دراسة تجريبية عن الأجهاد الصيفى فى الأرانب

١ـ التقييم الوصفى والكمي للدورة الخلوية المنوية فى الأرانب البلدى

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وصفت الدورة الخلوية المنوية فى الأرانب البلدى على حســـب شـكل وهجـــرة الاسبرماتيد ، وانطلاق الحيوانات المنوية . ووجـود المراحل الاخرالية لخلايـــا الاسـبرماتوسيتس .

وتم التقـيم الكمى لخلايا الدورة بحساب كل خلية ثم نسبتها الى عدد خـلايـــا سرتولى . مع قياس قطـر الأنابيب المنوية وقد درست هذه القيم الثلاث ، مراحـل الدورة نسـبة خلايا السرتولى ، وقطـر الأنابيب المنوية لتكون وسيلة للتقـــيم المرضى والوظيفى للدورة الخلوية المنوية فى الأرانب كممثـل لحيوان تجارب .

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EXPERIMENTAL STUDY OF SUMMER STRESS IN RABBITS

I- THE DESCRIPTION AND QUANTIFICATION OF THE SPERMATOGENIC CELL CYCLE IN NORMAL BALADI RABBIT.

(With One Table and 8 Figures)

By

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SUMMARY

Stages of spermatogenic cell cycle were described and classified in Baladi rabbits according to the shape, migration of the spermatid,release of spermatozoa and presence of meiotic figures of spermatocytes. Quantification of the cells of the cycles were carried by calculating each type and relating it to the number of Sertoli. The diameter of the seminiferous tubules was measured. The three indecies,stages of the cycle, the Sertoli cell ratio and the diameter of seminiferous tubules were presented for pathophysiological evaluation of the spermatogenesis in rabbit as an experimental animal.

INTRODUCTION

Although buffalo and cow bulls are not seasonal breeders, EL-SHERRY et al.(1977)had proved low rate and disturbed spermatogenesis in summer. For detailed studies of the pathogenesis of this process,. an experimental animal is needed to describe the testicular, endocrinal and nervous changes during summer stress conditions. Rabbit as non seasonal breeder was thought to be representative model experimental animal. SWIESTRA and FOOTE (1963) had described the different stages of the spermatogenic

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cell cycle of the seminiferous tubules of rabbit adopting the classification of OTRVANT (1958) in lambs. COURT, HOCHEREAU-DEREVIERS & OTRAVANT, (1970) in their review about spermatogensis substantiated that Sertoli cell, morphologically and metabolically coordinates and regulates the whole seminiferous epithelial cycle. SKAKKEBEAK and HELLER (1973) employed the Sertoli cell ratio for quantitative analyses of the seminiferous epithelial cycle.

The aim of this work is to describe the spermatogenic cell cycle in normal rabbits and it's quantification using the Sertoli ratio together with estimation of the diameter of the seminiferous tubules as a stander for patho-physiological evaluation of the cycle and as a biological assay for various hormonal influences.

MATERIALS AND METHODS

Four normal Baladi 1-1½ years old male rabbits weighing 1½-2 Kg were used. Testicles were fixed in Suza. From each block serial sections 5 u thickness were stained by Harris heamatoxylene and eosin. Typing of the seminiferous cycle stages were carried by classification based on the shape of spermatids, the migration of spermatids, the presence of meiotic figures and the release of spermatozoa in the lumen of seminiferous tubules. The scheme of the eight stages of the classification of the cycle applied was corresponding to that used by SWIESTRA and FOOTE (1963) in rabbits and by COURT, HOCHEREU DE reviers and OTRAVANT (1970) in mammals as a general.

For estimating the Sertoli cell ratio, the different types of cells occuping the whole C-S of ten rounded seminiferous tubules were calculated. The ten tubules were representing the eight stages of the cycle and stage one and eight as a repetetion. A stage was typed and calculated when all the cell population of the C-S of seminifierous tubules was in the same stage (i.e) passage between stages was not selected. For estimation of the diameter 30 rounded C-S from each 2 testicles were measured and the medium was calculated.

Typing the cells for calculation; the spermatogonia were classified into type A and B. Intermediate type claculated in type B. The preleptotene and leptotene figures of the first meiotic prophase were calculated as one type, also the diplotene and diakinesis.

RESULTS

The result of quantification of the different types of cells is presented in (Table 1). The different types of association of cells will be described for each stage as follow:

Stage 1:

(Fig.1) start from the release of mature spermatozoa into the lumen and end with the start of nuclear elongation of the spermatid of stage 2. On the basement membrane type A spermatogonia was predominant. It is a large cell with ovoid nucleus. The major axis of the nucleus is parallel to the basement membrane.The chromation is homogenous dust like. One or two nuclei are attached to the inner aspect of the nuclear envelop. The triangular shape large size Sertoli nucleus was near the basement membrane. It is characterised by large nucleolus associated with several chromatin masses. There are two generations of primary spermatocytes. The zygotene phase of the first meiotic prophase of the primary spermatocytes.Then higher up another generation,the pachytene phase of primary spermatocytes. Near the lumen there is one generation of rounded spermatids type B.

Stage 2:

(Fig. 2) This is the stage of nuclear elongation of the spermatid. It start with elongation of spermatid nuclei and with the formation of bundles of spermatids in the Sertoli Cytoplasm. On the basement membrane type A spermatogonia is still present beside the Sertoli cells. The two generation of primary spermatocytes are present. The zygotene spermatocytes and then higher the pachytene spermatocytes. The shape of elongated spermatid varies from ovoid to eleptical with centrally located

nuclei at the end of the elongattion phase.The elongated nucleus will be diffusely stained with euchromatin with accumulation of chromatin mass at the base of the head opposite to the achrosome.

Stage 3:

(Fig. 3): Start from the formation of bundles of elongated spermatids in the sertolian cytoplasm up to the appearance of the metaphase plates of the first maturation division. On the basement membrane type A spermatogenia are still present beside the Sertoli cells. Then the zygotene generation of the spermatocytes. Then this stage is characterised by the appearance of diplotene phase of spermatocytes from the previous pachytene of stage 2. Few figures of diakinesis could be encountered. The diplotene are characterised by large nuclei. The chromosomes of which dispersed forming tetrades. The clear zone of nuclei increases, the nucleolus disappears and the nuclear membrane also disappears at the end of this stage. Few diakinesis figure could be seen where paires of highly spiralised chromosome either apart or partly attached together. The elongated spermatids moved towards the Sertoli cytoplasm to be arranged in bundles.

Stage 4:

(Fig. 4): Start with the metaphase plates on the first meiotic division to the disappearance of the second maturation division. This stage is characterised by the appearance of type B Spermatogonia on the basement membrane. The target morphology for (the intermediate and) type B spermatogonia would be ovoid or rounded nuclei with centrally located nucleus and apppearance of course chromatin granules. The zygotene generation of spermatocytes are still present near the basement membrane. Above this zygotene generation,the configuration of metaphase plate will be seen with the appearance of secondary spermatocytes generation. The nuclei of secondary spermatocytes were smaller than that of primary spermatocytes and larger than that of spermatids. It is characterised by

thin network chromatin to which attachad fine chromatin granules. The second meiotic division gives rounded spermatids type A. The second metaphyseal plate is small than the first metaphyseal plate.

The elongated spermatids are in bundles in the Sertoli cytoplasm. Characteristic for this stage is higher up position of the Sertoli nuclei.

Stage 5:

(Fig. 5): Start from the end of last maturation division up to the appearance of dusty chromatin in the nuclei of young spermatids. The spermatids of this stage are classified as type A spermatids. They have small nucleus containing some karyosomes connected with chromatin network. From the stage five the cycle will be characterised with two generation of spermatids in bundles which appeared previously in stage three and still persisting in the maturation phase and the young spermatid type A which arised from the previous secondary spermatocytes of stage four. The one generation of spermatocytes are the pachytene which arised from the zygotene of stage four. The bouquet like arrangement of the chromosomes of the zygotene nucleus will now be lost. The chromosomes becomes thicker and are distributed through out the whole nucleus of the pachytene. There is prominant nucleolus.

On the basement membrane dark spermatogonia type B predominate beside type A.

Satge 6:

(Fig. 6): Start from the appearance of dusty chromatin in young spermatids up to the migration of the bundles of elongated spermatids towards the lumen of the seminiferous tubules. On the basement membrane, type B spermatogonia are predominating. There is one generation pachytene spermatocytes and there are two generations of spermatids. The differentiation of type B spermatids with dusty chromatin accumulation, and the elongated spermatids in bundles in the Sertoli cytoplasm which start to migrate the lumen.

Stage 7:

(Fig. 7): It start and end with the centripetal migration of the elongated spermatids towared the lumen. On the basement membrane type A is present beside type B. This stage is characterised by the appearance of leptotene spermatocytes from the dark spermatocgonia. The nucleus of leptotene is characterised by individualised thin filaments within the chromatin distributed in crusts and in later stage, it assume more diffuse euchromatin. The pachytene spermatocytes is persisting. Then the rounded type B spermatid and the elongated spermatids centripetaly arranged around the circumference of the seminiferous tubules.

Stage 8:

(Fig. 8): It is the stage of release of the mature spermatid type D from the Sertoli cytoplasm into the lumen as spermatozoa leaving the residual bodies. On the basement membrane individual number of type A is present and few number of type B spermatogonia is present. The majority of the basement membrane is occupied by leptotene spermatocytes, higher up the pachytene spermatocytes then the rounded spermatids type B arranged along the whole circumference of the seminiferous tubules.

DISCUSSION

Several quantitative methods have been introduced for the evaluation of the spermatogenic cell cycle. The number of spermatocytes generated from one stem cell was calculated by CLERMONT and LEBLOND (1953). The number of spermatids generated from one primary spermatocytes was calculated by OTRAVANT (1959). In rabbit the two indecies were calculated by SWIERSTRA and FOOTE (1963) to be 16-1 and 31-1 respectively. The Sertoli cell ratio introduced by SKAKKEBAEK and HELLER (1973) was in advantage because of the followings. There are regular degeneration of germ cells during the process of spermatogenesis. MONEI (1972) stated that at several points during the mitotic division of spermatogonia, the cells degenerates specially type A spermatogonia. Further degenerations occur during the

two mitotic divisions so that there is about 27-37% loss of spermatid. SWIERSTRA and FOOTE (1973) calculated 24% loss of spermatid in rabbit during normal spermiogenesis. Sertoli cells unlike germ cells are very resistance to various influences (CLERMONT and MORCENTALLER, 1968). The Sertoli cells did not divide (STEINBERGER and STIENBERGER, 1971). The Sertoli ratio for a given cell type of a given individual is smaller than inter individual variation and provides a constant which would compensate for variation due to the shrinkage caused by different fixative (SKAKKEBEAK and HELLER, 1973).

In this work the same basis of classifications has been used as that applied by SWIESTRA and FOOTE (1963). But the following difference can be noted. The spermatid classification used is type A spermatid corresponding to the young rounded spermatid arising from secondary spermatocytes of stage four. Type B spermatid is the young rounded spermatid aquiring the dusty chromatin in stage six, and continuing during stage seven, eight and one. Type C spermatid is the elongating spermatid of stage two. Type D spermatid, the elongatid maturing spermatid which is formed at the end of stage two and continue to stage eight of relase. Spermatozoa is applied to the spermatid when released into the lumen of the seminiferous tubule during stage eight. SWIESTRA and FOOTE (1963) applied the term spermatozoa to the spermatid from when the new generation of spermatids appeared. The associations described for stage three in SWIESTRA and FOOTE (1963) classification start from the end of elongation of spermatids to the begenning of the first maturation division of primary spermatocytes. An important moment had been missed. That is migration of elongated spermatid to form bundles in the Sertoli cytoplsm which is the start point of stage three in our observation. In SWISTRA and FOOTE (1963) observations the, elongated spermatids moved towards the Sertoli during stage five.

The diplotene type and few elements of diakinesis primary spermatocytes were forming the second generation with zygotene in the association of stage three in our Baladi rabbit.

In SWISTRA and FOOTE (1963) observation, the diplotene and diakinesis of prophase with the two meiotic divisions, secondary spermatocytes in between and the young spermatids at the end were regarded in one stage, the stage four with relative frequancy of 11 days.

In our observation stage four start with the presence of the metaphase of the first meiotic division, presence of secondary spermatocytes and end with the presence of metaphase plate of the second meiotic division and presence of young spermatid type A. The frequency of such association of stage four is very low. Peculiar to this stage is the high position of the Sertoli nuclei in the seminiferous wall and the inserted bundles of spermatid type D in the Sertoli cytoplasm.

That the diplotene and diakinesis phases of primary spermatocytes are found in separate stage agree with the classification given by ROOSEN-RUNGE (1962) for the spermatogenic cell cycle of rat, mouse and ram. This was true in bull (COURT, HOCHEREAU-DE REVIERS and ORTAVANT (1970).

The present observation agreed with SWIESTRA and FOOTE (1963) that type A spermatogonia are present in all stages. The begenning of appearnce of type B spermatogonia differed. It appeared from stage four, in our observation is counted as type B; the target morphology started with more or less rounded cells with centrally located nuclei with course chromatin granules. Also the preleptotene and leptotene were counted in one category. The leptotene was observed in the association of stage seven in our association instead of stage eight in SWIESTRA and FOOTE (1963) observation. The target morphology was the appearance of thin filamentous chromatin distributed all over the nucleus. The leptotene continued during stage eight with increasing intensity of euchromatin. The passage from leptotene to the zygotene stage started in stage one but was predominant in stage two with the characterestic bouquete like chromosome polarised to one side of the cell. The passage from the zygotene stage to pachytene stage was observed in stage five based on the transformation of the polarised bouquetes of the chromosemes of the zygotene nuclei to the

pachytene nucleus where the chromosomes become thicker and distributed throughout the whole nucleus. In SWIESTRA and FOOTE (1963) observation the passage from zygoene to pachetene was observed in stage four.

In conlusions, the different cell association in the above enumerated eight stages of the epithelial cycle of the seminiferous tubules together with the Sertoli cell number of each cell will be standered qualitative and quantitative indecies for detailed analyses of the spermatogenesis in rabbit under various pathophysiological influences.

REFERENCES

Clermont and Leblond (1953): Renewal of spermatogonia in the rat.Amer.J. Anat. 93, 475.

Clermont,Y., and Morcentaller,H. (1955): Quantitative study of the spermatogenesis in the hypophysectomised rat. J.Endocrinology. 57, 369.

Clermont,Y.; and Perey,B. (1957): Quantitative study of the cell population of the seminiferous tubules in immature rats. Amr.J.Anat. 100, 241.

Court,M., Hochereau de Reviers,M.T., and Ortavant,R. (1970): The testis part I, chapter 6 "Spermatogenesis" 365-375.

El-Sherry,M.I.; El-Naggar,M.A. and Sanna,M. Nassar (1977): Evaluation of the spermatogenic cell cycle in summer sterility of buffaloes. Assiut Vet.Med.J.Vol. 4 No 7. 134-147.

Heller,G.V. O'Keefe,K.B. and Heller,C.G. (1968): Effects of follicle stimulating hormone (F.S.H.) on Sertoli cells in the hypophyctomised rat.J.Clin.Res. 16-113.

Lacy,D. and Lofts,B. (1965): Studies on the structure and function of the mammalian testis. 1. Cytological and histochemical observations after continuous treatment with oestrogenic hormone and effects of F.S.H. and L.H. proc. R.Soc.B. 162. 188.

Monesi,V. (1972): Reproduction in mammals. Germ cells and fertilization. Austin,C.R., and Short,R.V. Cambridge University press, 1972. Section 31 spermatogenesis and spermatozoa, 46.

Monesi,V. (1967): Arch. Anat. Micr. Morph. Exp. 56-61. Cited by Monesi, V. (1972).

Ortavant,R. (1958): Le cycle spermatogénetique chez le bérlien. Thesis. University of Paris. Farance.

Ortavant,R. (1959): Spermatogenesis and morphology of spermatozoan. Reproduction in domestic animals Vol.2. P.1. Edited by Cole H.and Cupps, P.T. Academic press. New York.

Roosen-Rugne, E.C. (1962): The process of spermatogenesis on mammals. Biol. Rev. 37, 343.

Skakkeback,N.E. and Heller,C.G. (1973): Quantificantion of human seminiferous epithelium. I.Histological studies in twent-one fertile men with normal chromosome complements J.Reprod. Fert. 32. 379-389.

Steinberger,A. and Steinberger,E., (1971): Replication patteren of Sertoli cell in maturing rat testis in vivio and organ culture. Biol. Reprod. J. 4. 84.

Swiestra,E.E. and Foote,R.H. (1963): Cytology and kinetics of spermatogenesis in rabbit. J.Reprod. Fert. 5, 309.

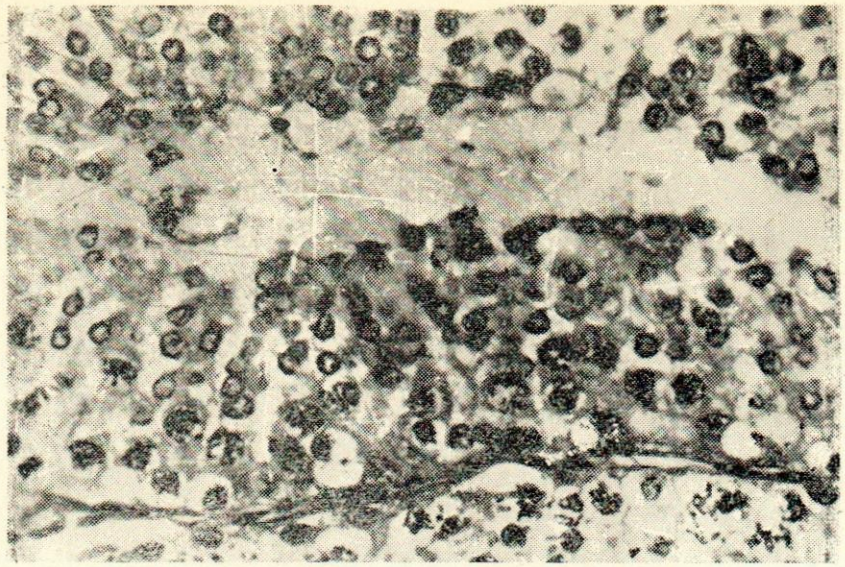
SUMMER STRESS

Table 1: Average number of cells, their Sertoli ratio and diameter of seminiferous tubules in normal

Case Number	Sertoli	Spermatogonia Type A	Spermatogonia Type B	Total Spermatogonia	Spermatocytes Leptotene	Spermatocytes Zygotene	Pachytene	diplotene diakinesis	Secondary Spermatocytes	Total Spermatocytes	Spermatids A	Spermatids B	Spermatids C	Spermatids D	Total Spermatids	Diameter of seminiferous tubules in μ.
1	8.5	10.8	4.3	15.1	2.3	10.8	16.3	3.5	3.2	36.1	6.4	29.6	12.9	25.5	74.4	221
2	5.1	8.0	8.1	16.1	3.1	13.7	22.5	1.8	2.0	37.8	4.0	38.9	9.7	1.5	73.1	167
3	6.3	10.1	7.2	17.3	4.0	10.8	25.4	2.0	1.1	39.3	9.2	52.9	7.4	29.2	98.7	178
4	9.3	12.6	3.7	16.3	3.7	9.1	27.2	4.2	2.6	46.8	2.3	46.9	8.3	28.4	89.5	179
Mean	7.3	10.3	5.8	16.2	3.3	11.1	22.9	2.9	2.2	40.0	5.5	42.0	9.6	25.2	83.9	186.3
S.D.	1.6	1.6	1.8	0.8	0.6	1.7	4.1	1.0	0.8	4.1	2.6	8.7	2.1	4.6	10.7	23.8
S.E.	±0.3	±0.3	±0.3	± 0.1	±0.1	±0.3	± 0.7	±0.2	±0.1	± 0.7	±0.4	± 1.4	±0.3	±0.7	± 1.7	± 2.2
Sertoli ratio	-	1.4	0.8	2.2	0.5	1.5	3.1	0.4	0.3	5.5	0.8	5.8	1.3	3.5	11.5	-

S.D. Standerd Deviation.

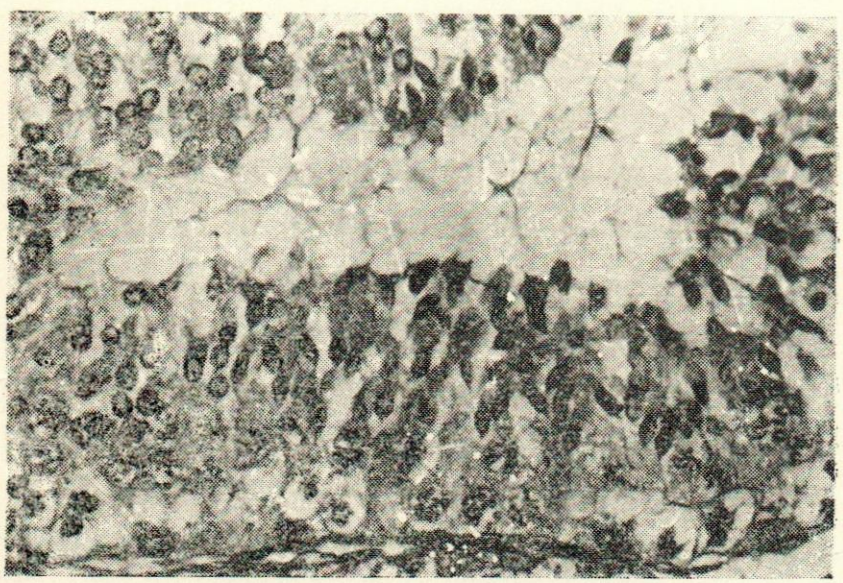
S.E. Standerd Error.



(Fig. 1) : Stage I

- Rounded spermatids type B.
- Pachytene spermatocytes.
- Zygotene spermatocytes.
- Sertoli cells & spermatogonia type A,

  H & E 40 X 12.5.



(Fig. 2) : Stage II

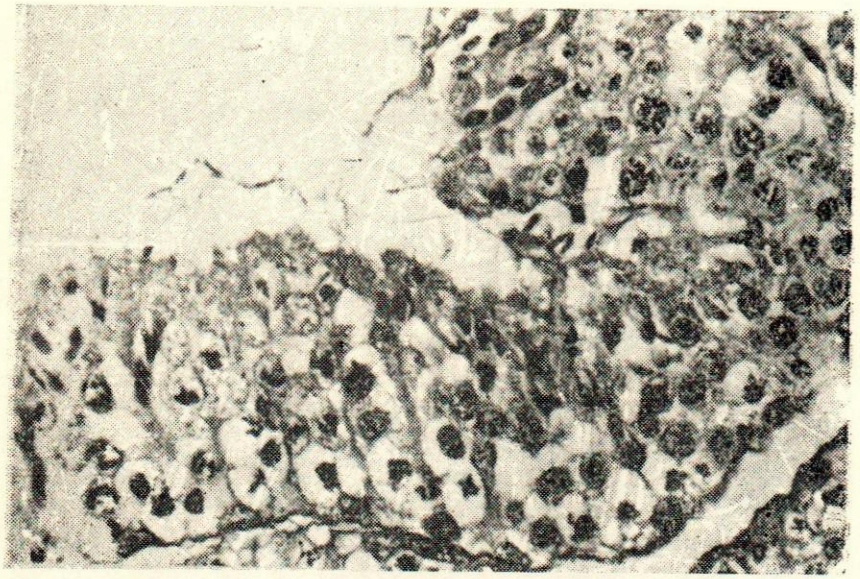
- Elonatiag spermatids Type C.
- Pachytene spermatocytes.
- Zygotene spermatocytes.
- Spermatogonia type A.

  H & E 40 X 12.5.

(Fig. 3) : Stage I I I

- Migration of spermatids to form bundles.
- Diplotene.
- Zygotene
- Sertoli cells and spermatogonia type A.

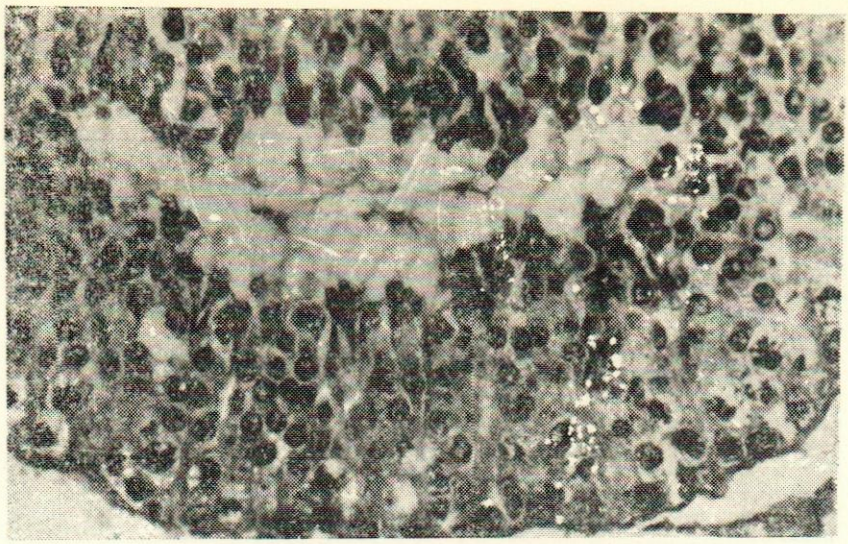
H & E 40 X 12.5.



(Fig. 4) : Stage I V

- Elongated spermatids type D in bundles.
- Secondary spermatocytes.
- Upper right corner diplotene spermatocytes.
- Lower left corner first metaphyseal plate.

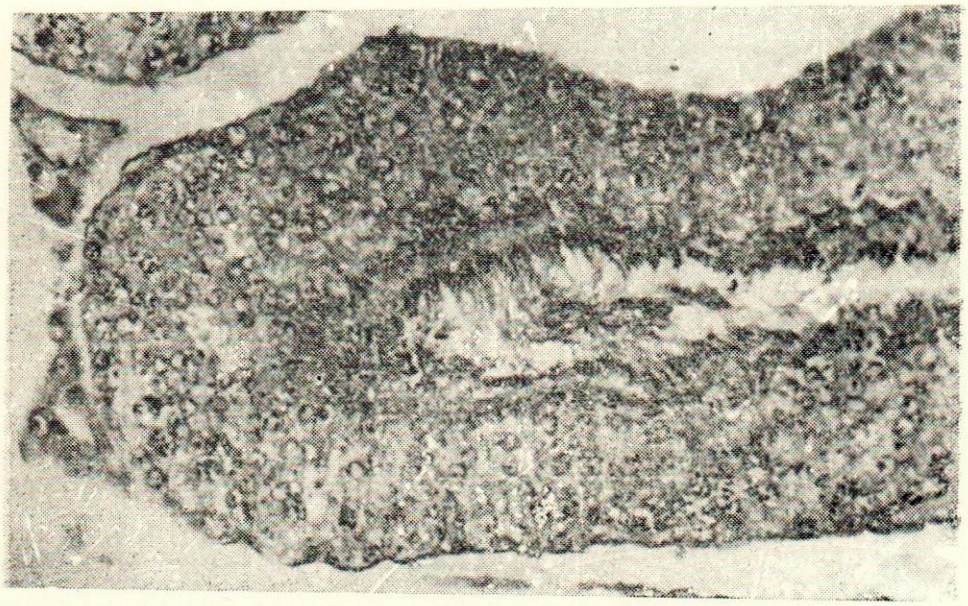
H & E 40 X 12.5.



(Fig. 7) : Stage V I I

- Start of centripetal arrangement of elongated spermatid.
- Rounded spermatid type B.
- Pachytene spermatocytes.
- Leptotene spermatocytes & spermatogonia type B.

H & E 40 X 12.5.



(Fig. 8) : Stage V I I I

of release

- The mature spermatozoa were released into the lumen of seminiferous tubules leaving residual bodies.

H & E 20 X 12.5.