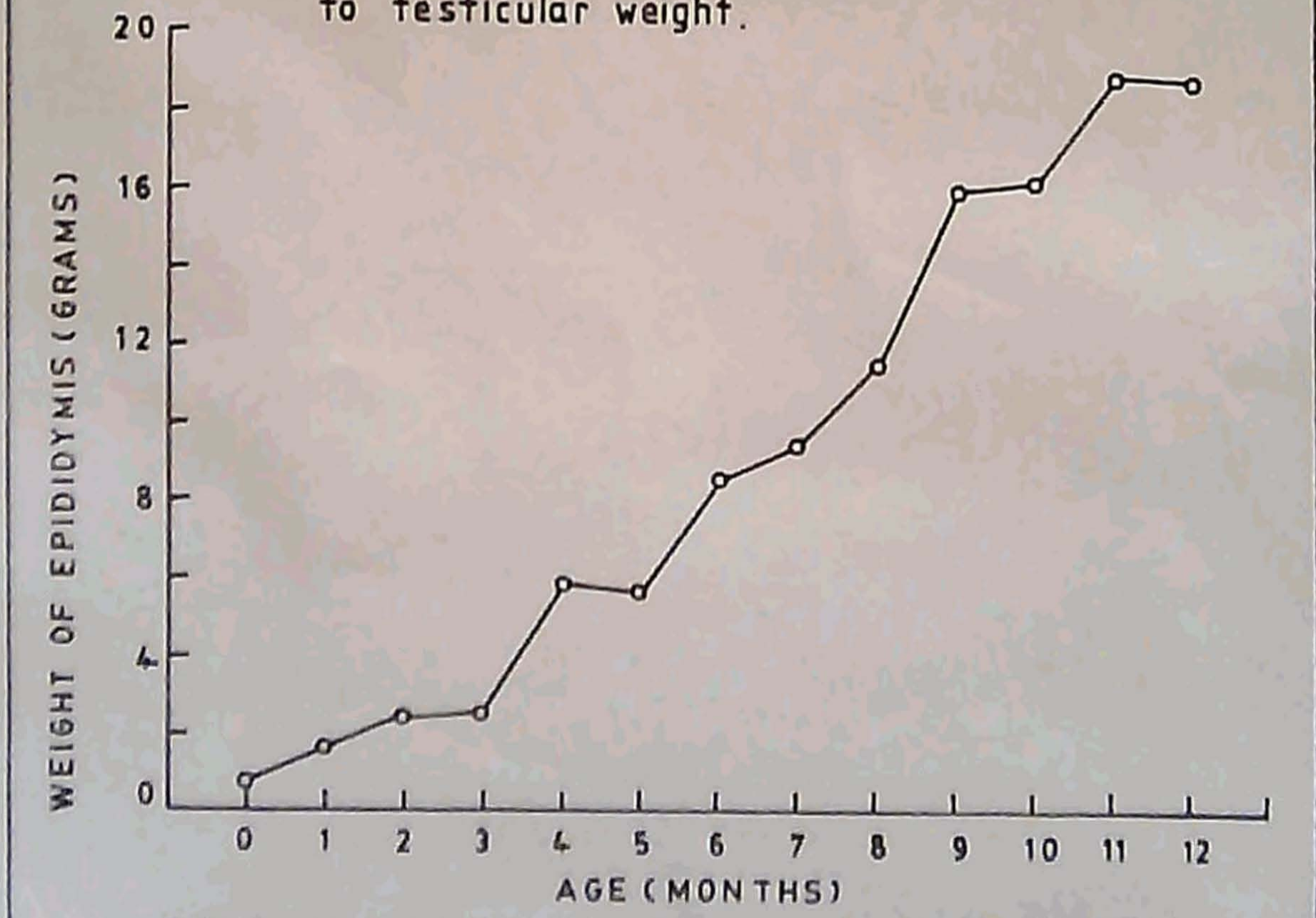

FIG.6. Weight of epididymis in relation to age.

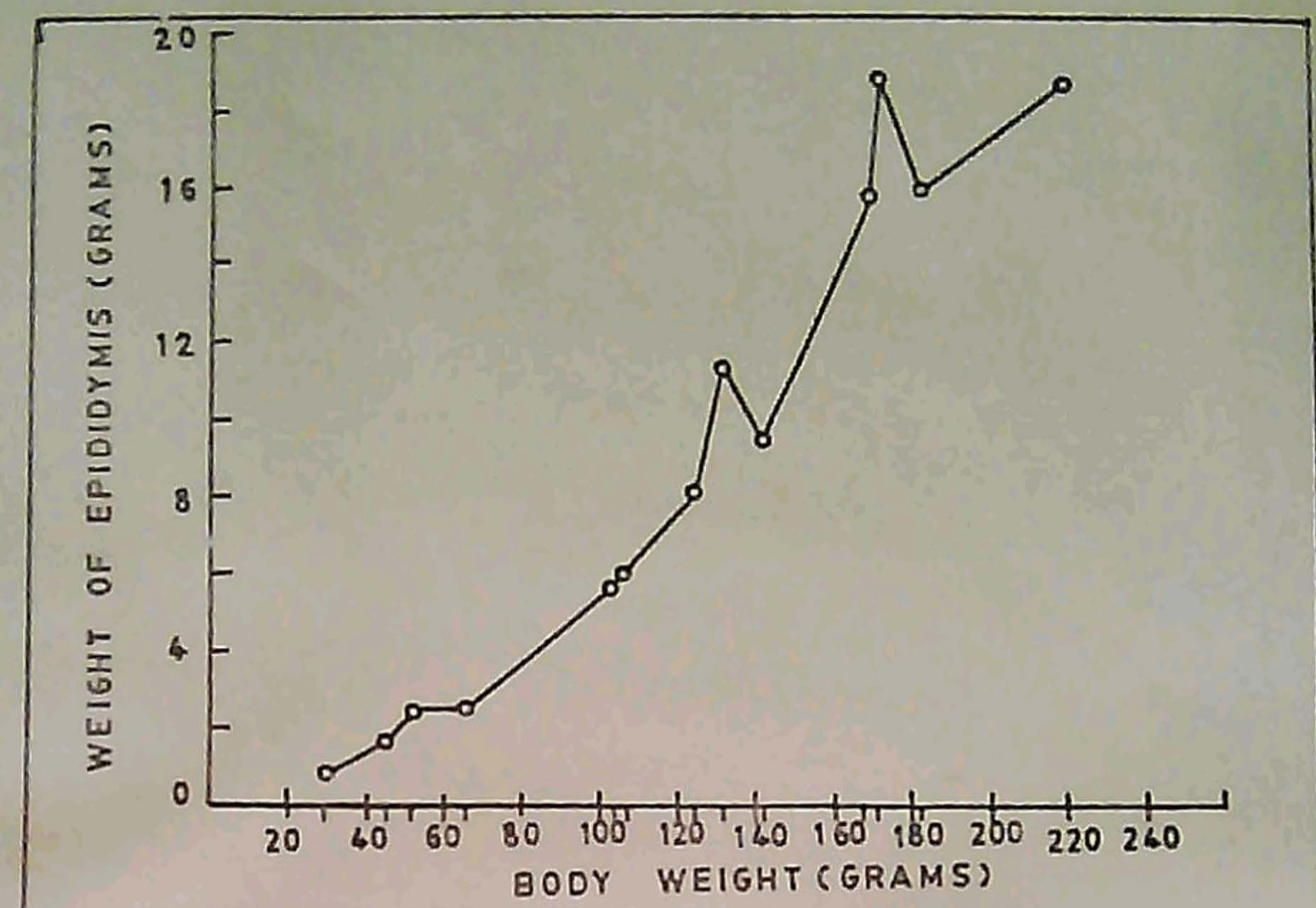

FIG.7. Weight of epididymis in relation to body weight.

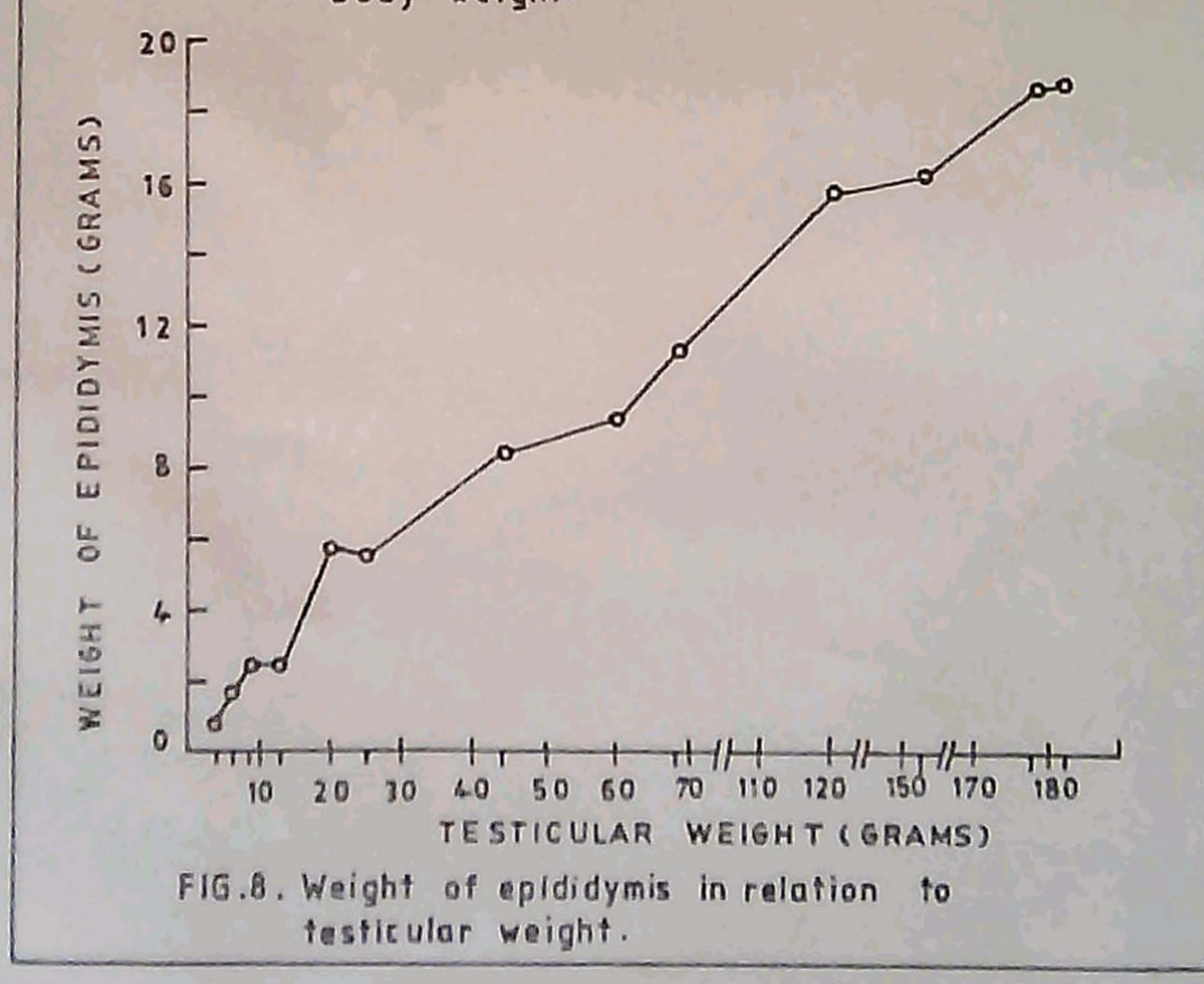

FIG.8. Weight of epididymis in relation to testicular weight.

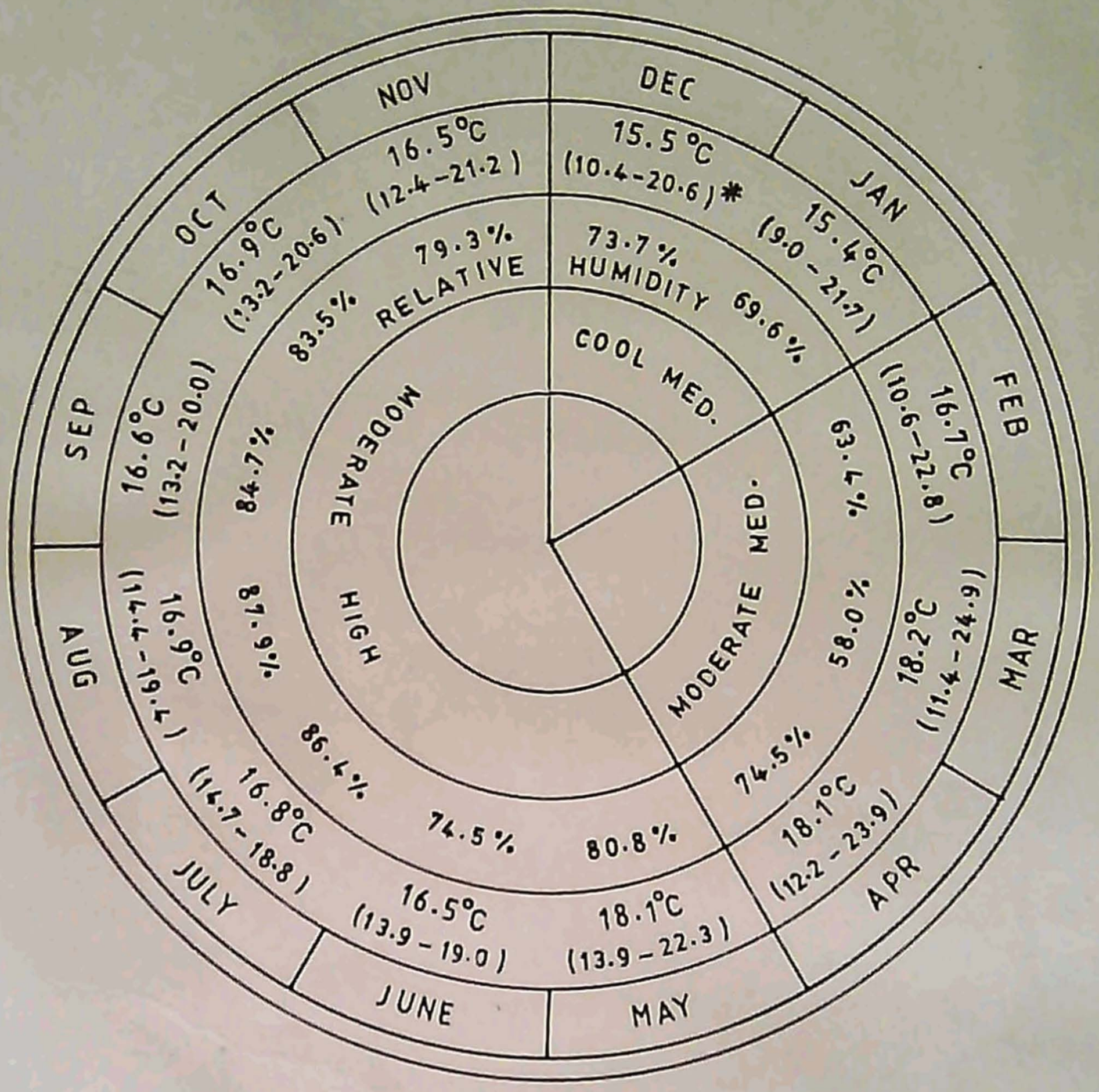

FIG.9. Schematic diagram of Macroclimate of Mattupatti (10°-10'N and 77°-0'E., 1700 MSL) based on data collected from Indo-Swiss project.

* In parenthesis - minimum and maximum temperature.

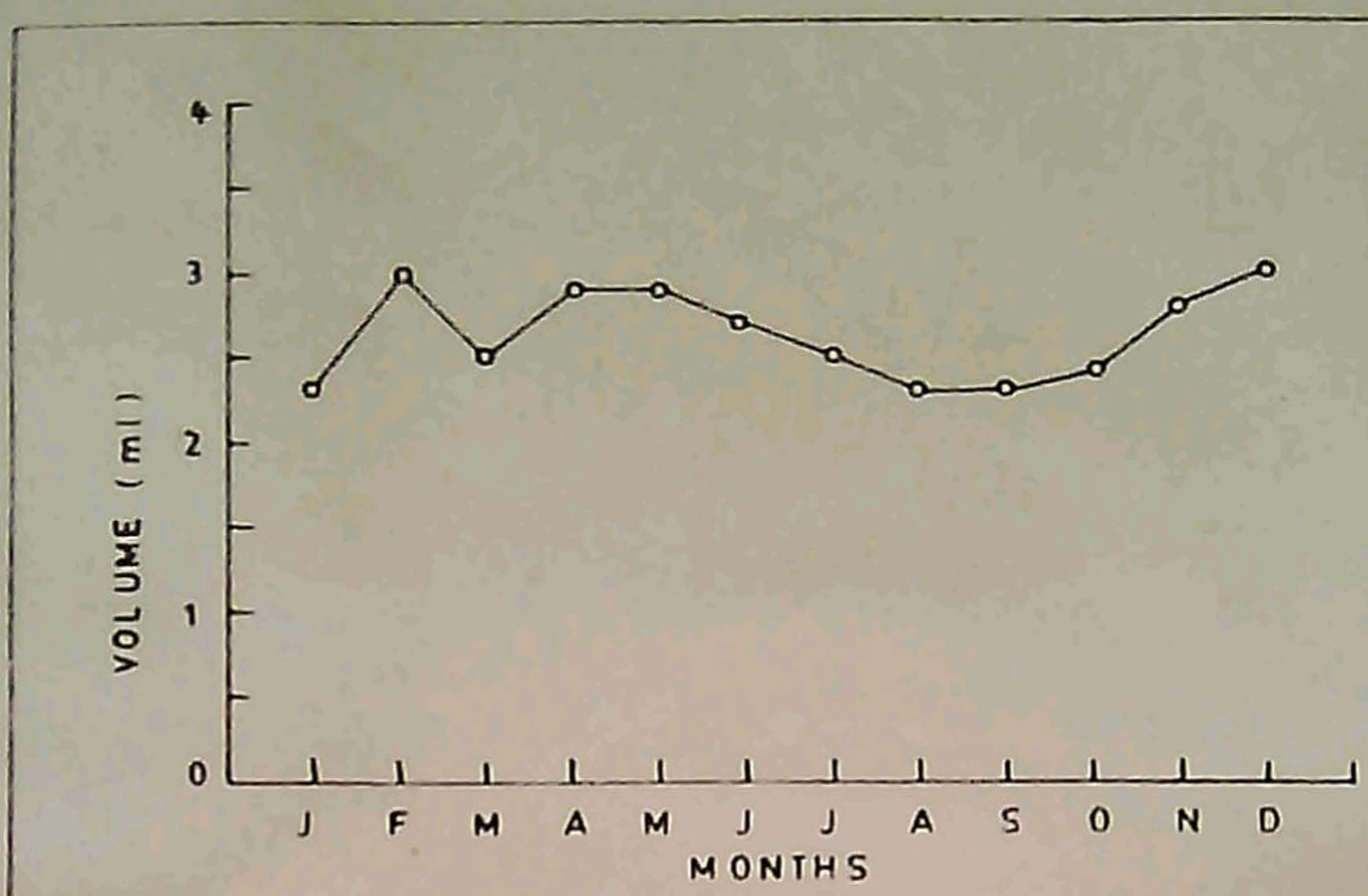

FIG.10. Ejaculate volume of crossbred bulls – month wise

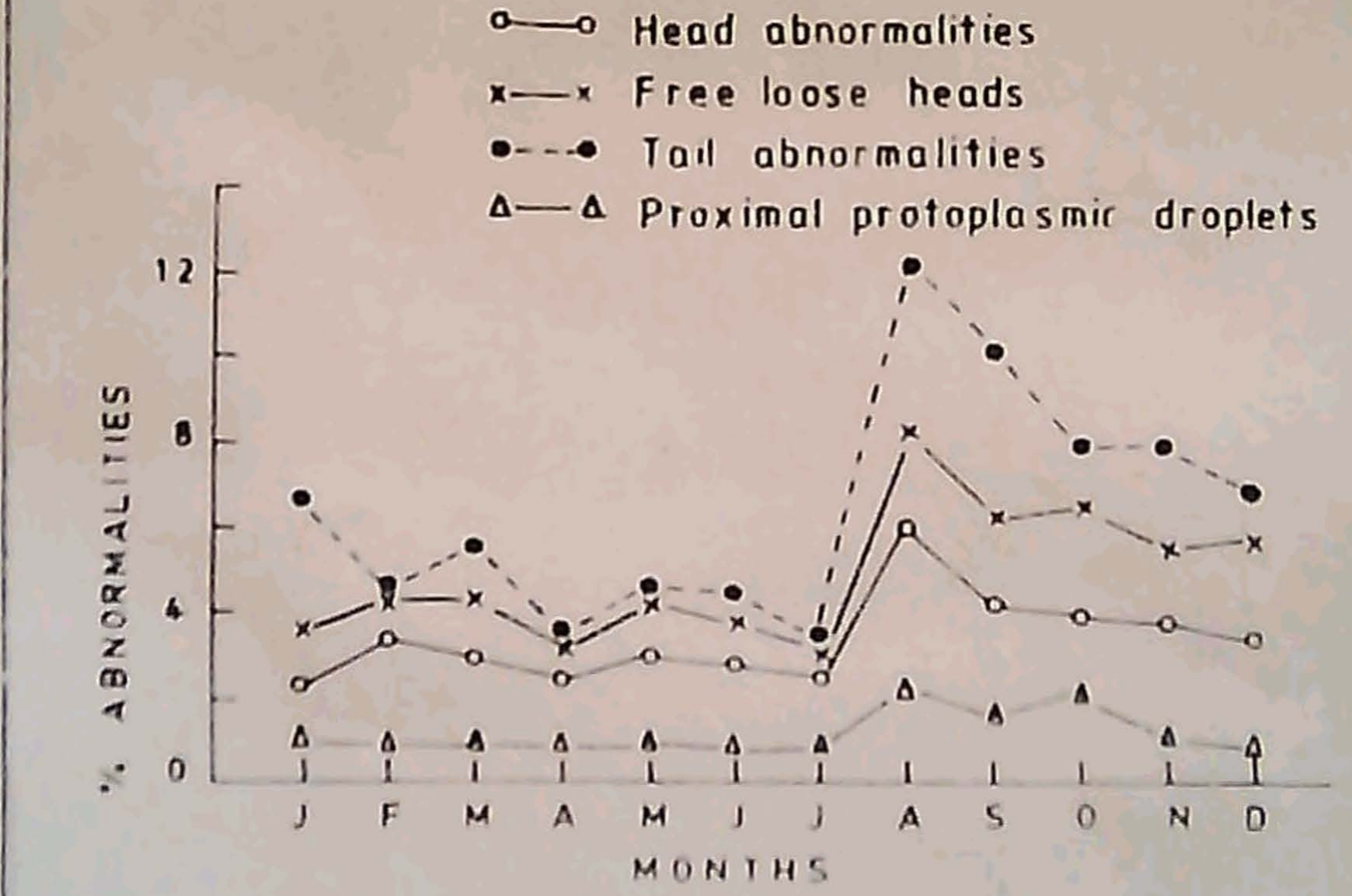

FIG.11. Incidence of sperm head abnormalities, free loose heads, tail abnormalities and proximal protoplasmic droplets - month wise

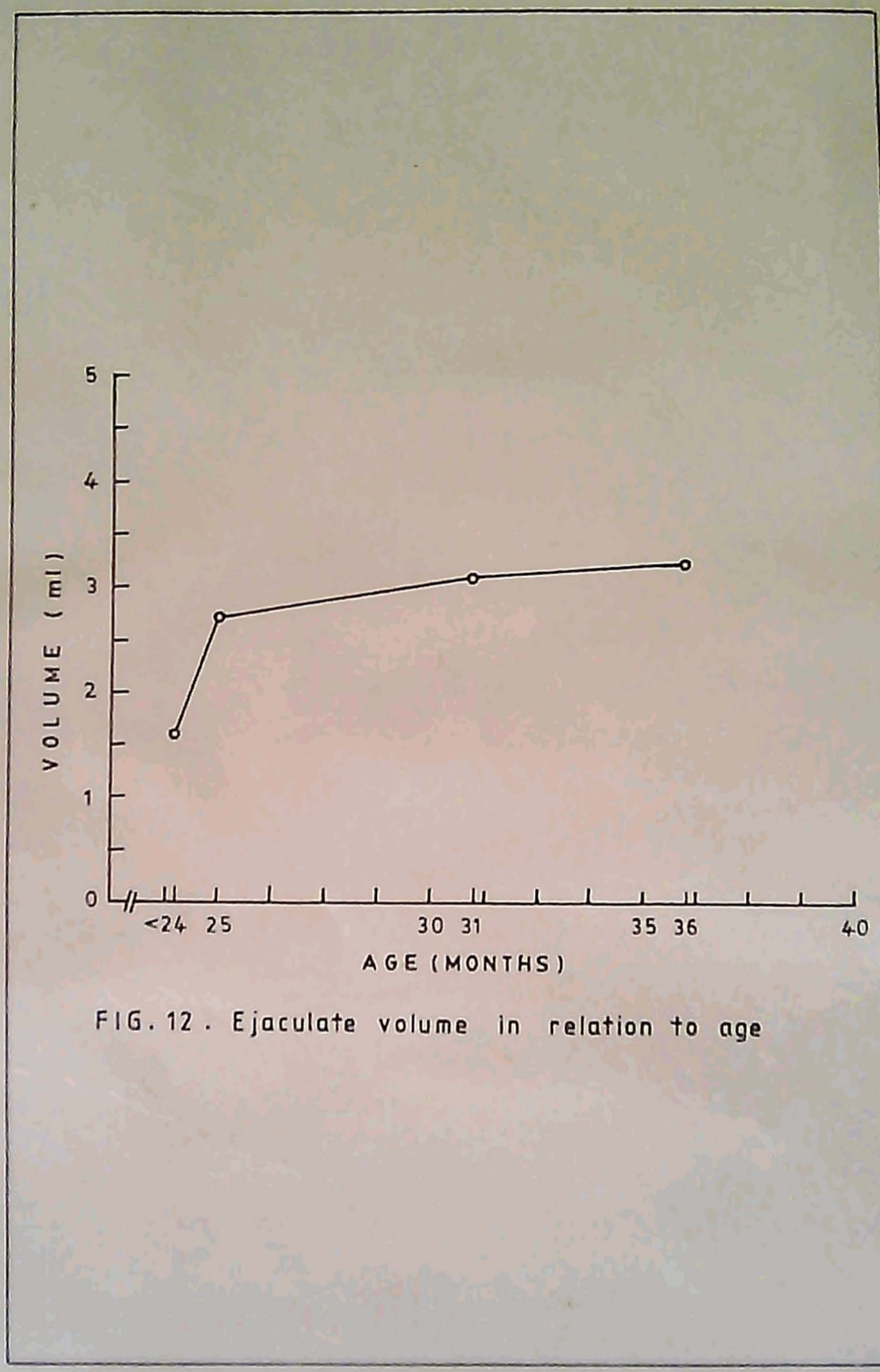

FIG. 12. Ejaculate volume in relation to age

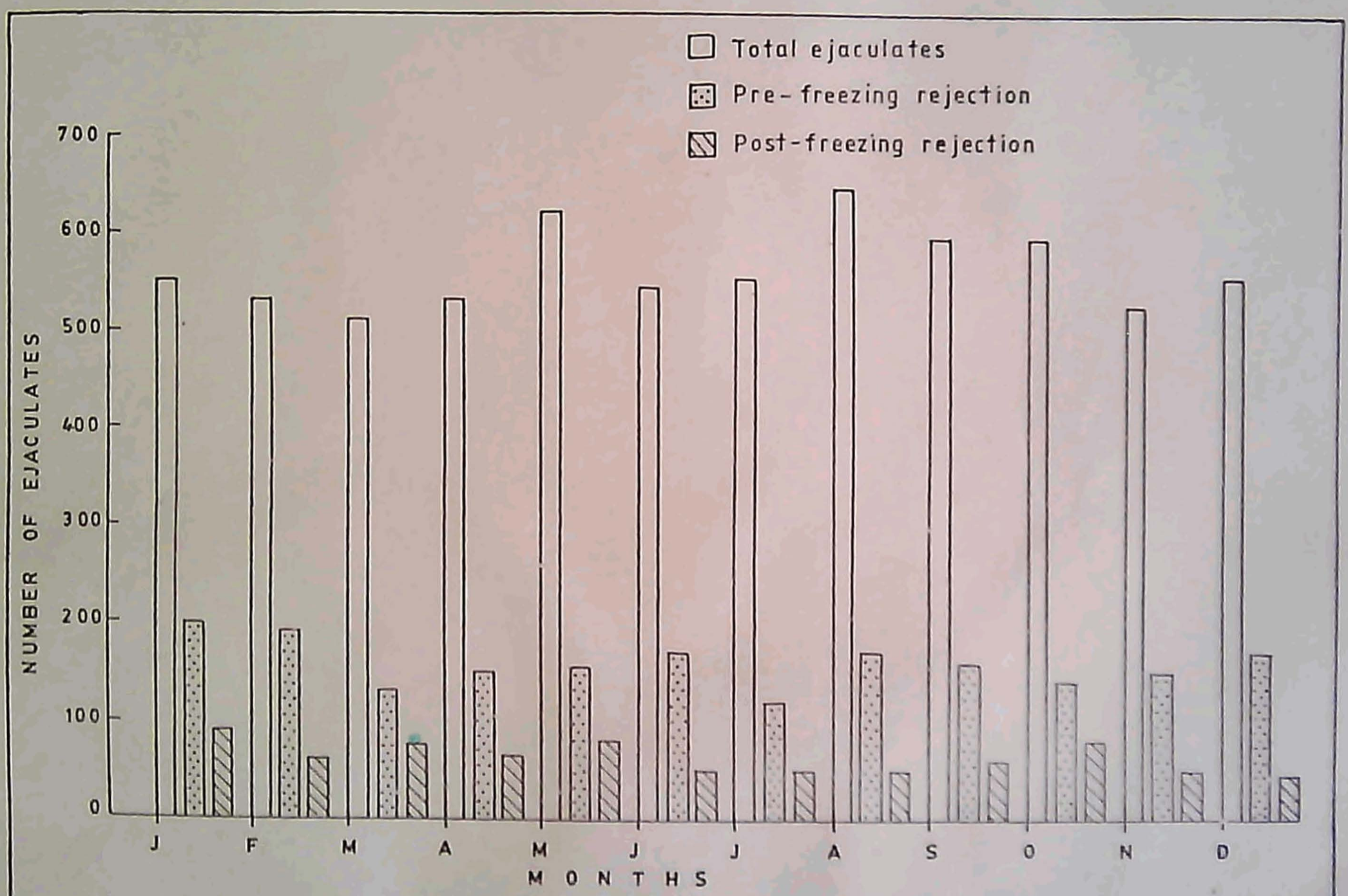

FIG. 13. Pre-freezing and post-freezing discard of ejaculates- month wise

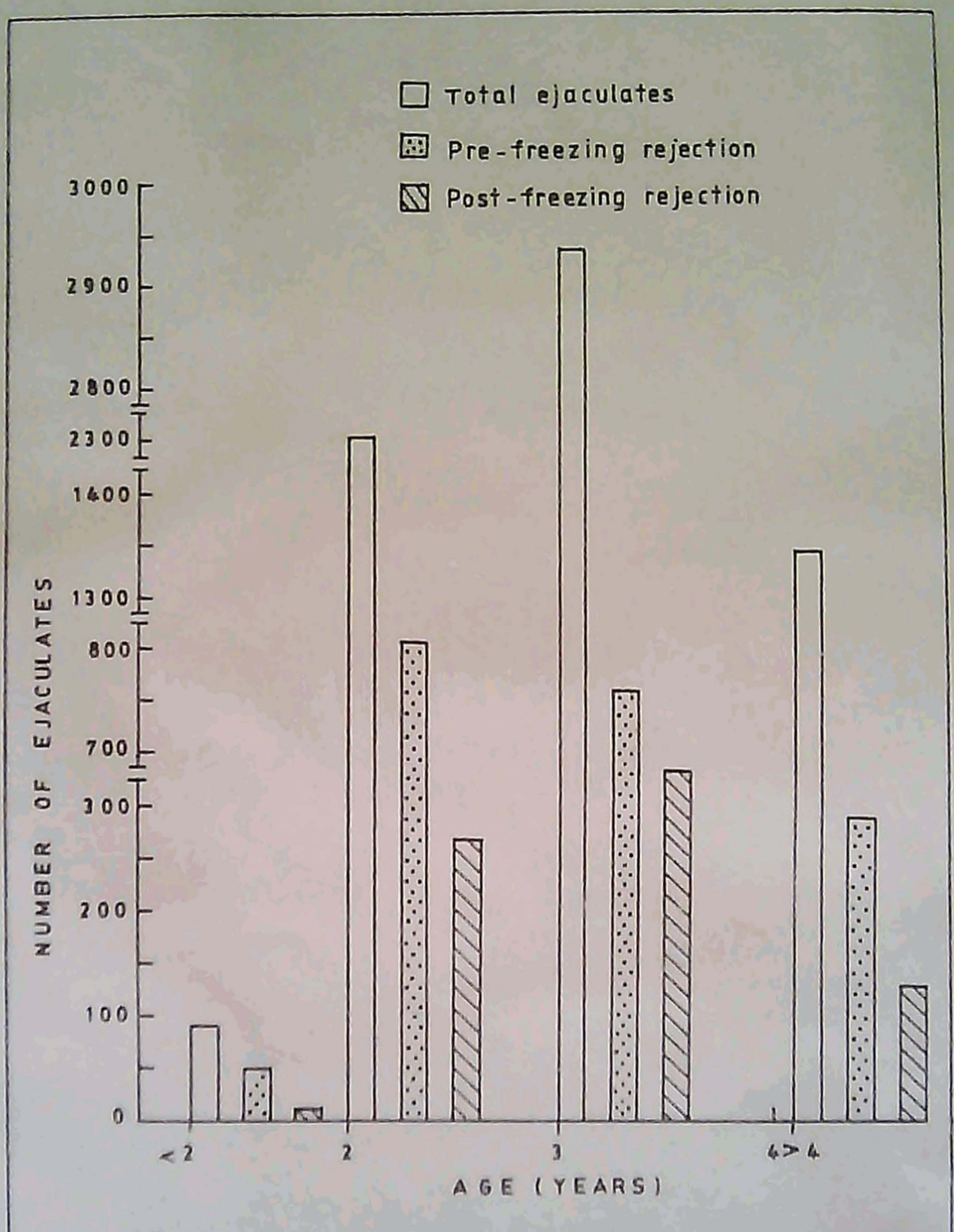

FIG.14. Pre-freezing and post-freezing discard of ejaculates - age wise

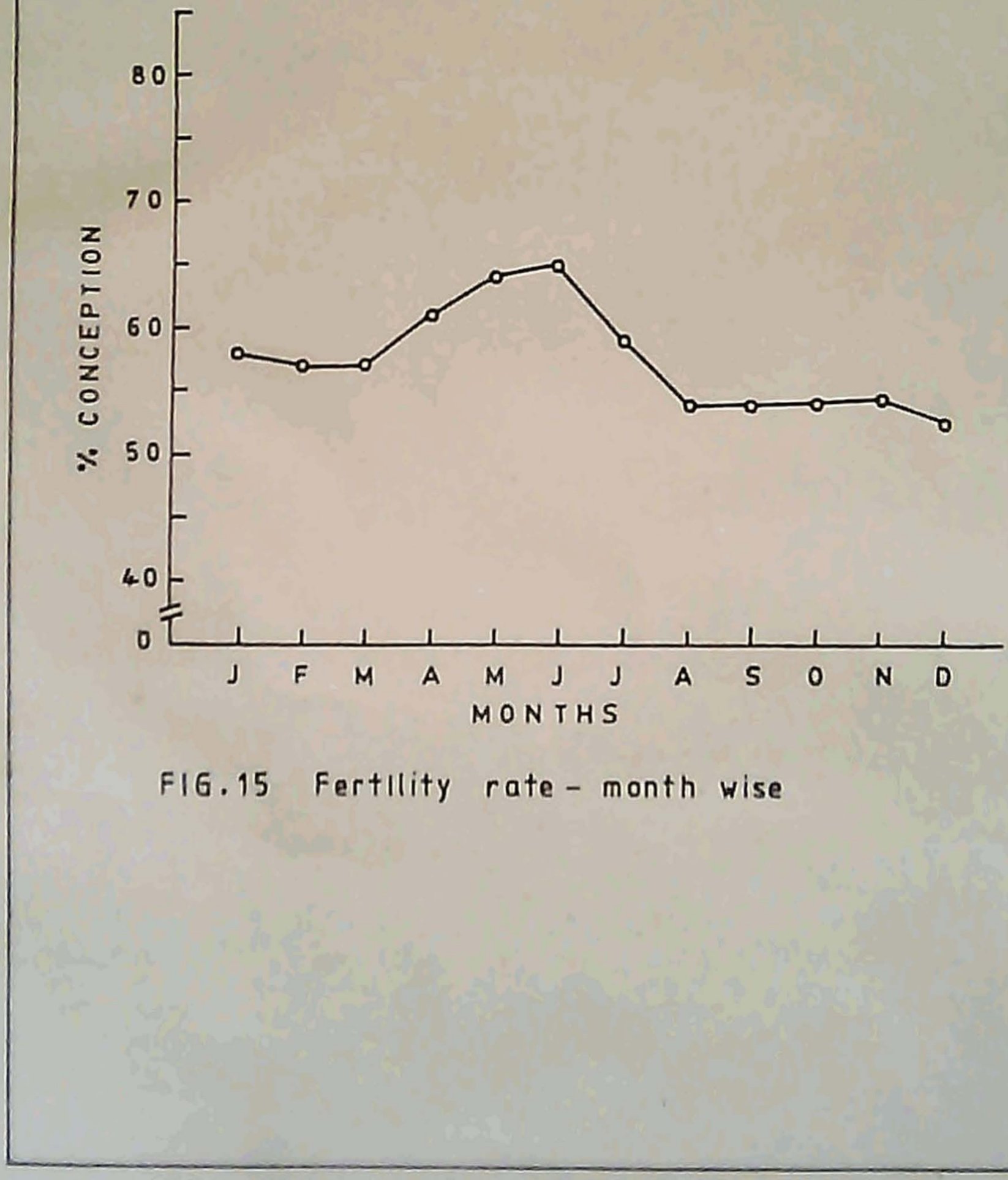

FIG.15 Fertility rate - month wise

Postnatal development of testis and epididymis:

Testis: Growth rate:

The mean testicular weights at different periods of growth are consolidated in Table 3. The gain in weight of testis was observed to be 3.87 gm between 0-120 days, 12.08 gm between 120-240 days and 27.50 gm between 240-360 days of age. The pattern of testicular growth was found to be curvilinear, when plotted against age (Fig. 1) as well as body weight (Fig. 2). The testicular weight was noted to be significantly correlated with the age ($P<0.01$) and body weight ($P<0.01$) of the animal (Table 4). The right testis was significantly heavier ($P<0.01$) than the left, the mean weights of the testes being 34.37 gm and 33.64 gm, respectively (Table 3).

The mean testicular measurements are furnished in Table 5. The testis was found to be more thick than broad in all age groups. The coefficient ratios of length:breadth, length:thickness, thickness:breadth and circumference:length were: 6.20%, 7.00%, 7.90% and 10.50%, respectively (Table 6).

Histology:

The data on the diameter of seminiferous cords/tubules at different ages/body weight are given in Table 7. The diameter of the cord increased slowly between 0-120 days (18.92 microns), rapidly between 120-240 days (66.38 microns)

and again reduced between 240-360 days of age (44.40 microns). The size of the cord/tubules was noted to be significantly correlated with age ($P<0.01$), body weight ($P<0.01$) and testicular weight ($P<0.01$) of the animal (Table 8 and Figs. 3 to 5). The histological features observed at different ages are detailed below:

<u>0 Day</u>: The section of the testis revealed widely separated seminiferous cords with abundant interstitium (Figs. 16 and 17). The sex cords measuring on an average 43.24 microns in diameter contained a homogenous unsegmented material with few gonocytes scattered in the centre. Occasionally, the gonocytes were seen located on the basement membrane interposed between the supporting cells. Some cords were devoid of gonocytes. At the periphery, the seminiferous cords were lined by a single layer of undifferentiated supporting cells.

Interstitium was wide and filled with mesenchymal cells having either spherical or oval nuclei.

<u>30 days</u>: The average diameter of the cord increased to 46.62 microns. There was increase in the number of supporting cells in the centre. The gonocytes were present in most of the tubules (Figs. 18 and 19).

There was no appreciable change in respect of interstitium.

60 days: The cords were still solid with a mean diameter of 49.95 microns. There was considerable increase in the number of supporting cells and more cords showed the presence of these cells in the centre. The gonocytes in some cords manifested signs of degeneration (Fig. 20).

Interstitium continued to remain as it was in the previous age group.

90 days: The diameter of cords increased to 57.50 microns. The supporting cells were present in the centre in many more cords. The gonocytes showed signs of degeneration (Fig. 21).

The interstitium was reduced.

120 days: The mean diameter of the cord was 62.81 microns. The cords were arranged closer to each other as compared with the previous age groups (Fig. 22). There was considerable increase in the number of supporting cells. Some of the basal supporting cells showed pyramidal shaped nuclei with cytoplasm tapering towards the interior--an indication of the initiation of the formation of Sertoli cells. Gonocytes were scanty. Many cords showed the presence of spermatogonia (Fig. 23).

The interstitium was reduced further.

<u>150 days</u>: The seminiferous cords (Fig. 24) measured on an average 68.29 microns in diameter. The tubular cytoplasm in some cords revealed irregular cracks radiating from the centre and in some others, there were even vacuolations. Sertoli cells had been formed in many tubules. In addition to spermatogonia, primary spermatocytes were present in few cords.

Interstitium was further reduced and the interstitial cells showed spindle shaped and spherical nuclei.

<u>180 days</u>: There was rapid increase in the diameter of the cords (107.84 microns). The tubules were in contact with each other (Fig. 25). The intratubular cells viz., Sertoli cells, spermatogonia, and primary spermatocytes were seen. Some tubules showed acidophilic fibriller net work.

Interstitium was scanty. The interstitial cells had spherical or oval nuclei and contained one or more nucleoli.

<u>210 days</u>: The mean tubular diameter was 120.76 microns. The tubules with well developed luminae in all were found to be in contact with each other (Fig. 26). There was appreciable increase in the intensity of concentration of the germinal cells which were seen arranged in 2 or 3 layers.

Interstitium showed similar changes as observed at 180 days.

240 days: The mean tubular diameter was 128.59 microns. Other changes were similar as observed as above (Fig. 27).

270 days: The mean diameter of the tubule was 148.39 microns. The histological picture was similar to that of the previous age group. Spermatids were detected for the first time in few tubules (Figs. 28 to 31).

300 days: The size of the tubules increased to 166.88 microns. Lumen was very clear. Sertoli cells, spermatogonia, primary spermatocytes and spermatids were arranged in 3 to 5 rows (Fig. 32). Development beyond spermatids was not observed in many tubules. In a few, spermatids showed signs of degeneration. For the first time, sperms were detected in some tubules.

330 days: The tubules measured on an average 174. 66 microns. All the tubules showed different spermatogenic cells. There was increase in the number of sperms which were seen attached to Sertoli cells (Fig. 33). A few tubules revealed the presence of sperms in the lumence as well (Figs. 34 and 35).

There was no appreciable change in Interstitium.

360 days: The mean tubular diameter was 172.79 microns. Different cell types of spermatogenesis were seen in all the tubules. There was also increase in the density of sperms (Figs. 36 and 37).

Interstitium contained well developed Leydig cells.

Epididymis:

Growth rate:

The mean epididymal weights at different ages/body weight are furnished in Table 9. The pattern of growth was found to be curvilinear, when plotted against age (Fig.6) and body weight (Fig. 7). The rate of growth was found to be slow upto the age of 240 days and rapid, thereafter. The epididymal weight was noted to be significantly correlated with age ($P<0.01$), body weight ($P<0.01$) and testicular weight ($P<0.01$) of the animal (Table 10). Figure 8 shows the growth curve of the epididymis in relation to testicular weight. The right epididymis (4.32 gm) was significantly heavier ($P<0.01$) than the left (4.46 gm) as can be seen from Table 9.

Histology:

Histometry of caput, corpus and cauda epididymis are furnished in Tables 11, 12 and 13, respectively and represented in Figs. 38 to 68. Both tubular diameter and height of epithelium increased with increasing age in all the three regions. But there were distinct regional differences. At birth (0 day) the greatest diameter of the tubules was observed in the cauda (119.88 microns), next in the corpus

(73.26 microns) and then in the caput (66.60 microns). The rate of growth was noticed to be gradual in the cauda. There was a spurt of growth in the corpus between 90-120 days of age and luteum 240-300 days of age in the caput as well. At birth (0 day) the highest epithelium was observed in the cauda (23.31 microns). The epithelium of the corpus and the caput had the same height at birth. The rate of growth of epithelium was gradual in the cauda while a spurt of growth was noticed in the corpus between 90-150 days of age and in the caput between 180-300 days of age. At 360 days of age, the epithelium showed maximum height in the caput (69.30 microns), next in the corpus (64.94 microns) and then in the cauda (62.29 microns). The simple columnar epithelium lining the tubules at birth changed into pseudostratified ciliated epitholium in all the regions with increasing age. This change took place earliest in the cauda (120 days of age), next in the corpus (150 days of age) and last in the caput (180 days of age).

Semen Characterization:

The data on the physical and morphological characteristics of semen/sperms are presented in Tables 14 to 22 and Figs. 10 to 12. The ejaculate volume, initial motility, sperm concentration, live sperm count, sperm head abnormalities, free loose head, middle piece abnormalities, tail abnormalities and proximal protoplasmic droplets were found to be 2.65 ±

0.084 ml, 66.24 ± 0.874%, 1599.64 ± 55.371 millions per ml, 72.55 ± 1.310%, 3.43 ± 0.300%, 4.91 ± 0.439%, 0.65 ± 0.045%, 6.47 ± 0.775% and 1.20 ± 0.148%, respectively. The analysis of variance between bulls as well as between months of collection in respect of physical characteristics of semen are given in Table 23 and those in respect of morphological characters of sperms, in Table 24. Significant variations ($P<0.01$) between bulls were observed with regard to sperm head abnormalities, free loose head, middle piece abnormalities, tail abnormalities, and proximal protoplasmic droplets. Variations between months were found to be significant ($P<0.01$) in respect of ejaculate volume, sperm head abnormalities, free loose head, tail abnormalities and proximal protoplasmic droplets.

The effect of season on the semen characteristics is illustrated in Tables 25 to 27. Significant ($P<0.05$) seasonal variations were observed only in the occurrence of tail abnormalities. The lowest percentage of abnormalities (4.79%) was noticed in season II (February to April) and highest in season III (May to November).

The effect of the age of the bull on the semen characteristics is evident from the data presented in Tables 28 to 30. Variations due to age was found to be significant ($P<0.01$) in the case of ejaculate volume, sperm concentration, free loose head, middle-piece abnormalities and tail abnormalities.

In general, the ejaculate volume increased and tail abnormalities decreased with advancing age. The highest sperm concentration was noted in the semen of bulls aged 25-30 months and the lowest in that of bulls aged below 24 months. The occurrence of free loose head and middle piece abnormalities showed a tendency to increase with advancing age.

Freezability of Spermatozoa:

The mean freezability of sperms expressed in terms of percent post-thawing motility was observed to be 36.66 (Table 31). Variations in the freezability were noted to be significant ($P < 0.01$) between the ages of the bulls (Table 32). The freezability of spermatozoa was not significantly altered between months of semen collection. The maximum freezability was observed in the month of December and the minimum during the month of January.

Pre-freezing and post-freezing discard of ejaculates:

The data on the effect of month, season of collection and age of the bull on the rate of prefreezing and post-freezing discard of semen are given in Tables 33 to 35 and represented in Figs. 13 and 14. The rate of discard before freezing was found to be 28.55%. The post-freezing discard was found to be 15.7% of all the frozen samples. The pre-freezing, the post-freezing and the total rejection rates of

the ejaculates was found to be significantly ($P<0.01$) influenced by the month and season of semen collection. There was significant negative correlation ($P<0.01$) between age of the bull and the rate of discard of the ejaculate.

Fertility Rate of Bulls:

The effect of month and season of insemination as well as of the age of the bull on fertility can be assessed from the data furnished in Tables 36 to 39 and Fig. 15. The fertility rate varied from 52.75% during December to 64.89% during June, with an overall mean of 56.66%. Significant variations ($P<0.01$) were observed in the fertility rate between months and between seasons of insemination. There were also differences ($P<0.01$) in the fertility rate between age groups. A significantly higher rate of fertility was obtained in bulls aged above 4 years (61.19%) when compared with those of 2 to 4 years of age (52.35%). As between bulls, the difference in fertility rate was found to be statistically significant (Table 40).

Discussion

Postnatal Development of Testes and Epididymis:

The size and the growth rate of the testes observed during the course of the present study were found to be lower than those reported in SMB bulls by Abdel Raouf (1960). The variations may be attributed to the breed differences as well as to the differences in the feeding and managemental practices followed in the two studies. The pattern of testicular growth was found to be curvilinear, the rate of increase being slow in the initial period, rapid between 120-240 days and more rapid between 240-360 days of age (Fig. 1). The present finding is in keeping with that reported in bull calves by Lagerlof (1934) and Abdel Raouf (1960). The relative weight of the testis increased with advancing age (Table 3). An observation similar to this has been made in SMB calves by Abdel Raouf (1960). Testicular weight was found to be significantly ($P<0.01$) correlated with the age of the animal (Table 4). Positive correlation between testicular weight and age of the animal has been reported in rams (Courot,1971), goats (Unnikrishnan, 1975) and boars (Thomas, 1973). Apparently, similar studies have not been carried out in bulls except for lone one of Brigatte (1951) who observed a proportionate increase in the weight of the testes with advancing age. The testicular weight was also noted to be significantly ($P<0.01$) correlated with the body weight of the animal (Table 4).

Identical observations have been made in various breeds of cattle by Hafez et al. (1952), Van Demark and Mauger (1954), Abdel Raouf (1960) and Aire and Akpokodje (1975). These findings are, however, at variance with those recorded in Italian breeds of cattle by Brigatti (1951) who did not notice any correlation between testis weight and body weight at any stage of development. The right testis was noted to be significantly heavier ($P<0.01$) than the left as can be seen from the data presented in Table 3. However, the variation in the weight between the right and the left testis was relatively small in very young animals and became conspicuous only on approaching puberty. Abdel Raouf (1960) who too observed asymetry in growth between the right and the left testis was of opinion that the bovines in general might be predisposed to have heavier right gonad than left in both the sexes.

The testis was found to be more thick than broad at all ages (Table 5). This is essentially in keeping with the observation of Abdel Raouf (1960) in Sahiwal bulls. The coefficient of variations of the ratio of different testicular measurements (Table 6) was noted to be within the normal range of 5-15% (Quinoville, 1953). It would appear that the rate of increase in the various testicular measurements is more or less similar indicating that the shape of the testis remains unaltered during the prepuberal period. On the other

hand, the shape of the testis in SRB bulls was found to change from cylindrical at birth to oval at maturity (Abdel Rauf, 1960).

The mean diameters of the seminiferous cords/tubules (Table 7) are comparable to the corresponding values reported in Friesian bull calves by May et al. (1960). The measurements presently made, however, are lower than the values recorded in SRB bull calves (Abdel Rauf, 1960). The variation in the tubular diameter can be attributed to breed differences. As mentioned earlier, the mean weight of the testis in the present study was found to be lower than that reported for SRB bulls. The growth curve of the seminiferous cords/tubules was noted to be sigmoid, when plotted against age of the animal (Fig. 3). The diameter of the tubules increased rather slowly between 0-120 days and rapidly, between 120-240 days of age. The rate of growth beyond this age was found to be slightly reduced. The growth pattern of the tubules was found to be more or less similar to that reported in bull calves by earlier workers (Michatsch, 1933; Lagerlof, 1934 and Abdel Rauf, 1960). It was observed that the diameter of the tubules increased about four times between birth and 360 days of age. Fossland (1954) also had reported a four-fold increase in the diameter of the seminiferous tubules in Jersey and Holstein calves between birth and 18 months of age. Abdel Rauf (1960) recorded a five-fold increase in

the size of the tubules in the case of SRB bulls during a similar period. Contrary to these observations, Aire and Akpokodje (1975) noticed marked difference in the size of the seminiferous tubules from 20 weeks of age in White Fulani (Bos indicus) calves. The differences in breed and in the plane of nutrition on which the animals were reared might have contributed for the variations observed. The diameter of the seminiferous tubules was also noted to be significantly correlated ($P < 0.01$) with age, body weight and testicular weight of the animal (Table 8). A positive correlation between testicular weight and tubular diameter has been recorded in White Fulani bulls as well (Aire and Akpokodje, 1975).

Initiation of lumen formation in the seminiferous cords was noticed for the first time at 150 days of age. The process of lumen formation was completed in most of the cords at 160 days of age. The present findings are akin to those reported by Michatch (1933), Hooker (1942) and Abdel Raouf (1960) in different breeds of exotic cattle. However, Santamarina and Mecca (1957) reported 63 days of age for the initiation and 127 days of age for the completion of lumen formation in bull calves. The canalization of the seminiferous cords in Bos indicus was found to occur around 7 months (Igboeli and Nakhe, 1971; Aire and Akpokodje, 1975). The variations in the onset and completion of the process of lumen formation might be attributed to breed differences as suggested by Abdel Raouf (1960).

The germinal cells were represented at birth by gonocytes which were mostly located at the centre of the cords (Fig.\3). Occasionally, these cells were spotted on the basement membranes also. There was progressive increase in the total number of gonocytes with advancing age, even though some underwent degeneration. The present findings are in general agreement with those recorded in SMB bull calves by Abdel Raouf (1960). Attal and Courot (1963) have reported mitotic division of gonocytes resulting in large reserve before the onset of spermatogenesis. The degeneration of gonocytes observed during the early stage of growth was believed to be due to the abrupt cut in the supply of gonadotrophic hormones through the maternal placenta immediately after birth (Abdel Raouf, 1960). Transformation of the gonocytes to spermatogonia was found to be a gradual process. It was initiated at about 120 days and completed by about 150 days of age. This is in accord with the findings of Abdel Raouf (1960) who recorded the presence of spermatogonia in all the tubules by about 20 weeks of age in SMB bull calves. The present observation, however, is at variance with that of Phillips and Andrews (1936) who detected the presence of loosely arranged rows of spermatogonia with occasional spermatocytes even at the age of 63 days in Guernsey, Holstein, Short-horn and Hereford calves. With the formation of spermatogonia, gonocytes started disappearing. But complete

disappearance of gonocytes was observed only when all the tubules were lined by spermatogonia. Similar observations have been made in bulls by Abdel Raouf (1960), Courot (1962) and Attal and Courot (1963). A definite spermatogonic series represented by spermatogonia and a few primary spermatocytes was observed at the age of 150 days. The reports on the age at which primary spermatocytes first appeared in bulls showed variations. The present finding is comparable to that recorded in SNB bulls by Abdel Raouf (1960). However, Phillips and Andrews (1936) observed the primary spermatocytes for the first time at the age of 63 days in different breeds of exotic cattle. Similarly, Gier and Marion (1970) were able to detect primary spermatocytes by 3rd month of age in bull calves. Contrary to these reports, Mann et al. (1949) recognised the primary spermatocytes in bull calves only at the age of 6 months. As far as the Bos indicus cattle are concerned there is only the report of Aire and Akpokodje (1975) available for comparison. According to these authors, primary spermatocytes are identified in White Fulani bull calves between 36 and 44 weeks of age.

Spermatids were observed for the first time in the present study at 300 days of age. Development beyond spermatids was not noticed in many tubules. In a few tubules, Spermatids showed evidence of degeneration. The sperms were seen formed between 300-330 days of age. The formation of spermatids and spermatozoa appeared to take place at a time earlier than the present in exotic bulls (Phillips and Andrews,

1936; Fossland, 1954; Abdel Raouf, 1960 and May et al., 1961). Mann et al. (1949) could not identify spermatids and spermatozoa in the tubules before the age of 12 months. In the case of Bos indicus cattle, the formation of spermatozoa was delayed upto 60 weeks in Brazilian calves (Godinho, 1970), 73 weeks in Angoli bulls (Igboeli and Rakha, 1971) and 48 weeks in White Fulani calves (Aire and Akpokodje, 1975). It is evident from the above reports that there are wide variations in the formation of spermatozoa between Bos taurus and Bos indicus bulls. While in Bos taurus it occurs at an early age, spermatogenesis is generally delayed in Bos indicus cattle. The observation made during the course of the present study suggests that in crossbred cattle spermatogenesis is completed at a period intermediary between the above two extremes. If the formation of sperms is taken as a basis to indicate the onset of puberty, it can be inferred that the animals under investigation attain puberty by about 300-330 days of age. Incidentally, this also coincides with the age of puberty as per the formula suggested by Pomeroy (1955). According to Pomeroy (1955), puberty is reached in bulls when the body weight is about 30% of mature weight. The adult weight of Brown Swiss crosses (62.5% exotic inheritance) is around 600 Kg as per the records maintained at Indo-Swiss Project, Hettupatty. Therefore, the attainment of puberty in these animals is to be expected when they gain a body weight of about 180 Kg. The data presented in Table 3 reveal that the

animals under study attained this weight between 300-330 days of age.

The onset of puberty has been reported to be a variable time factor as it is influenced within species by the plane of nutrition and breed of the animal (Roberts, 1971). The age of puberty both in males and females is not reached at the same time in Bos taurus and Bos indicus and in the intermediary type. Even among the Bos taurus cattle, small breeds like Jersey reach puberty earlier than the heavy breeds like Brown Swiss. The indigenous cattle in Kerala are notoriously slow in their growth and attainment of puberty. The delay in the onset of puberty of Brown Swiss crosses might be attributed to the genes they have inherited from the dam and the sire both of which have hereditary predisposition for delayed maturity. Another factor involved is the plane of nutrition on which the animals are maintained during the early stages of growth. There is accumulated evidence in proof that the onset of spermatogenesis can be brought about at an early age, if fed on high plane of nutrition (Abdel Raouf, 1960; Sichan and Hunter, 1961; McMillan and Hafs, 1969). It has been reported that the initiation of spermatogenesis is more related and better influenced by the body weight than by the age of the animal (Asdell, 1946; Kibler et al.,1943; Abdel Raouf, 1960 and Courot, 1971). The animals under the present study have a much lower body weight as compared with that of exotic bull calves. Studies

on the nutrient requirements of crossbred animals for specific physiological functions are meagre and the results of the present study point out the need to carry out systematic investigations in this regard with special reference to the needs of bull calves.

The formation of Sertoli cells from undifferentiated supporting cells was found to be a gradual process. The supporting cells which were few and arranged in a row at the basement membrane at birth progressively increased in number by mitotic division with advancement of age. The first observable change in the transformation of supporting cells to Sertoli cells was noticed at the age of 120 days. However, fully formed Sertoli cells could be detected in many tubules only at the age of 150 days. The process of transformation appeared to be associated with the appearance of primary spermatocytes. This is in keeping with the finding of Courot et al. (1970) who have stated that the development of supporting cells occurs when the sex cords are crowded by primary spermatocytes. The process of metamorphosis continued and by about 180 days all the tubules were seen lined by Sertoli cells. In the case of exotic cattle, the transformation of supporting cells into Sertoli cells has been reported to occur at an age earlier than that observed presently (Mcintosh,1933; Abdel Raouf, 1960 and Gier and Marion, 1970). The observation made during the course of the present study that no cell

division occurs once the Sertoli cells are formed is in agreement with that reported by earlier workers in this regard (Abdel Raouf, 1960 and Attal and Courot, 1963).

The interstitium that occupied a major portion of the testicular parenchyma at birth showed progressive reduction with corresponding increase in the tubular diameter. Transformation of immature Leydig cells to mature Leydig cells was found to be initiated at about 150 days and completed by 180 days of age. The onset of metamorphosis of Leydig cells in exotic bulls has been reported to occur earlier than 150 days of age (Hooker, 1944; Abdel Raouf, 1960).

The growth curve of the ductus epididymis as represented in Fig. 6 was found to be curvilinear, when plotted against the age of the animal. The pattern of growth resembled that of the testis, but the pace of growth was found to be slow, as compared to that of testis. The rate of growth was noted to be slow upto the age of 240 days and rapid, there after. A gradual increase in the relative weight with advancing age was also visible (Table 9). Similar observations have been made in SRB calves by Abdel Raouf (1960). Significant correlation ($P < 0.01$) between epididymal weight and age/body weight of the animal was observed in the present study. In contrast to the findings of Brigatti (1951), a positive correlation between the weight of epididymis and age/body weight had been recorded in rams (Terrill 1968).

goats (Harshan, 1975) and boars (Thomas, 1973). The weight of the epididymis was also noted to be correlated ($P < 0.01$) with testicular weight (Table 10). An observation similar to this has been made in SMB bulls by Abdel Raouf (1960). The right epididymis was observed to be significantly heavier than the left ($P < 0.01$). This is in keeping with the finding of Abdel Raouf (1960).

Distinct differences in the diameter of the tubules of the caput, corpus and cauda epididymis were observed in the present study (Tables 11-13). The diameter of the tubule was noted to be greatest in the cauda followed by the corpus and the caput in that order throughout the period of growth. The present findings are in general agreement with those of Abdel Raouf (1960) in bulls, Harshan (1975) in goats and Thomas (1973) in boars.

There were regional differences in the height of the epithelium lining the epididymis. At birth, the tallest epithelium was noticed in the cauda, but by the time the animals reached 360 days of age, the height of the epithelial cells was found to be maximum in the caput followed by the corpus and the cauda in that order, resulting from probable differences in the rate of growth of the epithelium in the three regions. Regional differences in the height of epithelium have been reported in bulls (Abdel Raouf, 1960), goats (Harshan, 1975) and boars (Thomas, 1973).

The degree of transformation of epithelial lining from simple columnar to pseudostratified type was found to vary between the three regions. Pseudostratification of epithelial cells was completed earliest in the cauda, later in the corpus and last in the caput. This is in accord with the findings of earlier workers in different species of animals (Abdel Raouf, 1960; Thomas, 1973 and Marshan, 1975). The differentiation of simple columnar epithelium to pseudostratified type is reported to be due to mitotic division of epithelial cells and subsequent rearrangement of the basal cells alternating with columnar cells on the basement membranes. In keeping with the findings of Abdel Raouf (1960) in bulls, the results of the present study indicate that the differentiation of epithelium in all three regions will be completed earlier than the adult height is reached.

It can be seen that acquiring the adult height and differentiation of the epithelial cells occur in ascending order, both the processes being completed first in the cauda, next in the corpus and last in the caput. According to Abdel Raouf (1960) who had reported similar findings the distal region of ductus epididymis had already advanced longer towards maturity at birth as compared with the proximal region. The present finding lends evidence to support this view.

Sperms were observed for the first time in the lumen of the caput, corpus and cauda epididymis at the age of 300 days.

This coincided with the formation of sperms in the testis as discussed earlier. Abdel Raouf (1960) detected sperms in the ductus epididymis at a period earlier than this. This variation may be attributed to the differences in the onset of spermatogenesis observed in the two studies. The density of sperm increased with advancing age and at about 360 days there was considerable increase in the concentration of sperms in all the three regions. The density of sperms, however, was found to be higher in the cauda then either in the corpus or in the caput. A similar observation has been made in SMB bulls by Abdel Raouf (1960) who attributed this regional variations in sperm density to the differences in the rate of passage of sperms through the different parts of ductus epididymis. According to the author (Abdel Raouf, 1960), the passage of testicular products is considerably more rapid in the proximal part than that in the distal region.

Semen Characteristics:

The volume of semen per ejaculate in the present study was found to be in the range of 0.5 to 6.00 ml with a mean of 2.65 ± 0.084 ml (Table 14). This value is lower than that reported by Mathew (1974) for the crossbred bulls of the same genetic make up, the variation in this regard probably being attributable to the differences in the ages of the animals used in the two studies. The average ejaculate volume observed

was also found to be lower than that reported for exotic (Salisbury, 1944; Anderson, 1945 and Mathew, 1974) and Indian breeds of cattle (Bhattacharya and Prabhu, 1954; Tomer et al., 1964 and Rao and Rao, 1980). On the other hand, a higher volume of semen per ejaculate than that observed during the course of the present study has been recorded in the case of various crossbred bulls of different exotic inheritance by Rao and Kotayya (1977), Saxena and Tripati (1978) and Rao and Rao (1978).

Roberts (1971) reported variations in the ejaculate volume not only between breeds but also between bulls within the same breed. Significant variations in the semen volume between bulls have been observed in the case of crossbred (Rao and Rao, 1978) and Ongole bulls (Rao and Rao, 1980). However, no significant difference in the ejaculate volume between bulls was evident in the present study (Table 23).

A highly significant difference in the volume of semen ($P<0.01$) was observed between months (Table 23), highest value being obtained during December and lowest during September. Variations in the ejaculate volume have been recorded in crossbred bulls by Rao and Rao (1978) and in Ongole bulls by Rao and Rao (1980). However, when monthly data were grouped and analysed on seasonwar basis, no significant difference was noted (Tables 25 and 26). This observation is akin to the findings of Swanson and Herman (1944),

Mukherjee and Bhattacharya (1952), Kodagali (~~1961~~; 1962) and Mathew (1974), but is at variance with those of several others (Erb et al., 1942; Mercier and Salisbury, 1947; Brown, 1959; Horie and Ishikura, 1964; Sinha and Prasad, 1966; Rao and Rao, 1965). Milicevic (1965), Rao and Rao (1975) and Rao and Rao (1978) have recorded a low ejaculate volume in different breeds of bulls during summer while Amann et al. (1966) and Tomar et al. (1966) obtained a significantly higher ejaculate volume during summer when compared with other seasons. Lack of evidence of seasonal effect on the ejaculate volume in the present study may be attributed to the absence of extreme temperature fluctuations at the place of study.

As between age groups, significant differences in the ejaculate volume ($P < 0.01$) were observed (Tables 28 and 29). The lowest volume was recorded in bulls below 24 months and highest in those above 36 months. A gradual increase in the ejaculate volume with advancing age was evident. An observation similar to this has been made in bulls of different breeds by earlier workers also (Zuliani, 1957; Povlicenko, 1964; Almquist and Cunningham, 1967 and Rao and Rao, 1979).

The initial motility of spermatozoa in the ejaculate was found to vary from 40 to 90% with a mean of 66.24 ± 0.87% (Table 15). The mean value observed is comparable to that reported for exotic bulls by Brown (1959). The percentage of motile sperms recorded in Bos taurus bulls by Lasley (1951)

is lower than the present value. However, a motility rating much higher than the one observed during the course of the present study has been reported in bulls by several others (Rao and Rao, 1975; Saxena and Tripati, 1978; Rao and Rao, 1978 and Rao and Rao, 1980). There was no significant variation in the initial motility of sperms between bulls.

The initial motility of spermatozoa was not significantly influenced either by month (Table 23) or by season (Table 26) of collection. Rao and Rao (1980) have made a similar observation in the case of Ongole bulls. The present finding, however, is at variance with several earlier observations in this regard (Swanson and Herman, 1944; Mukherjee and Bhattacharya, 1952; Johnston and Branton, 1953; Kodagali, 1962; Tomar et al., 1966; Rao and Rao, 1975 and Rao and Rao, 1978). According to Tomar et al. (1966) humid hot season does not appear to be conducive for the production of semen with high initial motility. The temperature at Mattupatty where the study was conducted was only moderate throughout the year and this might be the reason for the absence of variations in the spermatozoan motility between seasons.

A gradual increase in the percentage of motile sperms in the ejaculate was noted with advancing age (Table 26). The variations between the age groups were, however, not statistically significant. Similar improvement in initial motility of sperms with increasing age has been recorded in bulls of

various breeds by several workers (Dimitriev, 1964; Malberg, 1965; Singh et al., 1967; and Rao et al., 1979). Lindley et al.(1959) have reported significant correlation between initial motility of sperms and age of the bull. On the other hand, Maslov (1960) failed to observe in bulls any such correlation.

The average concentration of spermatozoa was found to be 1599.64 ± 55.371 millions per ml within a range of 820-2830 millions per ml of ejaculate (Table 16). Mathew (1974) has reported essentially the same value in the crossbred bulls of identical exotic inheritance. However, the mean value obtained in the present study is much higher than that recorded in pure bred bulls of both exotic (Lagerlof, 1934; Salisbury, 1944; Anderson, 1948; Blom,1950; Stone et al., 1950; Mathew, 1974 and Rao and Rao, 1975), and Indian cattle (Paul et al., 1966; Rao and Rao, 1975 and Rao and Rao, 1980). The sperm concentration per ml of semen in different crossbred bulls is also noted to be comperatively lower (Saxena and Tripati, 1978 and Rao and Rao, 1978). The single exception to these observations is the finding of Paul et al. (1966) who obtained 1729 and 1674 millions sperm per ml of semen in Sindhi and Gir bulls, respectively.

Contrary to the reports of Rao and Rao (1978) in cross-bred bulls, no significant variation in sperm concentra-

tion was observed between bulls in the study under report (Table 23). Essentially the same observation was made by Rao and Rao (1980) in Ongole bulls.

The sperm count in the ejaculate was not affected by the month of collection (Table 25). This influence is not in keeping with that reported earlier in pure bred (Rao and Rao, 1975; Rao and Rao, 1980) and crossbred bulls (Rao and Rao, 1978). The lowest sperm concentration of 1386.19 millions per ml of ejaculate was obtained during January and the highest value of 2073.09 millions, during February. No significant differences were observed in the sperm concentration between different seasons (Table 26). Salisbury (1944), Johnston and Branton (1953) and Mathew (1974) also did not observe any significant seasonal variation in sperm concentration. But significant seasonal variations in the sperm count of Bos taurus and Zebu bulls have been reported by several workers (Erb et al., 1942; Mukherjee and Bhattacharya, 1952; Kodagali, 1962; Horie and Ashikura, 1964; Tomer et al., 1966; Roy,1972; Igboeli and Rakha, 1971; Andreev, 1971 and Bhosreker et al., 1978). Stone et al. (1950) attributed the reduction in sperm concentration in bulls to sudden temperature variations associated with high relative humidity. According to Amann et al. (1966) production of sperms in Holstein bulls is observed during the period of rapidly increasing day length. In the area where the present study was conducted, the temperature

was moderate with little variation in day length throughout the year. This might probably explain the reason for not observing seasonal variations in the sperm count.

There was significant variation ($P \leq 0.01$) in the sperm count as between bulls of different age groups (Table 29). The lowest value was obtained in bulls aged below 24 months and highest, in those between 25-30 months (Table 28). But the sperm count in bulls beyond 24 months was not significantly correlated with age suggesting that the production of sperms probably gets stabilized by about 25-30 months. Rao et al. (1979) reported 23 months as the age at which nearly normal semen was obtained from crossbred bulls. A gradual improvement in the concentration of spermatozoa with advancing age has been recorded by Almquist et al. (1963) and Rao and Rao (1978). The observation made in the present study is at variance with the report of Lepard et al. (1941), Maslov (1960) and Singh et al. (1967) who were all of the opinion that the age of the bull exerts no influence on the sperm density of semen.

The average percentage of live spermatozoa in the ejaculate was noted to be 72.55 ± 1.310, within a range of 43 to 90 (Table 17). This value is comparable to that reported in bull semen by Bishop et al. (1954), Bratton et al. (1956) and Tomar et al. (1966) but lower than the same recorded in purebred bulls (Singh et al., 1967; Rao and Rao, 1975 and Rao

and Rao, 1980) and crossbred bulls of different exotic inheritance (Diwan et al., 1976; Rao and Rao, 1978 and Saxena and Tripati, 1978). The discripancy in this regard might be attributed to differences in the genetic make up of the animals employed in the various studies.

There was no significant variation in the live sperm count of the ejaculate between bulls (Table 23). While this observation is in keeping with the finding of Rao and Rao (1980) in Ongole bulls, it is at variance with that of Rao and Rao (1975) in purebred and of Rao and Rao (1978) in crossbred bulls.

Neither month of collection (Table 23) nor season (Table 26) was found to exort any significant effect on the percentage of live sperm in the ejaculate as reported by Rao and Rao (1980) in Ongole bulls. Significant seasonal variations in the percentage of live sperm in the ejaculate have, however, been reported in both exotic, Zebu and crossbred bulls by earlier workers (Lasley and Bogart, 1943; Tomar et al., 1966; Rao and Rao, 1975 and Rao and Rao, 1978). As in the case of initial motility, hot humid season is unfavourable to have high percentage of live sperm in the ejaculate (Tomar et al., 1966). The moderate temperature experienced at the area under study throughout the year might have contributed to the elimination of any seasonal influence on live sperm concentration.

Even though there was a gradual increase in the live sperm count with advancing age (Table 28), no significant variation was observed in the value between the different age groups (Table 29). This is akin to the observation of Tomer (1970) who reported that the age of the bull has no effect on the occurrence of live sperm count in the ejaculate. According to Singh et al. (1967), the bulls over 3 years of age alone will produce semen with higher percentage of live spermatozoa.

The percentage of sperm head abnormalities was found to be in the range of 0.00 to 12.00 with a mean of 3.43 ± 0.300 (Table 18). The mean value obtained is in near agreement with that recorded in exotic bull semen by Haq (1949) and Rollinson (1951). The frequencies of occurrence of sperm head abnormalities in the semen of Tharparkar and Jersey bulls (Rao and Rao, 1975) and crossbred bulls of different exotic inheritance (Biswas et al., 1976 and Rao and Rao,1978) are higher than the value presently reported for Brown Swiss crosses. The variation is attributable to the genetic differences of the bulls employed for the various studies.

The incidence of head abnormalities was found to vary significantly ($P < 0.01$) between bulls (Table 24). This is in agreement with the findings of Rao and Rao (1978) in crossbred bulls. In the case of Ongole bulls no such variation was observed by Rao and Rao (1980).

The percentage of head abnormalities was found to differ significantly ($P < 0.01$) between months of collection (Table 24). An observation similar to this has been made in respect of the semen of Tharparkar and Jersey bulls (Rao and Rao, 1975) crossbred bulls (Rao and Rao, 1978) and Ongole bulls (Rao and Rao, 1980). The highest count of sperm head abnormalities in the ejaculate was noted in August and lowest in the month of January. When the data were analysed on seasonwar basis, no significant variations could be observed (Table 27).

The percentage of sperm head abnormalities was found to decrease with age (Table 28) but the differences in this regard were not statistically significant (Table 30). Rao *et al* ~~and Rao~~ (1979) have reported that the incidence of head abnormalities decreases during the growth period in young crossbred bulls and becomes nearly normal at the age of 23 months.

The percentage of free loose head in the semen varied from 0.00 to 20.00 with a mean of 4.91 ± 0.439 (Table 19). The mean value observed is comparable to that reported in the semen of Tharparkar and Jersey bulls (Rao and Rao, 1975), crossbred bulls (Rao and Rao, 1978) and Ongole bulls (Rao and Rao, 1980).

There was significant variation ($P < 0.01$) in the frequency of occurrence of free loose head between bulls (Table 24). Variations in the incidence of free loose head between bulls have been reported earlier in pure bred Tharparkar and Jersey (Rao and Rao, 1975) and crossbred bulls (Rao and Rao, 1978), but not in Ongole bulls (Rao and Rao, 1980).

The incidence of free loose head was found to differ significantly ($P < 0.01$) between months in the present study (Table 24). Similar trend has been reported in crossbred bulls (Rao and Rao, 1978) and Ongole bulls (Rao and Rao,1980). The maximum concentration of free loose head was observed in August and minimum, in July. However, when the data were analysed seasonwise, no significant variation was apparent (Table 27).

Significant variation in the occurrence of free loose head ($P < 0.01$) was observed between age groups as well (Table 30). Contrary to the report of Rao 박선 [redacted] (1979), the incidence of free loose head was found to increase with age (Table 28) in the study under report. But in no age group the incidence crossed the limit prescribed for the normal bull semen (Blom, 1950).

Middle piece abnormalities were noted to be in the range of 0.00 to 4.00% with a mean of 0.65 ± 0.045% (Table 20)

These values are similar to those reported in crossbred (Rao and Rao, 1978) and Ongole bulls (Rao and Rao, 1980). A slightly higher value has been recorded in the ejaculates of Therparker and Jersey bulls (Rao and Rao, 1975).

The incidence of middle piece abnormalities was found to differ significantly ($P<0.01$) between bulls (Table 24). An observation similar to this has been made in crossbred bulls by Rao and Rao (1978).

Neither month (Table 24) nor season (Table 27) was found to exert any effect on the occurrence of middle piece abnormalities. This inference is in agreement with that of Rao and Rao (1975) in pure bred and Rao and Rao (1978) in crossbred bulls.

Age of the bull significantly ($P<0.01$) influenced the incidence of middle piece abnormalities (Table 30). A gradual reduction in the occurrence of abnormality with advancing age as reported by Rao *et al.* (1979) in crossbred bulls could not, however, be observed in all the age groups in the present study, the values obtained being well within the normal range suggested for bulls by Haq (1949) and Rollinson (1951).

The incidence of tail abnormalities ranged from 2.00 to 20.00% with a mean of 6.47 ± 0.773% (Table 21). The mean value obtained is comparable to that reported in crossbred

bulls by Rao and Rao (1978). It is lower than that reported for exotic bulls by Haq (1949), Blom (1950), Rollinson (1951) and Rao and Rao (1975) and higher than that for Tharparkar (Rao and Rao, 1975) and Ongole bulls (Rao and Rao, 1980).

Contrary to the reports of Rao and Rao (1980) in Ongole bulls, the incidence of tail abnormalities was noted to vary significantly ($P<0.01$) between bulls and between months of collection (Table 24). The higher incidence of abnormalities was observed in the month of August and lowest in July. Analysis revealed significant ($P<0.05$) seasonal variations also in the occurrence of tail abnormalities (Table 27).

The abnormalities of tail tend to decline with increasing age of the bull (Table 30). The maximum abnormality of 11.25% was noted in the semen of bulls aged below 24 months and the minimum of 4.06% in that of bulls aged above 39 months. The variations between age groups were noted to be significant ($P<0.01$).

The incidence of proximal protoplasmic droplets in the ejaculate ranged from 0.00 to 12.00% with a mean of 1.20 $\pm$ 0.14% (Table 22), which is lower than the mean value reported for purebred (Rao and Rao, 1975) and crossbred bulls (Rao and Rao, 1978) and suggested for normal fertile bulls by Lagerlof (1934), Blom (1950) and Rao (1971).

The percentage of incidence of protoplasmic droplets differed significantly ($P < 0.01$) between bulls (Table 24). This observation is similar to that of Rao and Rao (1975) in the case of Tharparkar and Jersey bulls. Rao and Rao (1980) did not observe in the case of Ongole bulls any significant variation in the occurrence of proximal protoplasmic droplets between bulls.

Significant variation ($P < 0.01$) in the incidence of protoplasmic droplets was observed also between months of collection (Table 24). This is in accordance with the findings of Rao and Rao (1978) in crossbred bulls and Rao and Rao (1980) in Ongole bulls. The highest value was obtained in August and lowest in June. Contrary to the findings of Rao and Rao (1975) no seasonal effect was observed in the incidence of proximal protoplasmic droplets (Table 27).

No significant variation was noticed in the occurrence of protoplasmic droplets between age groups of bulls (Table 30). In the ejaculate of growing bull calves, a gradual decline in the incidence of protoplasmic droplets with advancing age has been reported by Rao et al. (1979). According to these authors, nearly normal semen can be obtained from crossbred bulls at the age of 23 months. The observations made during the course of the present study lend evidence to support this view.

Freezability of Spermatozoa:

The mean freezability of spermatozoa expressed in terms of percent post-thawing motility was observed to be 36.86 (Table 31). This is higher than the mean value reported in the case of purebred and Brown Swiss crossbred (62.5% exotic inheritance) bulls by Mathew (1974). The present finding is also at variance with the observations of Reddy et al. (1980) and Satter et al. (1980) who recorded a post-thawing motility of over 55% in both exotic and crossbred bulls. The variations may be attributed to differences in the genetic make up of the animals employed in the studies.

There were no significant differences in the rate of freezability of sperms between months (Table 32). This observation is in accord with that of Mathew (1974) who has reported that season has little influence on the freezability rate of sperms of Brown Swiss bulls and their crosses. It may be pointed out that in the case of crossbred bulls used in the present study there was no evidence of seasonal effect on semen characteristics except for tail abnormalities.

Significant variations were observed in the freezability of sperms between the age groups of the bulls (Table 32). A general tendency towards increase in the rate of freezability with advancing age was apparent. A similar trend was

noticed in the case of semen characteristics also during the course of the present investigation.

Pre- and post-freezing discard of Semen:

The data presented in Table 33 reveal that out of a total of 6679 ejaculates, 2658 (39.80%) were discarded. Of these, 1907 (71.75%) were rejected before freezing and the rest (28.25%) after freezing. The present finding that the majority of semen samples were discarded on account of poor semen quality (viz. less than 60% of initial motility in the study under report) is in confirmity with the earlier report of the Milk Marketing Board, England (1973-1974).

The pre-freezing rejection rate of the ejaculation was noted to be 28.55%. This is comparable to the value reported for the Brown Swiss crosses of similar genetic make up by Mathew (1974). A pre-freezing discard rate of 23.4% has been recorded in the Annual Report of the Milk Marketing Board, England (1973-74). However, the rejection rates reported for the ejaculates of Jersey and Holstein bulls comparatively were much lower then the value observed in the present study. The post-freezing discard was noted to be 15.74% of all the frozen semen samples. This is lower then the value given by Mathew (1974) for Brown Swiss bulls of similar exotic inheritance. The Milk Marketing Board, England (1973-74) recorded only 4.2% of post-freezing discard of semen. The present

value is also higher then that reported by Sattar *et al.* (1978).

The pre-freezing, the post-freezing and the total discard rates of the ejaculates differed significantly ($P<0.01$) between months of collection, the lowest and the highest values having been obtained during the months of July and January respectively (Table 33). The rate of discard of the semen was also found to very significantly ($P<0.01$) between seasons (Table 34). Season I comprising of December and January showed the highest rejection rate and season II covering May to November, the lowest. While significant variations were observed between seasons I and II and Seasons I and III, there was no variation between seasons I and II.

A significant variation ($P<0.01$) was observed in the pre-freezing, post-freezing and total rejection rates between the different age groups of bulls (Table 35). There was a gradual and significant decrease in the rate of discard of the ejaculates with advancing age. This may be attributed to the general improvement in the semen quality with advancing age, as reported by Rao *et al.* (1979).

Fertility Rate of Bulls:

The overall fertility rate of bulls whose semen was frozen and used for insemination was observed to be 56.06%

(Table 36). The value obtained during the course of the present study is higher than that reported from India for purebred and crossbred bulls of different exotic inheritance (Nair, 1975; Sattar et al., 1976; Koul et al., 1979; Rao and Rao, 1979; Nautiyal et al., 1980; Rao and Murthy, 1980). The centres from where the present data were collected are those now being used for the implementation of progeny testing programme. Hence, it is obvious that in the matter of selection of females for insemination and in the adoption of varied steps involved in the handling and usage of frozen semen great care had been taken. It is probable that these factors might have been responsible for obtaining a high rate of fertility in the present study. The highest fertility rate (58.69%) was recorded in the second insemination, next in the first (56.36%) and then in the third (48.73%).

A significant ($P<0.01$) variation in the rate of fertility was observed between months of insemination (Tables 36 and 37). The analysis revealed significant ($P<0.01$) seasonal variation as well (Table 38). The present finding is in general agreement with that reported for the bulls of both exotic and Zebu cattle by several workers (Salisbury and Van Demark, 1961; Maule, 1962; Singh et al., 1963; Singh and Prasad, 1966; Tomar, 1966; Tomar et al., 1966; Bhosreker, 1973; Mukherjee, 1973), but is at variance with the observations of Kholi and Sarfi (1960), Nair (1975), Koul et al.(1979)

Sarush *et al*. (1980) and Rao and Murthy (1980). The highest conception rate was noticed in the descending order of summer (58.59%), winter (55.55%) and rainy season (55.00%).

The fertility rate showed significant variation (P 0.01) between the ages of the bulls (Table 39). The rate of fertility increased from 52.35% in bulls aged between 2-4 years to 61.39% in those aged above 4 years. This increase in fertility may perhaps be attributable to the general improvement in the semen quality associated with advancing age, as observed by Rao *et al*. (1979).

Significant variation (P 0.01) in the fertility rate was noted between bulls as well (Table 40). The highest fertility of 64.43% was observed in bull No. 110 and the lowest of 35.98% in bull No.206. Differences in the fertility rate between bulls within the same breed have been recorded earlier by Mathai *et al*. (1970) and Sattar *et al*. (1978).

From the considerations obtained in the foregoing paragraphs, it is evident that puberty in Brown Swiss crossbred bulls with 62.5 exotic inheritance, adjudged on the basis of the development of testis-epididymis and production of viable spermatozoa in the ejaculates, is attained at a time intermediary between the age of puberty of *Bos taurus* and *Bos indicus*. This inference lends evidence to the popular belief that crossbreeding hastens the onset of puberty of the

local cattle. Even though the animals show sexual desire and inclination to mount even at an age as early as 16 months, good quality semen suitable for breeding is not likely to be produced until they reach 24-30 months of age. However, the possibility of reducing the age of puberty of crossbred bulls through better feeding and management cannot be ruled out.

The ejaculates produced by the crossbred bulls at sexually matured age in general satisfy the criteria suggested for the normal bull semen. The comparatively low ejaculate volume obtained during the course of the present study appears to have been made good by the high sperm concentration in as much as the total output of spermatozoa remained unaltered. Individual differences observed in the quality of semen are in confirmity with the normal biological variations. Absence of seasonal variations in the semen quality in general and freezability in particular tends to suggest that the climatic conditions prevailing at the place of investigation viz., Indo-Swiss Project, Mattupatty, Kerala are conducive for the production of normal semen. The general improvement noticed in the quality of semen and freezability rate of spermatozoa with the advancement of age indicate that the quality of semen gets stabilized only at a period later than the actual attainment of puberty.

The pre-freezing and post-freezing rejection rates of the ejaculates seem to be rather high in crossbred bulls, the former accounting for more discard than the latter. From a recapitulation of the overall results, it can be summarised that the fertility rate of the crossbred bulls is quite satisfactory under the prevailing conditions of Kerala.

Summary

A systematic study involving the use of 24 Brown Swiss crossbred bull calves and 7 adult bulls with 62.5% exotic inheritance varying in age from 0-360 days and 22-40 months respectively, reared and maintained at Mattupatty, Kerala under Indo-Swiss Project, was carried out in order to (a) trace the postnatal growth and development of testis and epididymis and (b) assess the semen characteristics such as volume, initial motility, sperm concentration, live sperm count, and the incidence of abnormal sperms. As a corrolary to and as an integral part of this comprehensive investigation, 6679 semen collection particulars from the records maintained at the Indo-Swiss Project, Mattupatty and 7586 insemination data at the Crossbreeding Research Centre, Mavattupuzha were critically scrutinised to deduce freezability/discard rate of the ejaculates and fertility potency of the bulls respectively. The material used, the salient observations made and the valid inferences drawn are indicated below:

Twenty four pairs of testis-epididymis collected from the bull calves were processed to study the postnatal growth and development. The pattern of testicular growth was found to be curvilinear, exhibiting a slow growth rate in the beginning, rapid in the midway and more rapid while approaching puberty. The testicular weight was observed to be signi-

ficantly ($P<0.01$) correlated with the age and body weight of the animal. The right testis was significantly ($P<0.01$) heavier than the left, the difference in weight between the two being more conspicuous near about the attainment of puberty. The shape of the testis which was oval, remained unaltered throughout the period of growth. The growth curve of the seminiferous tubules appeared to be of sigmoid type. The diameter of the tubules was found to increase slowly between 0-120 days and rapidly, between 120-240 days of age. The rate of growth was reduced slightly between 240-360 days of age. The diameter of the tubules showed significant ($P<0.01$) correlation with the age, the body weight and the testicular weight of the animal. Lumen formation in the seminiferous cords was initiated at 150 days, and completed at 180 days of age. The germinal cells represented by gonocytes were found to transform themselves into spermatogonia with advancing age, the process of transformation having been initiated at 120 days and completed, at 150 days of age. The primary spermatocytes, spermatids and spermatozoa were spotted for the first time at 150 days, 300 days and 300-330 days of age, respectively. The first observable change in the transformation of supporting cells to sertoli cells was noticed at the age of 120 days. At the age of 150 days, fully formed sertoli cells could be observed in most of the tubules. The initiation of transformation of supporting cells into sertoli cells appeared to coincide with the formation of

primary spermatocytes. The process of transformation was seen completed in all the tubules by 150 days of age. The interstitium that occupied a major portion of the testicular parenchyma at birth showed progressive reduction with corresponding increase in the tubular diameter. The transformation of immature Leydig cells to mature cells was initiated at about 150 days and completed by 180 days of age.

The growth curve of the epididymis was noted to be curvilinear just as that of the testis. The rate of growth was found to be slow upto the age of 240 days and rapid, thereafter. A highly significant ($P<0.01$) correlation between the epididymal weight and the age/the body weight/the testicular weight of the animal was observed. The diameter of the tubules, height of the epithelium and the degree of transformation of epithelial lining from simple columnar to pseudo-stratified type have all shown distinct regional differences between the caput, the corpus and the cauda-epididymis. The tubular diameter was observed to be largest in the cauda, lesser in the corpus and least in the caput throughout the period of growth. The spurt of growth noticed in the corpus and the caput was not evident in the cauda where the rate of growth was gradual throughout. Similar trends were noticed in the epithelial height also. At birth the epithelium showed maximum height in the cauda but at 360 days it was the epithelium in the caput epididymis that was found to be the

tallest and that in the caude shortest. The degree of transformation of the epithelial cells from simple columner to pseudostratified type occurred first in the caude (120 days), then in the corpus (150 days) and last in the caput (180 days). The differentiation of epithelium was completed earlier than the adult height was reached. The distal part of ductus epididymis seemed to have attained the adult size at an age earlier than the proximal. Sperms were seen in all the three regions of the ductus epididymis at about 300 days of age. The density of sperms increased with advancing age. The concentration of sperms was more in the caude than either in the corpus or the caput.

Four hundred and five semen samples collected over a period of one year were used to assess the semen characteristics. The mean values of ejaculate volume, initial motility, sperm concentration, live sperm count, sperm head abnormalities, free loose head, middle piece abnormalities, tail abnormalities and proximal protoplasmic droplets were observed as 2.65 ± 0.84 ml, 66.84 ± 0.87%, 1599.64 ± 55.371 millions per ml of semen, 72.55 ± 1.310%, 3.43 ± 0.300%, 4.91 ± 0.439%, 0.65 ± 0.045%, 6.47 ± 0.775% and 1.20 ± 0.143%, respectively. Significant variations ($P<0.01$) were observed between bulls in respect of sperm head abnormalities, free loose head, middle piece abnormalities, tail abnormalities and proximal protoplasmic droplets. The differences between months were found

to be significant ($P<0.01$) in respect of ejaculate volume, sperm head abnormalities, free loose head, tail abnormalities and proximal protoplasmic droplets. Seasonal effect, however, was evident only in the incidence of tail abnormalities ($P<0.05$). Variations due to the age of the bulls were found to be significant ($P<0.01$) in the case of ejaculate volume, sperm concentration, free loose head, middle piece abnormalities and tail abnormalities. The ejaculate volume increased and tail abnormalities decreased with advancing age. The occurrence of free loose head and middle piece abnormalities increased with increasing age, but at no time the incidence crossed the limit prescribed for normal bull semen.

The freezability of sperms expressed in terms of per cent of post-thawing motility was observed to be 36.86. There was no seasonal effect on the freezability rate of spermatozoa. As between age groups, there were significant ($P<0.01$) variations, the rate of freezability increasing with the advancement of age of bulls.

The overall rejection of the ejaculates was observed to be 39.80%. Of the total samples discarded, 71.75% were rejected before freezing. The pre-freezing discard was found to be 28.55% of the total ejaculates. The post-freezing discard was 15.74% of the frozen samples. The pre-freezing, the post-freezing and the total rejection of the ejaculates

differed significantly ($P<0.01$) between months and between seasons. There were significant ($P<0.01$) variations in the rejection rates between different age groups of the bulls. The rate of rejection of the ejaculates decreased with advancing age.

The overall fertility rate of bulls was noted to be 56.06%. There was significant differences in the fertility rate between months/seasons of insemination, and between age of the bulls. The fertility rate improved with the advancement of age. The difference in the fertility rate was also significant ($P<0.01$) between bulls.

The significance of the observations and inferences indicated above and their relevance to the implementation of crossbreeding programmes in Kerala were discussed briefly.

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Title of the Thesis	:	POSTNATAL DEVELOPMENT OF TESTIS AND EPIDIDYMIS, SEMEN CHARACTERISTICS AND FERTILITY OF BROWN SWISS CROSSBRED BULLS.
Name of the student	:	Sri C.K. SURENDRA VARMA RAJA
Name and address of the Major Advisor	:	Dr. A. RAMAMOHANA RAO Professor & Head, Department of Animal Reproduction and Gynaecology, College of Veterinary Science, TIRUPATI.
Degree Awarded	:	Ph. D.
Year of award of degree	:	1981
Major Subject	:	ANIMAL REPRODUCTION & GYNAECOLOGY.

ABSTRACT

A systematic investigation involving the use of 24 Brown Swiss crossbred bull calves and 7 adult bulls with 62.5% exotic inheritance varying in age from 0-360 days and 22-40 months respectively, reared and maintained at Indo-Swiss Project, Mattupatty, Kerala, was carried out in order to (a) trace the postnatal growth and development of the testis and epididymis and (b) assess the semen characteristics such as volume, initial motility, sperm concentration, live sperm count and incidence of abnormal sperms; As a corrolary to and as an integral part of this comprehensive study, 6679 semen collection particulars from the records maintained at the Indo-Swiss Project, Mattupatty and 7886 insemination data at the Crossbreeding Research Centre, Muvathupuzha were critically scrutinised to deduce freezability/discard rate

of the ejaculates and the fertility potency of the bulls, respectively. The materials used, the salient observations made and the valid inferences drawn are given below:

Twenty four pairs of testis and epididymis collected from the bull calves were processed to study the postnatal growth and development. The pattern of growth of testis and of the epididymis was found to be curvilinear. The testicular and epididymal weights were observed to be significantly ($P<0.01$) correlated with the age, and body weight of the animals. A highly significant ($P<0.01$) correlation between epididymal weight and testicular weight was also observed. The growth rate of the seminiferous tubules appeared to be of sigmoid type. Formation of lumen in the seminiferous cords was initiated at 150 days and completed, at 180 days of age. The transformation of gonocytes to spermatogonia occurred at 150 days of age. The formation of primary spermatocytes, spermatid and spermatozoa occurred at the age of 150 days, 300 days and 300-360 days, respectively. The process of transformation of supporting cells was initiated at 120 days and completed at 180 days of age. The transformation of immature Leydig cells to mature cells was initiated at 150 days and completed, by 180 days of age. There were distinct regional differences between the caput, the corpus and the cauda epididymis in respect of the diameter of the tubules, height of the epithelium and the degree of transformation of

epithelial lining from simple columnar to pseudostratified type. The distal part of the ductus epididymis seemed to have attained the adult size at an earlier age than the proximal. Sperms were seen in all the three regions at about 300 days.

The mean values of ejaculate volume, initial motility, sperm concentration, live sperm count, sperm head abnormalities, tail abnormalities and proximal protoplasmic droplets were found to be 2.65 ± 0.84 ml, 66.24 ± 0.87%, 1599.64 ± 55.371 millions per ml of semen, 72.55 ± 1.310%, 4.91 ± 0.439%, 0.68 ± 0.045%, 6.47 ± 0.775% and 1.20 ± 0.143%, respectively. Significant ($P<0.01$) variations between bulls were observed in respect of sperm head abnormalities, tail abnormalities and proximal protoplasmic droplets and between months, in respect of ejaculate volume, sperm head abnormalities, free loose head, tail abnormalities and proximal protoplasmic droplets. Seasonal variation ($P<0.05$), however, was evident only in the incidence of tail abnormalities. Variations due to age of bulls were found to be significant ($P<0.01$) in the case of ejaculate volume, sperm concentration, free loose head, middle piece abnormalities and tail abnormalities.

The freezability of sperms expressed in terms of per cent of post-thawing motility was found to be 36.86. There was no seasonal effect on the freezability of sperms. The freezability rate increased significantly ($P<0.01$) with advancement of age of bulls.

The overall rejection rate of the ejaculates was noted to be 39.80%. Most of the rejection (71.75%) was done before freezing. The pre-freezing rejection was found to be 28.55% of the total ejaculate. The post-freezing discard to be 16.74% of the frozen samples. The rejection rates of the ejaculates differed significantly ($P<0.01$) between months/seasons/age of the bulls. The rate of rejection was found to decrease with advancing age.

The overall fertility rate of the bulls was found to be 56.06%. There were significant ($P<0.01$) differences in the fertility rate between months/seasons of insemination and between age of the bulls. As between bulls also, there was a significant variation ($P<0.01$) in the fertility rate.

The significance of the observations inferences indicated above and their relevance to the implementation of cross breeding programme in Kerala were discussed briefly.

Fig. 17. Testis (0 day) -- Supporting cells lining the basement membrane with few gonocytes in the centre of cords.
(H & E X 400).

Fig. 18. Testis (30 day) -- Widely seperated cords with abundant interstitium.
(H & E X 100).

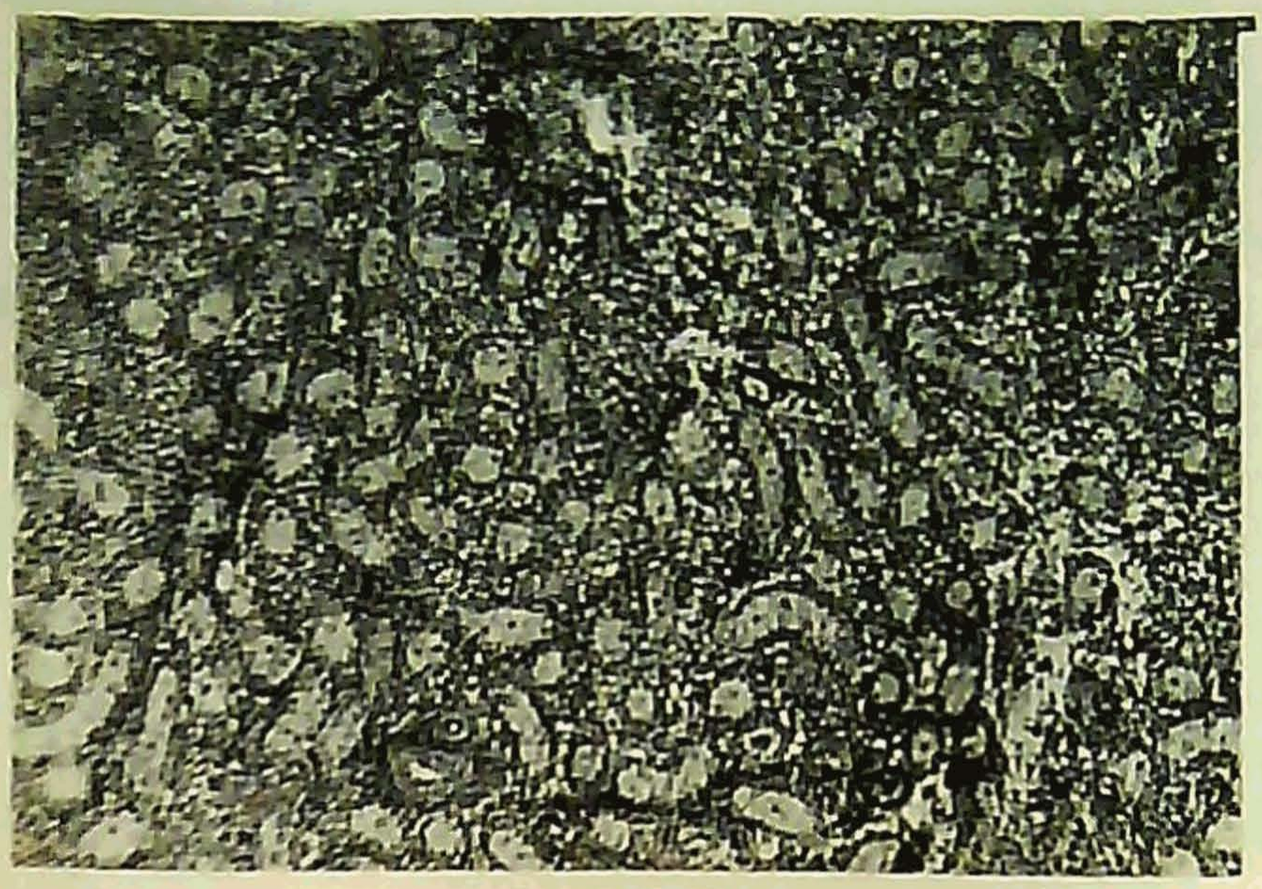

Fig. 16.

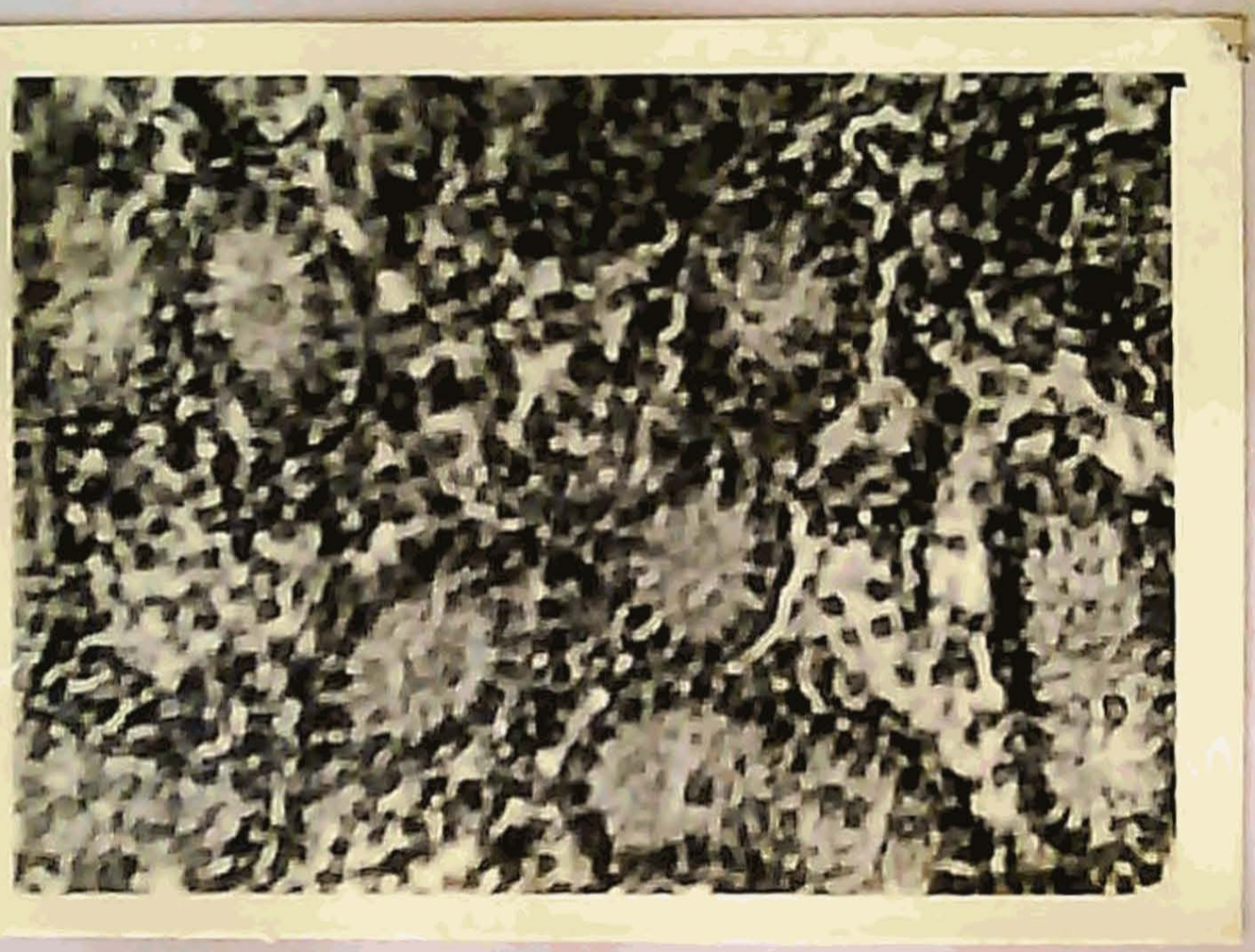

Fig. 17.

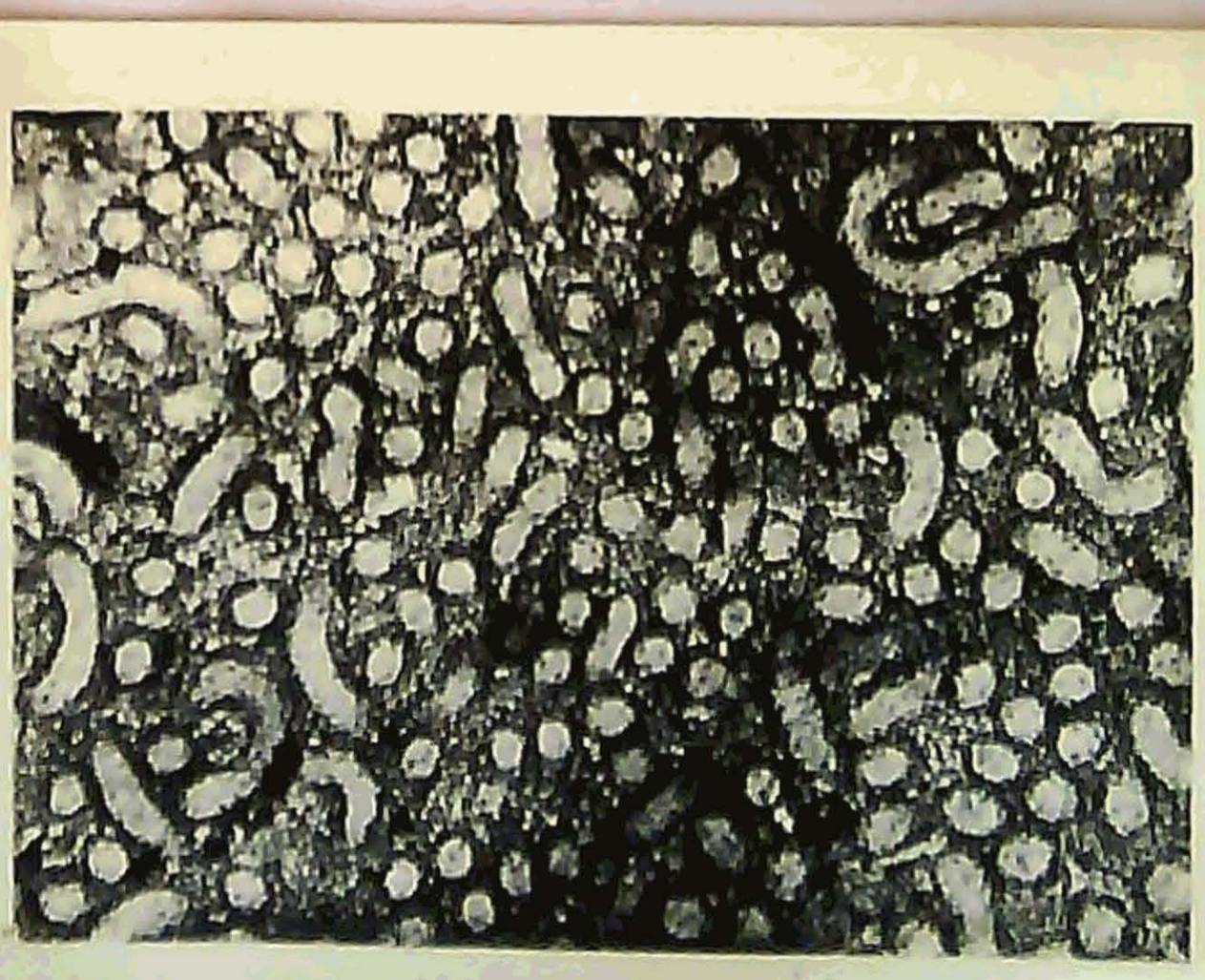

Fig. 18.

Fig. 19. Testis (30 day) -- supporting cells at the periphery and gonocytes in the centre of the cord. (H & E X 400)

Fig. 20. Testis (60 day) -- Few supporting cells in the centre of the cord and gonocytes showing signs of degeneration. (H & E X 400).

Fig. 21. Testis (90 day) -- Degenerating gonocytes in the centre of the cord. (H & E X 400).

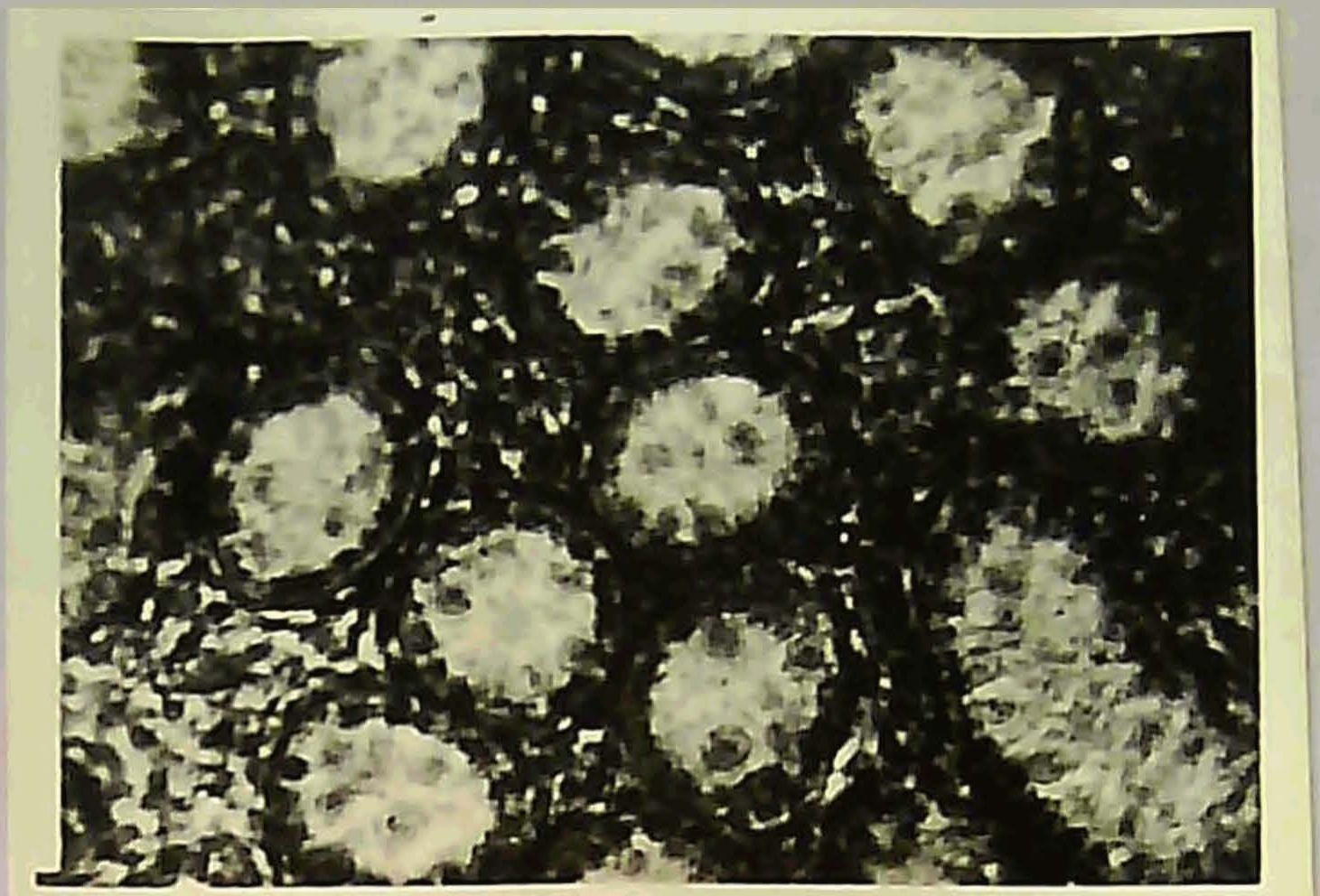

Fig.19.

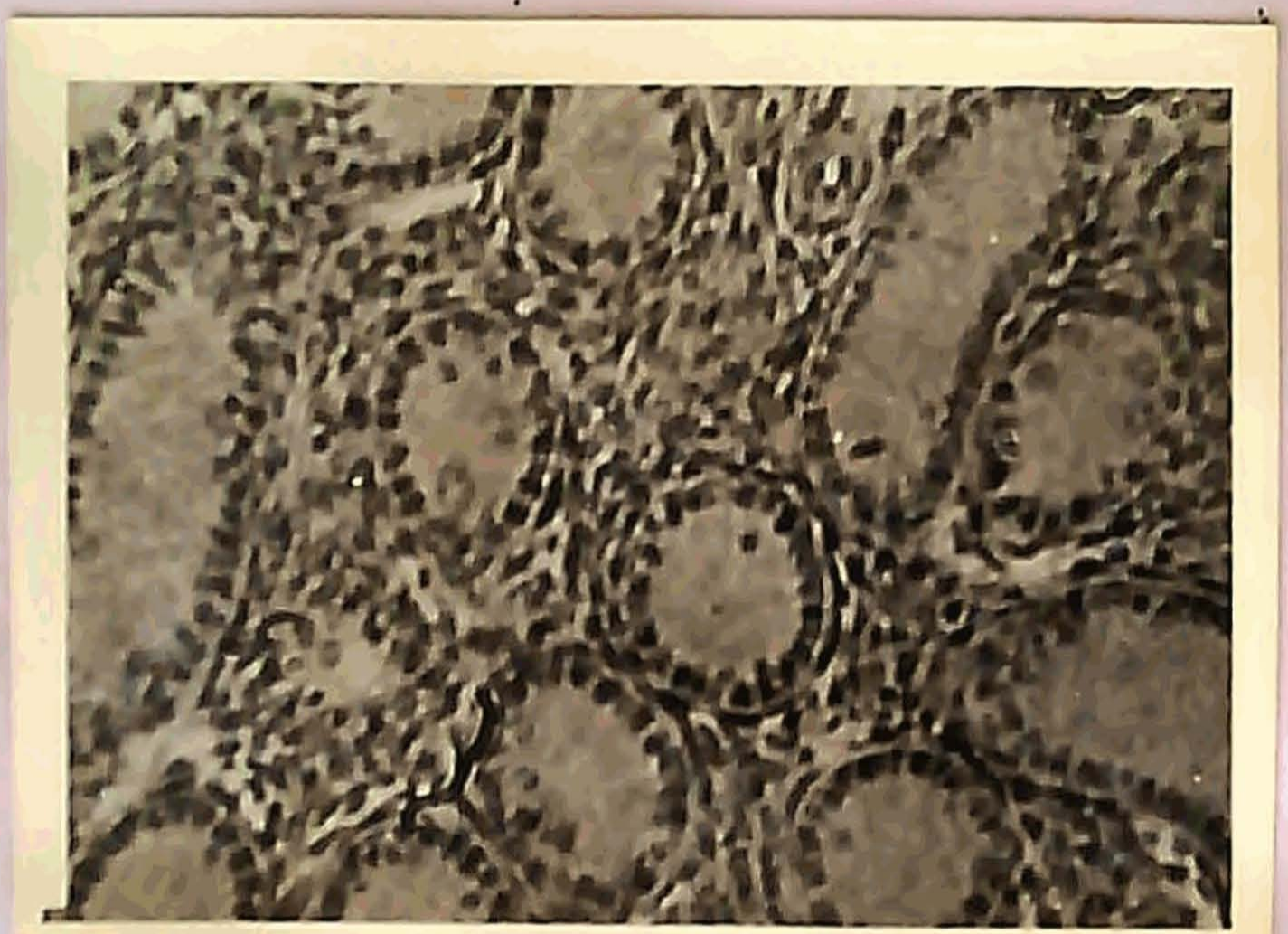

Fig. 20.

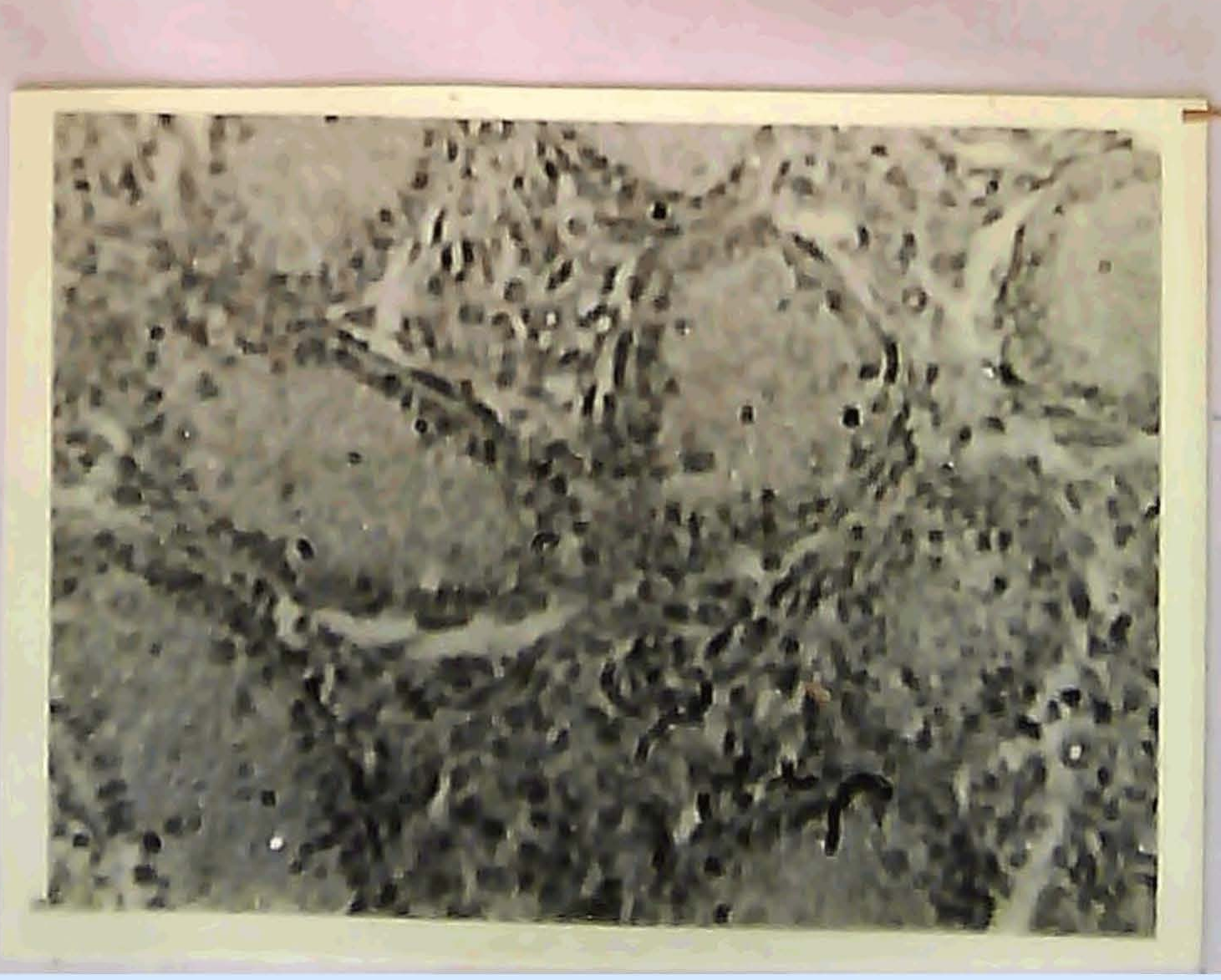

Fig. 21.

Fig. 22. Testis (120 day) -- Seminiferous cords showing Sertoli cells and spermatogonia at the basement membrane and degenerating gonocytes in the centre.(H & E X 400).

Fig. 23. Testis (120 day) -- Sertoli cells and spermatogonia. (H & E X 1000)

Fig. 24. Testis (150 day) -- Seminiferous cords close to each other - Spermatogonia. (H & E X 400).

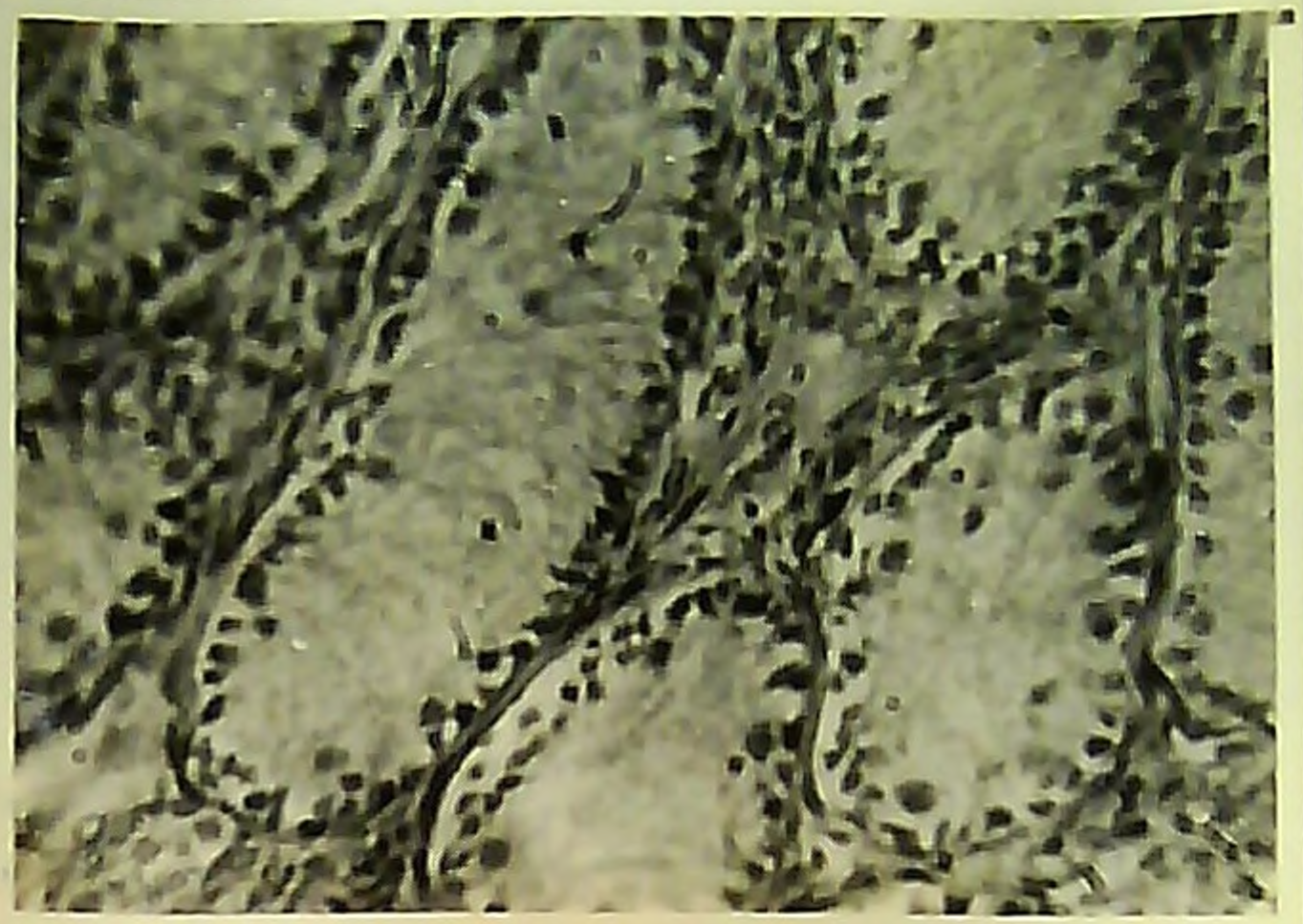

Fig. 22.

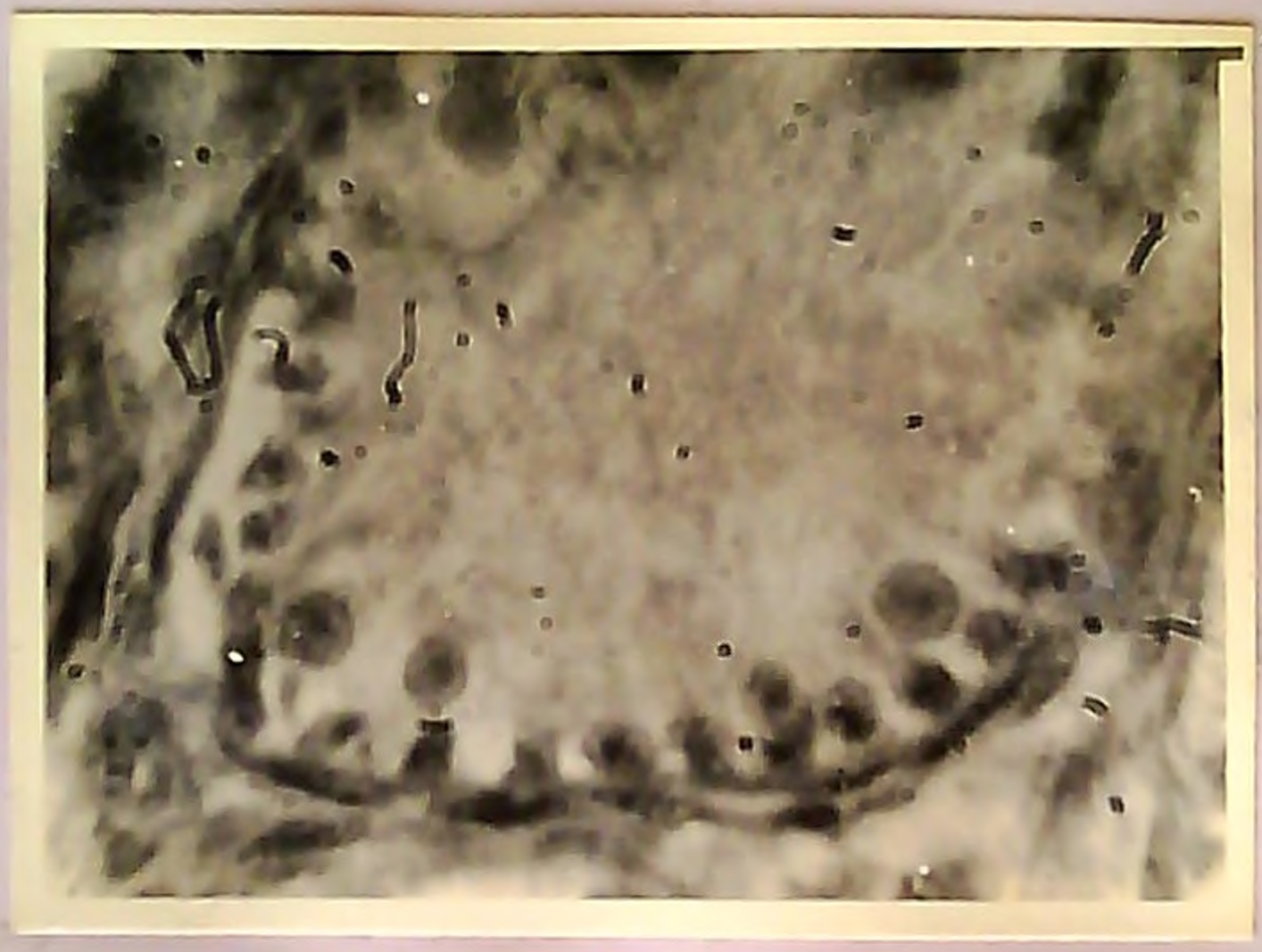

Fig. 23.

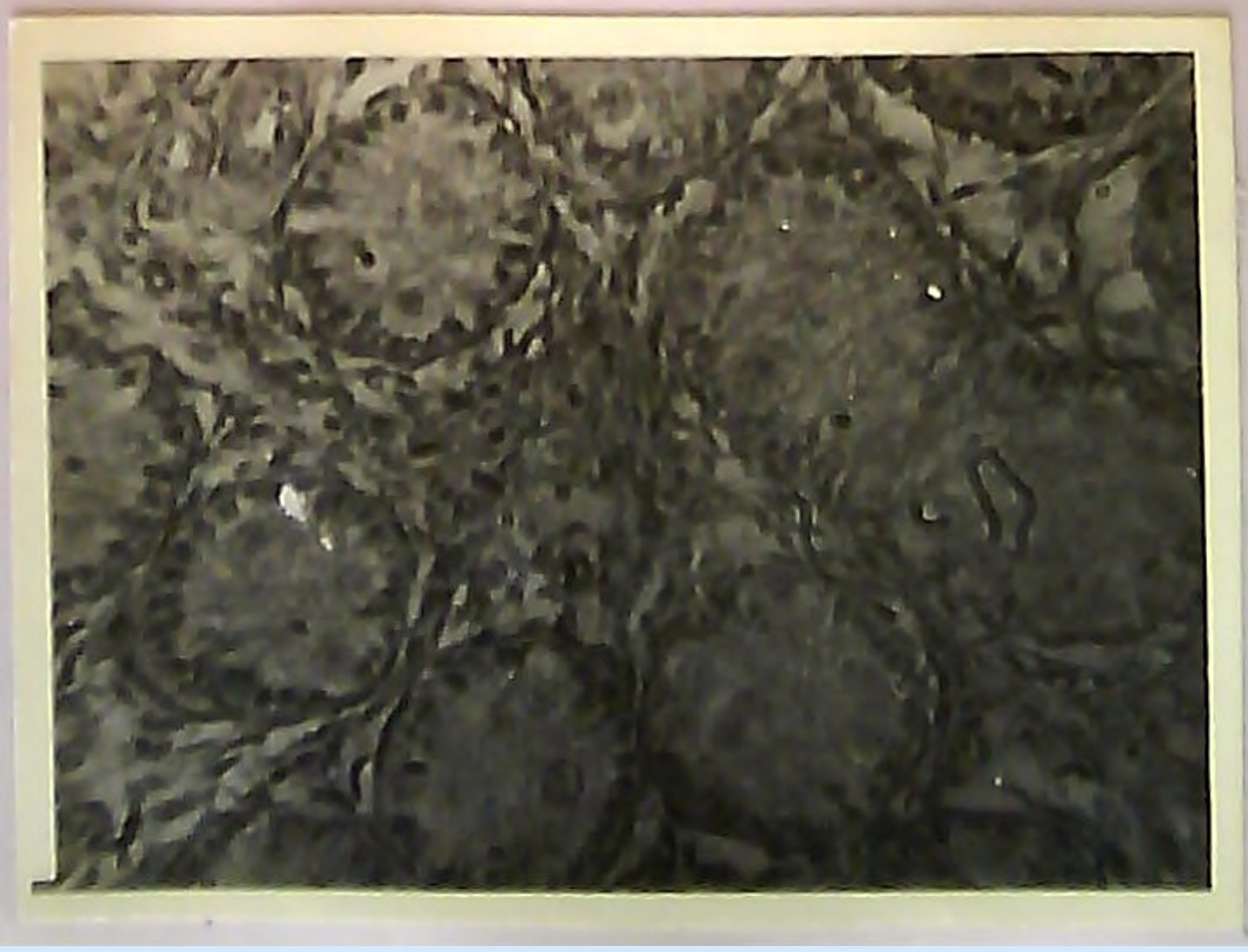

Fig. 24.

Fig. 25. Testis (180 day) -- Small cracks within the cord - Spermatogonia.

(H & E X 400).

Fig. 26. Testis (210 day) -- Tubules are in contact - Interstitium reduced - spermatogonia and primary spermatocytes.

(H & E X400)

Fig. 27. Testis (240 day) - Spermatogonia and primary spermatocytes. (H & E X 400).

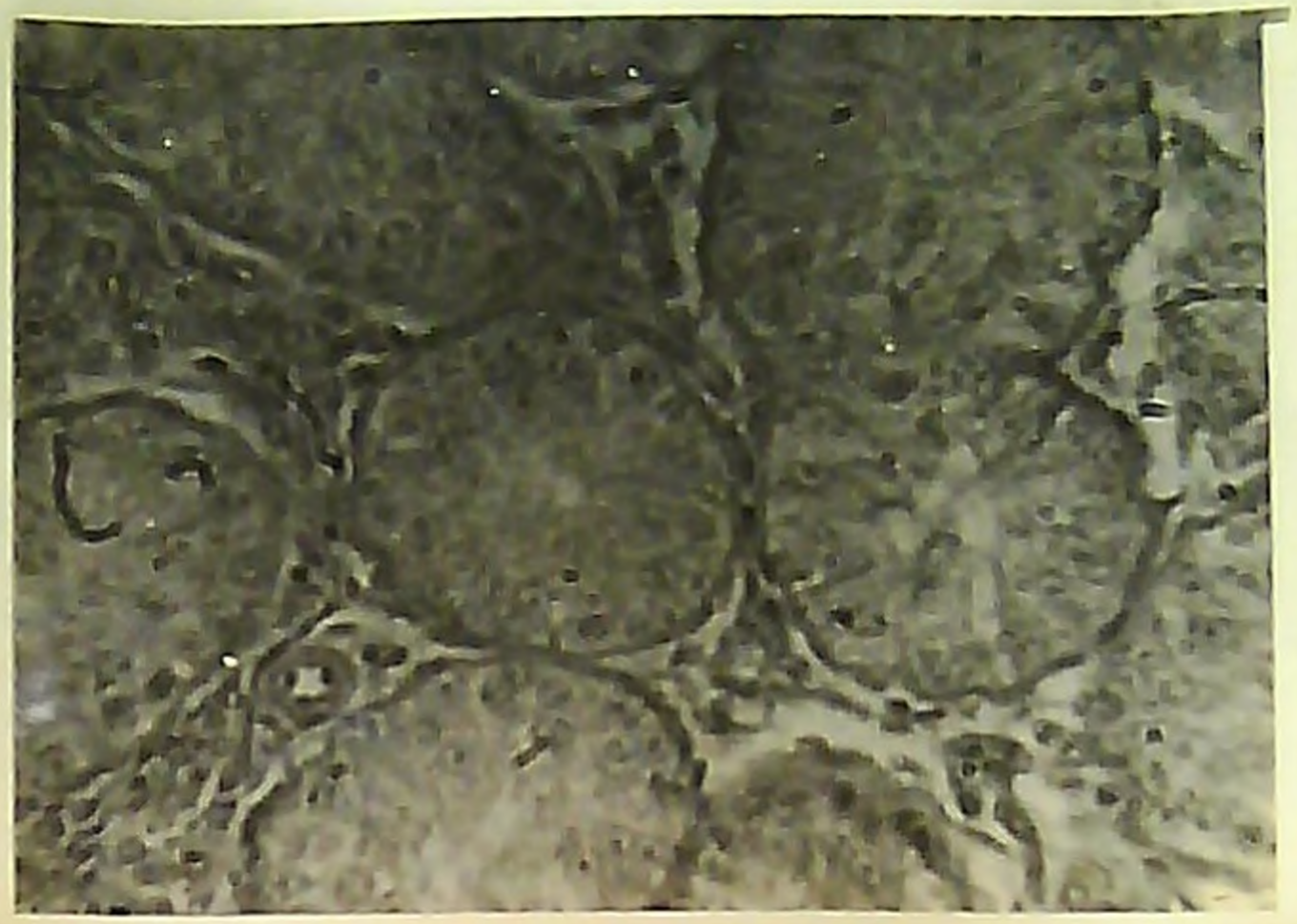

Fig. 25.

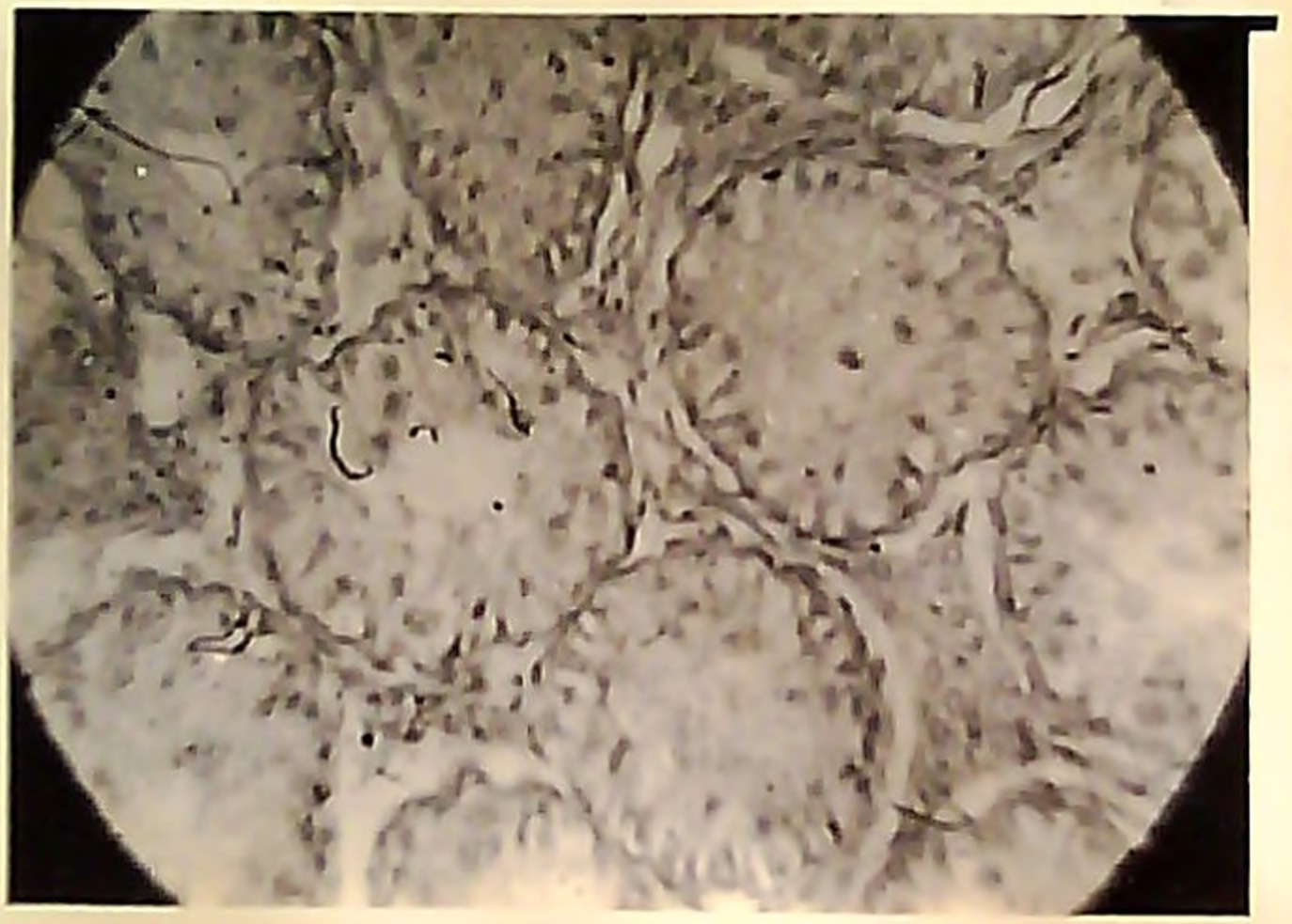

Fig. 26.

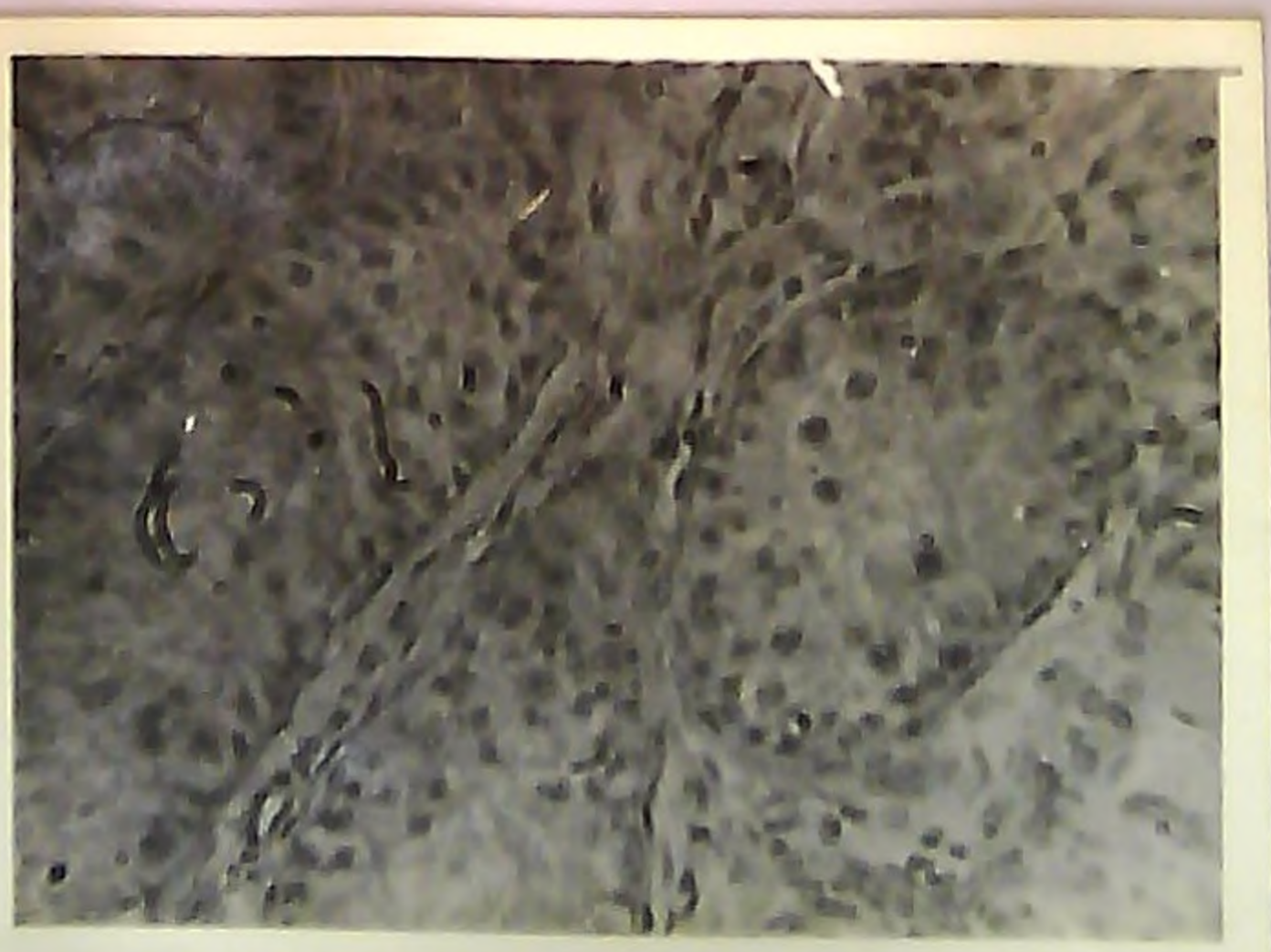

Fig. 27.

Fig. 28. Testis (270 day) -- Tubules in close contact with each other with scanty interstitium - multilayered germinal epithelium (H & E X 100).

Fig. 29. Testis (270 day) -- well developed tubules with multilayered germinal cells. (H & E X 400).

Fig. 30. Testis (270 day) -- Well developed lumen-- Sertoli cells, spermatogonia and primary spermatocytes in 2-3 layers. (H & E X 400).

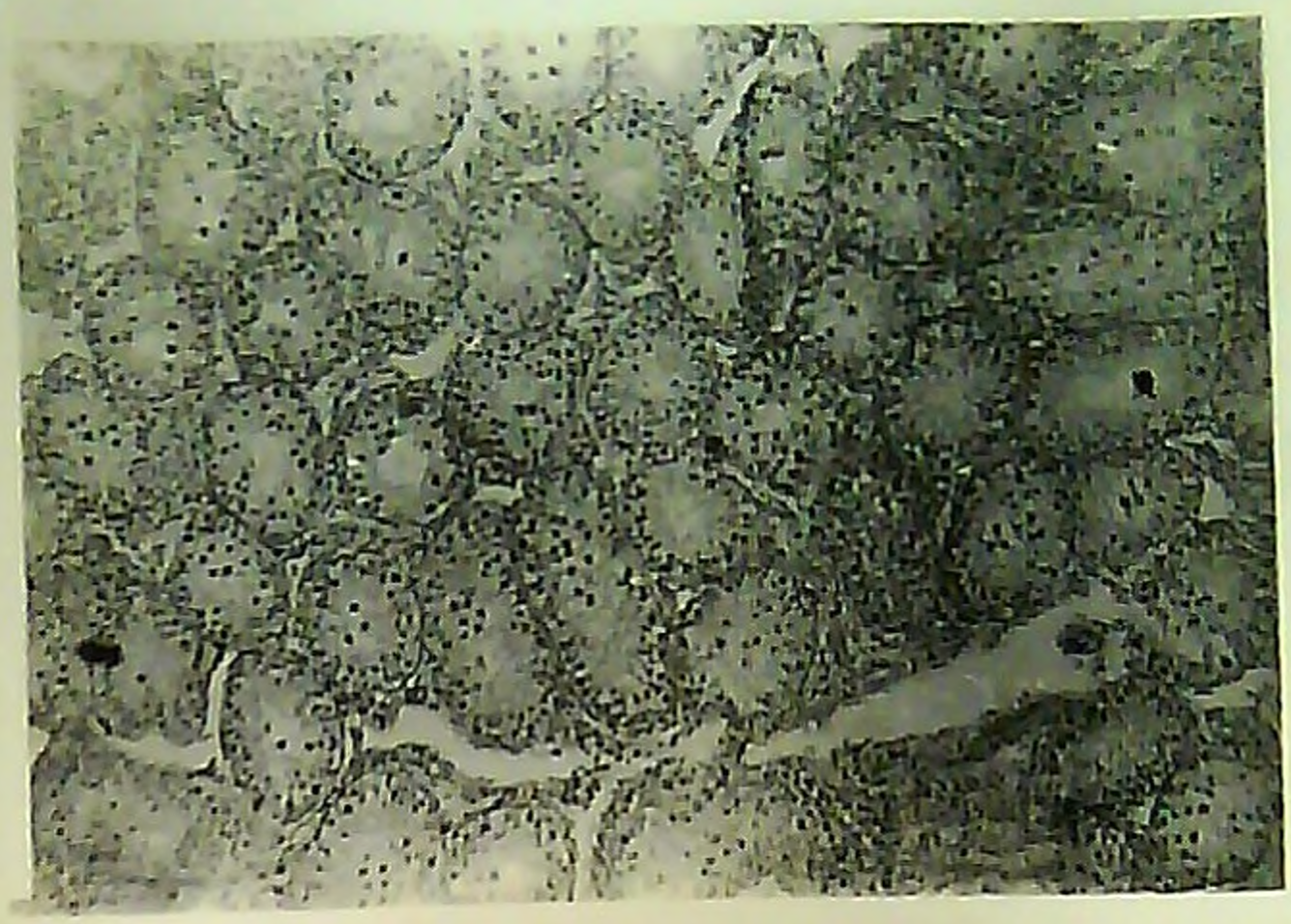

Fig. 28.

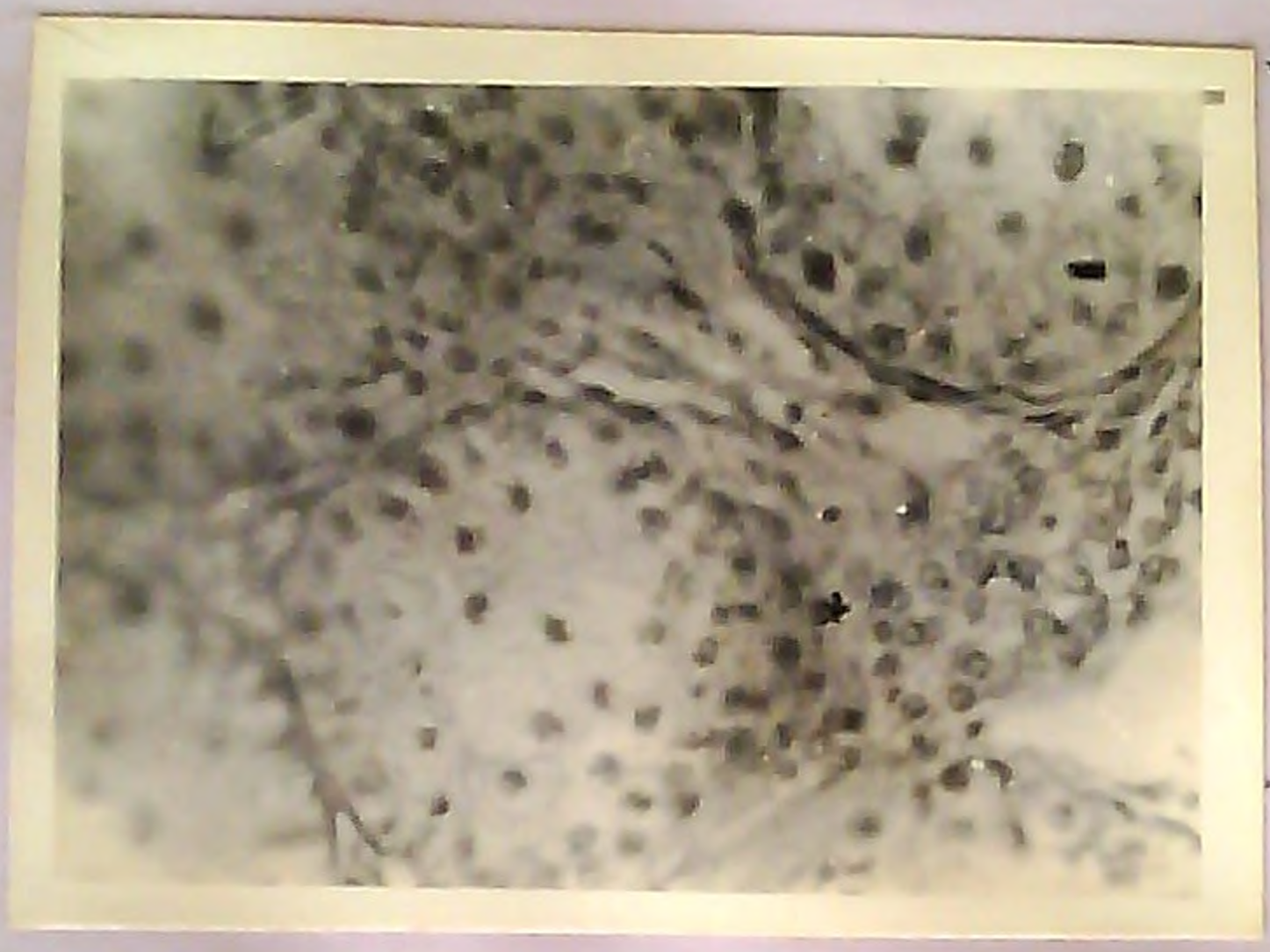

Fig. 29.

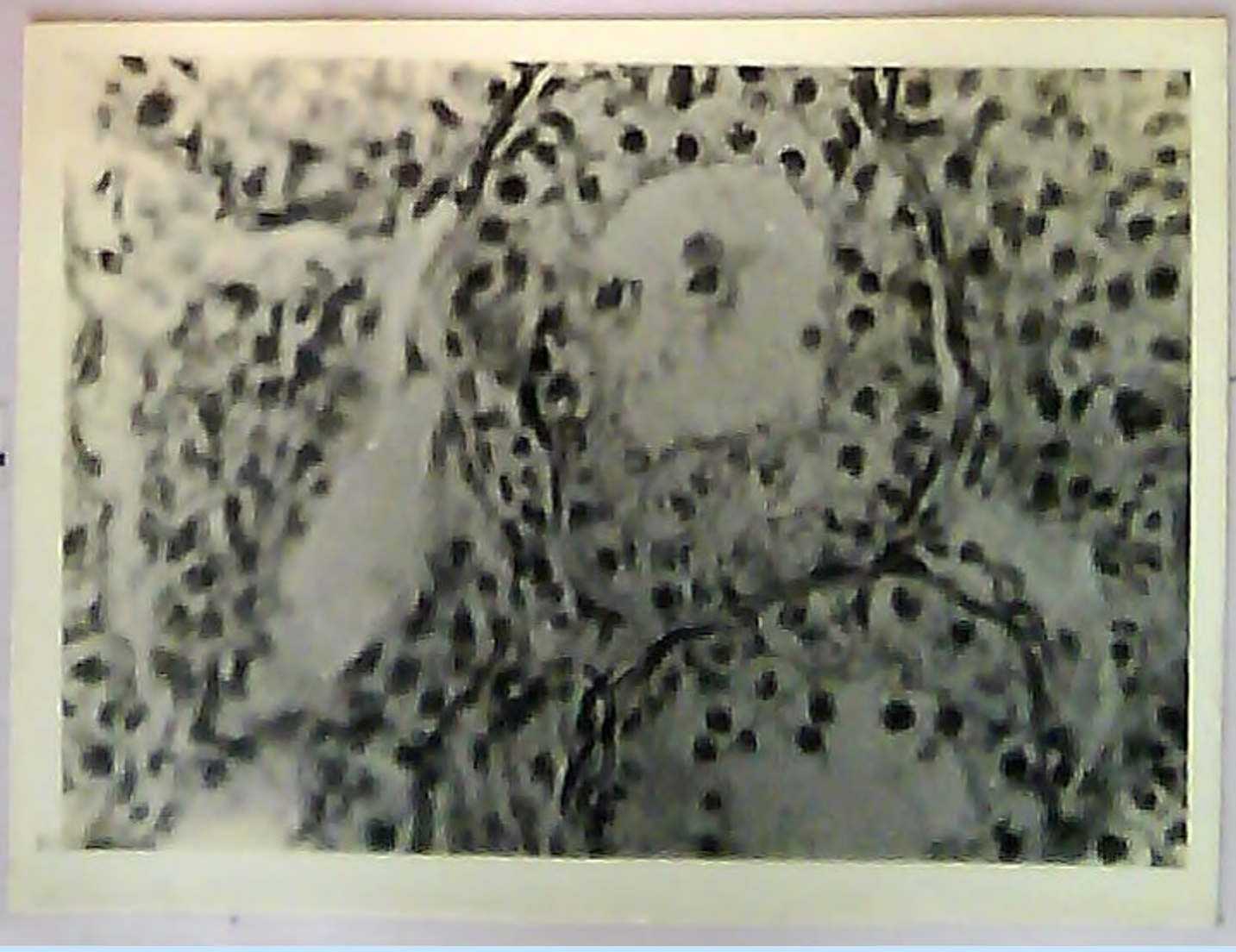

Fig. 30.

Fig. 31. Testis (270 day) -- Tubules showing Sertoli cells, spermatogonia and primary spermatocytes. (H & E X1000).

Fig. 32. Testis (300 day) -- Tubules showing spermatogonia, primary spermatocytes and round spermatids. (H & E X400).

Fig. 33. Testis (330 day) -- Elongated spermatids arranged in tufts in the Sertoli cells. (H & E X 400).

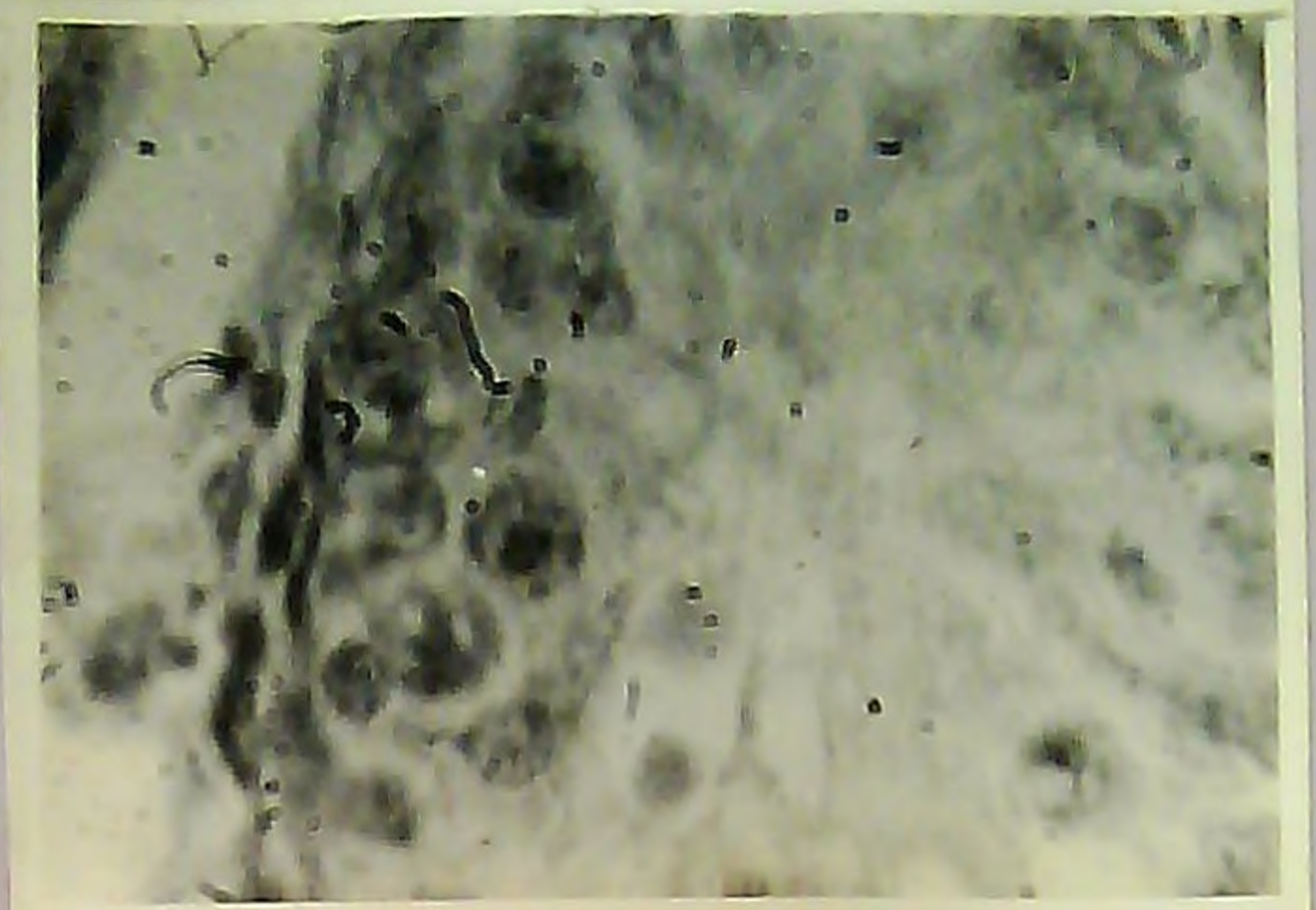

Fig. 31.

Fig. 32.

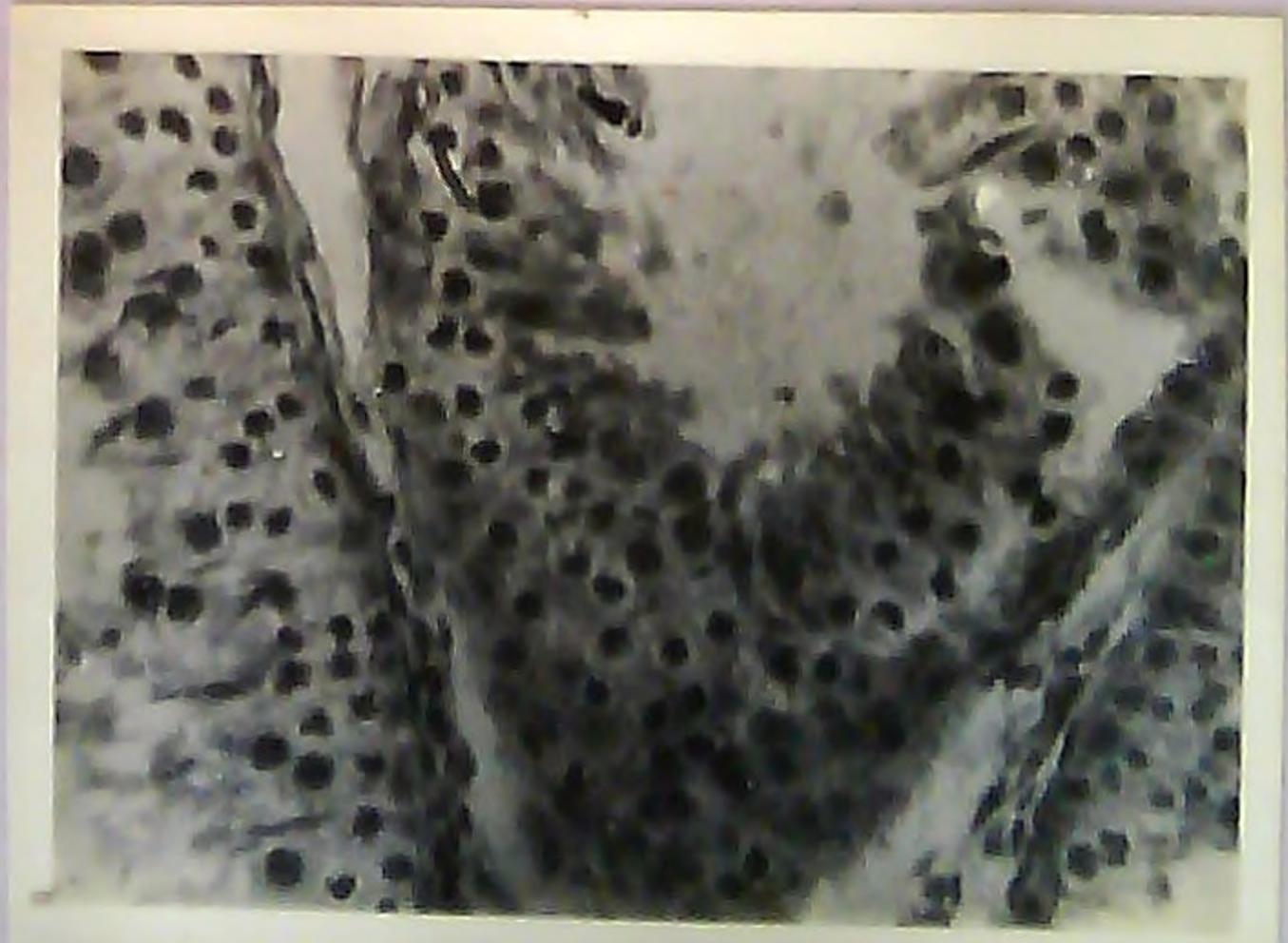

Fig. 33.

Fig. 34. Testis (330 day) -- Degenerating spermatids in the centre of the tubule.
(H & E X 400).

Fig. 35. Testis (330 day) -- All the germinal layers including elongated spermatids.
(H & E X 1000).

Fig. 36. Testis (360 day) -- Dense germinal layer with large number elongated spermatids and sperms. (H & E X 400).

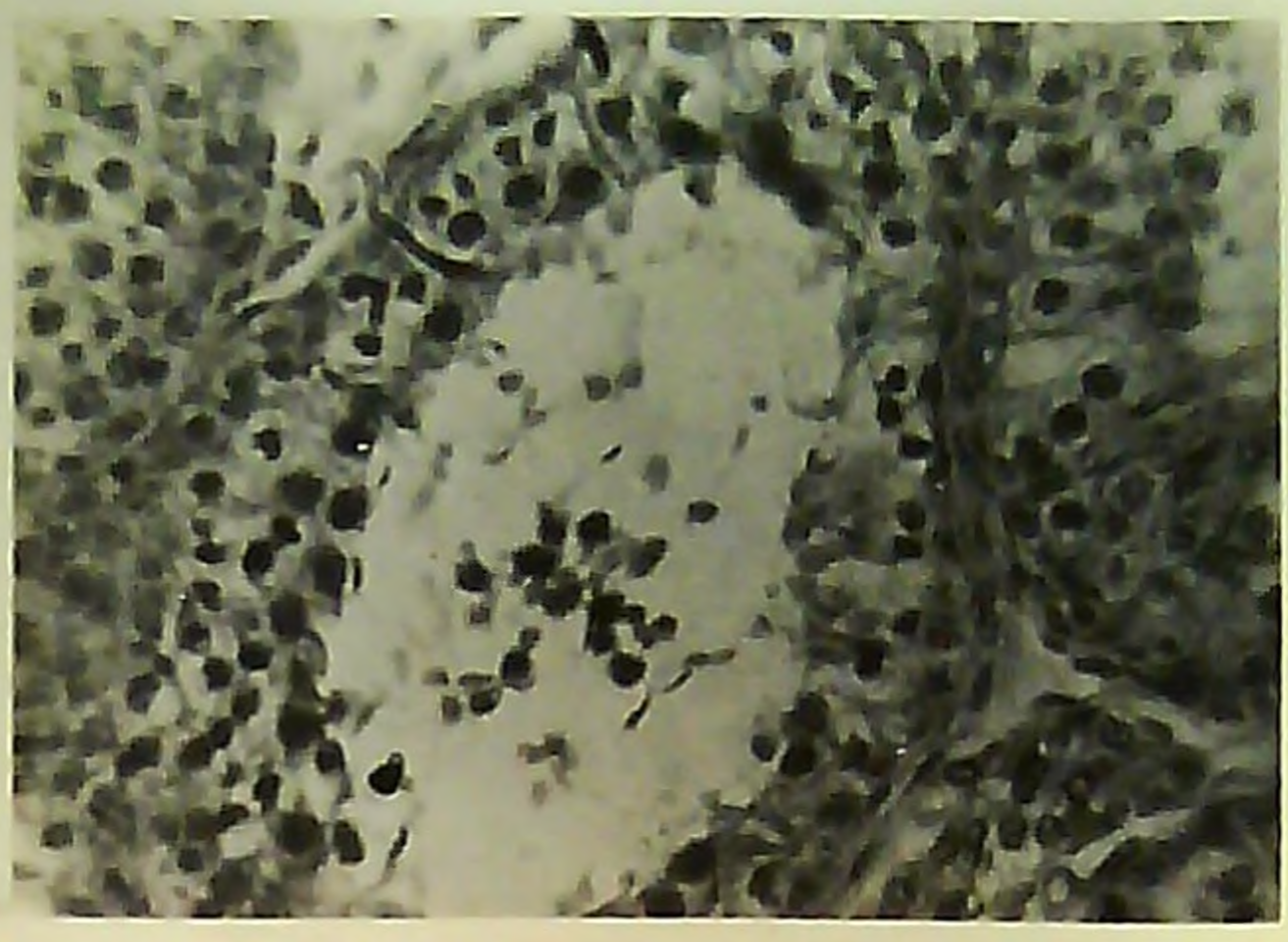

Fig. 34.

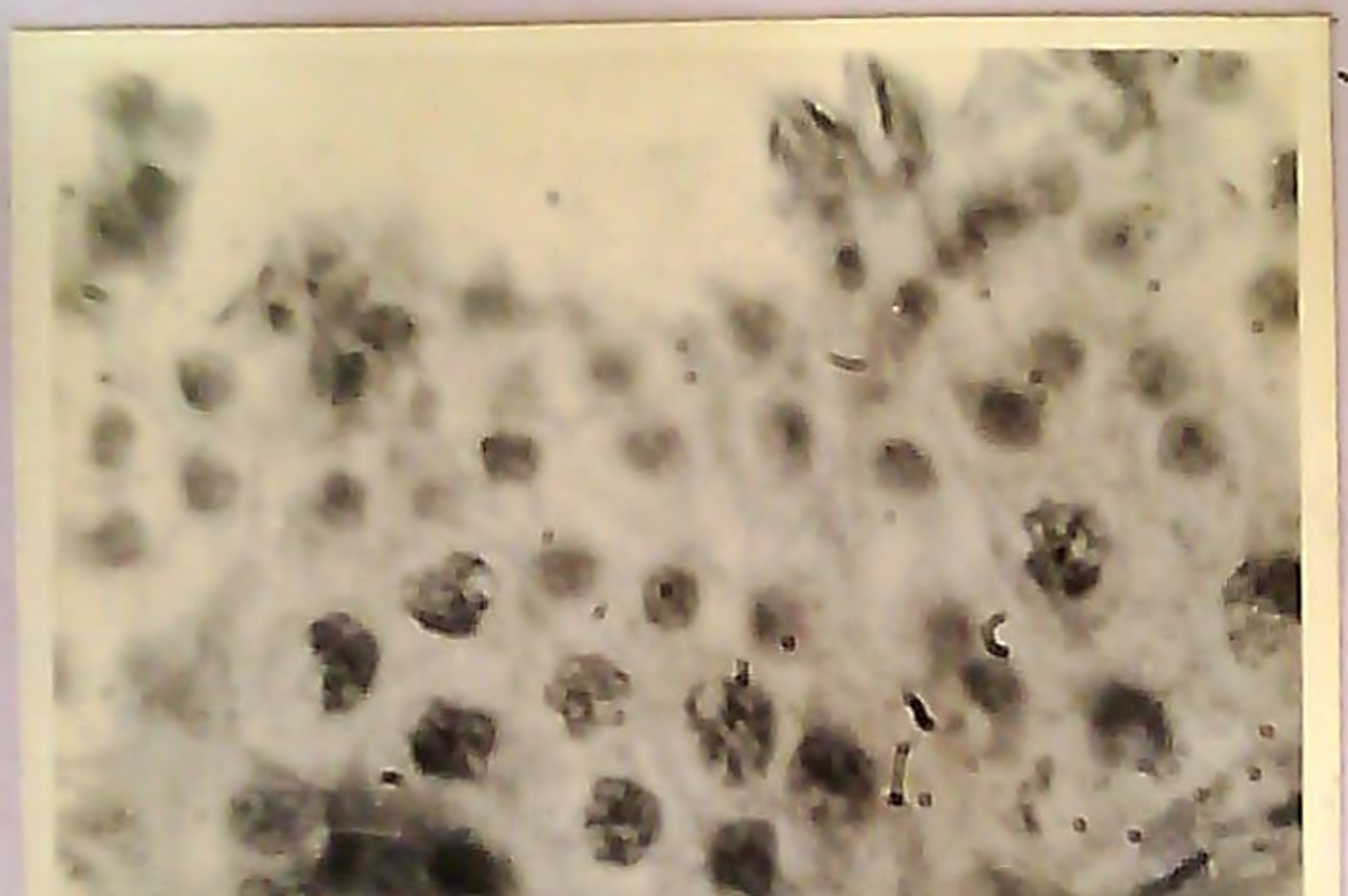

Fig. 35.

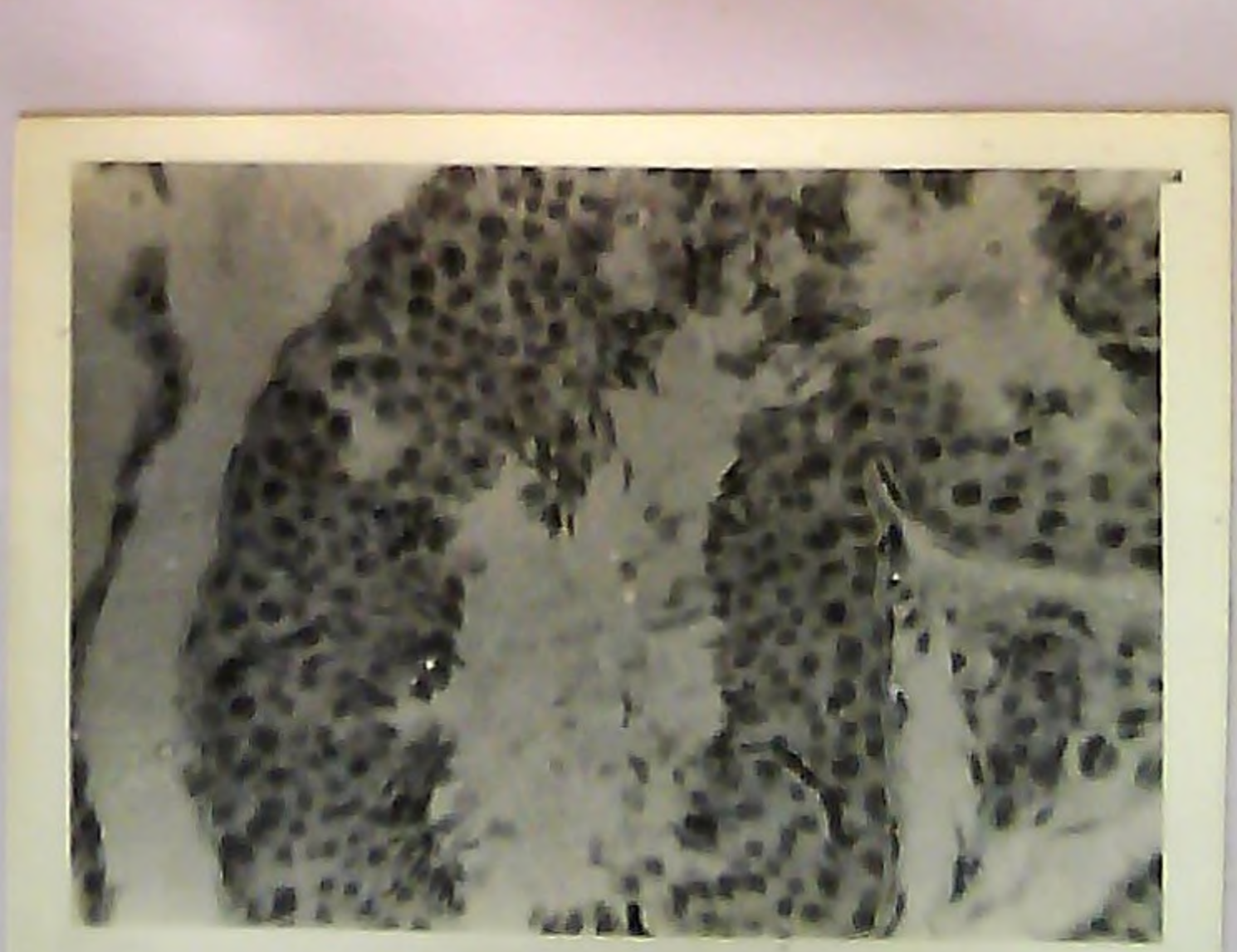

Fig. 36.

Fig. 37. Testis (360 day) -- Spermatozoa in the lumen of the tubules.

(H & E X 1000).

Fig. 38. Caput epididymis (0 day) -- Tubules lined by a single layer of simple columnar cells.

(H & E X 400).

Fig. 39. Corpus epididymis (0 day) -- Tubules lined by a single layer of columnar cells.

(H & E X100).

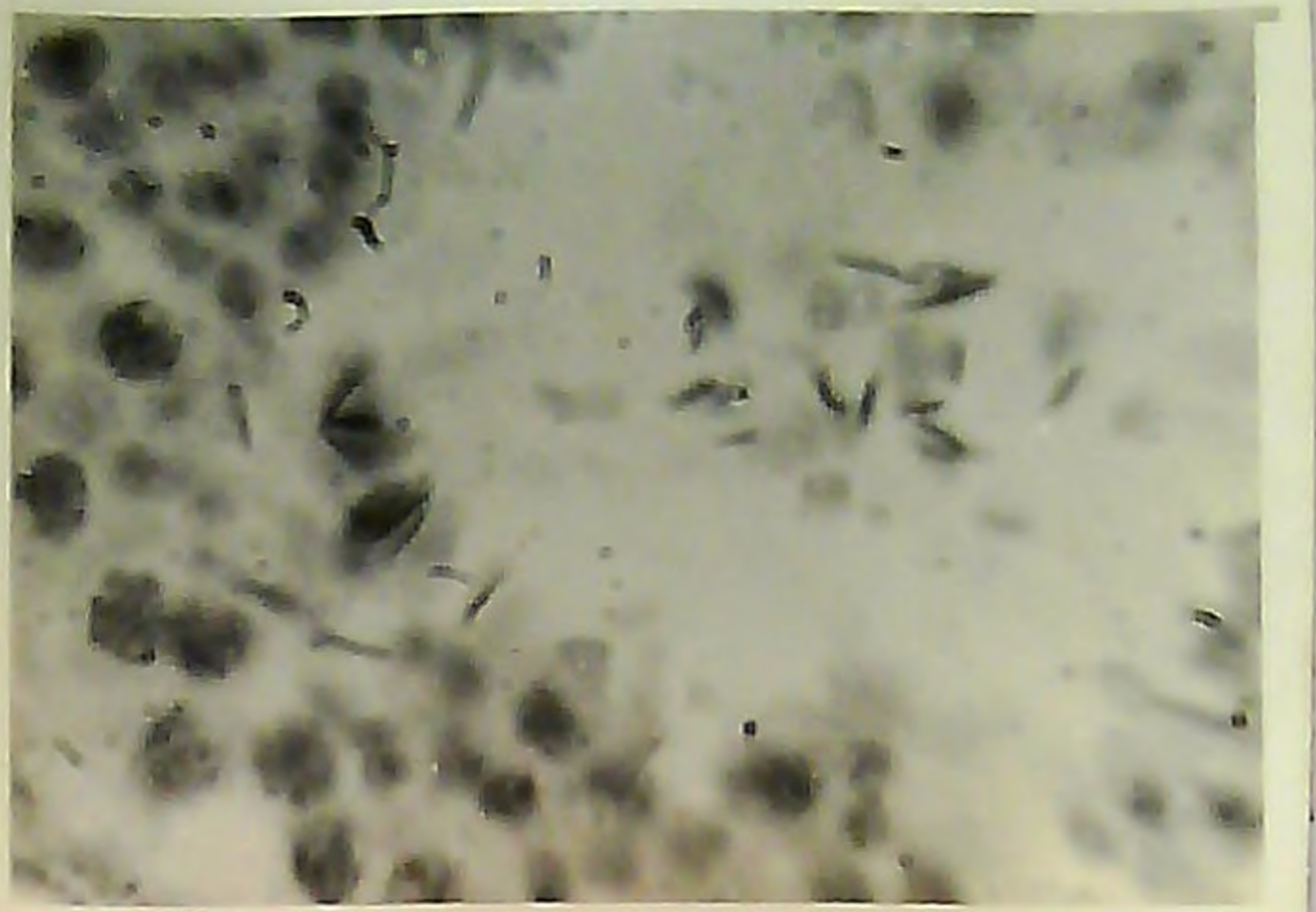

Fig. 37.

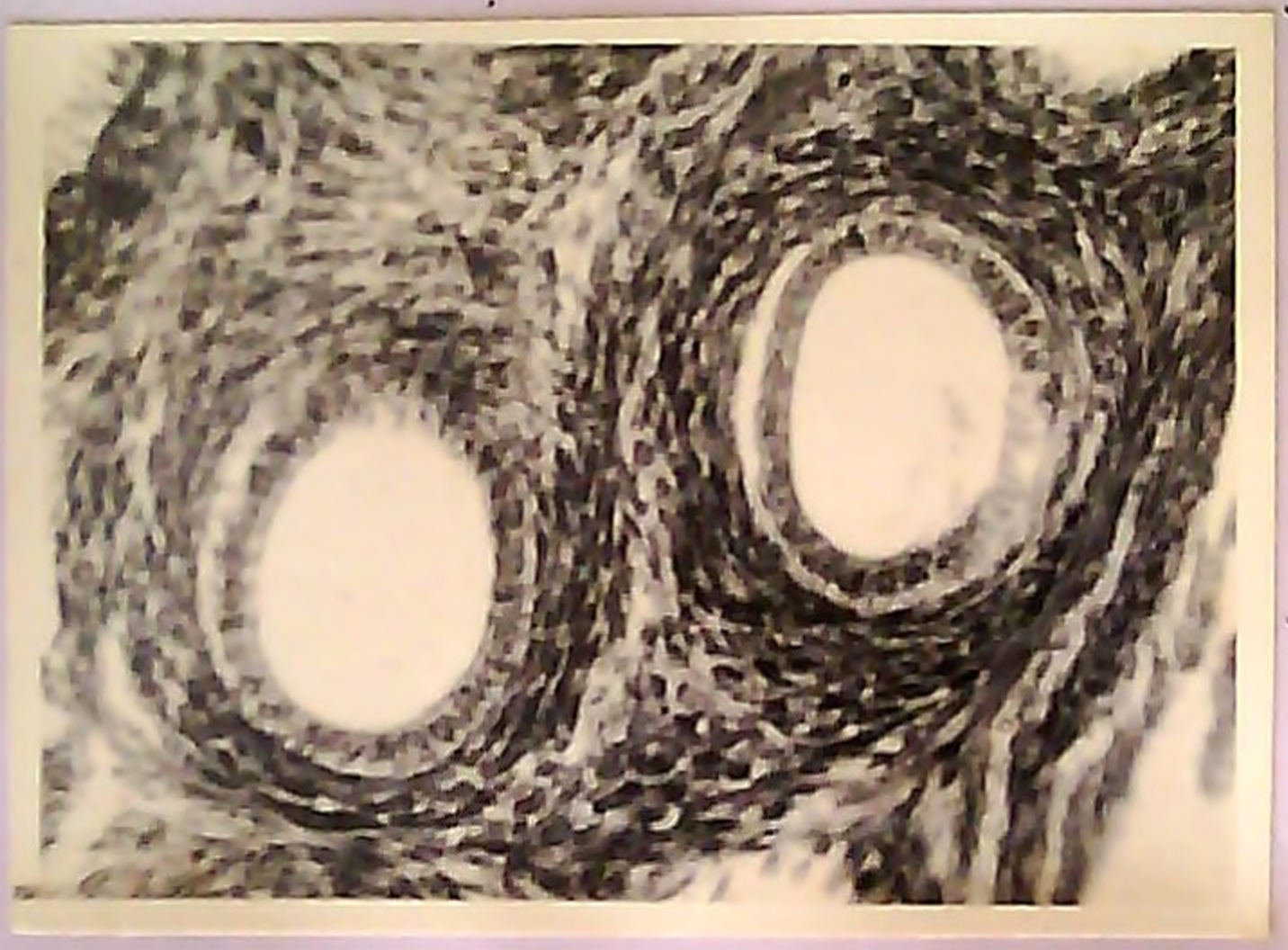

Fig. 38.

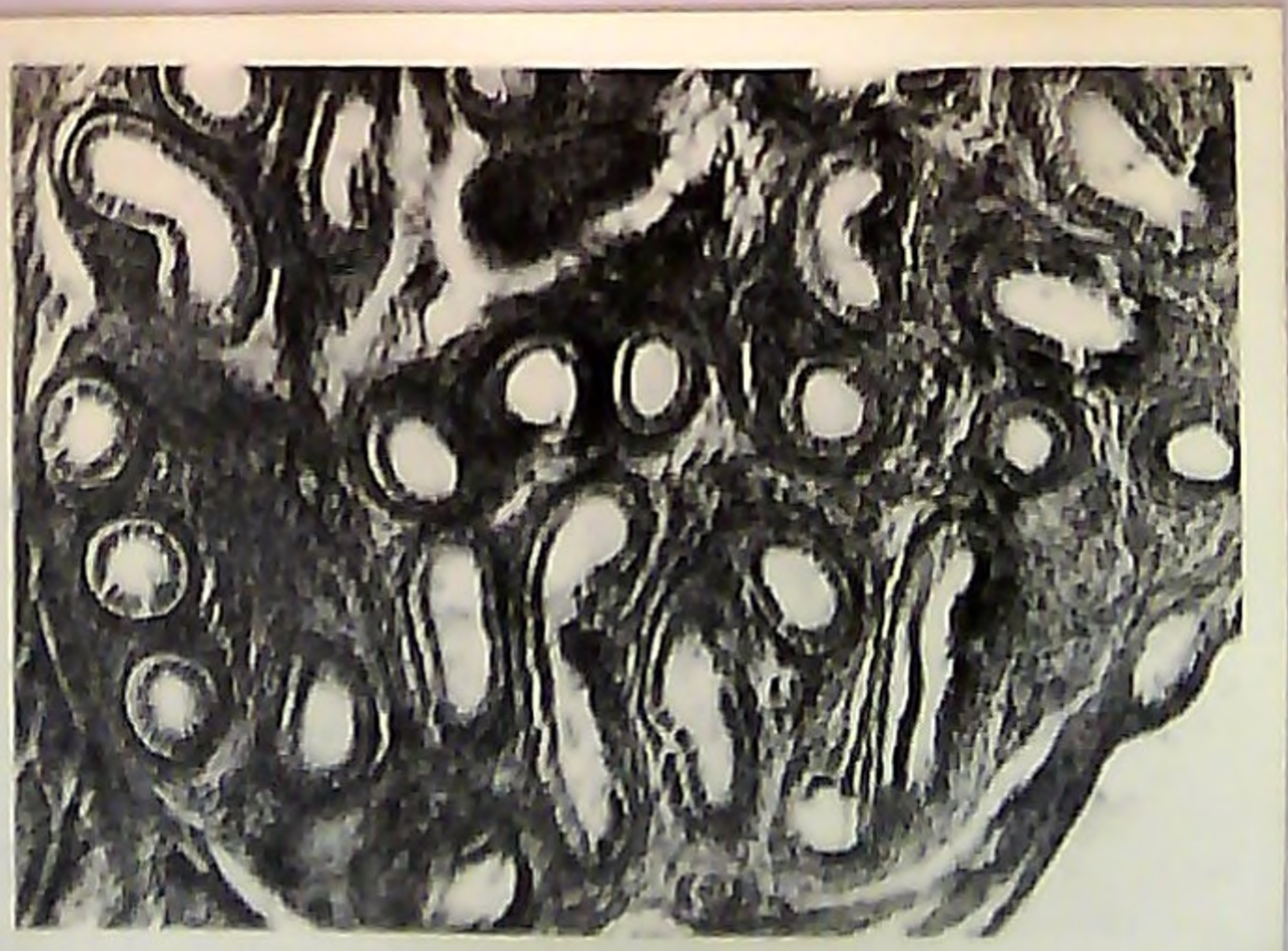

Fig. 39.

Fig. 40. Cauda epididymis (0 day) -- Tubules lined by columnar epithelium. (H & E X 400).

Fig. 41: Caput epididymis (30 day) -
(H & E X 100).

Fig. 42. Corpus epididymis (30 day).
(H & E X 100).

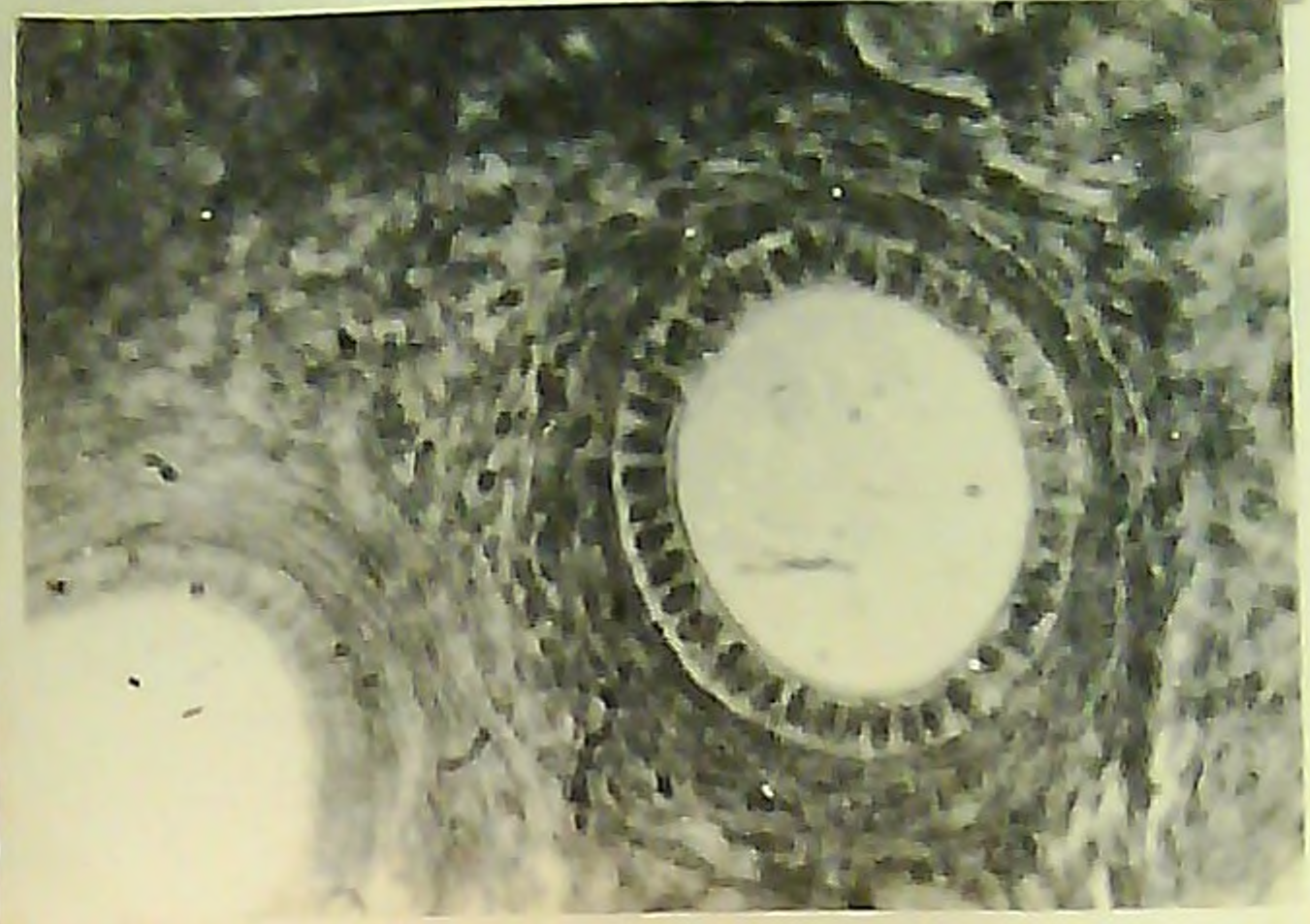

Fig. 40.

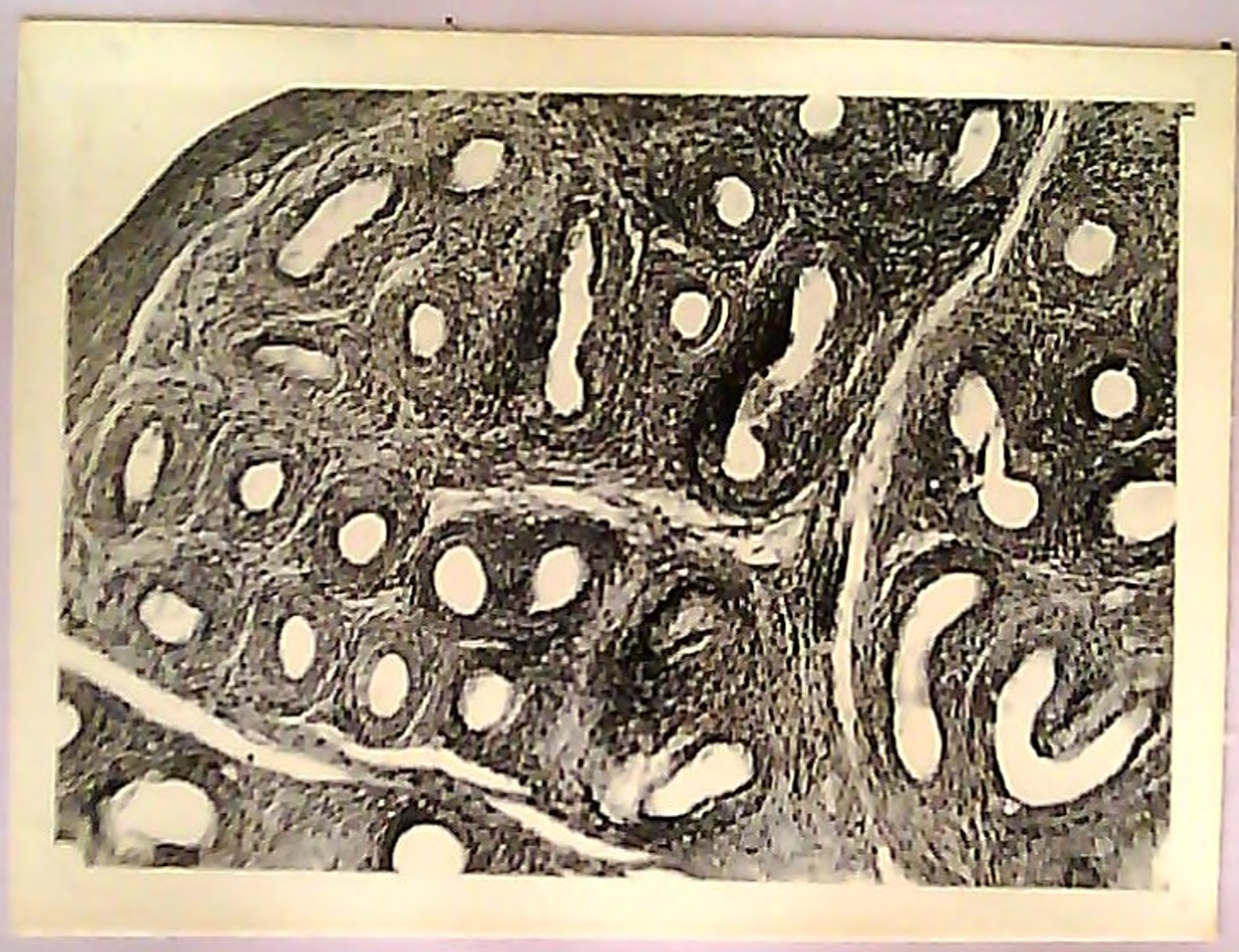

Fig. 41.

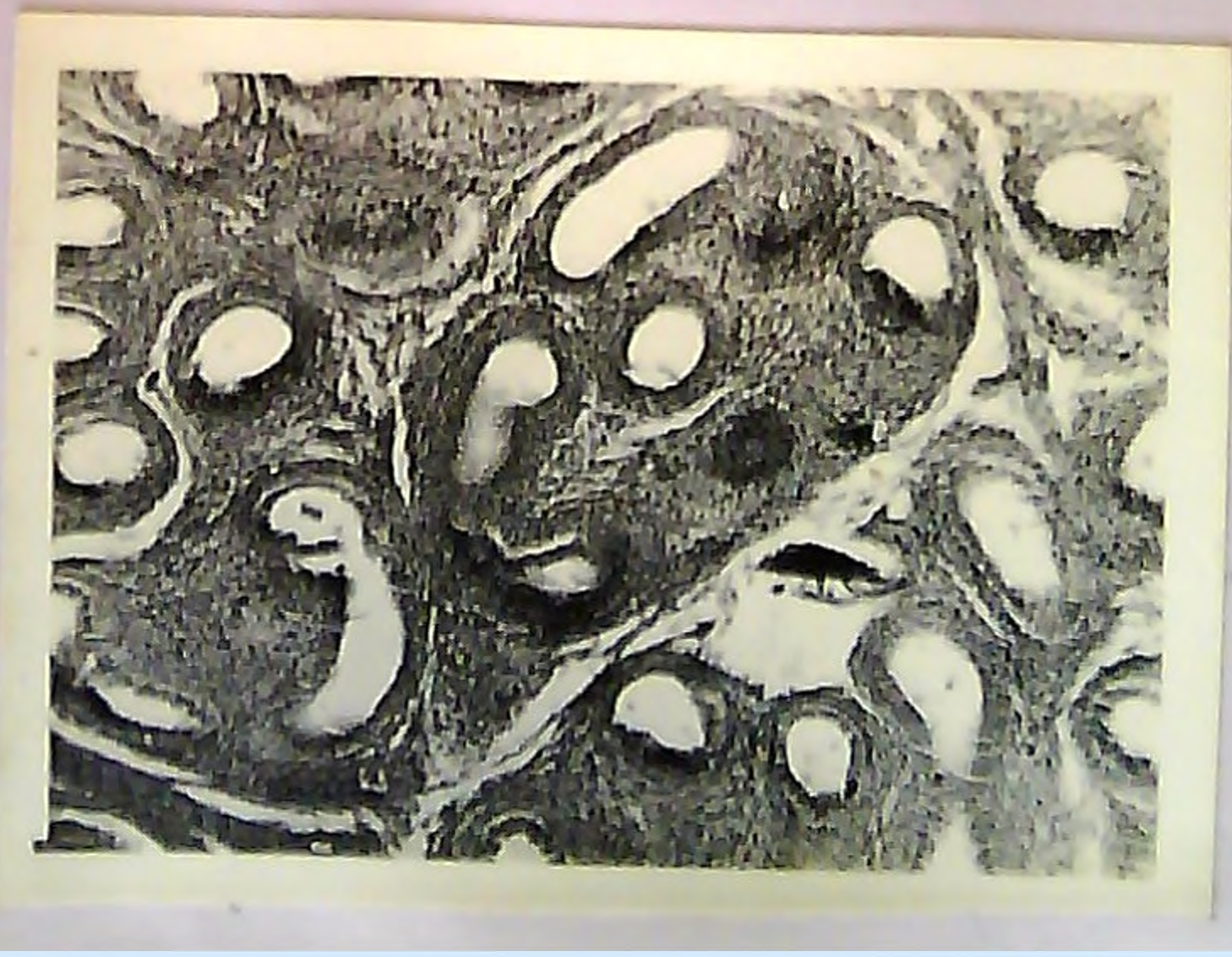

Fig. 42.

Fig. 43. Caudo epididymis (30 day).
(H & E X 100).

Fig. 44. Caput epididymis (60 day) -- Tubules
lined by columnar epithelium.
(H & E X 400).

Fig. 45. Corpus epididymis (60 day) -- Tubules
showing pseudostratification of epithelial
cells. (H & E X 400).

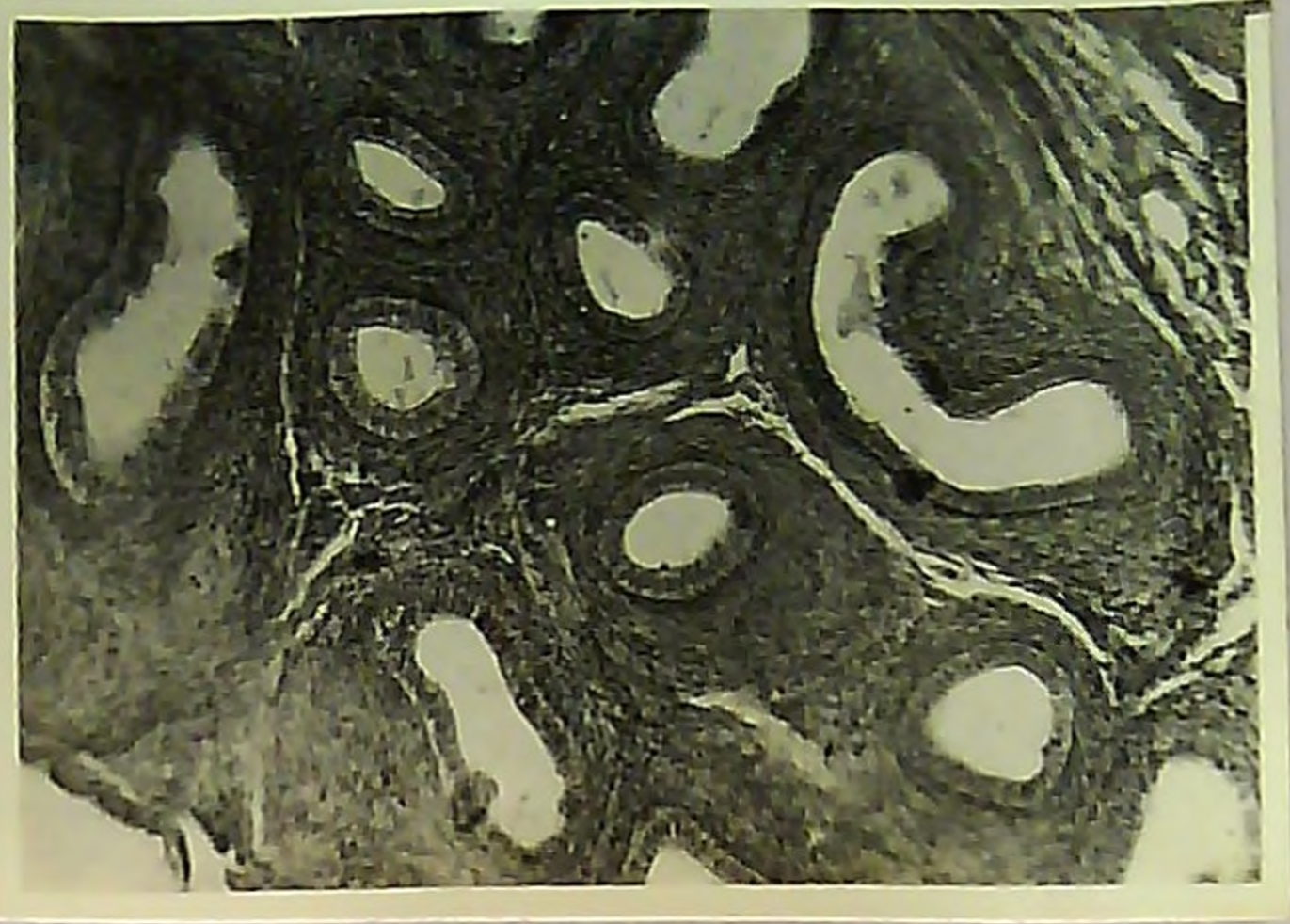

Fig. 43.

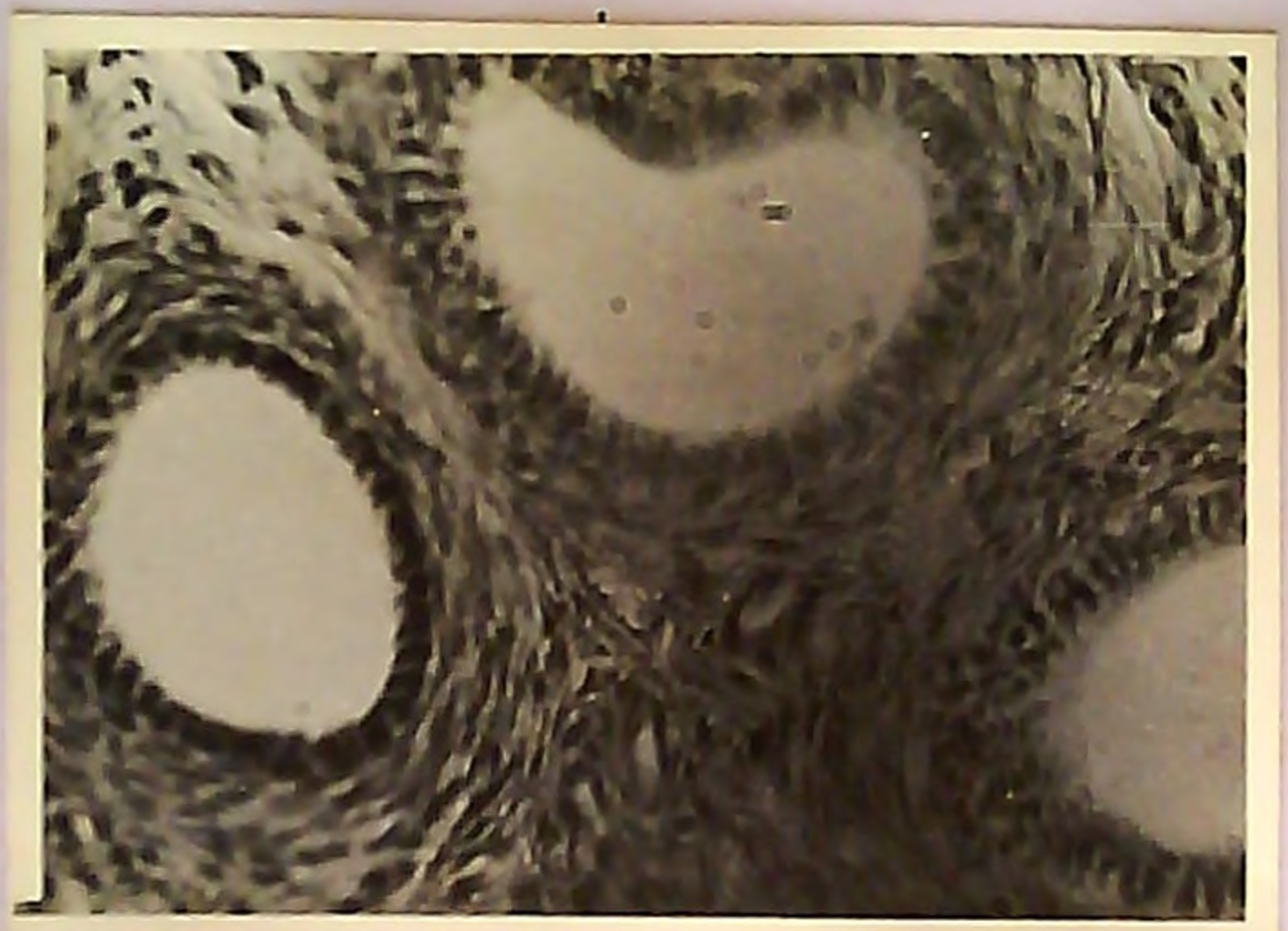

Fig. 44.

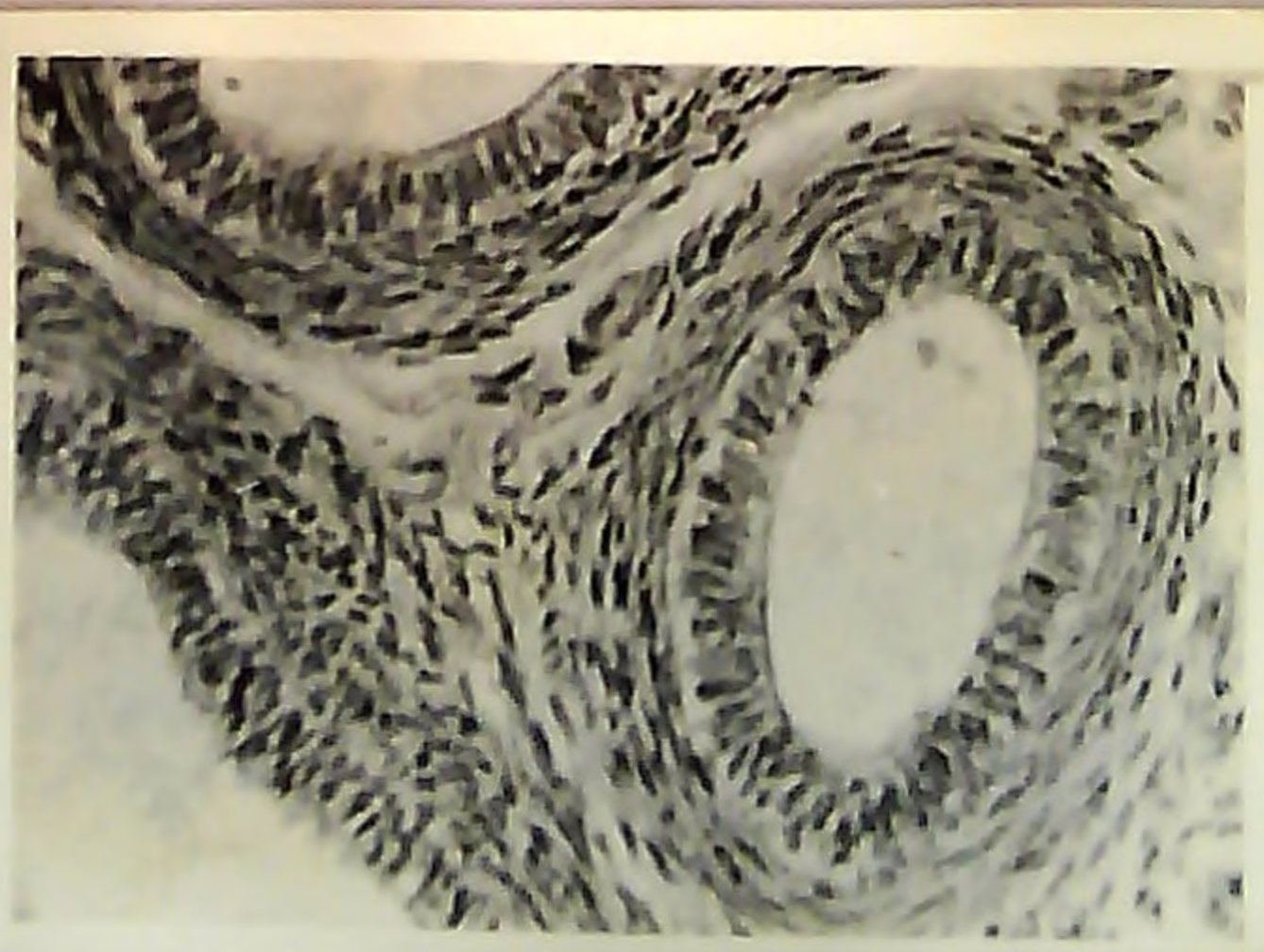

Fig. 45.

Fig. 46. Caude epididymis (60 day) -- Tubules showing pseudostretification of epitholial cells.
(H & E X 400).

Fig. 47. Caput epididymis (90 day) -- Tubules lined mostly by simple columnar epitheliai cells-- some showing pseudostratifiontion of cells.
(H & E X 400).

Fig. 48. Corpus epididymis (90day) -- Tubules lined by pseudostratified epithelial cells.
(H & E X 400).

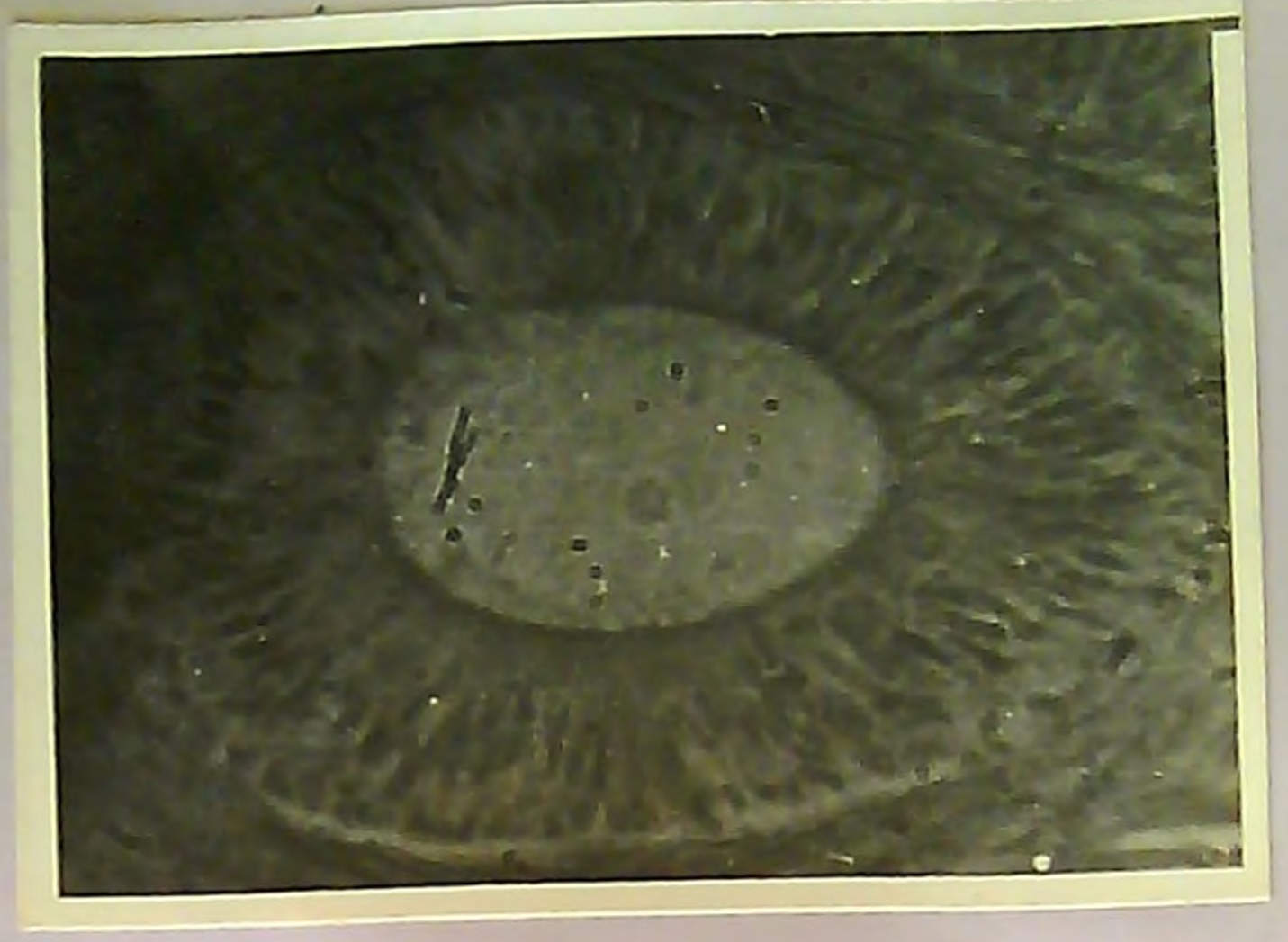

Fig. 46.

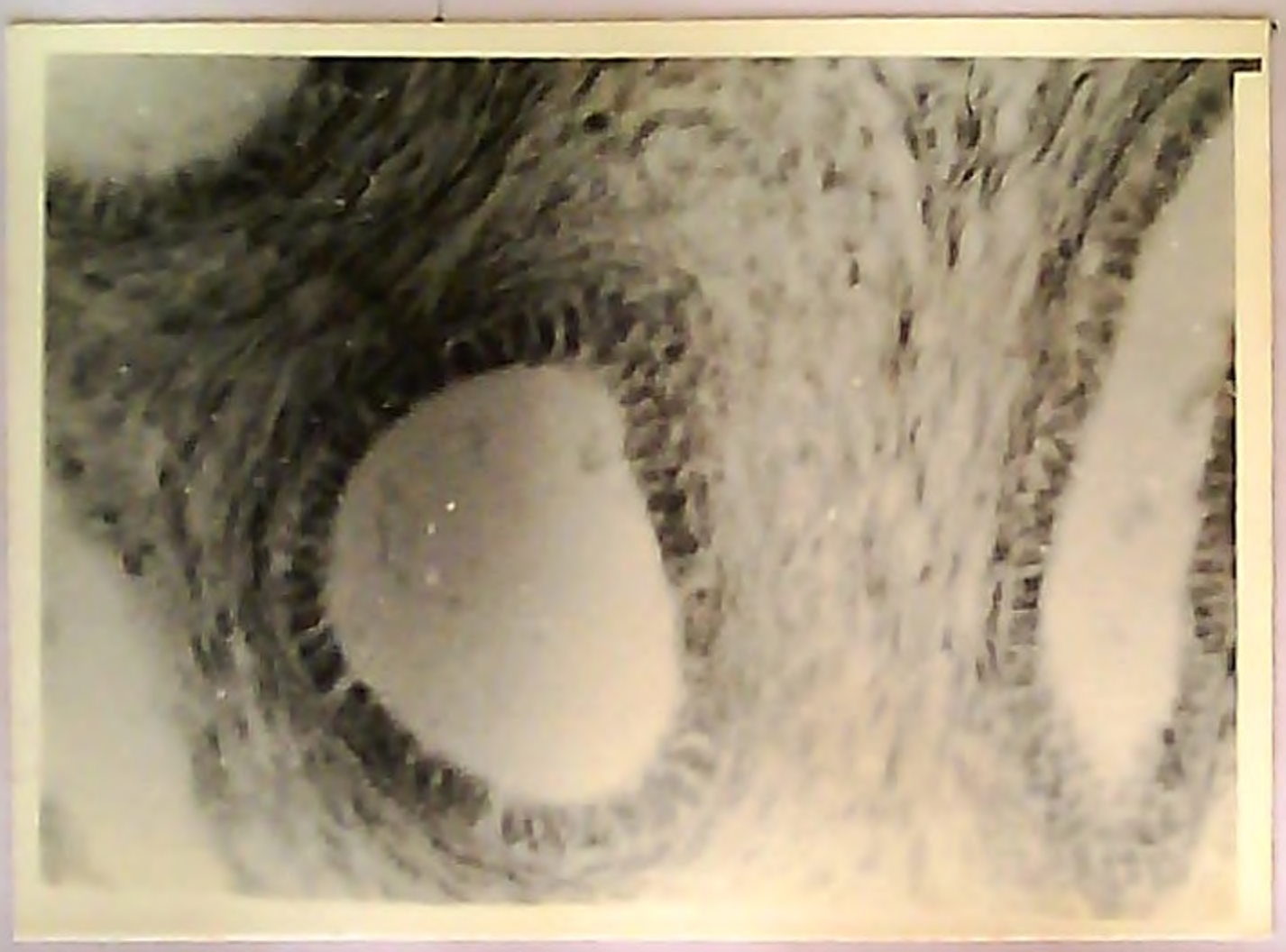

Fig. 47.

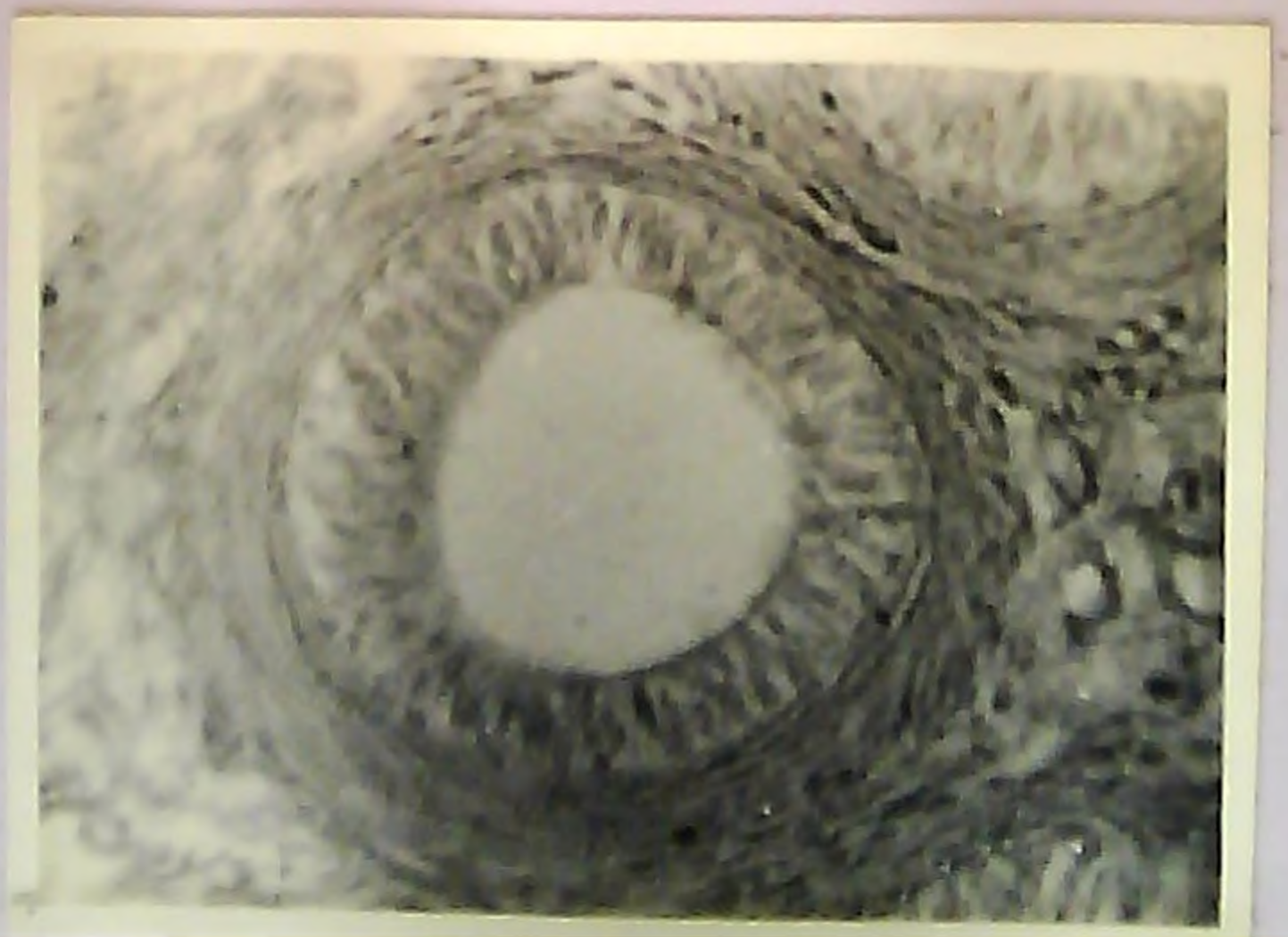

Fig. 48.

ig. 49. Cauda epididymis (90 day) -- Tubules lined by pseudostratified epithelial cells.
(H & E X 400).

ig. 50. Caput epididymis (120 day) -- Tubules lined by pseudostratified epithelium.
(H & E X 400).

ig.51. Corpus epididymis (120 day) -- tubules lined by pseudostratified epithelium.
(H & E X 400).

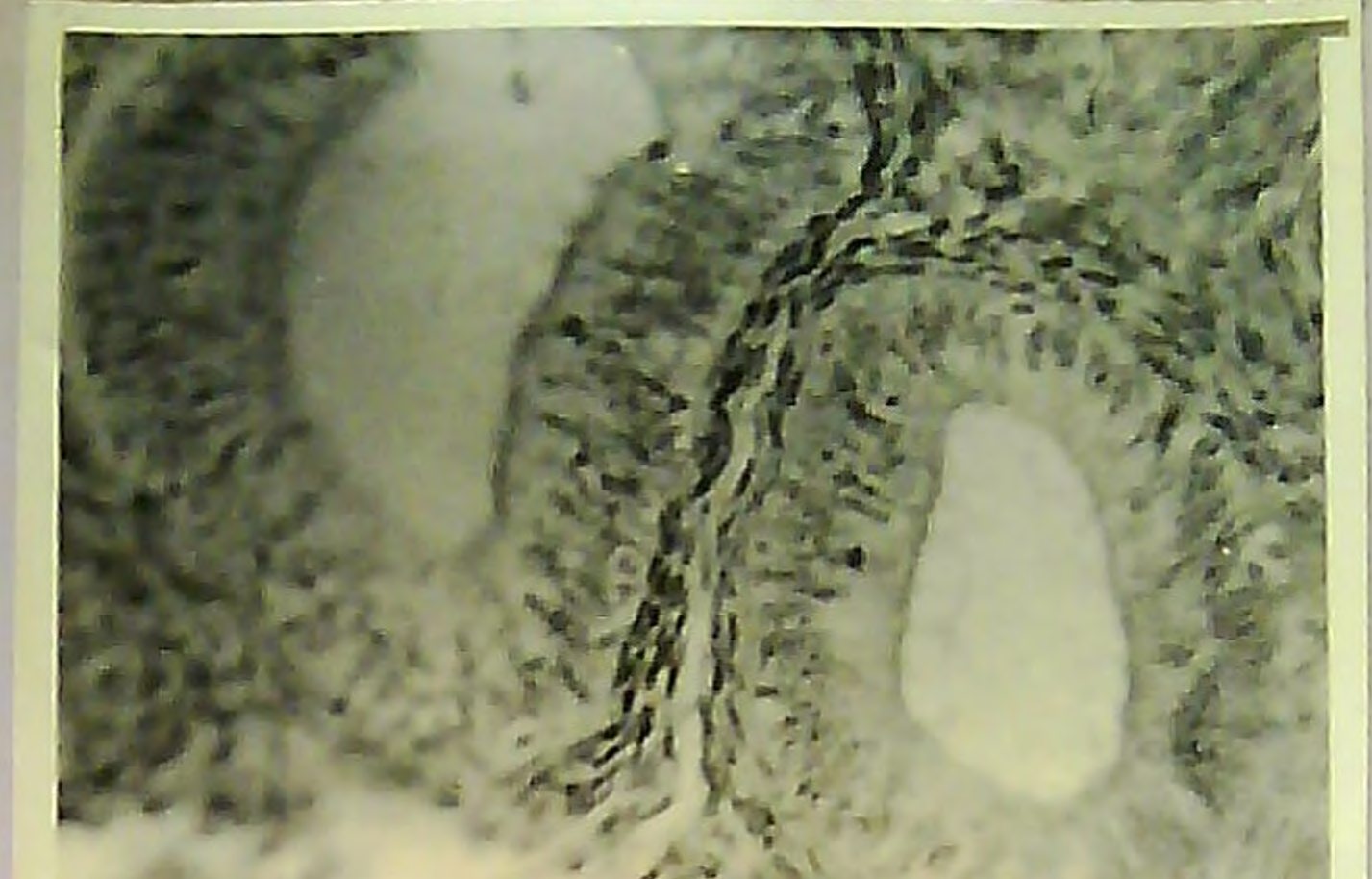

Fig. 49.

Fig. 50.

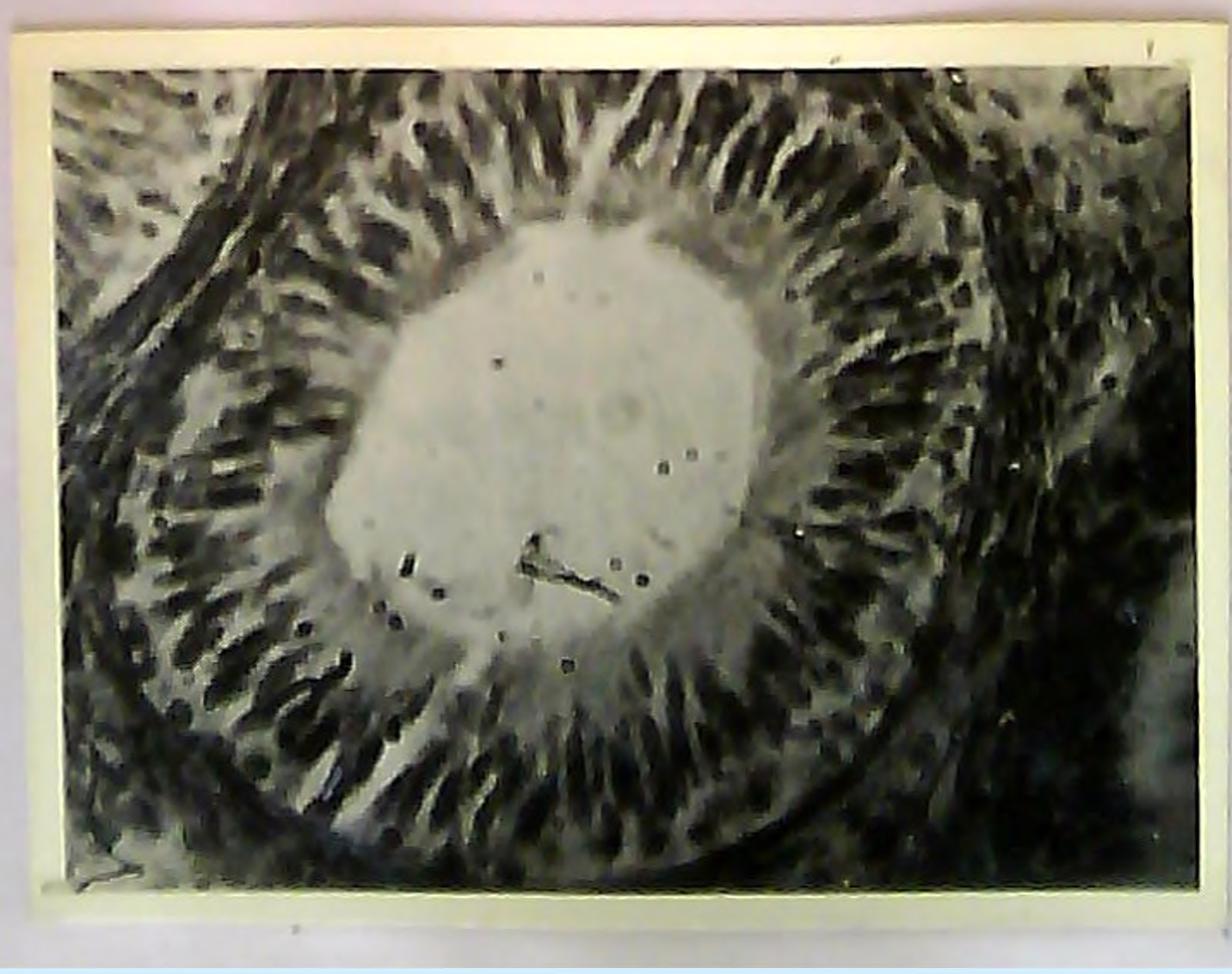

Fig. 51.

Fig. 52. Cauda epididymis (120 day) -- tubules lined by pseudostratified epithelium.
(H & E X 400).

Fig. 53. Corpus epididymis (210 day) -- tubules lined by pseudostratified epithelium.
(H & E X 400).

Fig. 54. Corpus epididymis (240 day) -- Tubules lined by Pseudostratified epithelium.
(H & E X 400).

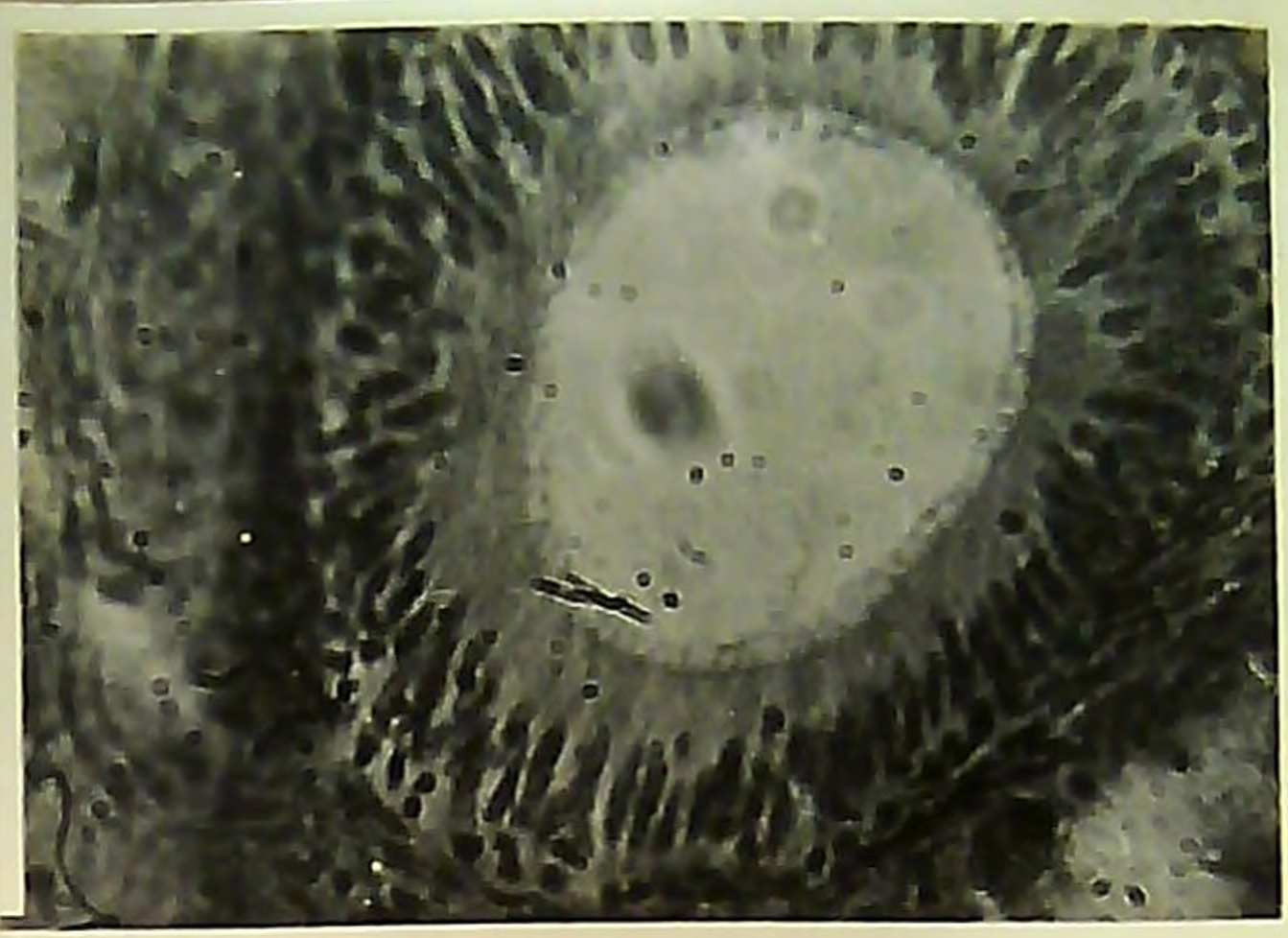

Fig. 52.

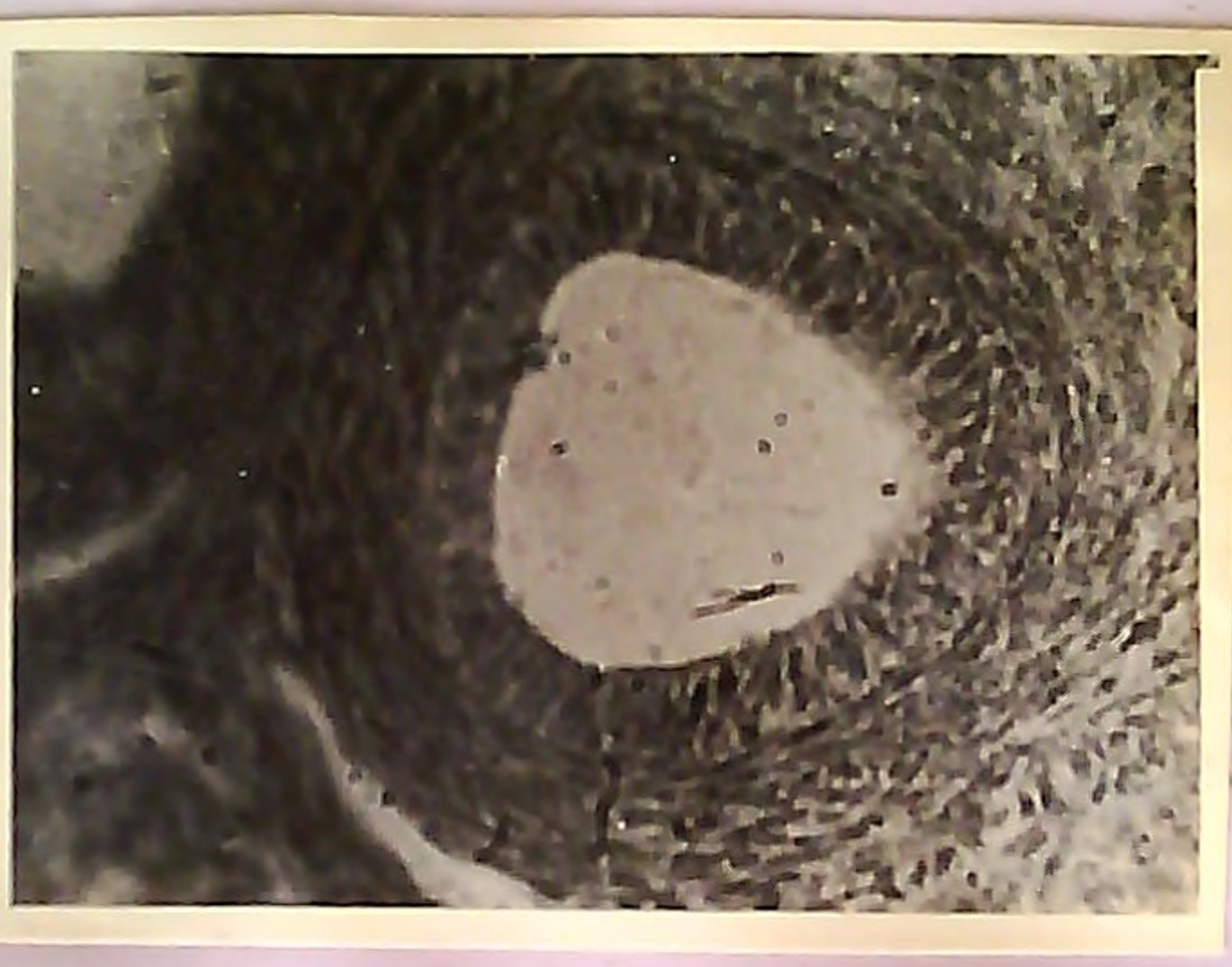

Fig. 53.

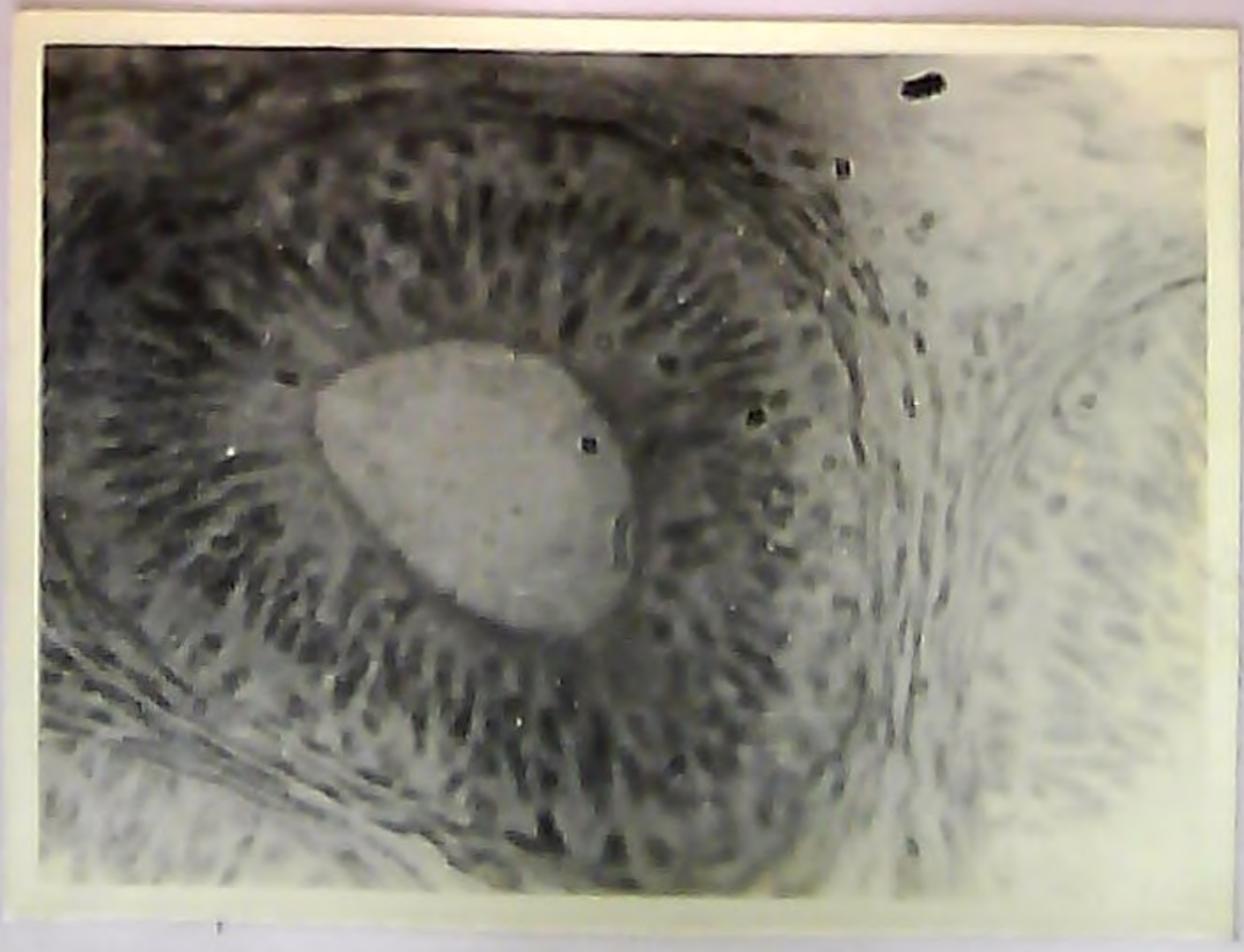

Fig. 54.

Fig. 55. Caude epididymis (240 day) -- Pseudo-stratified ciliated epithelium lining the tubules. (H & E X 400).

Fig. 56. Caput epididymis (270 day) -- Pseudo-stratified epithelium lining the tubules. (H & E X 400).

Fig. 57. Corpus epididymis (270 day) -- Pseudo-stratified epithelium lining the tubules. (H & E X400).

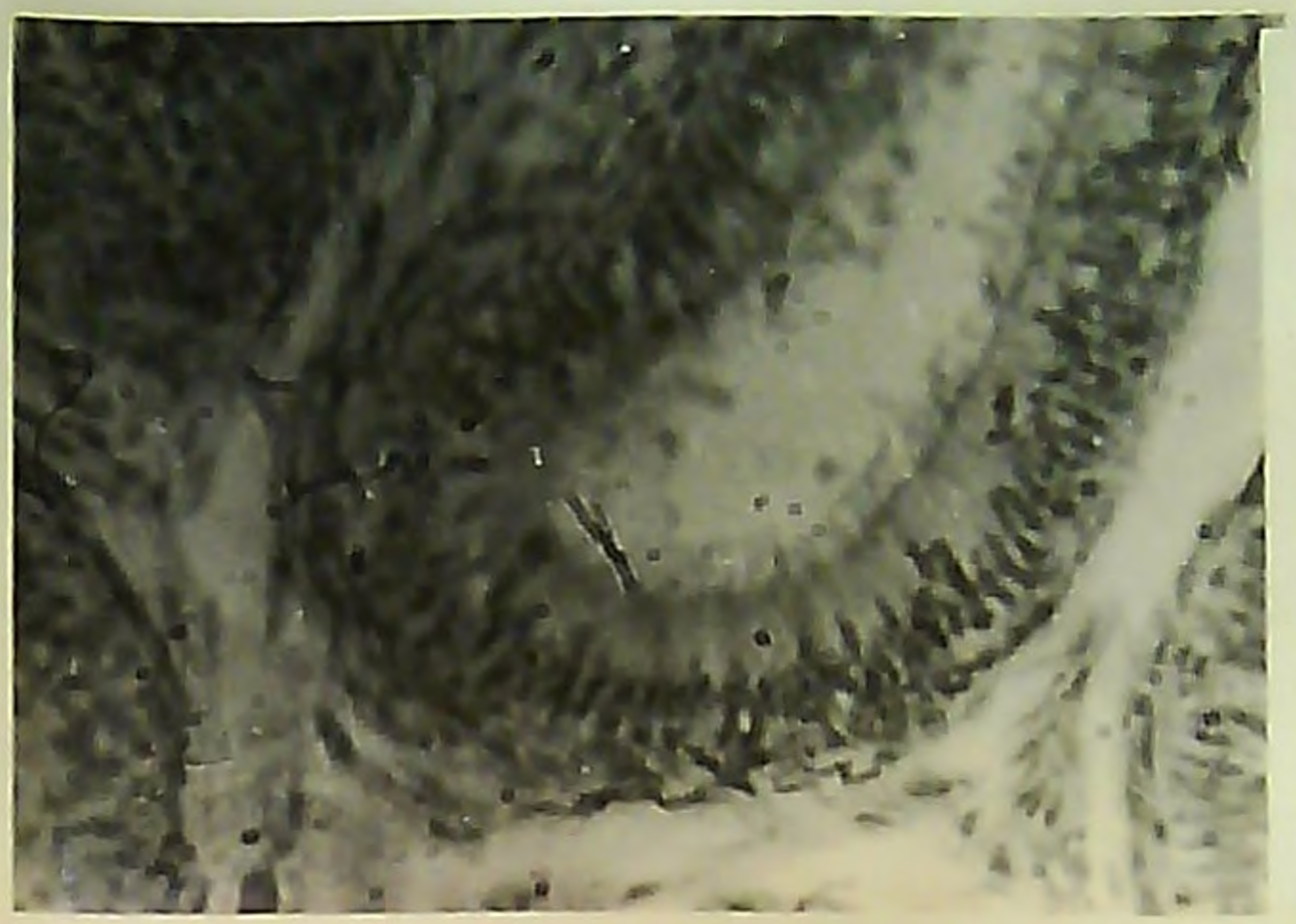

Fig. 55.

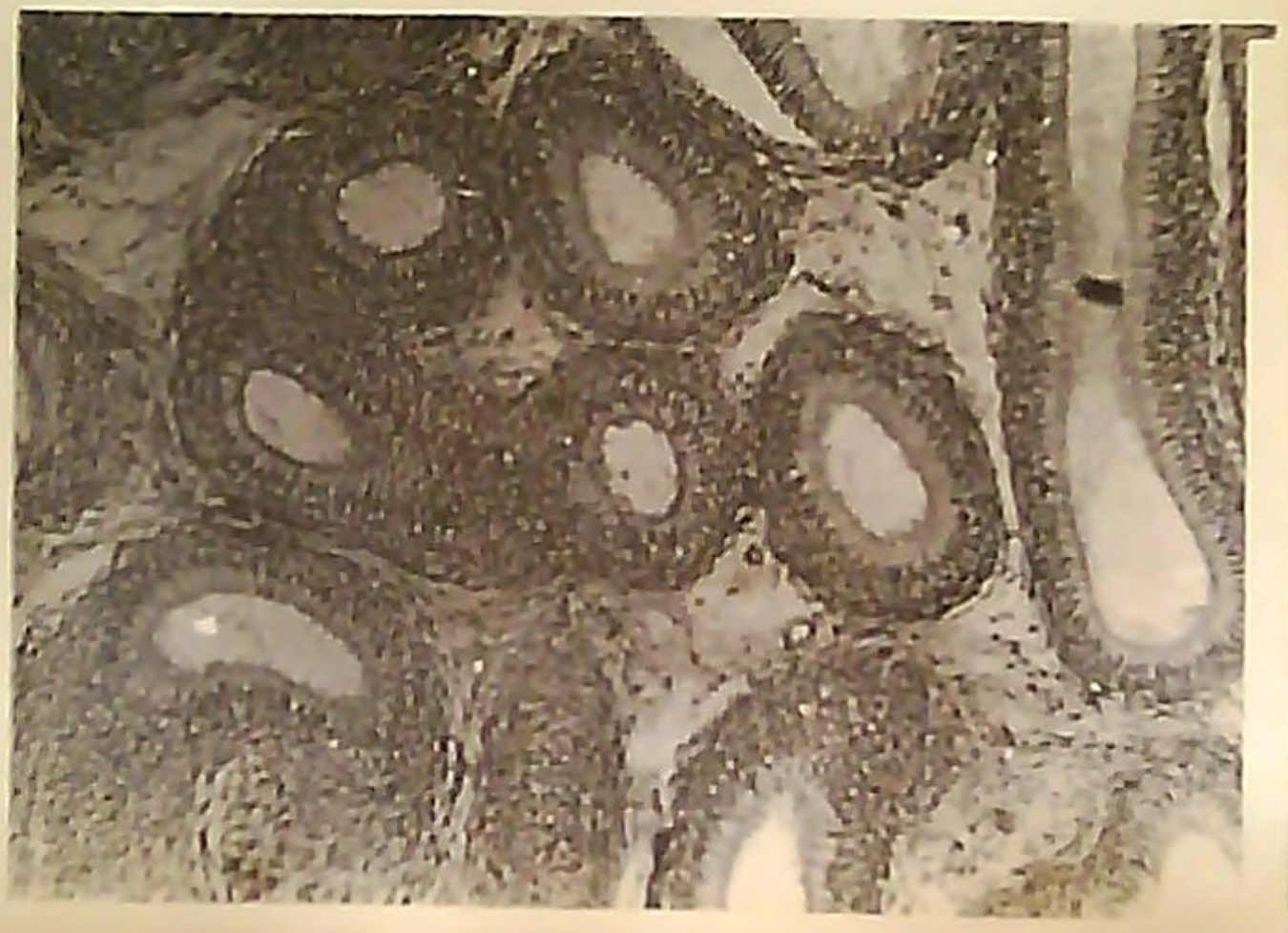

Fig. 56.

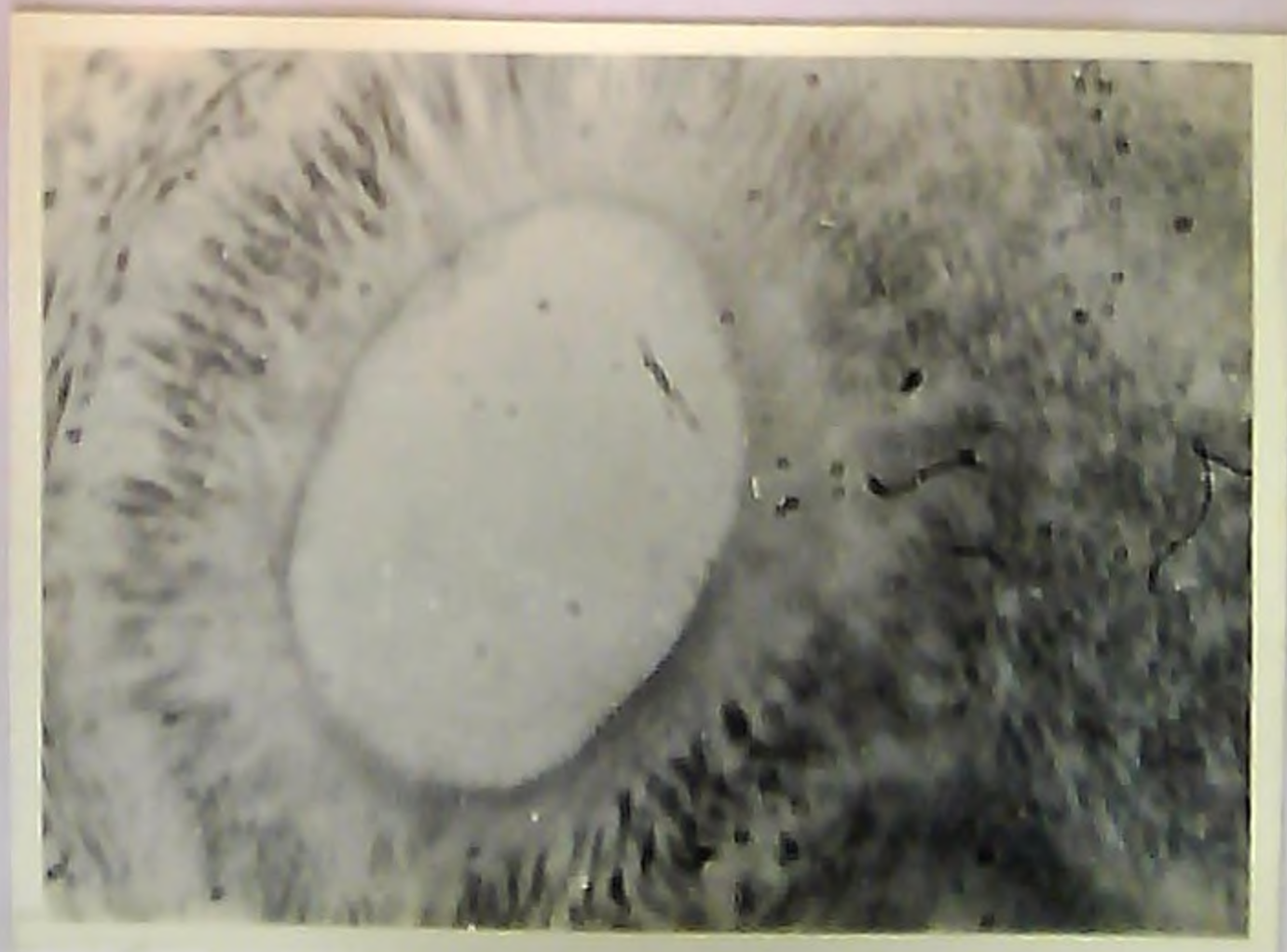

Fig. 57.

Fig. 58. Caude epididymis (270 day) -- Pseudo-stratified ciliated epithelium lining the tubules. (H & E X 400).

Fig. 59. Caput epididymis (300 day) -- Pseudo-stratified epithelium lining the tubules (H & E X 400).

Fig. 60. Corpus epididymis (300 day) -- Pseudo-stratified epithelium lining the tubules (H & E X 400).

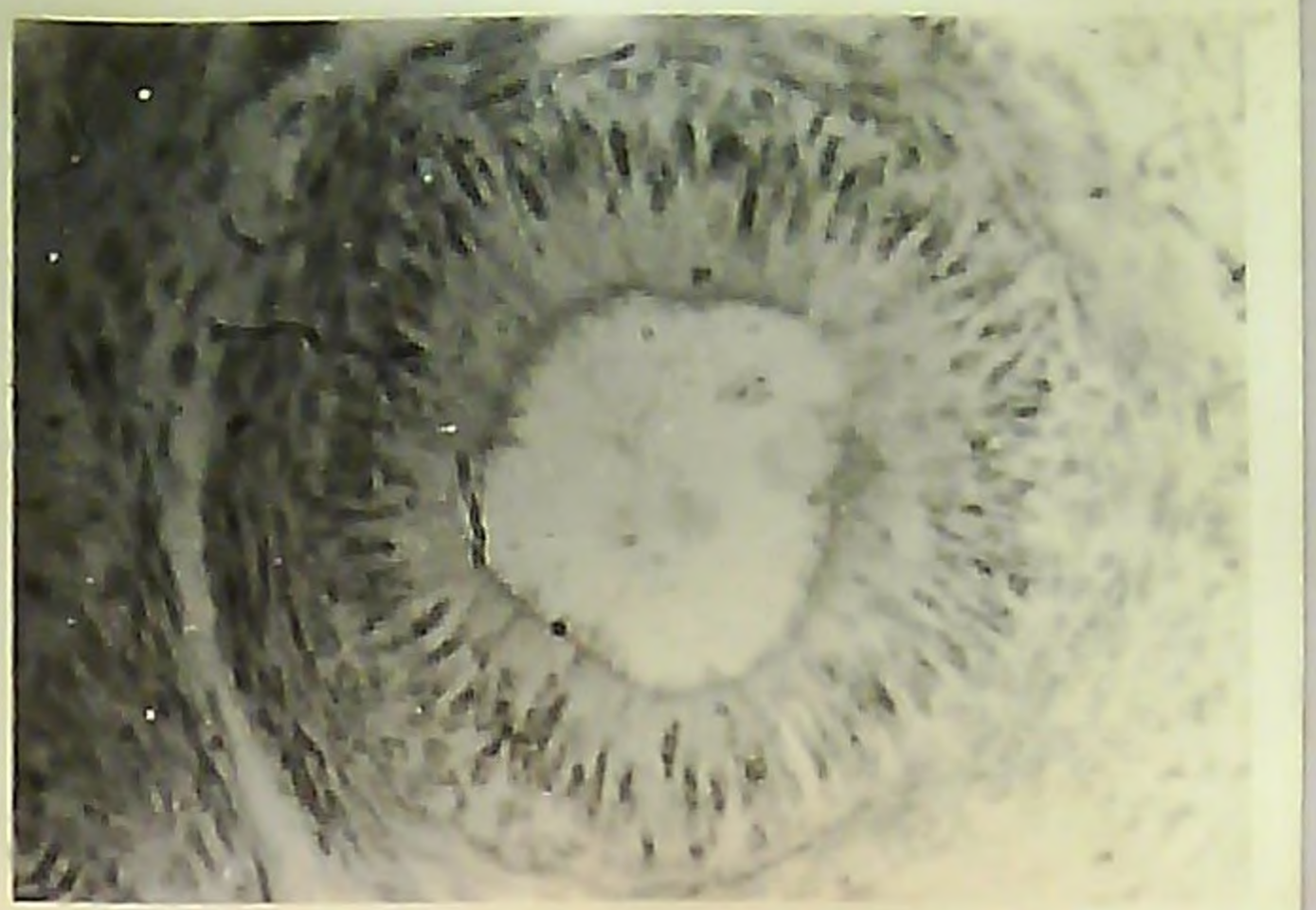

Fig. 58.

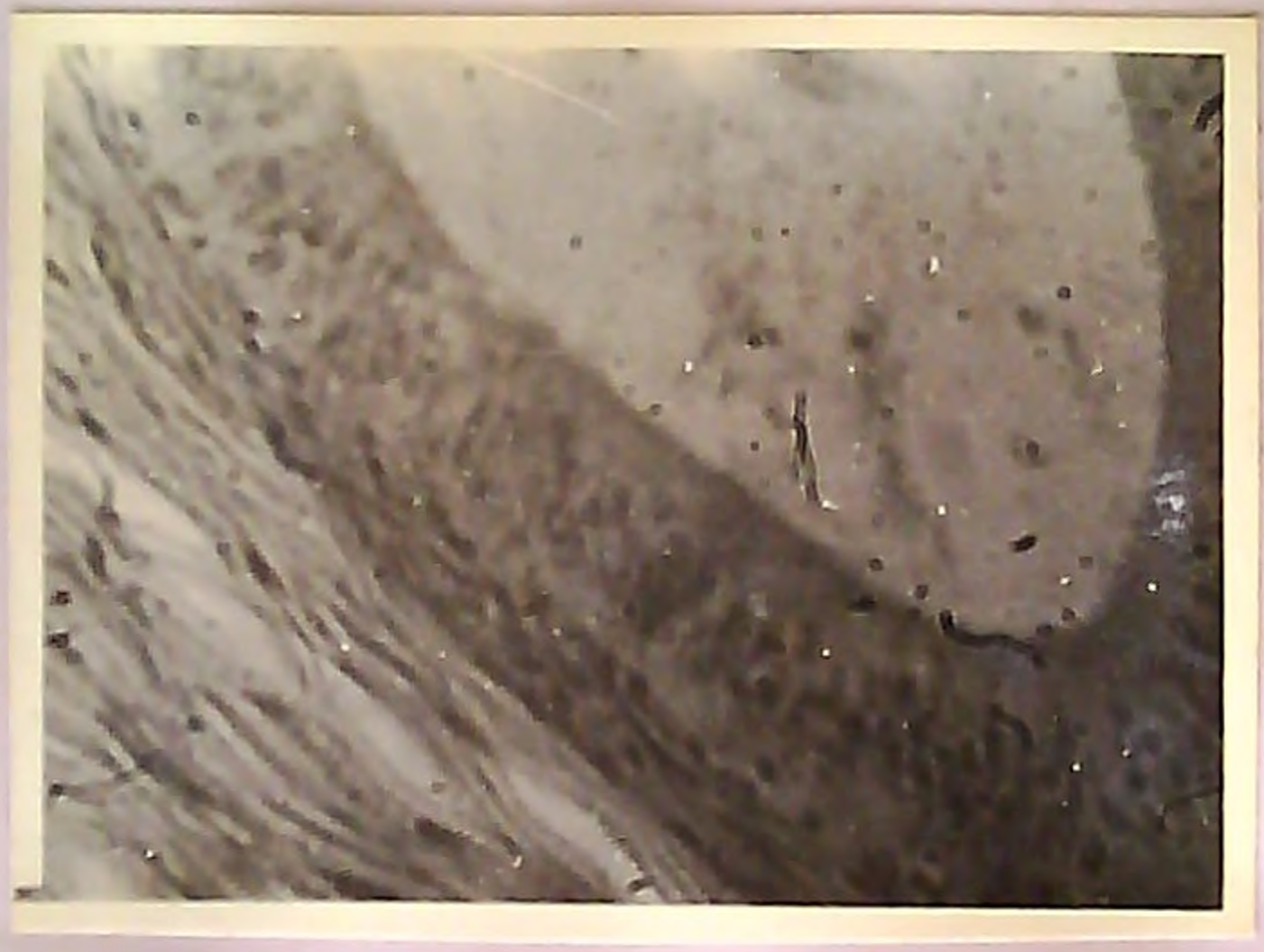

Fig. 59.

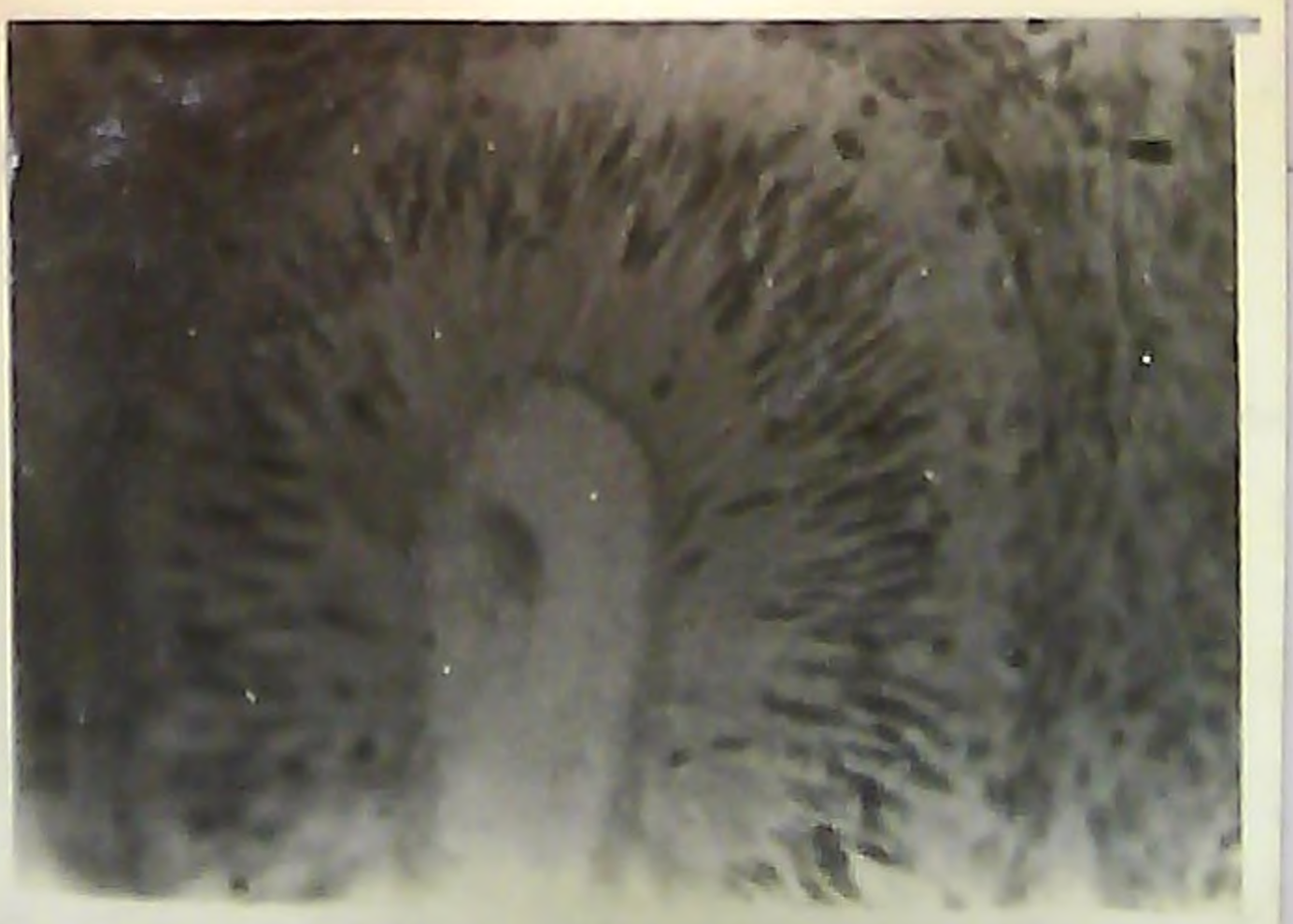

Fig. 60.

Fig. 61. Cauda epididymis (300 day) -- Pseudo-stratified epithelium with developed cilia. (H & E X 400).

Fig. 62. Caput epididymis (330 day) -- Pseudo-stratified epithelium with cilia - sperms in the lumen. (H & E X 400).

Fig. 63. Corpus epididymis (330 day) -- Pseudo-stratified ciliated epithelium lining the tubules. (H & E X 100).

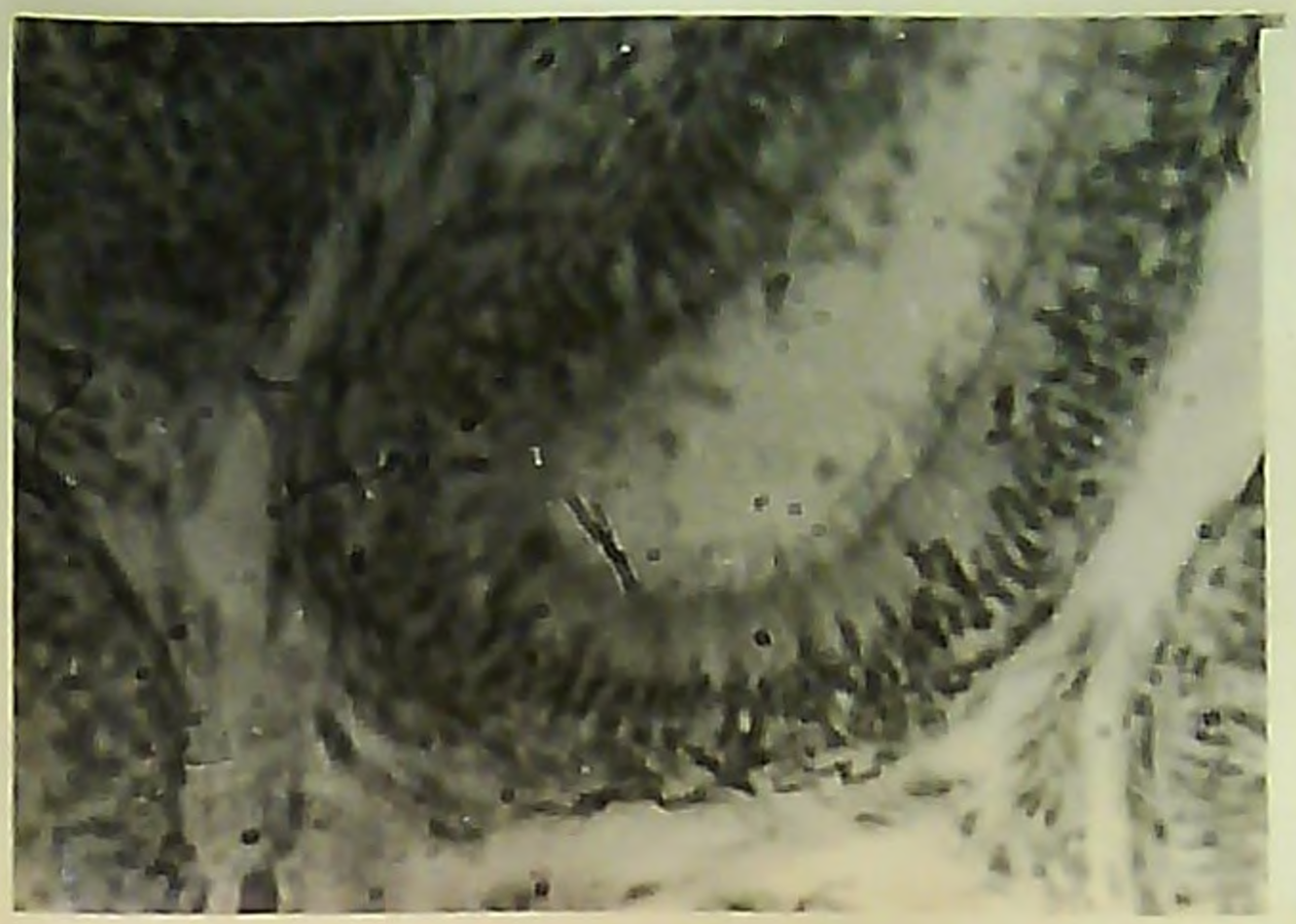

Fig. 55.

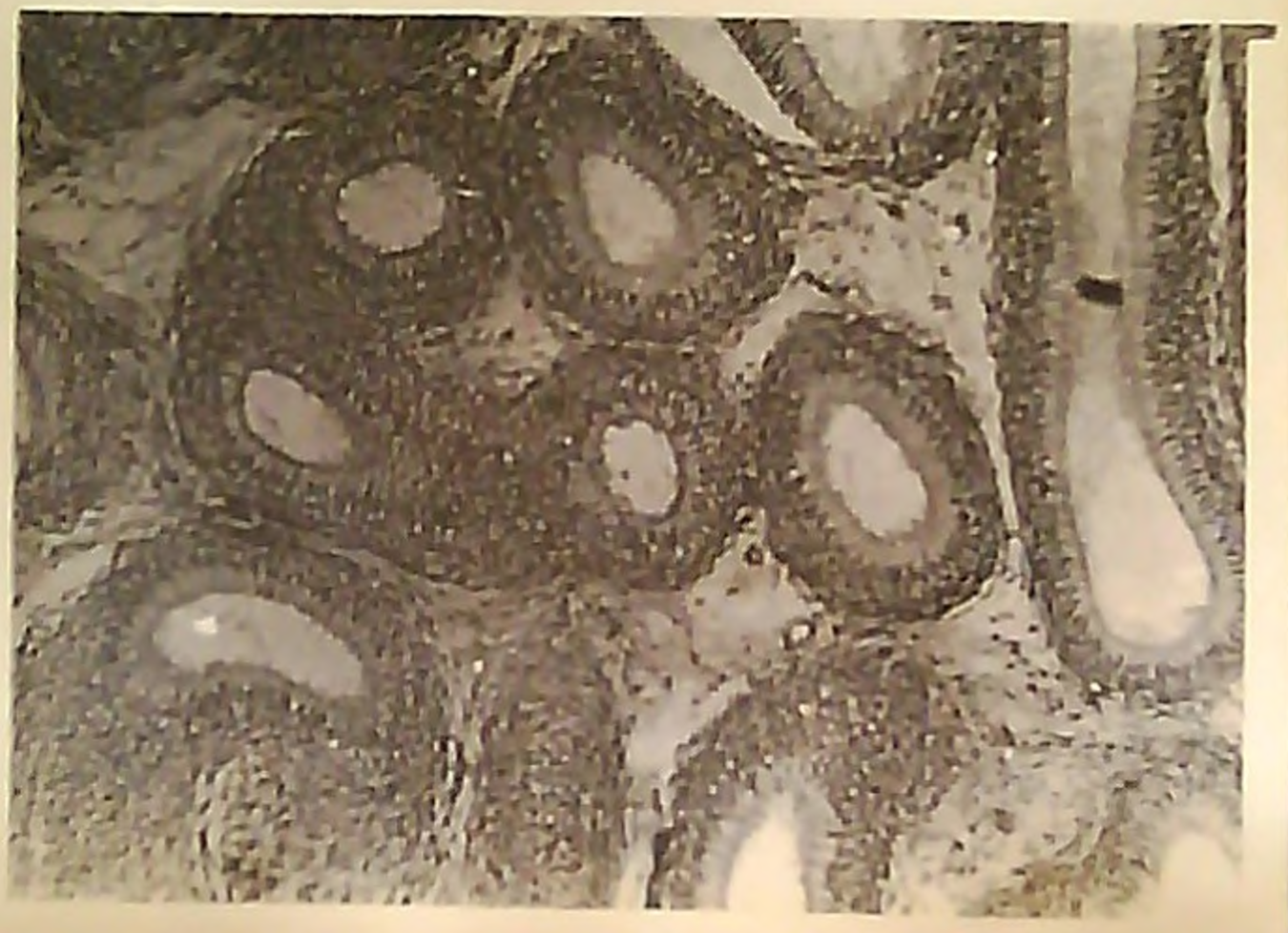

Fig. 56.

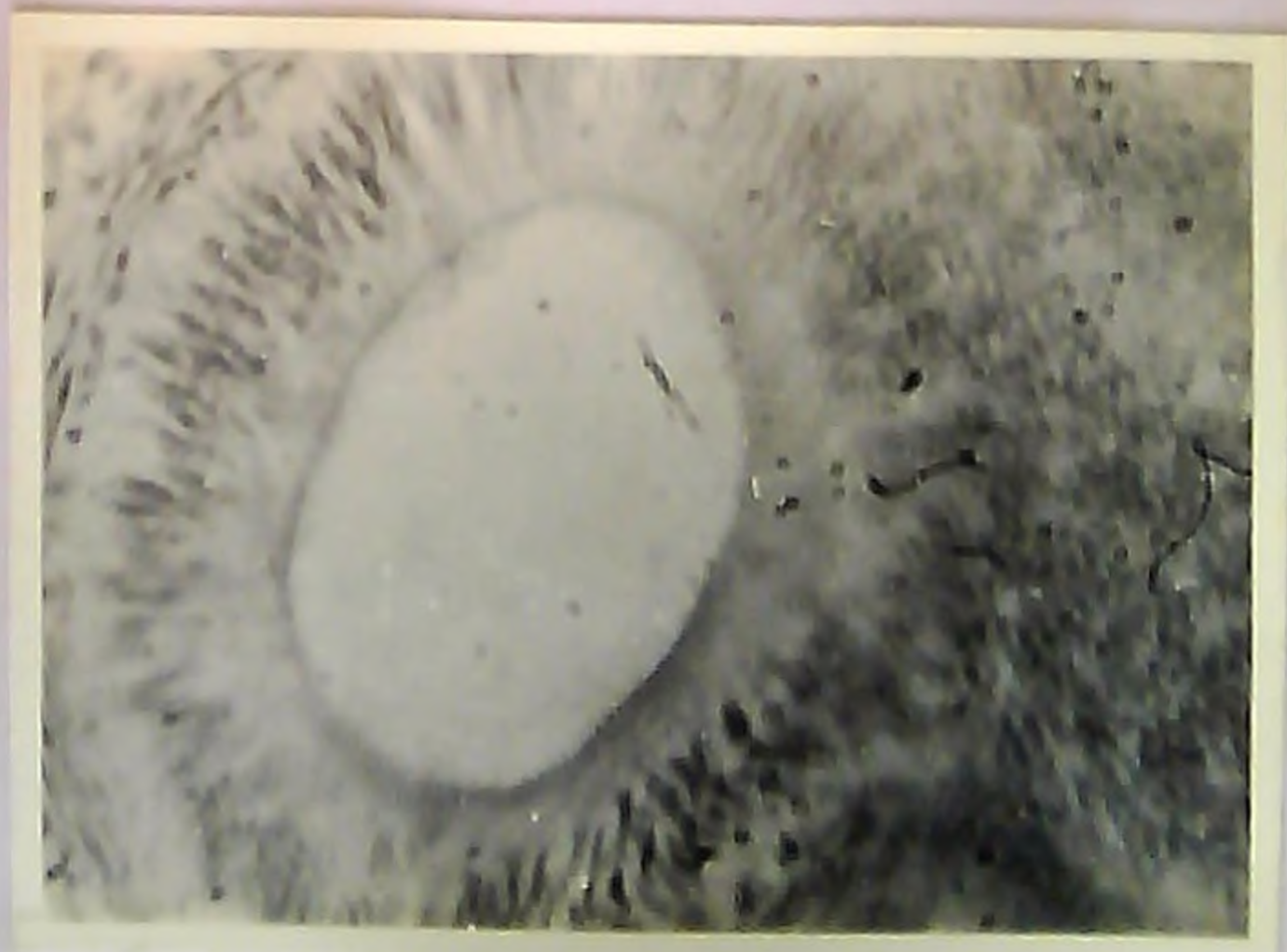

Fig. 57.

Fig. 58. Caude epididymis (270 day) -- Pseudo-stratified ciliated epithelium lining the tubules. (H & E X 400).

Fig. 59. Caput epididymis (300 day) -- Pseudo-stratified epithelium lining the tubules (H & E X 400).

Fig. 60. Corpus epididymis (300 day) -- Pseudo-stratified epithelium lining the tubules (H & E X 400).

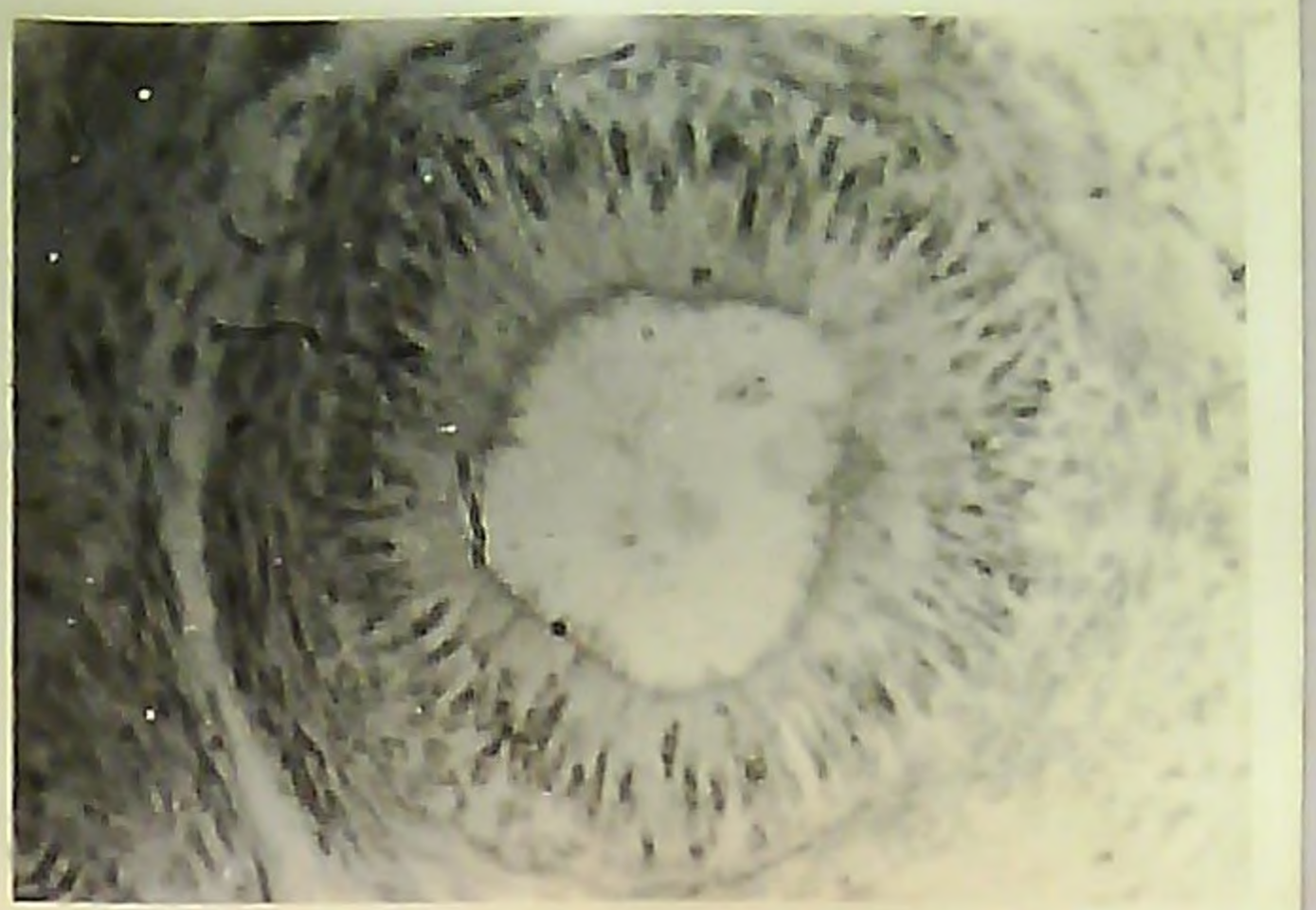

Fig. 58.

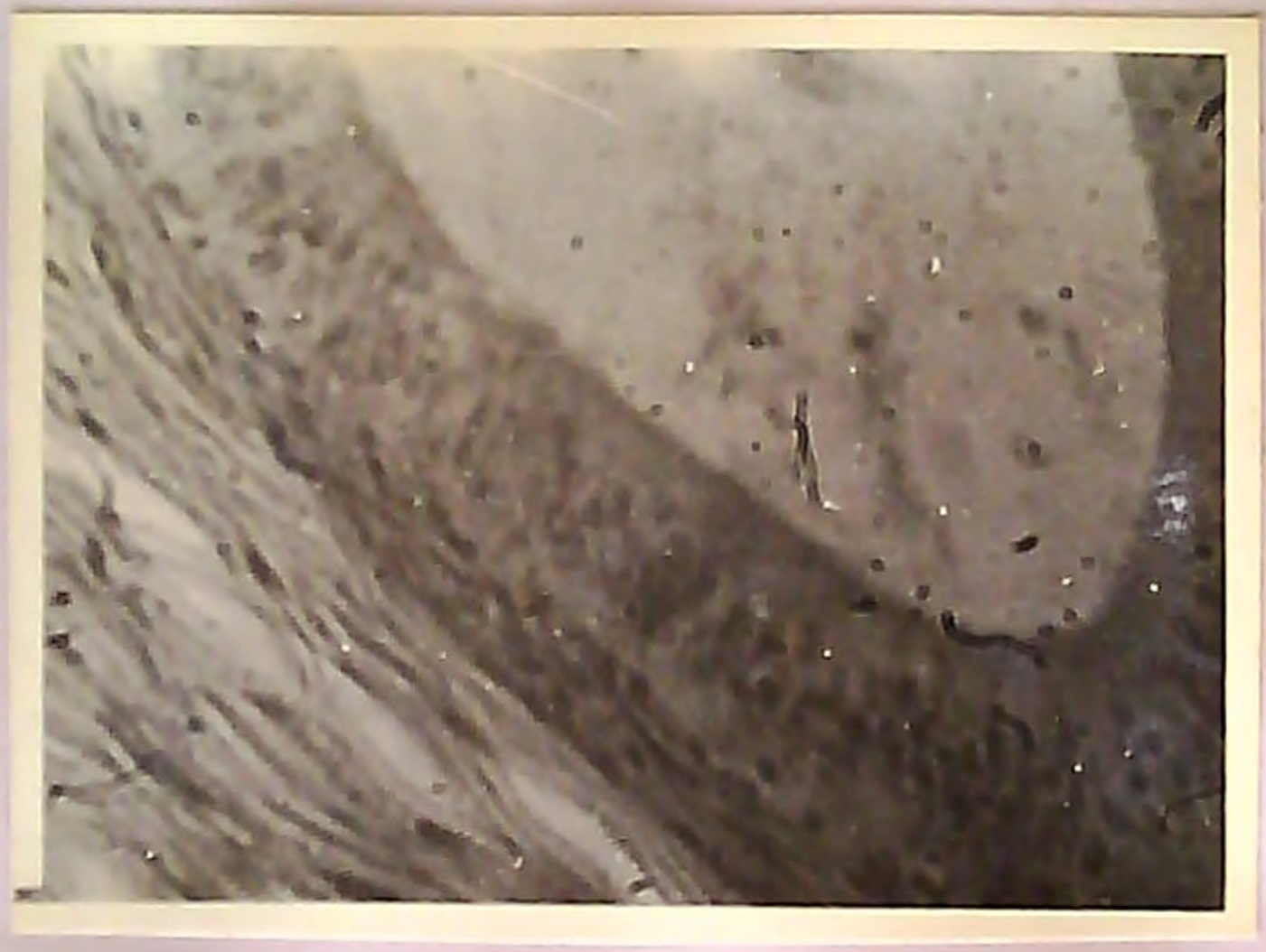

Fig. 59.

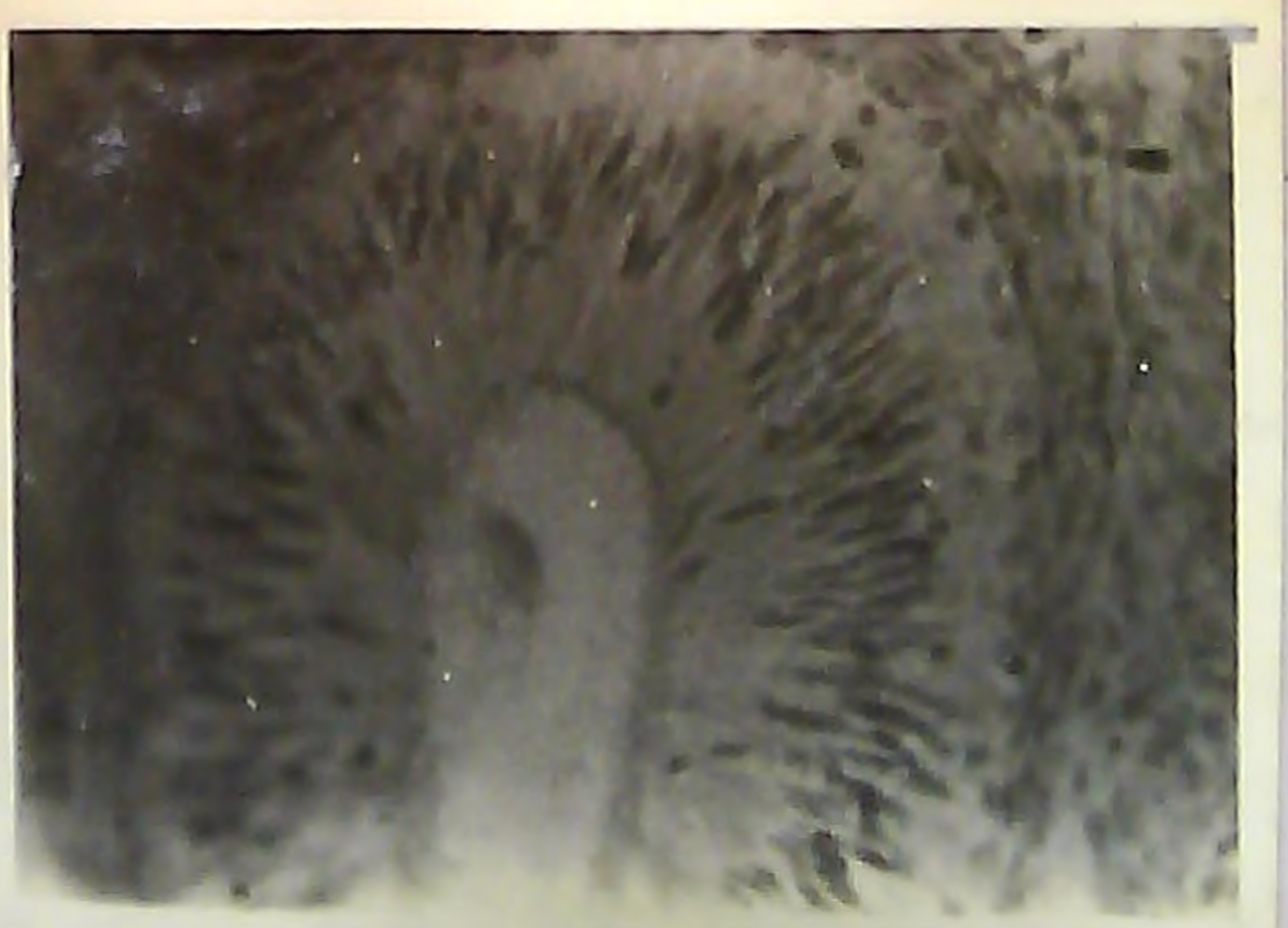

Fig. 60.

Fig. 61. Cauda epididymis (300 day) -- Pseudo-stratified epithelium with developed cilia. (H & E X 400).

Fig. 62. Caput epididymis (330 day) -- Pseudo-stratified epithelium with cilia - sperms in the lumen. (H & E X 400).

Fig. 63. Corpus epididymis (330 day) -- Pseudo-stratified ciliated epithelium lining the tubules. (H & E X 100).

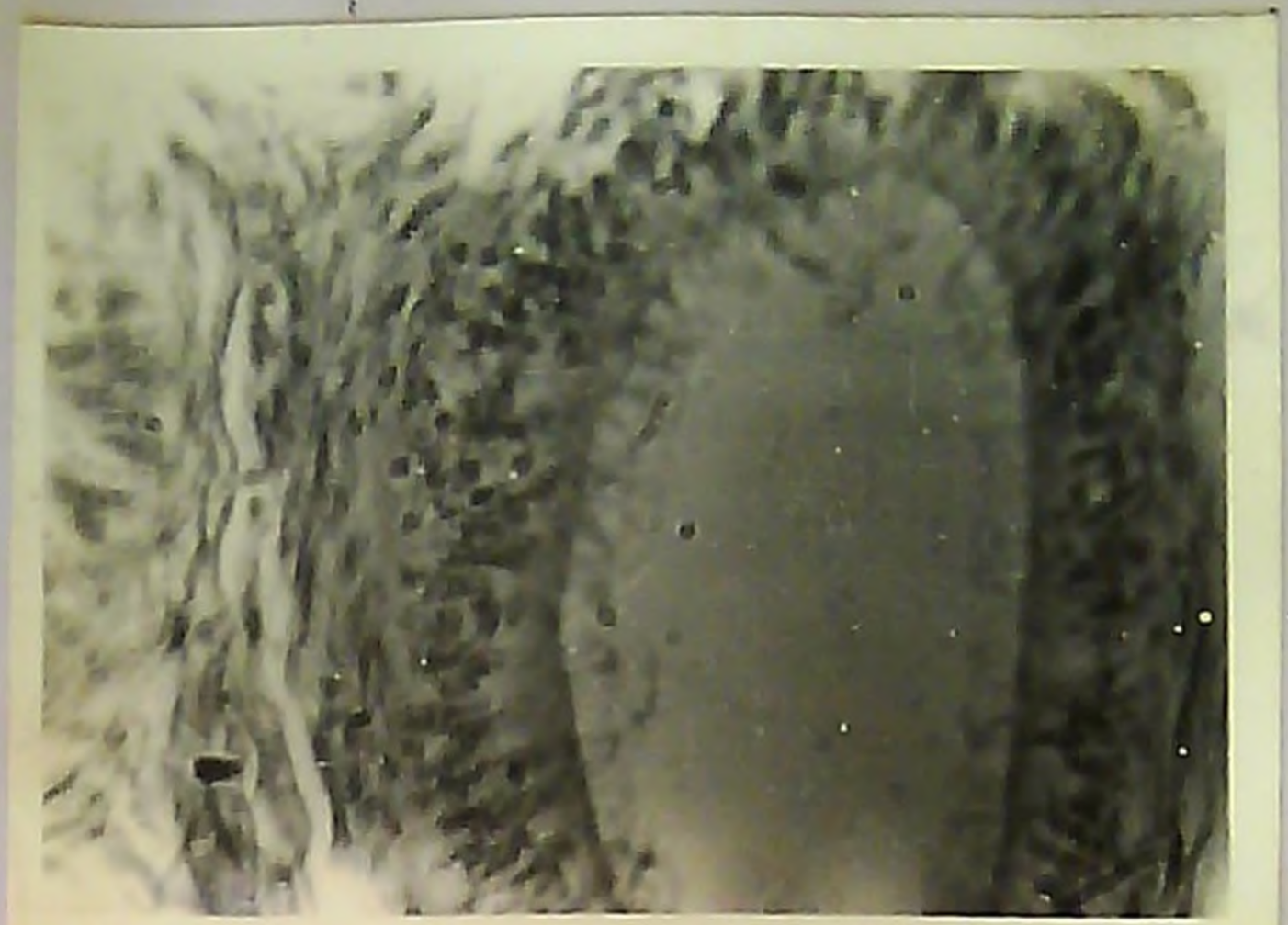

Fig. 61.

Fig. 62.

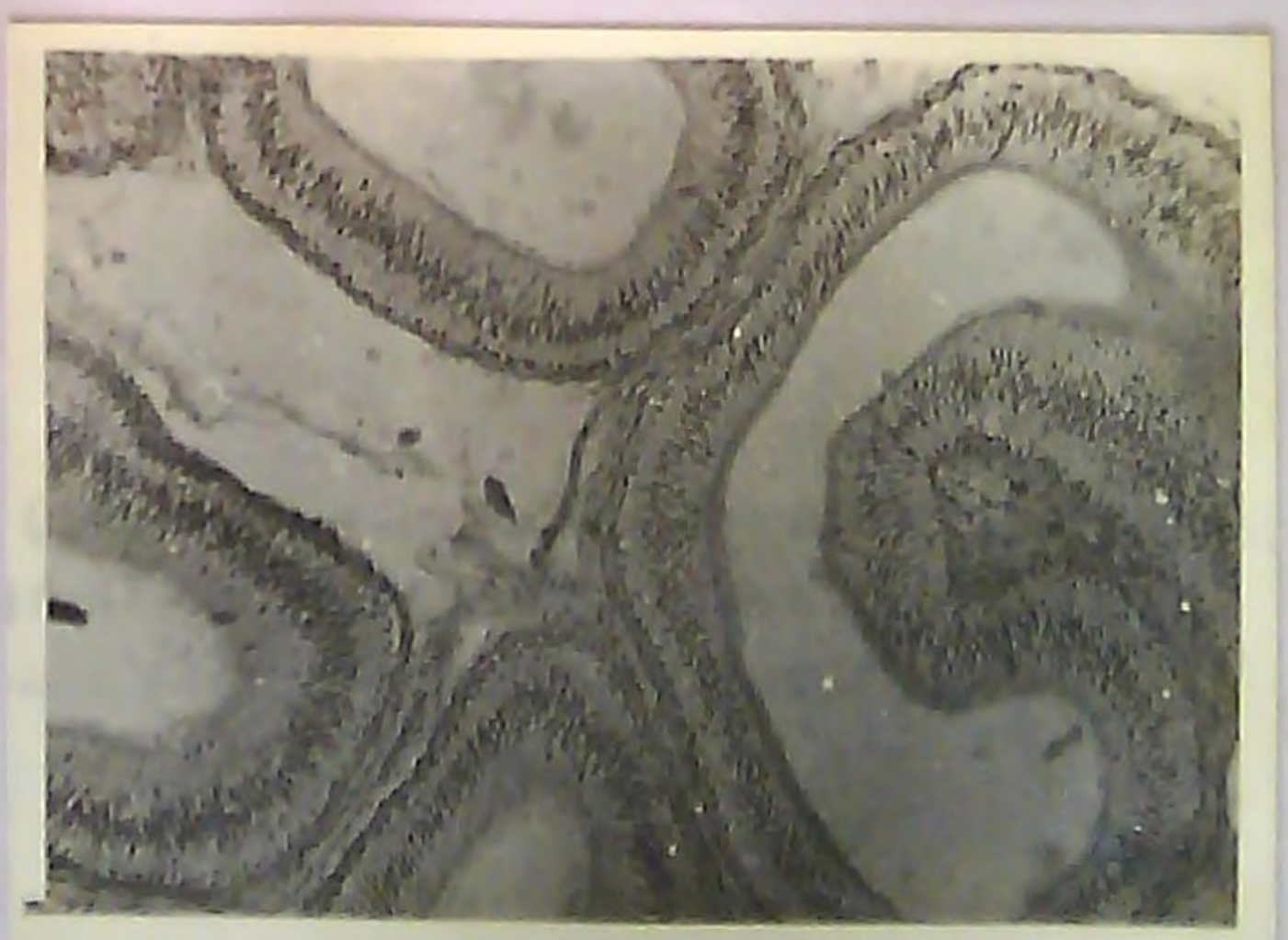

Fig. 63.

Fig. 64. Caudo epididymis (330 day) - Masses of sperm in the lumen. (H & E X 400).

Fig. 65. Caput epididymis (360 day) -- Masses of sperm in the lumen. (H & E X 100).

Fig. 66. Corpus epididymis (360 day) -- Masses of sperm in the lumen. (H & E X 400).

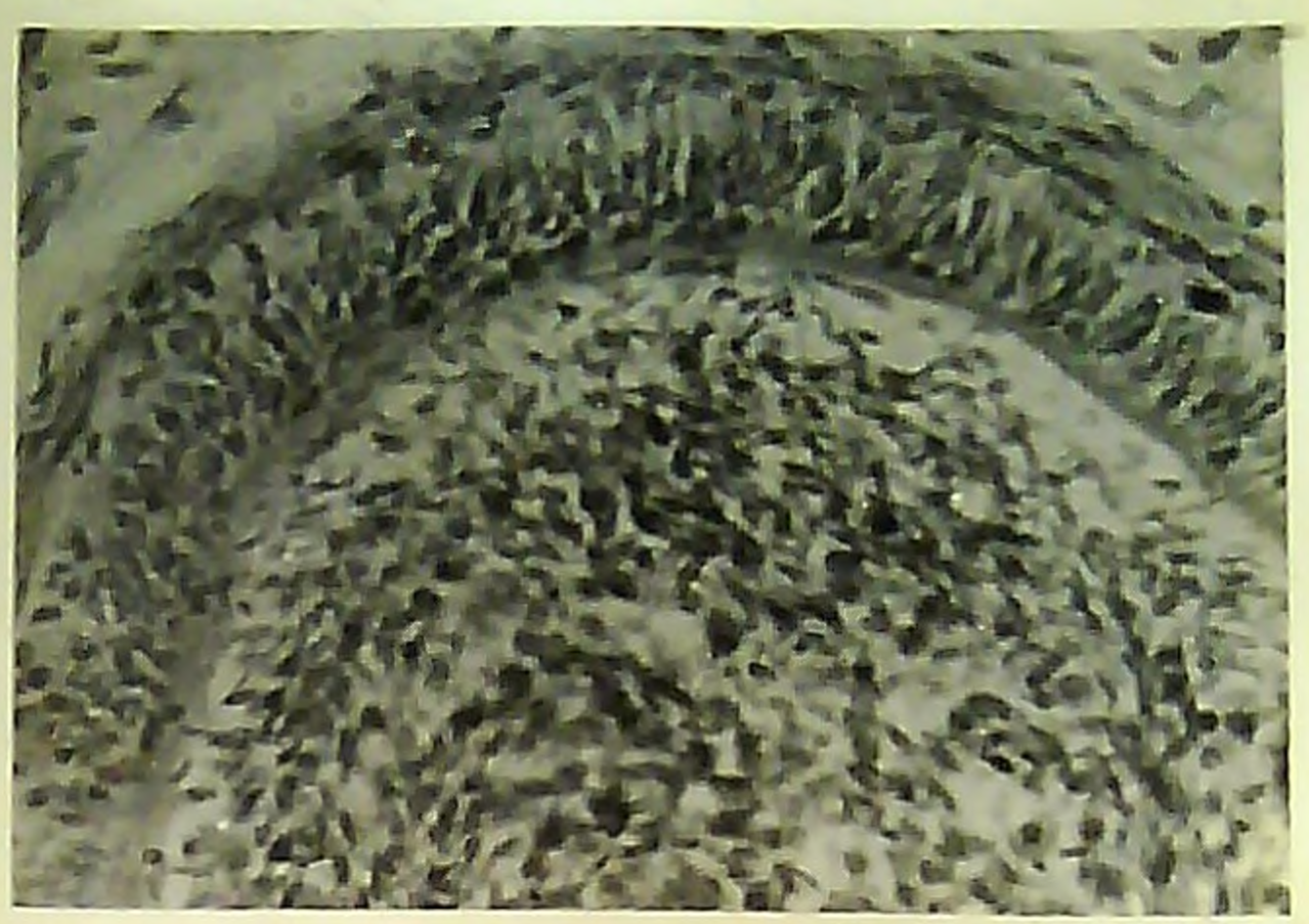

Fig. 64.

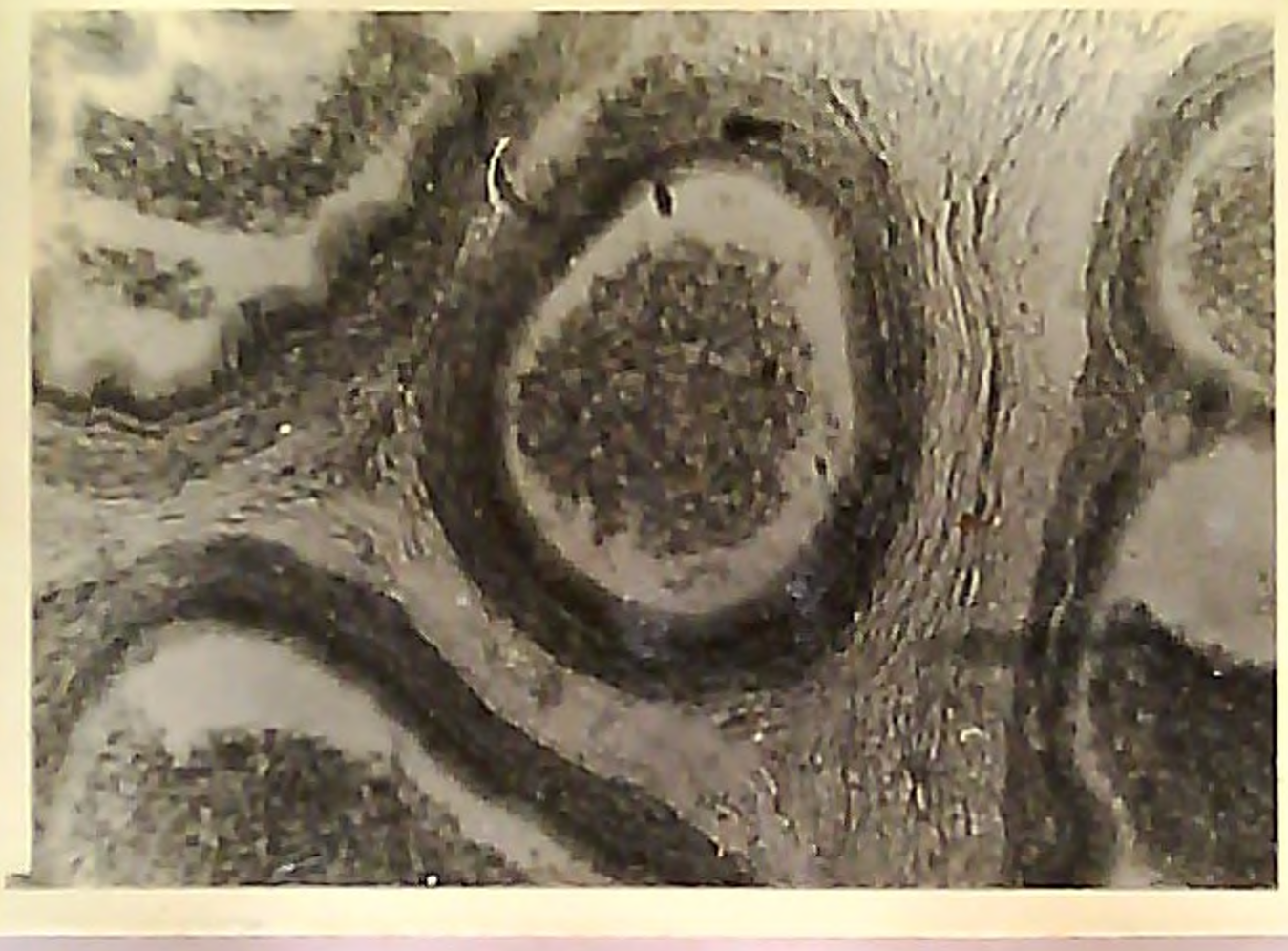

Fig. 65.

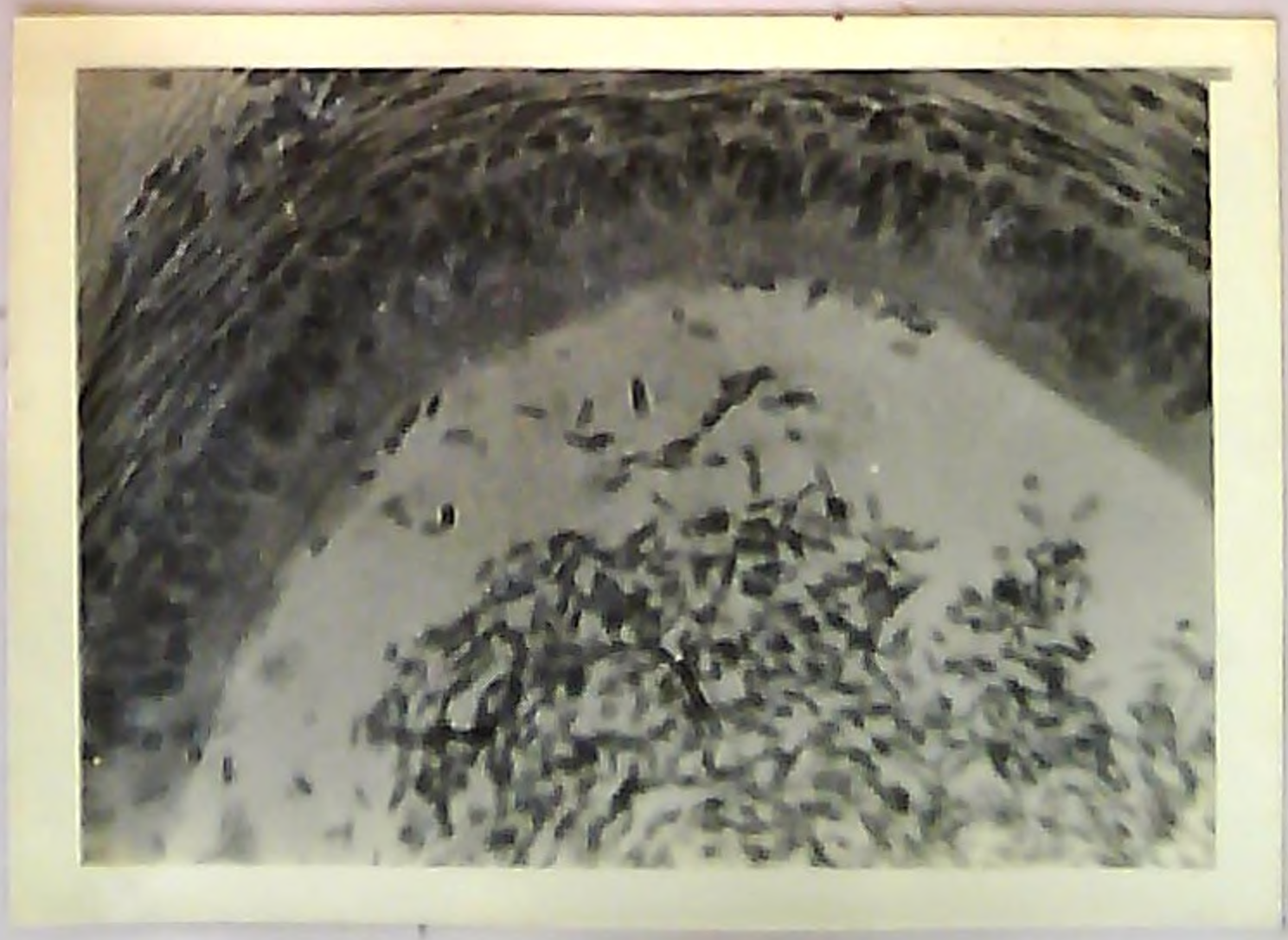

Fig. 66.

Fig. 67. Corpus epididymis (360 day) -- moderate sperm content in the lumen.

(H & E X 400).

Fig. 68. Cauda epididymis (360 day) -- Masses of sperm in the lumen.

(H & E X 400).

Fig. 69. Sperm abnormalities - Bent tail.

(X 1000).

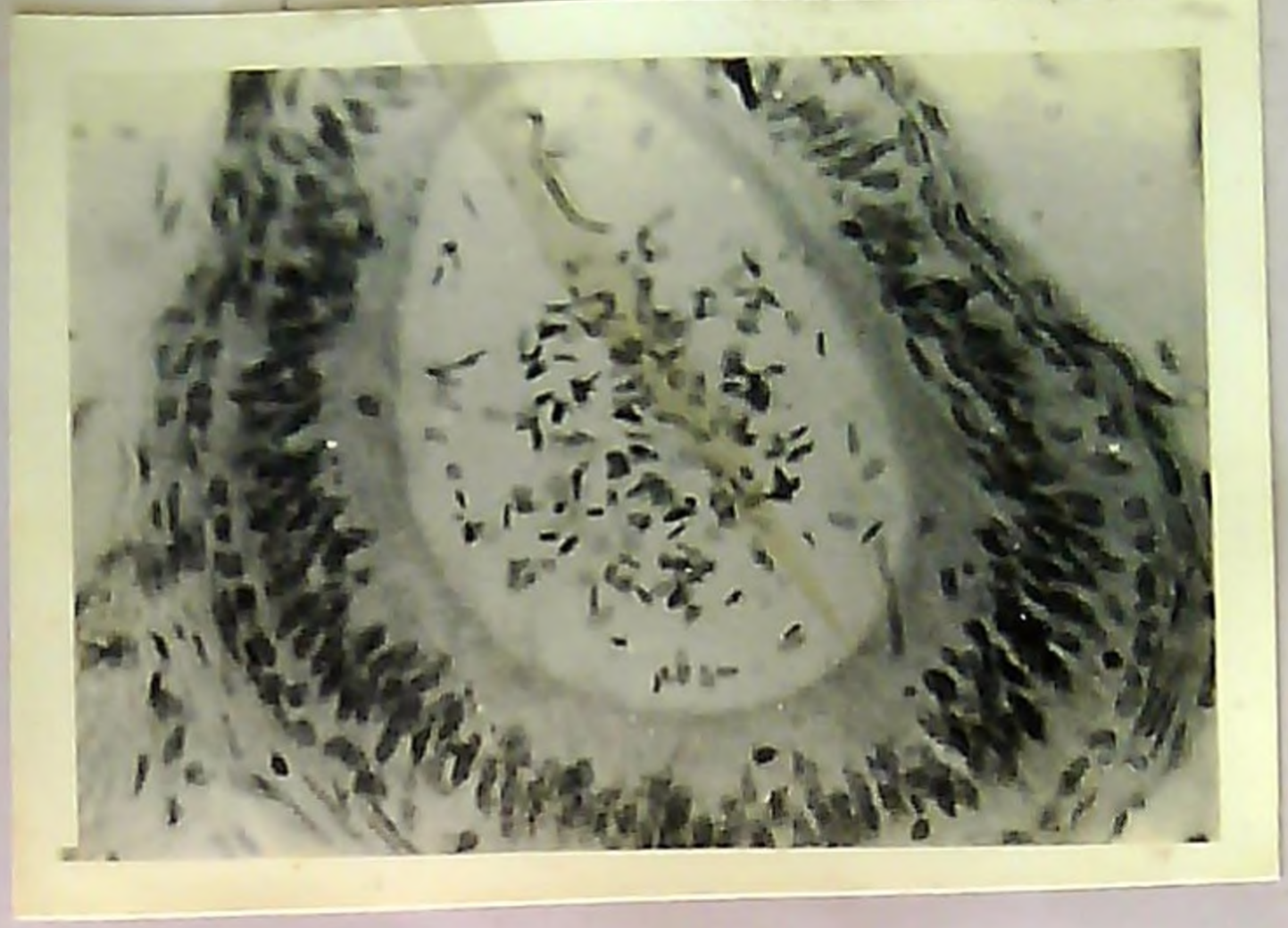

Fig. 67.

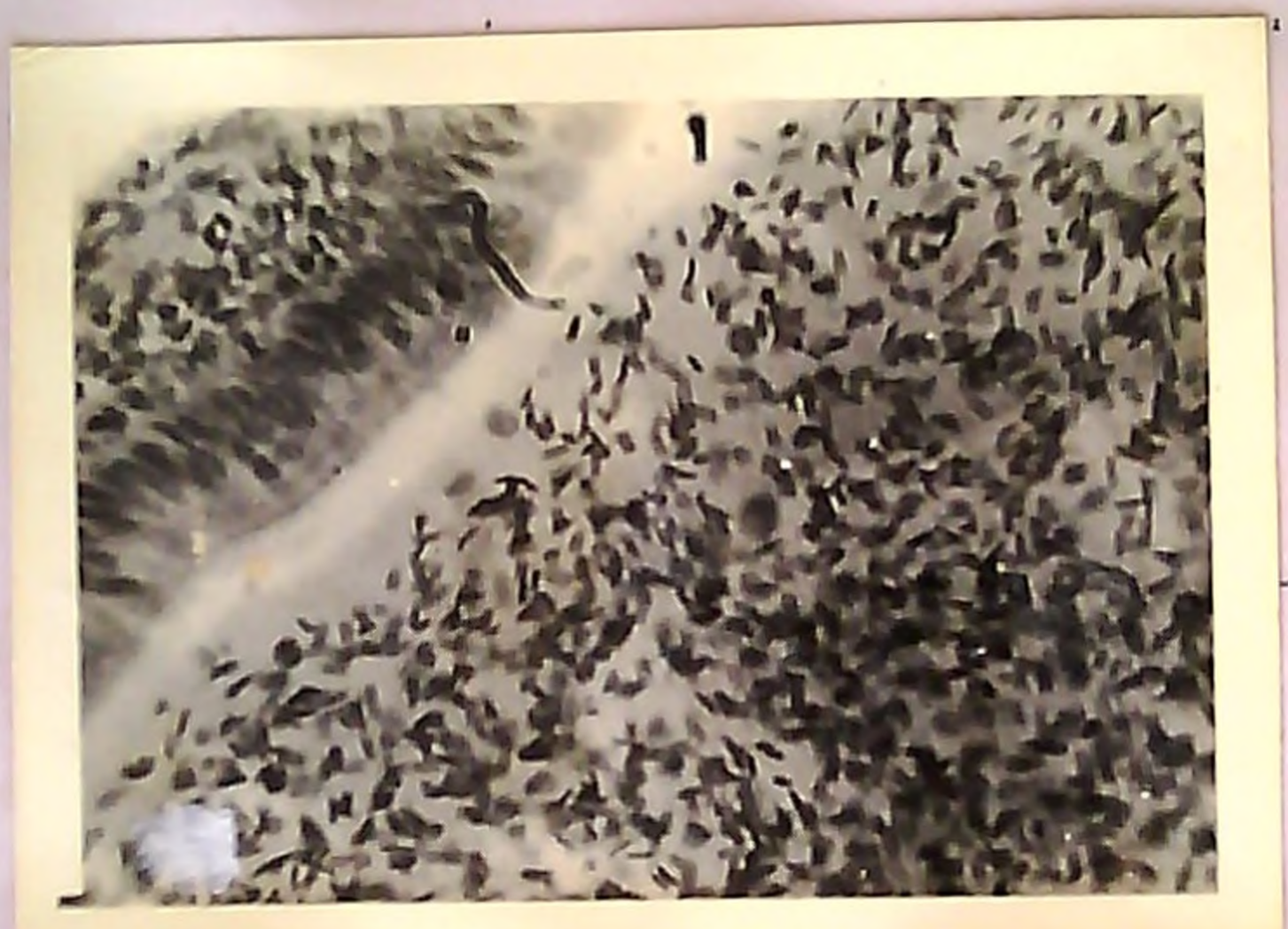

Fig. 68.

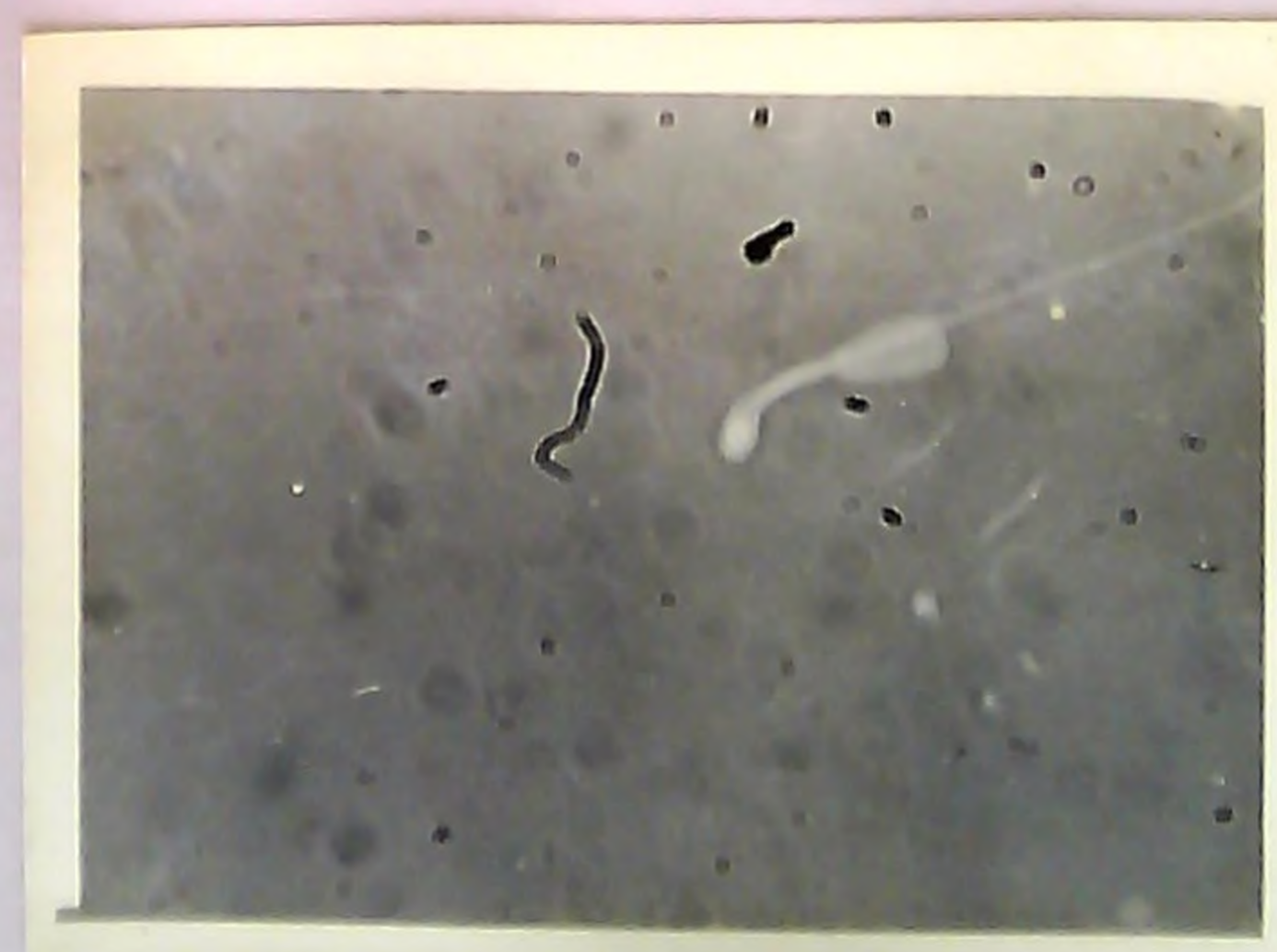

Fig. 69.

Fig. 70: Sperm abnormalities - Proximal protoplasmic droplets. (X 1000).

Fig. 71. Sperm abnormalities - Coiled tail. (X 1000).

Fig. 72. Sperm abnormalities - Free loose head. (X 1000).

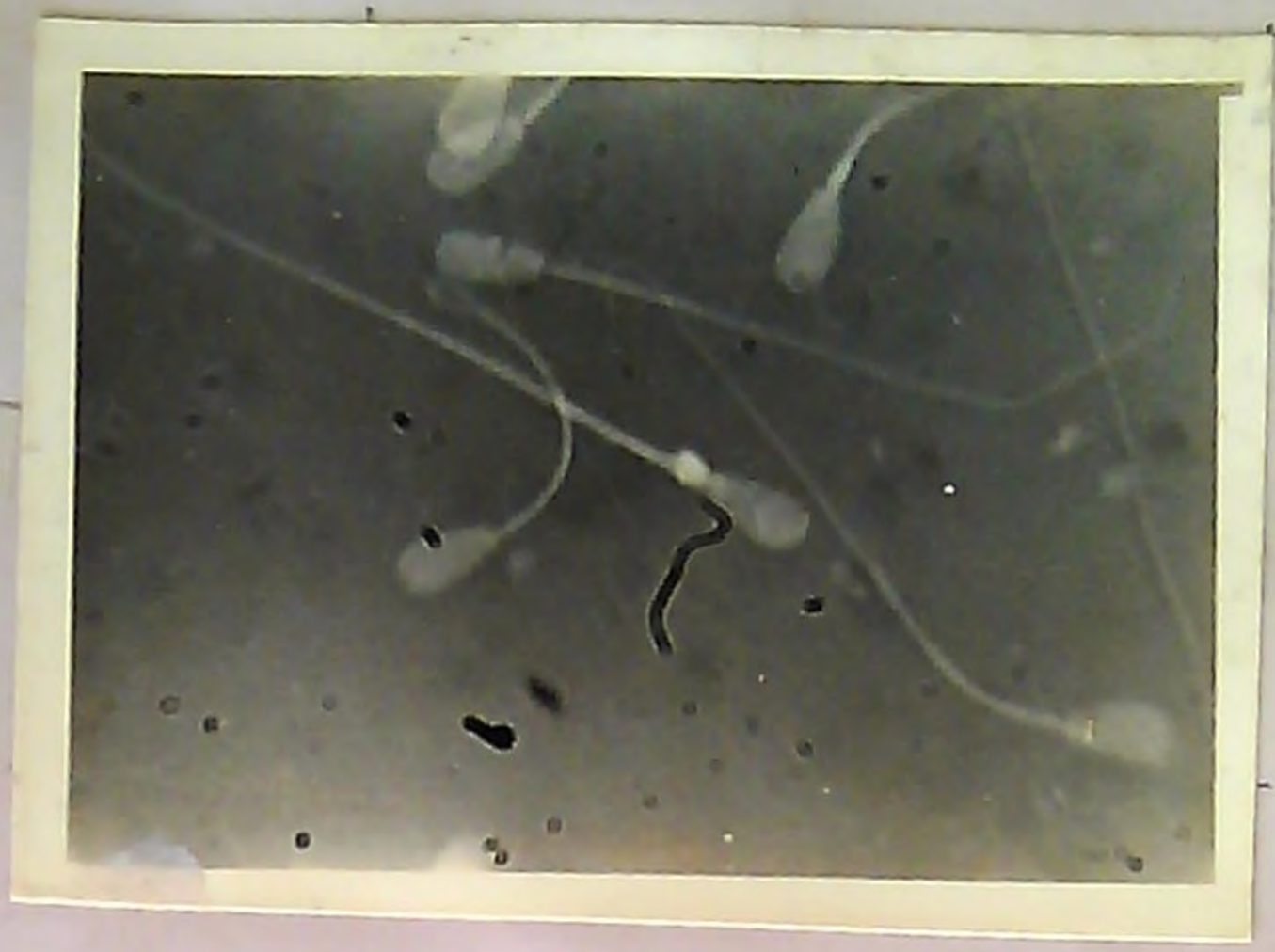

Fig. 70.

Fig. 71.

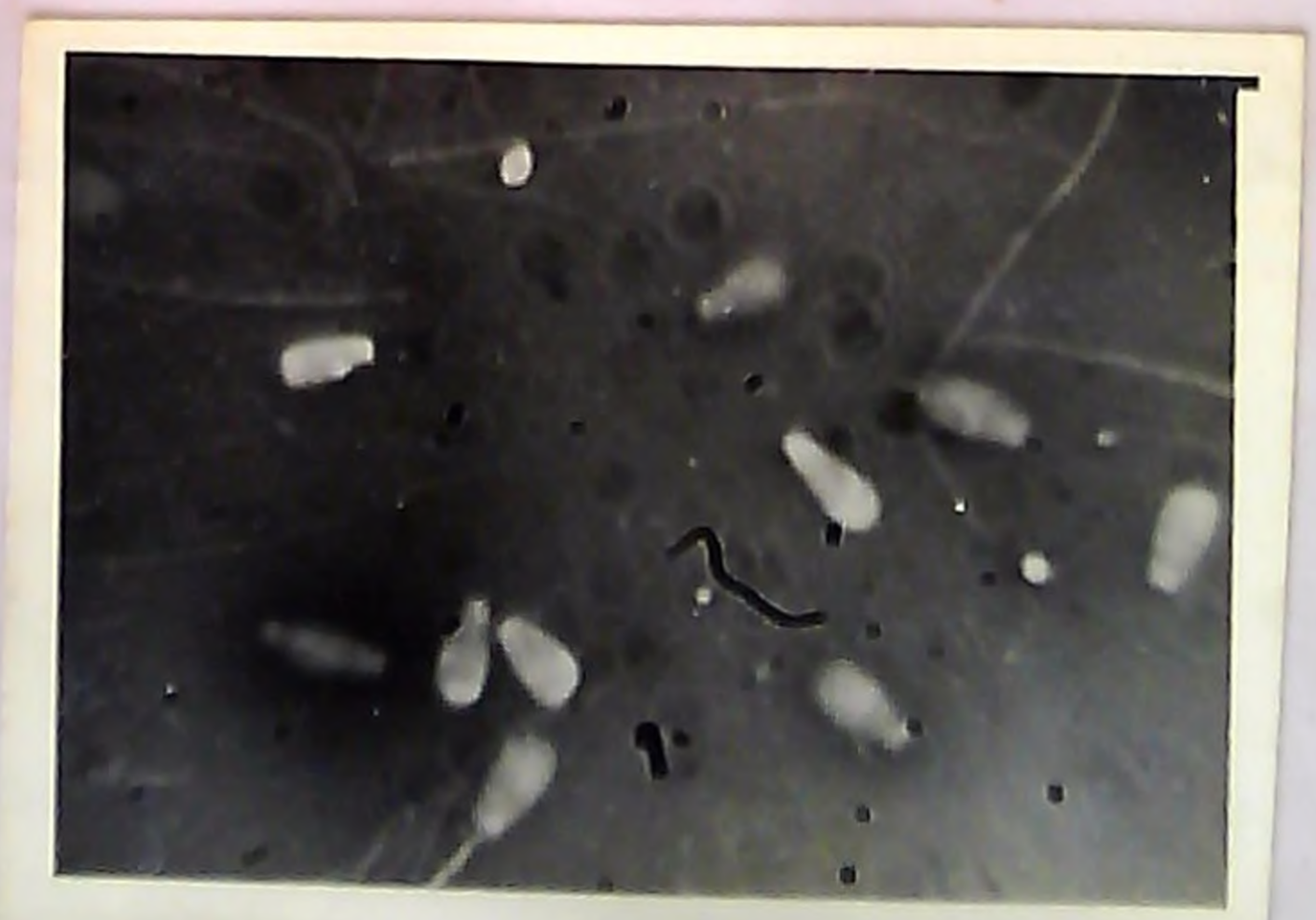

Fig.72.

Tables

Table 1: Age and body weight of the experimental animals.

Sl. No.	Calf No.	Date of birth	Birth weight (Kgs)	*Age at slaughter (days)	Body weight at slaughter (Kgs)
1.	427	25-5-80	30	0	30
2.	473	1-5-80	29	30	45
3.	448	12-3-80	23	60	52
4.	A	20-3-79	31	90	65
5.	B	28-3-79	32	90	65
6.	D	1-4-79	27	90	68
7.	410	8-11-79	45	120	103
8.	431	10-1-80	34	120	110
9.	295	23-10-78	31	150	102
10.	A 381	18-8-79	32	150	105
11.	289	9-9-78	25	180	123
12.	406	6-11-79	29	180	125
13.	286	14-8-78	40	210	146
14.	287	20-8-78	34	210	136
15.	372	1-8-79	34	240	124
16.	378	13-8-79	40	240	130
17.	278	6-6-78	34	270	174
18.	380	18-8-79	41	270	165
19.	327	20-3-79	35	300	185
20.	368	23-7-79	28	300	180
21.	315	16-2-79	41	330	180
22.	338	26-4-79	31	330	162
23.	306	13-12-78	22	360	209
24.	313	25-1-79	31	360	230

*Day of birth "0" day.

Table 2: Feeding schedule of the experimental animals

Age in days	Colostrum Milk	Concentrate	Green grass	Hay	Vitamins, Minerals
1 - 8	Colostrum (about 4 Kg)	-	-	-	-
9 - 45	Milk 4 Kg	100 gm CSM*	Little tender grass	Little hay	Vitamins**
46 - 90	Milk 3 Kg	200 gm CSM	-do-	-do-	-do-
91 -105	Milk 2 Kg	-do-	5 Kg	-do-	-
106- 120	Milk 1 Kg	250 gm CSM	10 Kg	¼ Kg	-
121 -240	-	1½ Kg concentrates	20 Kg	-	-
241 -365	-	2 Kg	25 Kg	-	-

The animals were allowed to graze for 1-2 hrs from 90 days onwards

* CSM: Corn-Soya Milk powder. ** Rovimix.

Table 3: Mean testicular weight in relation to age and body weight.

Age (days)	No. of observations	Mean body weight (Kg)	Mean testicular weight Left (g)	Right (g)	Total (g)	(Relative wt) Testis wt(g) / Body wt.(Kg)
0	1	30.00	2.31	2.31	4.62	0.16
30	1	45.00	3.37	3.37	6.74	0.15
60	1	52.00	4.52	4.52	9.04	0.17
90	3	66.00	6.32	6.82	13.14	0.19
120	2	106.50	9.93	10.18	20.11	0.19
150	2	103.50	11.90	13.21	25.11	0.24
180	2	124.00	19.98	24.06	44.04	0.35
210	2	141.00	29.07	31.00	60.07	0.43
240	2	131.50	33.67	34.77	68.44	0.52
270	2	160.50	60.2 3	59.70	119.93	0.70
300	2	182.50	75.28	76.81	152.10	0.83
330	2	171.00	89.40	93.01	182.41	1.03
360	2	219.50	91.33	87.11	178.44	0.81
Mean		33.64	34.37	36.01		

t = 3.400**

** $p < 0.01$

Table 4: Analysis of correlation between testicular weight and age/body weight of the animals.

S.No.	Particulars	r-Value	t-Value	Variation explained (%)
1.	Testicular weight and age of the animal	0.884	6.270**	78.14
2.	Testicular weight and body weight of the animal	0.572	2.312*	32.71

** $P < 0.01$

* $P < 0.05$

Table 5. Mean testicular measurements during growth.

Age (days)	No. of observations	Length (cm)	Breadth (cm)	Thickness (cm)	Circumference (cm)
0	1	3.30	1.60	1.80	4.10
30	1	3.60	1.70	2.00	4.50
60	1	3.70	1.80	2.20	5.10
90	3	3.83	1.87	2.43	5.20
120	2	4.53	2.00	2.65	6.58
150	2	4.85	2.18	2.98	6.33
180	2	5.70	2.50	3.29	8.05
210	2	5.58	2.63	2.95	8.38
240	2	6.15	2.93	3.85	9.63
270	2	7.08	3.75	4.25	11.85
300	2	7.75	3.75	4.38	12.50
330	2	7.95	4.25	4.65	13.95
360	2	7.70	4.03	4.43	12.35

Table 6. Coefficient of variation of the ratios of testicular measurements.

Age (days)	Length / Breadth	Length / Thickness	Thickness / Breadth	Circumference / Length
0	2.06	1.83	1.13	1.24
30	2.12	1.80	1.18	1.25
60	2.06	1.68	1.22	1.38
90	2.05	1.58	1.20	1.36
120	2.26	1.71	1.33	1.45
150	2.22	1.63	1.37	1.31
180	2.28	1.43	1.32	1.41
210	2.12	1.89	1.12	1.52
240	2.09	1.59	1.31	1.55
270	1.86	1.67	1.13	1.67
300	2.07	1.77	1.17	1.61
330	1.87	1.71	1.09	1.75
360	1.91	1.81	1.09	1.60
Average	2.07	1.70	1.21	1.47
C.V.	6.20	7.00	7.90	10.50

Table 7: Diameter of the seminiferous tubules in relation to age, body weight and testicular weight.

Age (days)	Body weight (Kgs)	Testicular weight (gm)	Diameter of tubule (μ)
0	30.00	4.62	43.29
30	45.00	6.74	46.62
60	52.00	9.04	49.95
90	66.00	13.14	57.50
120	106.50	20.11	62.61
150	103.50	25.11	68.29
180	124.00	44.04	107.84
210	141.00	60.07	120.76
240	131.50	68.44	128.59
270	169.50	119.93	148.39
300	182.50	152.10	166.88
330	171.00	182.41	174.66
360	219.50	178.44	172.99

Table 8: Analysis of correlation between the diameter of seminiferous tubules and age/body weight/testicular weight.

S.No.	Particulars	r-value	t-value	Variation explained (%)
1.	Seminiferous tubule diameter and age of the animal	0.99	205.56**	98.40
2.	Seminiferous tubule diameter and body weight of the animal	0.69	4.37**	47.60
3.	Seminiferous tubule diameter and weight of testicle	0.96	36.87**	91.49

** $P < 0.01$

Table 2: Mean epididymal weight in relation to age/body weight/ testicular weight.

Age (days)	No. of observations	Body weight (Kgs)	Testicular weight (g)	Epididymal weight(gm) Left	Right	Total	Epididymal weight(mg) / Body wt.(kg)
0	1	30.00	4.62	0.42	0.41	0.83	27.7
30	1	45.00	6.74	0.86	0.84	1.70	58.6
60	1	52.00	9.04	1.19	1.21	2.40	46.2
90	3	66.00	13.14	1.24	1.25	2.49	37.7
120	2	106.50	20.11	2.46	2.44	5.90	47.8
150	2	103.50	26.11	2.65	2.95	5.60	54.1
180	2	124.00	44.04	3.93	4.54	8.47	68.3
210	2	141.00	60.07	4.56	4.81	9.37	66.5
240	2	131.50	68.44	5.56	5.86	11.41	86.8
270	2	160.50	119.93	8.13	7.69	15.82	93.3
300	2	182.50	152.10	7.88	8.19	16.07	88.1
330	2	171.00	182.41	9.14	9.74	18.88	110.4
360	2	219.50	178.44	9.93	8.79	18.72	85.3
		Mean		4.46	4.52	8.98	

t = 3.398**

** $p < 0.01$

Table 10: Analysis of correlation between epididymal weight and age/body weight/testicular weight.

S.No.	Particulars	r-value	t-value	Variation explained (%)
1.	Epididymal weight and age of the animal	0.707	4.690**	49.98
2.	Epididymal weight and body weight of the animal	0.963	16.635**	92.73
3.	Epididymal weight and testicular weight	0.689	3.152**	47.47

** $P < 0.01$

Table 11: Histometric details of caput epididymis.

Age (days)	Mean tubular diameter (μ)	Mean height of epithelium (μ)	Type of epithelium
0	66.60	16.65	Simple columnar
30	69.93	16.65	-do-
60	76.59	16.65	Mostly simple columnar - In few pseudostratification has been initiated. Very few reveals pseudostratified ciliated epithelium.
90	90.58	18.32	More regions reveals pseudo-stratification.
120	96.57	20.98	-do-
150	101.90	22.81	Mostly pseudostratified ciliated epithelium - few still shows simple columnar.
180	109.89	24.29	All are pseudostratified ciliated epithelium.
210	120.24	42.31	-do-
240	145.65	49.91	-do-
270	185.80	55.32	-do-
300	201.17	62.62	-do-
330	203.56	67.96	-do-
360	223.78	69.30	-do-

Table 12: Histometric details of corpus epididymis.

Age (days)	Mean Tubular Diameter (μ)	Mean height of epithelium (μ)	Type of epithelium
0	73.26	16.65	Simple columnar
30	97.57	17.98	-do-
60	95.60	18.64	Mostly simple columnar. Pseudostratification is initiated. Few shows pseudostratified ciliated epithelium.
90	106.56	20.12	-do-
120	159.84	32.97	Mostly pseudostratified ciliated epithelium.
150	165.89	41.27	All are pseudostratified ciliated epithelium.
180	180.51	45.29	-do-
210	196.53	48.12	-do-
240	204.80	51.63	-do-
270	215.55	53.27	-do-
300	210.24	52.97	-do-
330	225.70	60.27	-do-
360	229.62	64.94	-do-

Table 13: Histometric details of cauda epididymis.

Age (days)	Mean tubular diameter (μ)	Mean height of epithelium (μ)	Type of epithelium
0	119.88	23.31	Simple columnar
30	149.85	29.97	Pseudostratification initiated. Few shows pseudostratified ciliated epithelium.
60	160.00	33.36	More shows pseudostratified ciliated epithelium.
90	167.03	35.40	Mostly pseudostratified ciliated and few simple columnar.
120	184.82	39.96	All are pseudostratified ciliated epithelium.
150	205.82	41.31	-do-
180	230.44	43.30	-do-
210	241.78	45.64	-do-
240	250.46	50.97	-do-
270	278.22	52.26	-do-
300	267.31	58.27	-do-
330	283.72	60.50	-do-
360	271.74	62.29	-do-

Table 14: Mean ejaculate volume (ml) of crossbred bulls.

Month	Bull Number							
	136	139	143	204	1254	1282	1286	Mean
August	1.72	1.80	1.63	1.72	2.86	3.50	2.86	2.29
September	1.60	2.86	1.50	1.60	1.65	3.13	3.58	2.27
October	1.64	2.50	1.83	1.64	2.64	2.63	4.08	2.42
November	1.69	2.85	3.63	1.75	3.38	2.81	3.38	2.78
December	2.00	2.50	2.44	1.86	4.00	3.25	5.25	3.04
January	2.25	2.60	2.05	2.25	--	3.25	3.75	2.31
February	2.67	2.67	3.25	2.67	3.42	2.33	4.17	3.03
March	2.05	3.00	3.30	2.05	2.88	2.50	2.00	2.54
April	3.25	3.56	2.31	3.38	--	4.30	3.75	2.94
May	2.41	3.22	3.18	2.41	2.35	3.29	3.44	2.90
June	2.46	2.67	3.04	1.25	2.95	3.13	3.63	2.73
July	2.17	2.19	2.25	--	3.19	3.63	4.45	2.55
Mean	2.16 ±0.142	2.70 ±0.14	2.53 ±0.207	1.88 ±0.179	2.44 ±0.202	3.15 ±0.155	3.69 ±0.232	2.65 ±0.084
Range	0.5-5.001	1.00-6.00	1.00-4.50	0.50-5.00	0.50-6.00	1.00-5.75	1.00-6.00	0.5-6.00

Table 15: Percentages of initially motile spermatozoa of crossbred bulls.

Month	Bull Number							Mean
	136	139	143	204	1254	1282	1286	
August	70.33	75.33	62.00	70.33	66.75	57.83	73.25	67.83
September	72.00	88.25	52.00	72.00	77.00	52.00	60.75	67.72
October	63.25	65.13	68.67	63.88	63.88	65.13	64.50	64.92
November	62.00	63.86	70.75	60.75	70.75	59.50	59.50	63.88
December	66.17	71.00	69.50	63.25	72.83	65.33	62.00	67.15
January	71.38	66.00	67.50	71.38	--	55.33	67.00	68.65
February	69.50	66.17	68.25	72.83	64.50	72.00	62.00	67.80
March	68.00	72.50	67.00	70.00	57.00	67.00	69.50	67.29
April	67.00	75.13	68.25	73.25	--	64.50	73.25	61.91
May	66.69	79.19	70.57	76.58	74.00	70.93	66.00	61.99
June	77.42	80.75	64.08	72.00	76.50	60.08	71.00	62.97
July	74.33	74.50	77.00	--	73.25	61.00	77.33	62.77
Mean	69.00 ±1.28	73.15 ±2.111	67.13 ±1.736	64.85 ±1.474	59.12 ±2.052	63.30 ±1.806	67.17 ±1.65	66.24 ±0.874
	45.00-85.00	45.00-90.00	45.00-90.00	45.00-85.00	45.00-85.00	40.00-75.00	45.00-80.00	40.00-90.00

Table 16: Mean sperm concentration (X 10^6 per ml) of crossbred bulls

Month	Bull Number							Mean
	136	130	143	204	1254	1282	1286	
August	1748.33	1151.67	1340.00	1501.67	1735.50	1530.00	1535.00	1519.17
September	1753.33	1532.50	1435.00	1326.66	1350.00	1475.00	1710.00	1511.79
October	1688.75	2012.50	1616.66	1725.00	1495.00	1585.00	1460.00	1654.70
November	1661.25	1677.00	1755.00	1577.00	1577.00	1395.00	1227.50	1552.82
December	1438.33	1394.00	1925.00	1533.75	1733.33	1265.00	1810.00	1585.63
January	1691.25	2050.00	1835.00	1688.75	--	1198.33	1240.00	1386.19
February	2586.67	1848.33	2060.00	2586.67	1656.67	1895.00	1878.33	2073.09
March	1833.00	1737.00	1583.00	1852.00	1575.00	1496.25	1400.00	1639.46
April	1855.00	1535.75	1740.00	1885.00	--	1476.67	1477.50	1428.27
May	2139.00	1952.00	1763.30	2099.17	1742.00	1395.00	1587.00	1811.07
June	1482.50	1632.50	1422.50	1170.00	1751.00	1312.50	2192.00	1580.43
July	1541.67	1976.25	1985.00	--	1730.00	1494.00	1452.00	1464.13
Mean	1795.76 ± 88.356	1707.46 ± 99.627	1705.04 ± 66.599	1586.31 ±115.198	1582.29 ± 42.42	1459.81 ± 51.504	1580.78 ± 80.197	1589.64 ± 55.371
Range	870-2830	870-2800	965-2340	880-2770	890-2570	890-2190	820-2660	820-2830

Table 17: Percentage of live spermatozoa of crossbred bulls.

Month	Bull Number							Mean
	136	139	143	204	1254	1282	1280	
August	75.33	74.66	72.50	72.83	71.36	67.33	77.50	73.36
September	77.00	84.00	60.00	71.66	83.00	56.25	62.75	70.67
October	64.50	71.88	75.00	69.63	70.38	70.50	72.50	70.63
November	65.25	69.60	73.13	71.90	78.13	64.75	70.00	70.39
December	72.50	71.80	87.38	72.88	75.83	76.66	66.00	74.72
January	75.75	73.80	72.40	84.50	--	66.33	71.25	65.58
February	72.33	78.33	72.25	82.50	70.00	80.33	70.50	75.18
March	70.90	78.10	72.50	75.80	62.75	71.13	79.50	72.95
April	71.50	79.00	80.25	80.50	--	68.50	79.25	67.71
May	80.58	80.00	86.06	83.00	78.30	78.35	71.80	80.31
June	83.50	82.50	69.77	85.10	83.40	73.00	83.66	80.03
July	85.66	77.00	82.00	--	71.50	66.70	83.60	68.78
Mean	74.65 ±1.88	77.23 ±1.285	75.22 ±2.192	72.09 ±1.768	64.56 ±1.824	68.99 ±1.745	74.03 ±1.925	72.55 ±1.310
Range	50.00-90.00	45.00-90.00	45.00-90.00	45.00-85.00	45.00-85.00	45.00-80.00	45.00-85.00	45.00-90.00

Table 18: Percentage of head abnormalities of spermatozoa of crossbred bulls.

Month	Bull Number							Mean
	136	139	143	204	1254	1282	1286	
August	11.17	0.50	3.50	4.33	1.25	13.00	8.25	6.00
September	6.83	2.13	0.50	4.67	1.00	10.00	5.00	4.30
October	8.25	1.25	2.17	1.63	2.38	8.00	5.33	4.14
November	7.75	1.00	1.88	3.20	3.88	4.50	4.25	3.78
December	4.67	1.60	1.63	3.25	4.00	3.16	6.50	3.54
January	3.38	1.10	1.10	3.63	--	3.83	3.25	2.33
February	4.00	2.50	1.75	4.17	4.66	4.17	2.66	3.42
March	5.00	2.00	1.20	4.00	2.25	4.13	2.00	2.94
April	4.75	1.50	1.25	3.50	--	4.17	2.25	2.49
May	5.38	1.50	2.07	2.00	1.90	3.79	3.90	2.93
June	6.00	1.42	1.08	2.50	1.90	3.50	2.75	2.79
July	6.33	1.88	1.00	--	2.13	3.50	2.60	2.49
Mean	6.12 ±0.622	1.53 ±0.158	1.59 ±0.224	3.08 ±0.292	2.11 ±0.389	5.48 ±0.906	4.06 ±0.550	3.43 ±0.300
Range	2.50-12.00	0.00-3.00	0.50-4.00	0.00-6.00	0.00-6.50	2.50-16.00	1.50-9.00	0.00-12.00

Table 19: Percentage of Free loose head of spermatozoa of crossbred bulls.

Month	Bull Number							Mean
	136	139	143	204	1254	1282	1286	
August	3.67	0.33	2.00	1.50	12.88	16.67	20.00	8.15
September	2.67	0.38	1.00	1.50	9.00	12.25	17.00	6.26
October	6.38	0.88	1.70	0.50	9.00	12.25	15.00	6.53
November	3.50	0.30	0.88	0.50	9.63	12.00	11.25	5.44
December	5.50	1.40	0.50	0.63	10.67	10.33	10.50	5.65
January	5.50	0.67	1.10	0.38	--	9.17	9.25	3.72
February	2.12	0.33	1.25	0.00	8.00	9.00	10.33	4.43
March	4.90	0.50	0.50	0.70	7.50	8.38	8.00	4.35
April	4.30	0.75	1.30	1.13	--	7.50	7.75	3.25
May	4.69	1.06	2.07	0.19	9.30	6.64	6.30	4.32
June	3.83	1.50	1.42	0.00	7.80	6.33	5.50	3.77
July	4.00	1.88	2.00	--	2.13	5.90	5.80	3.10
Mean	4.26 ±0.354	1.43 ±0.152	1.31 ±0.158	0.59 ±0.161	7.16 ±0.873	9.70 ±0.912	10.56 ±1.33	4.91 ±0.439
Range	0.50-12.50	0.00-2.50	0.00-5.00	0.00-3.00	1.50-16.00	5.00-20.00	4.50-20.00	0.00-20.00

Table 20: Percentage of middle piece abnormalities of spermatozoa of crossbred bulls.

Month	Bull Number							Mean
	136	139	143	204	1254	1282	1286	
August	0.33	0.17	0.00	0.00	0.38	2.67	0.75	0.61
September	0.17	0.25	0.50	0.33	0.00	2.00	0.00	0.46
October	0.75	0.25	0.50	0.50	0.13	2.13	2.17	0.92
November	1.50	0.50	0.00	0.10	0.00	2.25	0.50	0.69
December	1.00	0.50	0.13	0.38	0.50	2.17	1.00	0.81
January	0.75	1.10	0.00	0.25	--	0.83	1.125	0.50
February	1.17	0.00	0.50	0.00	0.00	2.33	0.67	0.67
March	1.10	0.20	0.20	0.00	0.00	2.25	0.00	0.54
April	1.00	0.25	0.50	0.13	--	1.00	1.75	0.66
May	1.69	0.31	0.00	0.19	0.00	1.07	0.50	0.54
June	1.92	0.40	0.20	0.00	0.10	0.50	0.40	0.52
July	2.33	0.63	1.00	--	0.00	0.90	0.50	0.77
Mean	1.14 ±0.182	0.38 ±0.084	0.29 ±0.089	0.16 ±0.055	0.09 ±0.055	1.66 ±0.217	0.79 ±0.190	0.65 ±0.045
Range	0.00-3.00	0.00-1.50	0.00-1.50	0.00-2.00	0.00-1.00	6.00-3.50	0.00-4.00	0.00-4.00

Table 21: Percentage of tail abnormality of spermatozoa of crossbred bulls.

Month	Bull Number							Mean
	136	139	143	204	1254	1282	1286	
August	9.33	14.33	14.75	18.00	6.13	8.83	13.75	12.16
September	7.83	12.63	13.50	9.83	6.50	7.75	12.50	10.08
October	6.25	10.88	8.33	9.13	5.50	5.50	9.50	7.87
November	6.50	9.00	8.38	9.38	5.50	6.16	10.25	7.89
December	9.50	8.90	5.63	4.63	4.33	5.50	8.50	6.72
January	9.50	8.70	7.70	5.75	--	4.66	10.75	6.72
February	3.50	3.33	4.75	3.66	7.66	3.33	5.50	4.53
March	4.40	5.30	4.60	3.00	4.00	6.88	11.00	5.60
April	3.50	5.63	4.25	3.63	--	4.17	4.25	3.63
May	3.40	3.33	4.30	5.40	4.40	4.10	7.70	4.66
June	3.20	4.40	4.60	6.00	4.90	3.92	3.66	4.38
July	3.83	4.00	4.00	--	4.50	3.10	4.40	3.40
Mean	5.89 ±0.746	7.53 ±1.084	7.07 ±1.405	6.53 ±1.194	4.45 ±0.365	5.32 ±0.519	8.48 ±0.989	6.47 ±0.775
Range	2.50-12.50	2.00-16.00	2.00-16.00	2.00-20.00	2.00-9.00	2.50-10.00	3 .00-14.50	2.00-20.00

Table 22: Percentage of proximal protoplasmic droplets of spermatozoa of crossbred bulls (%).

Month	Bull Number							Mean
	136	139	143	204	1254	1282	1286	
August	3.17	0.16	0.75	0.33	0.00	10.67	0.75	2.26
September	3.67	1.00	0.00	0.33	0.00	7.00	0.25	1.75
October	4.25	0.36	0.17	0.00	0.00	7.00	1.00	2.03
November	2.63	0.20	0.25	0.00	0.00	4.25	0.25	1.08
December	1.66	0.40	0.00	0.13	0.00	3.50	0.00	0.81
January	1.63	1.30	0.30	0.38	--	2.50	1.25	1.05
February	2.67	0.83	0.00	0.00	0.00	2.50	0.66	0.95
March	2.60	0.20	0.10	0.10	0.00	4.25	0.50	1.11
April	2.75	0.13	0.25	0.00	--	2.67	0.50	0.89
May	2.00	0.63	0.14	0.13	0.00	2.64	1.40	0.99
June	2.00	0.58	0.17	0.00	0.00	1.50	0.83	0.73
July	1.67	0.88	0.00	--	0.00	2.20	0.50	0.75
Mean	2.66 ±0.089	0.56 ±0.11	0.18 ±0.063	0.12 ±0.032	0.00 ±00	4.22 ±0.778	0.66 ±0.118	1.20 ±0.148
Range	0.50-8.00	0.00-2.00	0.00-1.50	0.00-5.00	0.00--	0.50-12.00	0.00-2.50	0.00-12.00

Table 23: Analysis of variance of Physical characteristics of semen of crossbred bulls.

S.No.	Source	d.f.	Mean sum of square with F values			
			Ejaculate volume	Initial motility	Sperm concentration	Live sperm count
1.	Between months	11	2.430 (4.006**)	96.250 (0.671NS)	245762.218 (1.658NS)	144.293 (0.870NS)
2.	Between bulls	6	1.073 (1.768NS)	288.860 (2.014NS)	275342.810 (1.858NS)	231.766 (1.397NS)
3.	Error	66	0.607	143.417		
	Total	83				

** ($P < 0.01$) Figures in parenthesis indicate F value

NS : Non significant.

Table 24: Analysis of variance of morphological characteristics of sperms of crossbred bulls

S.No.	Source	d.f.	Mean sum of square with F values				
			Head abnormality	Free loose head	Middle piece abnormality	Tail abnormality	Proximal protoplasmic droplets
1.	Between months	11	7.620 (2.774**)	16.152 (2.782**)	0.126 (0.504NS)	50.305 (11.837**)	4.013 (4.095**)
2.	Between bulls	6	41.390 (15.067**)	217.256 (37.426**)	4.135 (16.540**)	22.500 (5.294**)	37.650 (38.423**)
3.	Error	66	2.747	5.805	0.250	4.250	0.979
	Total	83					

**: P<0.01 Figures in parenthesis indicate F value

NS: Non significant.

Table 25: Effect of season on the semen characteristics of crossbred bulls.

Seasons	Ejaculate volume (ml)	Initial motility (%)	Sperm concentration (X 10^6)	Livability (%)	Head abnormality (%)	Free loose head (%)	Middle piece abnormality (%)	Total abnormality (%)	Proximal protoplasmic droplets (%)
Season I (December & January)	2.64	63.90	1485.93	70.84	3.15	4.91	0.70	7.02	1.04
Season II (Febry, March & April	2.79	66.65	1724.61	72.63	3.16	4.23	0.62	4.79	1.09
Season III (May to November)	2.52	68.16	1588.38	74.15	3.99	5.59	0.64	7.51	1.48
Mean	2.65	66.25	1599.64	72.54	3.43	4.91	0.65	6.44	1.20

Table 26. Analysis of variance on the seasonal effect of the physical characteristics of semen of crossbred bulls.

Source	d.f.	Mean sum of square with F value			
		Ejaculate volume	Initial motility	Sperm concentration	Live sperm count
Between season	2	0.442 (0.858^{NS})	36.419 (0.422^{NS})	91351.892 (1.072^{NS})	19.350 (0.222^{NS})
Error	18	0.514	86.128	85149.173	86.88
Total	20				

Figures in parenthesis indicate F value

NS: Non significant.

Table 27: Analysis of variance on the seasonal effect of the morphological characteristics of spermatozoa of crossbred bulls.

Source	d.f.	Mean sum of square with F value				
		Head abnormality	Free loose head	Middle piece abnormality	Tail abnormality	Proximal protoplasmic droplets
Between seasons	2	1.610 (0.514^{NS})	3.210 (0.189^{NS})	1.773 (1.172^{NS})	13.565 (3.555*)	0.460 (0.240^{NS})
Error	18	3.132	16.942	1.512	3.815	1.950
Total	20					

Figures in parenthesis indicate F value

* ($P < 0.05$)

NS: Non significant.

Table 28: Effect of age on the semen characteristics of crossbred bulls.

Age groups (months)	Volume (ml)	Initial motility (%)	Sperm concentration (X 10^6)	Livability(%)	Head abnormality (%)	Free loose head(%)	Middle piece abnormality(%)	Total abnormality (%)	Proximal protoplasmic droplets(%)
I									
Below 24	1.56	64.84	1506.02	70.01	3.67	1.99	0.40	11.25	0.84
II									
25 to 30	2.70	65.89	1695.66	72.20	3.61	4.16	0.62	6.48	1.40
III									
31 to 36	3.10	66.44	1623.32	73.58	3.40	6.09	0.98	4.21	1.40
IV									
Above 36	3.25	67.73	1575.57	74.51	3.04	7.41	0.60	4.06	1.09

Table 29: Analysis of variance of the effect of age on the physical characteristics of semen of crossbred bulls.

Source	d.f.	Mean sum of square with F value			
		Ejaculate volume	Initial motility	Sperm concentration	Live sperm count
Between age groups	3	2.329 (10.17**)	5.087 (0.532^{NS})	44175.108 (4.462*)	15.217 (1.405^{NS})
Error	15	0.229	9.570	9887.971	10.830
Total	18				

Figures in parenthesis indicate F value

* ($P < 0.05$)

** ($P < 0.01$)

NS: Non significant.

Table 30: Analysis of variance of the effect of age on the morphological characteristics of spermatozoa of crossbred bulls.

Source	d.f.	Mean sum of square with F value				
		Head abnormality	Free loose head	Middle piece abnormality	Total abnormality	Proximal protoplasmic droplets
Between age groups	3	0.286 (0.920^{NS})	21.452 (9.640**)	0.478 (10.380**)	55.263 (15.900**)	0.352 (1.345^{NS})
Error	15	0.311	2.225	0.046	3.475	0.262
Total	18					

Figures in parenthesis indicate F value

** ($P < 0.01$)

NS: Non significant.

Table 31: Freezability of spermatozoa of crossbred bulls. Month/age wise.

Month	Post thawing motility (Percentage)			
	2-3 Yrs	3-4 Yrs	4 Yrs	Mean
January	33.64	31.40	32.85	32.63
February	37.37	36.67	36.30	36.78
March	38.00	39.27	38.83	38.70
April	35.60	38.33	36.27	36.73
May	25.53	39.87	37.27	34.22
June	28.85	43.80	42.40	38.35
July	35.75	34.67	40.20	36.87
August	35.33	37.43	38.20	36.99
September	29.00	42.00	41.17	37.39
October	33.67	39.17	39.80	37.55
November	35.67	38.67	38.20	37.51
December	36.75	36.80	42.67	38.74
Mean	33.76	38.19	38.67	36.86

Table 32: Analysis of variance of freezability of spermatozoa of crossbred bulls.

S.No.	Source	d.f.	Mean sum of square with F value
1.	Between the months	11	9.6003 (0.7729)NS
2.	Between the age groups	2	87.7569 (7.0647)**
3.	Error	22	12.4219

** $P < 0.01$

NS: Non significant.

Table 33: Pre-freezing, Post-freezing and total rejection of ejaculates of crossbred bulls (month wise).

Month	Pre-freezing rejection			Post-freezing rejection			Total rejection		
	No. of ejaculates	No. rejected	Percent rejection	No. of freezable ejaculates	No. rejected	Percent rejection	Total ejaculates	Total rejection	Percent rejection
January	562	204	36.96	348	88	25.29	562	292	52.90
February	529	189	35.73	340	61	17.94	529	250	47.26
March	510	131	25.69	379	75	19.79	510	206	40.40
April	530	148	27.92	382	64	16.75	530	212	40.00
May	619	156	25.20	463	81	17.49	619	237	38.29
June	543	167	30.76	376	48	12.77	543	215	39.60
July	553	123	22.24	430	50	11.63	553	173	31.28
August	590	166	28.14	424	53	12.50	590	219	37.12
September	587	160	27.26	427	59	13.82	587	219	37.31
October	589	141	23.94	448	79	17.63	589	220	37.35
November	524	154	29.39	370	48	12.97	524	202	38.55
December	553	168	30.38	385	45	11.69	553	213	38.52
Total	6679	1967	28.55	4772	751	15.74	6679	2658	39.80

$\chi^2 = 58.04^{**}$ $\chi^2 = 52.88^{**}$ $\chi^2 = 74.71^{**}$

** $P < 0.01$

Table 34: Pre-freezing, Post-freezing and Total rejection of ejaculates of crossbred bulls (Season wise)

Month	Pre-freezing rejection			Post-freezing rejection			Total rejection		
	No. of ejaculates	No. rejected	Percent rejection	No. of freezable ejaculates	No. rejected	Percent rejection	Total ejaculates	Total rejection	Percent rejection
Season I (December & January	1105	372	33.67	733	133	18.14	1105	505	45.70
Season II (February, March & April)	1569	463	29.83	1101	200	18.17	1569	663	42.57
Season III (May to November)	4005	1067	26.64	2938	418	14.23	4005	1485	37.08
Total	6679	1907	28.55	4772	751	15.74	6679	2653	39.80
		X^2= 22.57**			X^2= 13.12**			X^2=120.85**	

Season I X II = 2.580 NS
Season I X III = 27.087**
Season II X III = 14.366**

** ($P < 0.01$)
NS: (Non significant)

Table 35: Pre-freezing, Post-freezing and Total rejection of ejaculates of crossbred bulls. (Age wise).

Age-group	Pre-freezing rejection			Post-freezing rejection			Total rejection		
	No. of ejaculates	No. rejected	Percent rejection	No. of freezable ejaculates	No. rejected	Percent rejection	Total ejaculates	Total rejected	Percent rejection
< 2 Yrs	91	48	52.75	43	12	27.91	91	60	65.93
2 - 3 Yrs	2305	808	35.05	1497	271	18.10	2305	1079	46.81
3 - 4 Yrs	2936	761	25.92	2175	336	15.45	2936	1097	37.36
> 4 Yrs	1347	290	21.53	1057	132	12.49	1347	422	31.33
Total	6679	1905	28.55	4772	751	15.74	6679	2658	39.80
			X^2=127.27**			X^2= 19.31**			X^2= 74.716**

Age groups

I	X	I	=	12.846**
I	X	III	=	30.511**
I	X	IV	=	45.808**
II	X	III	=	88.087**
II	X	IV	=	84.070**
III	X	IV	=	23.022**

** $P < 0.01$

Table 36: Fertility of crossbred bulls (month-wise).

Month	First insemination			Second insemination			Third insemination			Total insemination		
	No. of AI followed up	No. of positive	Conception rate (%)	No. of AI followed up	No. of positive	Conception rate (%)	No. of AI followed up	No. of positive	Conception rate (%)	No. of AI followed up	No. of positive	Conception rate (%)
January	537	321	59.77	264	150	60.22	104	55	52.88	905	535	58.01
February	438	252	57.53	270	149	55.18	99	56	56.57	807	457	56.63
March	338	200	59.17	192	115	59.89	84	35	41.67	614	350	57.00
April	224	136	60.71	99	64	64.65	34	18	52.94	357	218	61.04
May	127	81	63.78	63	43	68.25	15	7	46.67	205	131	63.90
June	125	84	67.20	46	29	63.04	17	9	52.94	188	122	64.89
July	131	73	55.72	42	31	73.81	25	13	52.00	198	117	59.09
August	444	243	54.73	212	117	55.19	71	35	49.29	727	395	54.33
September	442	242	54.75	271	158	58.30	132	56	42.42	845	456	53.96
October	552	291	52.72	233	130	55.79	122	65	53.28	907	486	53.86
November	533	288	54.03	307	184	59.93	157	73	46.49	997	545	54.66
December	454	238	52.42	257	145	56.42	126	58	46.40	836	441	52.75
Total	4345	2449	56.36	2256	1324	58.69	985	480	48.73	7586	4253	56.06

$X^2 = 156.1017^{**}$

Table 37: Comparison of fertility of crossbred bulls between months.

Month	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.
January		1.084	0.6710	0.404	1.5950	2.1670	0.0001	3.7627	4.7216*	5.6375*	5.5062*	7.1460**
February			0.019	1.9880	3.5553	4.2823*	0.4326	0.8178	1.3731	1.6033	0.6987	1.7382
March				1.533	3.018	3.700	0.267	0.961	1.328	1.730	0.842	2.381
April					0.445	0.769	0.207	4.415*	5.136*	5.810*	4.378*	6.992**
May						0.0377	0.985	5.956*	6.609*	7.208**	5.896*	8.369**
June							1.377	6.778**	7.453**	8.066**	6.727**	9.142**
July								1.425	1.702	1.988	1.310	2.589
August									0.021	0.912	0.185	0.391
September										0.025	0.090	0.248
October											0.223	0.1209
November												0.669
December												

Table 38: Fertility of crossbred bulls season-wise.

Season	First insemination			Second insemination			Third insemination			Total inseminations		
	No. of AI followed up	No. of positive	Conception rate(%)	No. of AI followed up	No. of positive	Conception rate(%)	No. of AI followed up	No. of positive	Conception rate(%)	No. of AI followed up	No. of positive	Conception rate(%)
Summer (I) (February to May)	1127	669	59.36	624	371	59.46	232	116	50.00	1973	1156	58.59
Rainy (II) (June to October)	1604	933	55.08	804	465	57.84	367	178	48.50	2865	1576	55.00
Winter (III) (November to January)	1524	847	55.58	828	488	58.94	386	186	48.19	2738	1521	55.55
Total	4345	2449	56.36	2256	1324	58.69	985	490	48.73	7586	4253	56.06

χ^2= 30.344**

Season I & II 5.147*
Season I & III 4.186*
Season II & III 0.167 NS

NS: Non significant

Fertility of crossbred bulls age-wise.

	First insemination			Second insemination			Third insemination			Total inseminations		
	No.of AI followed up	No. of positive	Conception(%)	No.of AI followed up	No. of positive	Conception(%)	No.of AI followed up	No. of positive	Conception(%)	No.of AI followed up	No. of positive	Conception
rs	2814	1501	53.34	906	496	54.75	679	306	45.07	4399	2303	52.3
Crs	1531	948	61.92	1350	828	61.33	306	174	56.86	3187	1950	61.1
	4345	2449	56.36	2256	1324	58.69	985	480	48.73	7586	4253	56.0

$X^2 = 58.5$

.01

Table 40: Conception rate between bulls.

Sl. No.	Bull No.	Total AI followed up	Total pregnant	Conception (%)
1.	100	264	167	63.26
2.	101	261	157	60.15
3.	102	256	161	62.89
4.	103	233	144	61.80
5.	104	239	147	61.51
6.	105	257	143	55.64
7.	108	248	154	62.09
8.	110	194	125	64.43
9.	111	349	214	61.32
10.	112	275	166	60.36
11.	201	681	411	60.35
12.	202	355	150	42.25
13.	203	320	135	42.18
14.	204	653	391	59.88
15.	205	744	426	57.26
16.	206	328	118	35.98
17.	208	769	438	56.96
18.	211	557	289	51.89
19.	212	603	317	52.57
	Total	7585	4253	56.06

Chisquare (χ^2) = 156.101**

** $P < 0.01$