دراسة تجريبية عن الأجهاد الصيفى فى الأرانب
٢- التقييم الباثولوجى الكمى والكيفى للدورة الخلوية المنوية فى الأرانب

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بمقارنة نسبة خلايا سيرتولى ، وقطر الأوعية المنوية ، والمراحل الثمانية للدورة الخلوية المنوية فى مجموعة تحت الأجهاد الصيفى التجريبى ومجموعة أخرى طبيعية وجد أن خلايا الاسبرماتوجونيا أ تعتبر خلايا مقاومة للحرارة. بينما خلايا الاسبرماتوجنيا ب شديدة التأثر بالحرارة وقد وجد ثلاث مراحل حساسة لتأثير الحرارة :-

١- المرحلة الثالثة من الدورة الخلوية المنوية وهى مرحلة تحول الباكيتين الى د پلوتين .

٢- المرحلة الخامسة من الدورة الخلوية المنوية وهى مرحلة تحويل الزيجوتين الى الباكتين .

٣- الاسبرماتوسيت الثانوية .

وكانت الزيجوتين خلية مقاومة للحرارة . وبالرغم من التأثير الشديد للحرارة على عملية تخليق الحيوانات المنوية لم يتأثر نوع أ من الاسبراتيد وكانت عملية تراكم الكروماتين الترابى (نوع ب) وعملية استطالة الاسبرماتيد (نوع ج) ونضوج الاسبرماتيد (نوع د) شديد التأثير بالحرارة.

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EXPERIMENTAL STUDY OF SUMMER STRESS IN RABBITS
II- THE QUANTITATIVE AND QUALITIVE PATHOGENESIS OF SPERMATOGENIC CELL CYCLE IN RABBIT

(With 2 Tables and 10 Figures)

By

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SUMMARY

Comparing the Sertoli cell ratio,diameter and quality of the eight stages of seminiferous epithelial cycle in stressed rabbit to the normal control group. It was resistant to heat, type B sharply affected. In the process of spermatocytogenesis, there were three target stages to heat stress application. Stage three (pachytene transformation) and stage five (zygotene to pachytene transformation) and the secondary spermatocytes. The zygotene cells were found to be heat resistant. Although the spermiogenesis were severely affected, type A rounded spermatids were unchanged. The processes of accumulation of dusty chromatin (Type B), the spermatid eleongation (Type C) and spermatid maturation (Type D) were severely damaged.

INTRODUCTION

Summer sterility was a field problem in cow and buffalo bulls under our climatic conditions in Upper Egypt (EL-SHERRY et al. 1977). This experiment is a representation for the three factors operating in summer stress (i.e) temperature (VAN DEMARY and FREE,1970),photoperiod (COUROT, GAFFAUX and ORTAVANT, 1968) and relative humidity (PATRICK, JOHNSTON, KELLGREN, FAYE, D'ARENSBOURG and BRANTON, 1954). The detailed pathogenesis of the stress changes of the nervous, endocrine and parenchymatous

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organs specially the testicles will help in future correction or trials for treatment of the stress syndrom. The present work will described the pathogenesis of the spermatogenic cell cycle qualitatively and quantitatively in rabbit as a model non seasonal breeder experimental animals for herbivorous.

MATERIALS AND METHODS

The experiment included groups of male adult Baladi rabbit of 1.5 - 2 Kg. body weight. Each group consisted of four rabbits. The first was pilot group contained in a large thermostate with glass doors, partitionally divided into four chambers one for each rabbit. Ventillation was specially adjusted and dishes of water were included to produce relative high humidity. Continuous artificial illumination was provided by 400 watt lambs. The temperature of thermostate were adjusted to give 40°C with the day and night illumination.

The second experimental group was constructed when the rabbits of the first pilot group did not survive more than three days. The illumination was modified to be at only from 6 Oclock a-m to 7 Oclock p.m. to represent the medium duration of summer day light. The temperature was modified to 39°C day and night. The animal survived under these condition and was slaughtered at the end of the week.

The third group was constituted of clinically healthy rabbits housed normally under the normal enviromental condition prevailing at the time our experiment and was slaughtered as a control model after a week. The result of this group (Table 1) was previously puplished (EL-SHERRY et al. 1980). Specimens, in suiza and carnoy were taken from all organs and endocrine glands from the three groups. In the presented work the result of investigations of only testicular sections stained by H & E. will be discussed.

The spermatogenic cell cycle qualitatively was evaluated. For their quantitative evaluation 10 rounded C-S of seminiferous tubules representing the eight stages of the cycle and a repetition of stage one and

eight was selected. The nubmer and Sertoli cell ratio for each type of cells were calculated.

For evaluation of the diameter 30 rounded C-S were selected and measured. The results were statistically analysed and compared to the result of the control group by T test according to (CEPETLIEV, 1968).

RESULTS

In the first experimental group all the rabbits died. One after 8 hrs. and three at the end of the third day. The testicles of three animals were normally producing testicles till the time of death. All the stages of the seminiferous epithlial cycle were present. Hyperaemia and interstitial oedema were observed. The cytoplasm of all cells of seminiferous tubules was swollen and granulated. In only one rabbit "died at the third day" Spermatid giant cells were observed indicating disturbed spermiogenesis, i.e. The seminiferous epithelial cycle more or less can persist three days of severe stress before deleterious effect can be observed.

The rabbits in the second experimental group survived untill slaughtered at the end of the week. The testicular response to stress was variable. Two cases showed severe testicular degeneration. The majority of seminiferous tubules were affected and lined by Sertoli and spermatogonia only. Some of the Sertoli cells were lysed. The spermatogonia in one case were flatened with hyperchromatic nuclei. (Fig. 1). Few sporadic tubules showed persisting elements of zygotene spermatocytes, rarely pachytene (Fig. 2). Sproadic tubules showed rounded spermatid of stage one beside the zygotene and pachytene spermatocytes. The testicular degeneration was mild in the third rabbit. Few seminiferous tubules showed functional stages up to the stage six of the cycle. Stage seven and eight were absent. Few tubules were lined by Sertoli and spermatogonai. The majority of the tubules were lined by the different type of spermatocytes showing that spermatocytogenesis were more or less proceeding (Fig. 3). The spermiogenesis were not complete. Few tubules showed

round spermatid of stage one or elongated spermatid of stage two and three. The epididymis contained unmature spermatids (Fig. 4). All the seminiferous tubules were producing in the fourth rabbit although cytoplasmic swelling, granulation and partial Vacuoulation were observed. The following irregularities of the cycle were observed. In stage one failure of cytokinesis lead to appearance of spermatid giant cells (Fig. 5).Stage to was more or less normal(Fig.6).In some tubules showing stage three, the spermatocyte differentiation was retarded. The bundles of spermatids were gathered with pachytene instead of diplotene. All the tubules in stage four showed coaggulative necrosis and hyalinization of the secondary spermatocytes. (Fig. 7). Stage five showed karyolysis of some pachytene spermatocytes (Fig. 8). The spermatids were necrosed in stage seven and eight of release. (Fig. 9).

The interstitium of the four cases was hyperaemic. The interstitial cells were more or less normal.

The numbers of cells and their Sertoli ratio were presented (Table 2). Stress decreased the nubmer of Sertoli ($P < 0,999$) as some of them were suffering cytolysis. The spermatocgonial production in stress diffred according to their types. Type A spermatogonia were increased in number ($P < 0,999$) and ratio while type B sharply decreased ($P < 0,999$) in number and ratio.The increase of type A spermatogonia reflects a compansatory proliferation beside retarded transformation to type B. In the two cases of severe testicular degeneration the whole circumference of tubles were lined mainly by hyperchromatic spermatogonia A as a compansatory replacement for other type of cells occupying the basement membrane in normal tubules. The total nubmer of spermatogonia decreased ($P < 0,999$).

The process of spermatocytogenesis was damaged by stress. The total number and Sertoli cell ratio of spermatocytes decreased. ($P < 0,999$). This was true for all types of spermatocytes except the zygotene cells where their number was not affected and their ratio increased. This is

explained by the relative resistance of zygotene cells as they were the persisting spermatocytes in severe and mild testicular degenerations.

The spermiogeneses in general was sharply affected in stress than in the spermatocytogenesis. The number and Sertoli ratio of the total spermatids were sharply decreased (P $\angle$ 0,999). Inspite of the presence of spermatid giant cells of stage I (Failure of cytokinesis) and the necrosis of secondary spermatoctes of stage four (in the slightly degenerated testicle, there is no change in the number of type A spermatid and it's Sertoli ratio increased. This is only explained by retarded differentiation of type A type B. Confirmaory is the sharp decrease in the type B Spermatids (P $\angle$ 0,999) (Stage of accumulation of dusty chromatin D.N.A.). The decrease in nubmer and ratio of type C spermatids (P $\angle$ 0,999) also indicating retardation in the process of spermatid elongation. As in mild testicular degeneration all the tubules were lined by one generation of rounded spermatids only. Type D spermatids were sharply decreased (P $\angle$ 0,999) partly by prevention of new generation of previous type and partly by necrosis of already present spermatids of stage 7 and stage 8 (of release). The calculated elongated spermatids were those of stages 3, 4, 5 of the cycle in slightly degenerated testicles.

DISCUSSION

During the pilot test, the rabbits did not servive under 40°C with illumination and relative humidity. When temperature lowered to 39°C the animal survived. Increase of temprature over 40°C for any length of time causes an unreversable destruction of the phospholipid components of all mammalian cells (CHAMPMAN, 1967). Also increase of temperature 6-10°C above the body temperature causes non-specific lethal effect (STEINBERGR and DIXON, 1959). The seminiferous epithelial cycle persists three days before severe effect could be observed at the end of the week.

The spermatogonial production in stress differed according to their types. Type A were increased in number and Sertoli ratio while type B

sharply decrease in nubmer and ratio. ASDELL and SALSIBURY,(1941) reported alteration of spermatogonia of rabbit after experimental cryptorchidism for one day. PLOEN (1973) stated that the pathological changes are rare although the cryptorchydism experiment continued for 35 day and he stated that the spermatogonia is the most heat resistant germ cells. The resistance of spermatogenia to heat appears to be general low among other species. Local application of heat and experimental cryptorchidism did not affect the spermatogenia in mouse (PAYNE, 1956), ram (WAITES and ORTRAVANT, 1968), rat (COLLIN and LACY, 1969), pig (MAZZARI,et al.1970), buffaloe bulls (SHERRY et al. 1970).

Differential among the types of the spermatogonia, type A is the most resistant. Their survival during short period of temperature elevation is responsible for the continuation of spermiogenesis after the heat application (DUTT and HAMM, 1957; MOULE and WAITES, 1963; WAITS and SETCHELL, 1964; BOWLER, 1967 and ROCK and ROBENSON, 1965). Type A increaseed in bulls after heat application (SKINNER and LOUW, 1966).

In our experiment the increase of type A spermatogonia reflects a compansatory proliferation beside retarded transformation to type B. As in the two cases of severe testicular degeneration, the whole circumference of seminiferous tubules were lined mainly by hyperchromatic spermatogonia type A replacing the other types of cells occuping the basement membrane in normal tubules. The total nubmer of spermatogonia decreased more or less on the expenses of type B damage.

The process of spermatocytogenesis was damaged by stress. The Sertoli ratio for spermatocytes were decreased ($P \leq 0.999$). This was true for all types of spermatocytes except the zygotene cells where their number was not affected and their ratio increased. Many authors had demonestrated the general heat sensetivity of the meiotic prophase of the primary spermatocytes in different species, genia pigs (YOUNG, 1972), rat (NIEMT and KORMANO, 1965), mouse (PYANE, 1956), pig (MAZZARI et al. 1968) ram (WAITES and ORTOVANT, 1968), rabbit (ASDELL and SALLSBURY

1941, JOHNSON et al. 1968; ZOGT et al. 1968, IGBOELI and FOOTE, 1969 and PLOEN, 1973).

There were individual variation between the four rabbit spermatocytogenesis in this experiment. In two severely affected cases the seminiferous tubules were lined by zygotene and pachytene spermatocytes (i.e) the spermatocytogenesis did not proced after the pachytene of stage two. The diplotene, diakinesis and secondary spermatocytes were not produced. This agree with work of STEINBERGER and DIXON (1959), CHOWDHURY & STEINBERGER (1963 & 1964), COLLINS and LAGY (1967) in rat, that the primary spermatocytes from the pachytene to the secondary spermatocytes were damaged by heat. The same was stated by STEINBERGER et al. (1967) in cryptochidism. In sheep and pigs the heat sensetive spermatocytes were reported to be the pachytene at the end of stage seven and begening of stage eight (WAITES and ORTRAVANT, 1967, 1968 and MAZZARI et al. 1970).

In the third and fourth case all types of spermatocytes were produced although showing coaggulation of the cytoplasm and pycnosis of stage four, retarded differentiation form the pachytene to diplotene in stage three and karyolysis of pachytene of stage five (the stage of transformation from zygotene to pachytene). Pycnosis of secondary spermatocytes and abnromal meiotic figure due to heat effect were observed in sheep by ORTAVANT (1959). In rat, by CLERMONT (1962), ROSSEN-RUNGE (1962) and COLLINS and LACY (1969). In mouse by OAKBERGE, (1956) and in bull by ATTAL and COURT, 1963. The findings of ROOSEN-RUNGE (1955) in rat and ORTAVANT (1956) in ram and AMMAN (1962) in bull that the end of the zygotenes and the befenning of the pachetene stages are particularly sensetive agreed with karyolyses of pachytene of stage five (transformation from zygotene to pachytene).

In our observation the Sertoli ratio for zygotene was increased. This is explained by the fact that with the arrest of the cycle at earlier stages most of the tubules were lined by zygotene , pachytene

spermatocytes, Subsequently their frequency, number and ratio were inincreased. But this is true for the zygotene, but not true for pachytene. This allows to conclude that the zygotene cells are probably heat resistant cells. This result did not agree with ploen Statment (1973) that there are no resistant stages of the meiotic prohase in rabbit. The auther has categorized three critical heat sensetive periods. The first was the midpachytene (stage Eight and stage One). The second was between the two maturation divisions. The third was the spermatoleosis. In our observation the critical target point for heat stress during spermatocytogenesis were: The stage of zygotene-pachytene transformation (stage five) and pachytene-diplotene transformation (stage three) and the secondary spermatocytes.

The spermiogensis was sharply affected in stress than the spermatocytogenesis. The Sertoli cell ratio of the total spermatids were sharply decreased. The sensetivity of spermatids to temperature elevation was demonestrated in several species: rabbit (ASDELL and SALISBURY, 1941, ZOGY et al. 1968, JOHNSON, et al. 1968, IGBOELI and GOOTE, 1969, PLOEIN, 1973), bull (SKINNER and LOUW, 1966), buffaloe bull (EL-SHERRY et al. 1977), ram (WAITES and ORTRAVANT, 1968), mouse (PAYNE,1959) and rat (KANWAR, 1971).

Spermatid giant cells were observed in cross sections of seminiferous tubules of stage one. Multinucleated Spermatids have been found after temperature elevation of the testicle in the rabbit by ASDELL, SALISBURY (1941), ZOGG et al., (1968), JOHNSON et al., (1968), IGBOELI and FOOTE (1969).

PLOEN (1973) had an openion that the formation of multinucleated spermatids are non specific reaction as it occures after application of variable pathological conditions.

In the presented work, inspite of the presence of spermatid giant cells, and the necrosis of secondary spermatocytes, there is no change in number of type A spermatid and their Sertoli ratio increased. These

results is in contrast with work of CHOWDURY and STEINBERGER (1963 & 1964) NIEMI and KORMANO, (1968) in sheep and MAZZARRI et al. (1970) in pig who reported immediate losses among the rounded spermatids soon after heat elevation.

In our openion early stages of spermatids are less sensetive than advanced stages of spermatid. The increase of Sertoli ratio of type A is explained by decrease in Sertoli number and retarded differentiation of type A to B. Confirmatory was the sharp decrease of type B (stage of accumulation of D.N.A. dusty chromatin.). Also the process of spermatid elongation was affected as indicated by the decrease in the number and ratio of spermatid type C. Advanced stages of spermatid maturation type D were sharply decreased, Necrosis of already stage Seven and Eight were observed.

REFERENCES

Amann,R.P., and Aimquist,JOO. (1962): Reproductive capacity of dairy bulls. VIII. Dierect and indirect measurement of testicular sperm production. J.Dairy Sci., 45, 774.

Asdell, S.A., and Salisbury, G.W. (1941): The viability of spermatozoa the abdominal epididymis and the failure of motile sperms to fertilize ova. Am. J. Physiology. 132. 791.

Asdell, S.A., Salisbury, G.W. (1941): The rate at which spermatogenesis occurs in the rabbit. Anat.Rec. 80, 145-153.

Attal, J. and Corurot.M. (1963): Development testiculaire et ablissement de la spermatogenese chez le Toureau. Ann. Biol. Animale , Biophys. 3, 219.

Bowler, K. (1967): The effects of repeated temprature application to the testis on fertility in male rats. J.Reprod.Fertility. 14, 171.

Chapman, D. (1967): The effect of heat on membranes and membrane constiuents in "Thermobiology" (A.H. Rose ed.)). P. 123 Academic Press. New York.

Chowdhury,A.K., and Steinberger E. (1963): Selective damage induced by heat to the testicular germinal epithelium of rats. Anat . Record. 145, 217.

Chowdhury,A.K. and Steinberger,E. (1964): A quantitative study of the effect of heat on germinal epithelium of rat testis. Am. J. Anat. 115, 506.

Clermont,Y. (1962): Quantitative analysis of spermatogensis of the rat. Am. J. Anat. 111, 111.

Collins,P.M. and Lacy,D. (1967): Effect of heat on the germinal epithelium and steroid metabolism of the seminiferous tubules and interstitial cells of the rat. Gen. Comp. endocrinol. 9, 500 (Abstr).

Collins,P.M. and Lacy,D. (1969): Studies on the structure and function of the mamalian testis. 2- Cytological and histochemical observations on the testis of the rat after a single exposure to heat applied for different lengths of time. Proc.Roy.Soc. B. 172, 17.

Cepetliev,D. (1968): Statistical methods for scientific medical researchs. Ed. Medecine, Moscow.

Courot,M., Goffaux,M. and Ortavant,R. (1968): Analysis of seasonal variations in the Fertility of cattle in the Frech. Jara.Ann.Biol. Anim. Biochim. Biophys., 8, 2091216.

Butt,R.H. and Hamm,P.T. (1957): Effect of expesure to high environmetal temprature and sheering on semen production of rams in winter. J. anim. Sci. 16, 328.

El-Sherry,M.I., El-Naggar,M.A. and Sanaa,M. Nassar. (1977): Evaluation of the spermatogenic cell cycle in summer sterility on buffaloes. Assiut. Vet.Med.J.Vol. 4 No7. 135-147.

El-Sherry,M.I., Sanaa,M. Nassar. and El-Naggar.M.A. (1980): Experimental study of summer stress in rabbit. I. The description and quantification of the spermatogenic cell cycle in normal Baladi rabbit. (in press).

Igboeli,G, Foote,R.H. (1969): Changes in epididymal spermatozoa and in the testes of rabbits after experimetnal cyptorchidism. J. exp. Zool. 170, 489-498.

Johnson,A.D., Gomes,W.R., Free,M.J. and Vandemark,N.L. (1968): Testicular lipids. 3- Effect of surgery and unilateral or bilateral cryptorchidism. I. Reprod. Fertility. 16, 409.

Johnson,A.D., Gomes,W.R., and Vandemark,N.L. (1969): Effect of elevated ambient temperature on lipid levels and cholesterol metabolism in the ram testis. J. Animal, Sci. 29, 469. (1971).

Kanwar,K.C., Bawa,S.R., Singol,P.K. Mode of formation of giant cells in testicular hyperthermia. Fertil. and Steril. 22, 778-783.

Moule,G.R., and Waites,G.M.H. (1963): Seminal degeneration in ram and its relation to the temperature of the scrotum. J. Reprod. Fertility. 5, 433.

Mazzarri,G., du Mesnil du Buisson,F., and Ortavat R. (1968): Action de la lumiere et le pouvion Fecondant du sperme chez le verrat. Proc. 6th intern. Congr.Animal reprod.Arif. insem. Paris. Vol. 1. 305-308.

Mazzarri,G., du Mesnil du Buis son,F; and Ortavant R. (1970): Action de la temperature et de lumiere sur la spermatogenese, La productive et al. pouvion fecandant due sperme chez le verrot. Ann. biol. Animal, Biochim, Biophys.

Niemi,M. and Kormano,M. (1965): Response of the cycle of the seminiferous epithelium of the rat testis to artificial cryptorchidism Fertility, sterility. 16, 236.

Ortavant,R. (1956): Action de la dure d'eclariement sur les processus spermatogenetioues chez le belier. Compt. Rend.Soc.Biol. 150, 471.

Ortavant,R. (1959): Spermatogenesis and morphology of the spermatozoon. In "Reproduction in Domestic Animals" (H.H.Cole and P.T.Cupps, eds), 1st ed., Vol. II. P.1. Academic press, New York.

Oakberg,E.F. (1956): A description of spermiogenesis in the mouse and its use in analysis of the cycle of the seminiferous and germ cell renewal Am. J. Anat. 29, 391.

Patrick,T.E., Johnston,J.E., Kellgren,H.C., Frye,J.B. D'Arenshourg, G.F. and Branton, C. (1954): effects of that weather and high Humidity on semen production fertility and physiological responses of Dairy Bulls. J. Anim. Sci. 13, 1028.

Payne,J.M. (1956): The degenerative changes in the adult mouse testis returned to the abdominal cavity J. Pathol. and bacteriol.71. 117.

Ploen,L. (1973): A light Microscope study of the immediate and delayed effects on rabbit spermatogenesis following experimental cryptocrhidism for twenty four hours. Virchows Arch. Abt.B. Zellpath. 14. 185-196.

Rock,J., and Robinson,D. (1965): Effect of induced intrascrotal hyperthermia on testicular function in man Am. J. Abster.Gynecol. 93. 793.

Roosen-Runge, E.C. (1955): Quantitative studies on spermatogenesis in the albino rat. III. Volume changes in the cells of the seminiferous tubules. Anat. Record. 123, 385.

Skinner and Louw, T.N. (1966): Heat stress and spermatogenesis in Bos indicus and Bos taursus Cattle. J. Appl. Physiol. 21, 1784.

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.

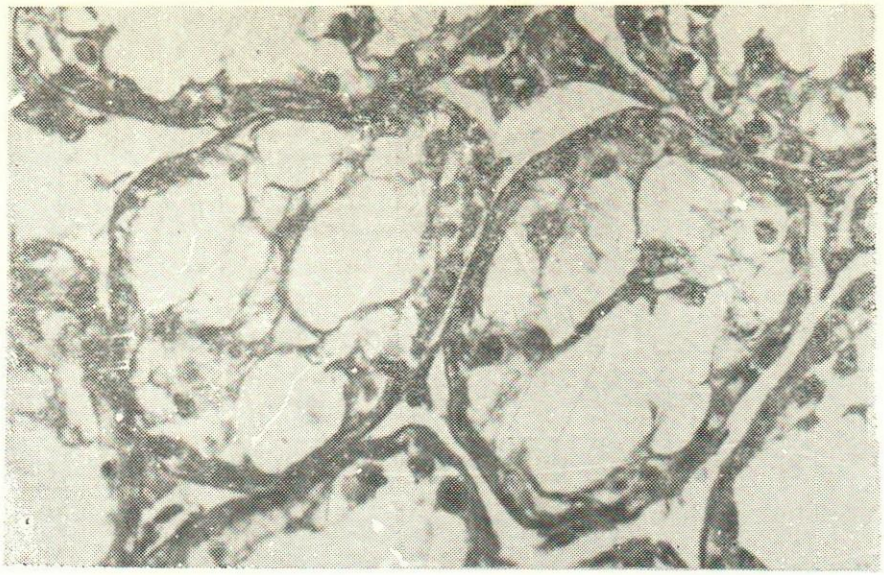
S.E. Standerd Error.

Table 2: Average number of cells, their Sertoli ratio and diameter of seminiferous tubules in stress.

Case Number	Sertoli	Spermatogonia Type A	Spermatogonia Type B	Total Spermatogonia	Spermatocytes Lepto-tene	Spermatocytes Zygo-tene	Spermatocytes Pachy-tene	Spermatocytes diplo-tene diaki-nesis	Secondary Spermato-cytes	Secondary Total Sperma-tocytes	Spermatids A	Spermatids B	Spermatids C	Spermatids D	Total Sperma-tids	Diameter of semi-niferous tubules in U
1	6.6	9.4	1.8	11.2	1.9	4.5	2.2	0	0	8.6	5.8	0	0	0	5.8	161
2	1.5	18.8	0	18.8	0	7.1	4.9	0	0	12.0	0	0	0	0	0	99
3	5.8	9.3	1.4	10.7	0	19.4	15.6	0.9	2.9	38.8	10.9	4.8	6.2	14.5	36.4	149
4	6.0	7.2	4.1	11.3	5.4	13.2	32.3	5	0.5	56.4	5.3	42.1	10.8	16.8	75	189
Mean	5	11.2	1.8	13.0	1.8	11.1	13.8	1.5	0.9	29.0	5.5	11.7	4.3	7.8	29.3	149.5
S.D.	2.0	4.5	1.5	3.4	2.2	5.8	11.8	2.1	1.2	19.7	3.9	17.7	4.6	7.9	29.8	32.6
S.E.	±0.3	±0.7	±0.2	±0.5	±0.3	±0.9	±1.9	± 0.3	± 0.2	±3.1	±0.6	± 2.8	±0.7	±1.3	± 4.7	± 2.9
Sertoli ratio	-	2.2	0.4	2.6	0.4	2.2	2.8	0.3	0.2	5.8	1.1	2.3	0.9	1.6	5.9	-

S.D.: Standerd Deviation

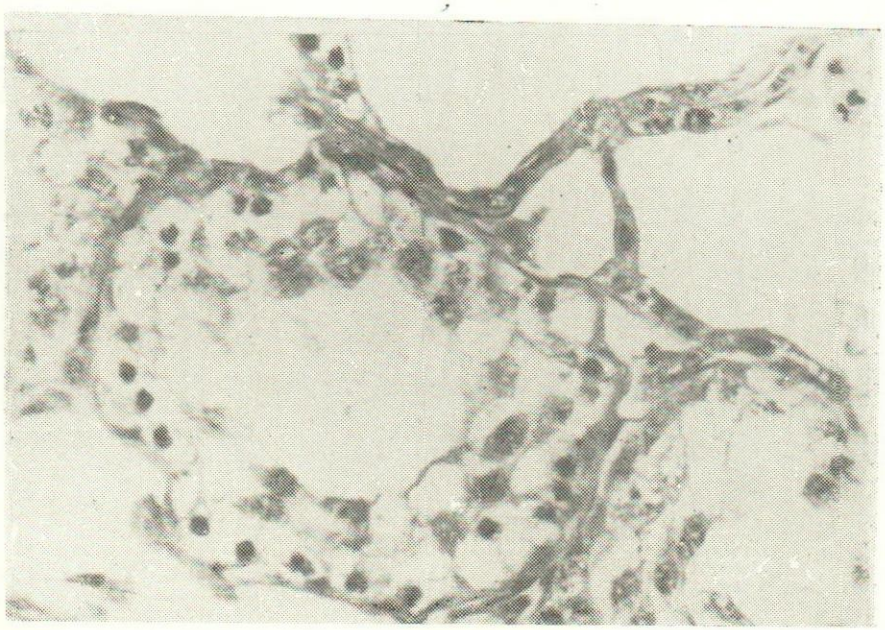
S.E.: Standerd Error.



(Fig. 1)

The seminiferous tubules are lined mainly by flattened spermatogonia with hyperchromatic nuclei.

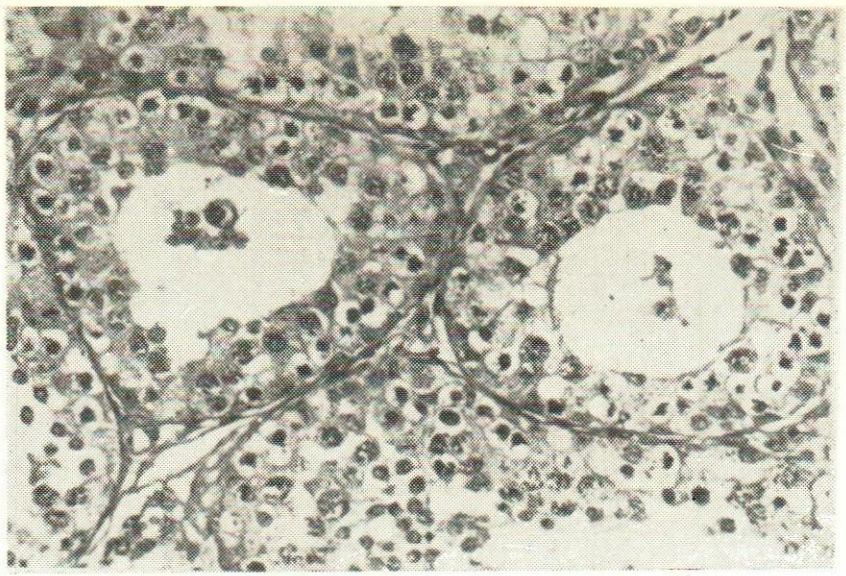
H & E 20 X 12.5.



(Fig. 2)

The s miniferous tubules are lined by persisting elements of zygotene spermatocytes & rarely pachytene.

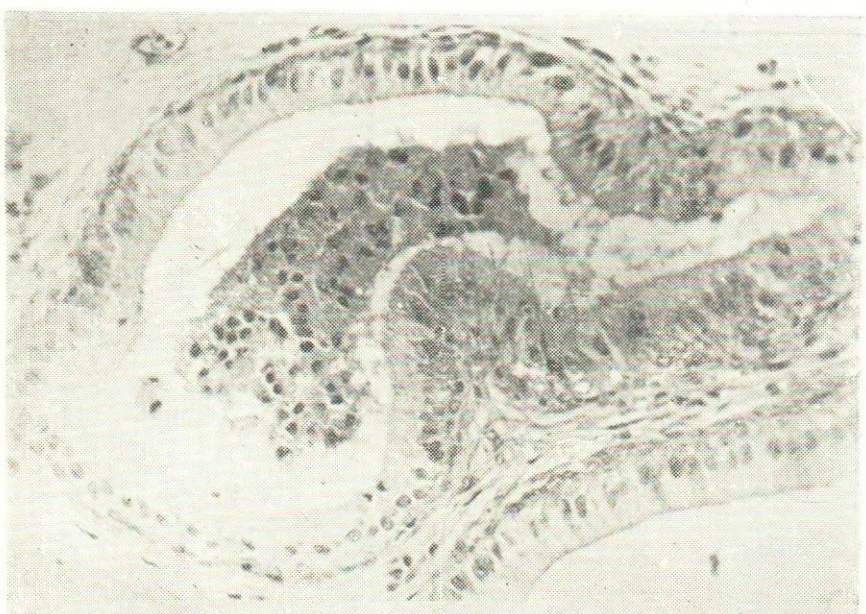
H & E 20 X 12.5.



(Fig. 3)

The process of spermatocytogenesis is going with absence of any spermatid generation.

H & E 20 X 12.5.

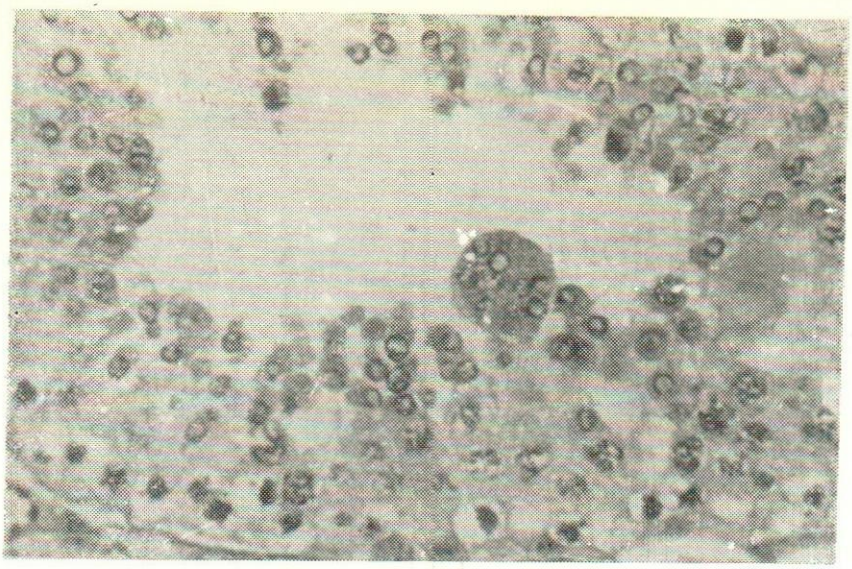


(Fig. 4)

The epididymis contains immature spermatids.

The spermatid cytoplasm is not detached.

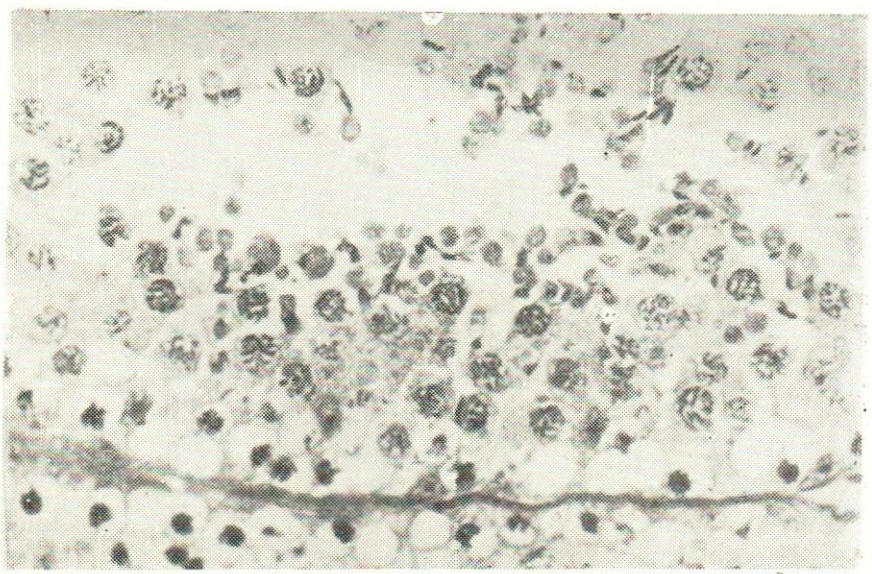
H & E 20 X 12.5.



(Fig. 5)

Stage one : Spermatid giant cell

H & E 40 X 12.5.

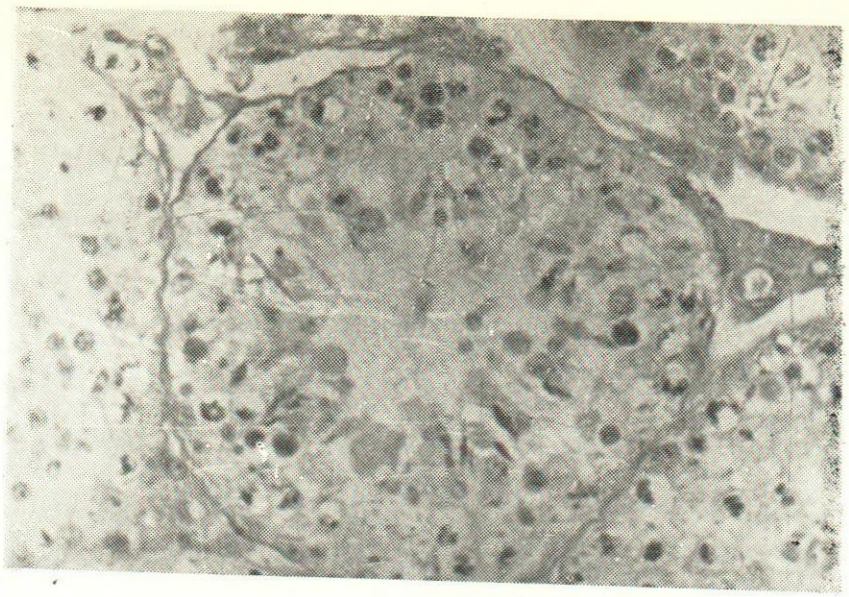


(Fig. 6)

Stage tow : Cytoplasmic swelling & granulation,

The association more or less normal.

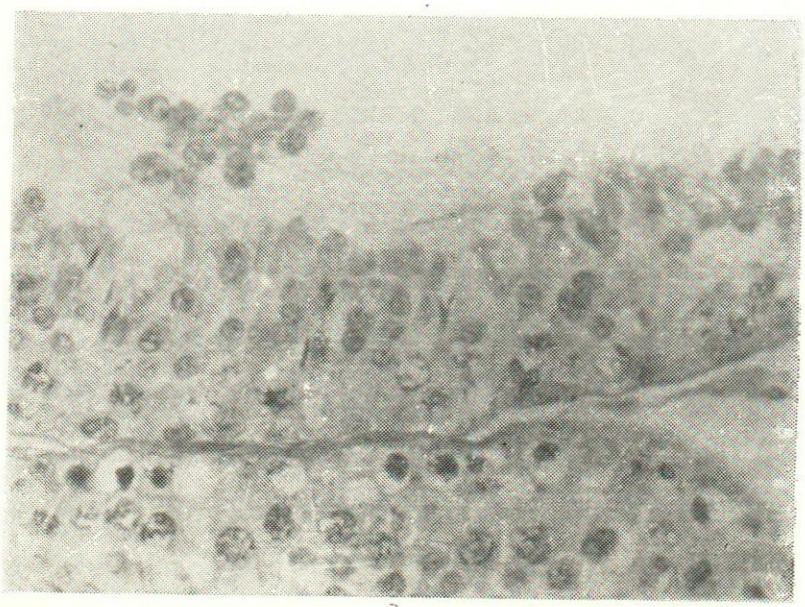
H & E 40 X 12.5.



(Fig. 7)

Stage four : Coaggulative necrosis & hyalinization of secondary spermatocytes.

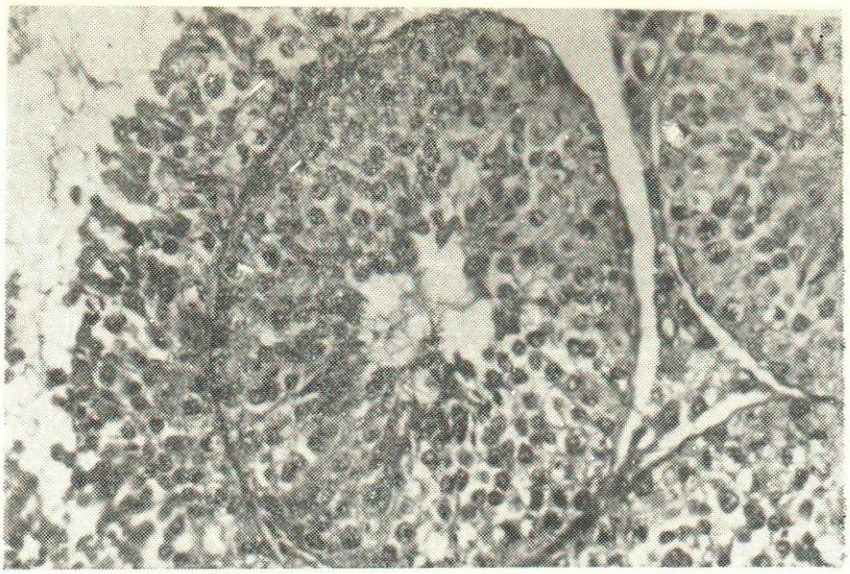
H & E 20 X 12.5.



(Fig 8)

Stage five : Karyolysis of some pachytene spermatocytes.

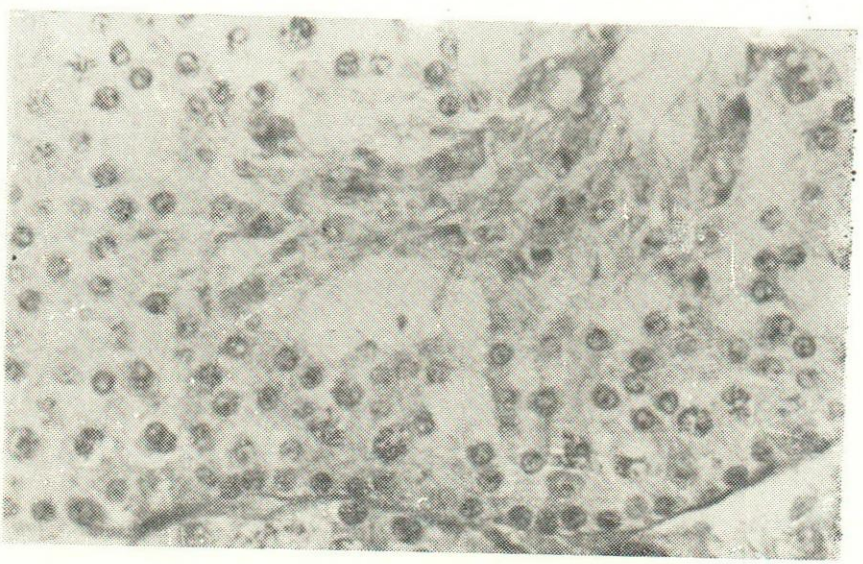
H & E 40 X 12 5.



(Fig. 9)

Stage seven : Necrosis of the spermatids.

H & E 20 X 12.5.



(Fig. 10)

Stage eight : Release, necrosis of the spermatids.

H & E 40 X 12.5.