دراسة تجريبية على الأجهاد الصيفى فى الأرانب

٤- التقييم الكمى والكيفى لتأثير الوتينيزنج هرمون على الدورة الخلوية المنوية للأرانب الطبيعية والأرانب تحت الأجهاد الصيفى التجريبى

م. الشرى ، سناء نصار ، م. النجار

تم حقن الوتينيزنج هرمون فى مجموعة من الأرانب الطبيعية ككنترول ومجموعة أخرى وضعت تحت الأجهاد الصيفى التجريبى بواسطة رفع درجة الحرارة واطالة مدة التعرض الضوئى ونسبة الرطوبة العالية . وتم التحليل الكيفى والكمى بواسطة نسبة خلايا سارتولى . وقد وجدت زيادة فى خلايا الاسبرماتوجونيا (نوع ب) وقد تم تحسين عملية تخليق خلايا الاسبرماتوستيس بتنشيط الأقسام الاخيرة فى المجموعة الطبيعية ، كذلك أصلحت عملية تخليق الاسبرماتوسيت فى مجموعة الأجهاد الصيفى . وبالرغم أن اللوتيينيزنج هرمون قد أثر بايجابية على عملية تخليق الحيوانات المنوية ولكنه كان هناك خلل فى النوعية عن طريق فشل الانفصال السيتوبلازمى وسرعة التميز الوظيفى والشكلى . كذلك عدم النضوج التام لخلايا الاسبرماتيد .

Depts. of Pathology, Gynaecology and Physiology,
Faculty of Vet.Med. Assiut University,
Heads of Depts. Prof.Dr.A.R.Khater, Prof.Dr.M.Osman & Prof.Dr.Y.Hamed.

EXPERIMENTAL STUDY OF SUMMER STRESS IN RABBIT

IV- THE QUANTITATIVE AND QUALITATIVE EFFECT OF L.H. INJECTION ON SPERMATOGENIC CELL CYCLE OF NORMAL AND STRESSED RABBIT

(With 4 Tables and 10 Figures)

By

M.I. EL-SHERRY, SANAA M. NASSAR*, and M.A. EL-NAGGAR.

SUMMARY

L.H. was injected in normal control rabbit group and group stressed experimentaly by elevated temperature, long photoparіod and relative humidity. Qualitative and quantitative analysis using the Sertoli cell ratio revealed that the type B spermatogonia increased. The process of spermatocytogenesis was improved through activation of meiosis in normal conrol group and was corrected in the stressed group. Although L. H. positively influenced the process of spermiogenesis but it was disturbed qualitatively through failure of cytockinesis and rapid differentiation and in stressed group by incomplete maturation of type D spermatids.

INTRODUCTION

The three foctors of summer stress (i.e.) elevated temperature, longevity of photoperiod and relative humidity had been demonestrated experimentally and naturally to cause decreased level of testosterone and L.H. hormone (RHYIVES and SWING 1973; KATONGOLE et al. 1974). In rabbits like many other animals, the secretion of L.H. is episodic and requlate the release and level of testosterone (VANDE WIEIE and FERIN, 1974). LINCOLN (1975) had prooved that each individual discharge can

* Faculty of Medicine, Assiut University.

result in transient stimulation of the testis with the associated increase in plasma testosterone levels. KATANGOLE, et al. (1974) stated that the changes in the frequency of L.H. discharges rather than the amplitituded is responsible for the flactuation of the level of testosterone with seasonal changes.

Based upon the above information, the aim of the present work is to study the morphological effect of L.H. injection on the spermatogenic cell cycle of normal rabbits and a trial to correct the pathologically altered spermatogenic cell cycle of experimentally stressed rabbits by L.H. injection.

MATERIALS AND METHODS

Two groups of adult male baladi rabbits (1½-2 years old) weighing 1½:2 Kg. Each group was composed of four animals. One group was injected by L.H. (chorion gonadotropin - Mucos, Emulsionsges llschaft mb H. 8022 Grunwald near Munich). Three mellilitters (600 I.U.) were injected sub/cut every three days i.e. During the week period, the animal received two doses.

The second group was put in a thermostate with a 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. Artificial illumination was provided by 400 watt lamb. The illumination started from 6 Oclock a.m. to 7 Oclock pm to represent the medium duration of summer day light.

The temperature was adjusted to 39°C for day and night. The animals were injected by L.H. as the first group. At the end of the week, the animals were slaughtered. Testicular specimens were fixed in Suza. From each block serial sections 5 microm thickness were stained by Harris haematoxylene and eosin. The spermatogenic cell cycle was qualitatively 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 number and Sertoli cell ratio for each type of cells were calculated. The Sertoli cell ratio of stressed rabbits group without treatment and normal control group were taken from previous work (EL-SHERRY et al. 1980).

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 (SEPETLIEV, 1968).

RESULTS AND DISCUSSION

I- The Effect Of L.H. On The Normal Control Group.

The testicles were normally producing in three cases. In the fourth case the cycle stoped at earlier stages with spermatid giant cell formation. In the three cases the following observations were recorded all over the stages of the cycle.Association between two stages was frequent in the cross section of the seminiferous tubules.

In stage one,elongation early started as elongating spermatids were present beside rounded spermatids. There was close aggregation of rounded and elongated nuclei of the spermatids together. In focal places of the wall of the seminiferous tubules, the aggregation of the nuclei was seen on a synthetium of cytoplasm indicating disturbed cytokinesis but without formation of giant cells (Fig. 1). The nuclei of zygotene and pachytene spermatocytes were aggregated together and of higher number.

In the cross sections of the seminiferous tubules of stage two. The elongated spermatid with hyperchromatic nuclei and distinct acrosome (of stage three) were frequently seen in association with the elongating spermatid (of stage two). The aggregation of the zygotene and pachytene spermatocyte nuclei was observed (Fig. 2). In stage three, the migration of spermatids to form bundles was focally retarded (Fig.3). The zygotene

and diplotene spermatocyte nuclei were aggregated. In stage four, the migration to form bundles was still focally retarded with aggreation of young spermatid nuclei and focal failure of cytokinesis (Fig. 4). In stage five focal part of the wall of the seminiferous tubules showed protruding synthetium of cytoplasm with aggregated nuclei of elongated spermatids indicating failure of cytokinesis (Fig. 5).

In stage six,seven and eight there was focal matting of the elongated spermatids together. The rounded spermatids and spermatocytes nuclei were higher in number and aggregated. (Fig. 6).

The interstitial cells of the three cases showed swollen nuclei and swollen foamy cytoplasm. The cells of many islands showed vacoulated or lysed nuclei on irregular vaculated ragged cytoplasm (Fig. 7). The interstitial cells are the target cells for L.H. hormone(CATT et al.1974). L.H. stimulates spermatogenesis by increasing androgen production by lydje cells (FRENCH et al. 1974). The above changes in the interstitial cells are probably over stimulation by L.H.

The result of quantification of the spermatogenic cell cycle and it's Sertoli ratio is presented in (Table 1,2,3 & 4). The number of Sertoli cells slightly decreased from normal.

By analysis of the number and Sertoli ratio of the spermatogonia and comparison to the normal control group (Table 1). It was found that there was a decrease in type A and insignificant increase in type B. The Sertoli ratio of the total number of spermatogonia was the same as the normal control group.

The total number and Sertoli ratio of the spermatocytes increase above the normal group. This is true for all types of spermatocytes except the pachytene type where their numbers and ratio decreased.

The enhancing role of L.H. on spermatogensis is mainly through androgen effect. The increase number of spermatocytes in our results can be evaluated on the light of the fact given by DORRINGTON and FRITZ (1973),

that spermatocytes showed the typical target tissue metabolism of C_{14} testosterone and 5 androsterone. EWLNG (1973) showed that the formation of these metabolites by the rabbit testis is greatly reduced after selective destruction of sperm cell by heat.

The rate of spermiogenesis was also increased. The Sertoli cell ratio and total number of spermatids increased above normal. This true for all types specially type A "Highly increased" with exception of type B which significantly decreased.

Androgens released by L.H. are necessary for sperm maturation. FRENCH et al.(1974) stated that testosterone bound to cytoplasmic receptors then transported to the cell nuclei. The androgen complex bind to the chromatin and initiate metabolic processes necessary for sperm maturation.

It can be consluded from these observation that:

I- There is rapid rate of meiotic divisions. This is proved by the following facts:

1) Although the total number of spermatogenia (the mother cell of spermatocytes) under the L.H. is the same as the normal group, the total number of spermatocytes is significantly increased than normal.
2) The generation of the secondary spermatocytes is highly increased than normal.
3) The high increase in the number of type A spermatid is a sequllae for increased number of spermatocytes.
4) The rapid rate of division is indicated by the aggregation of the nuclei of the spermatocytes and spermatids all over the different stages of the cycle.
5) Rapid rate of division probably may be responsible for the retarded cytokinesis as observed by the focal formation of synthetium of cytoplasm with spermatid nuclei all over the stage of the cycle without giant cell formation.

6) Migration of spermatids to form bundles was focally retarded from stage three up to stage eitht. This is probably due to disturbed cytokinesis.

II- Rapid rate of differentiation may probaly be prooved by the following facts.

1) The decrease in the number and ratio of pachytene cells (stage of D.N.A. doubling of the chromosomes) is only explained by rapid transformation to the diplotene diakinesis stages of the prophase of the spermatocytes meiosis.
2) In the process of spermiogenesis although type A highly increased than normal as well as type C, type D and the total number of spermatids. Type B (stage of aquiring D.N.A. as dusty chromatin) sharply decrease than normal which can be explained only by rapid transformation to advanced differentiated types.
3) Rapid differentiation may be responsible for the frequency of the presence of association between two following stages in the cross sections of the seminiferous tubules.

2- The Effect Of L.H. On Heat Stressed Testicles.

The result of L.H. stressed treated group revealed 3 cases with generally well producing testicles, where all the epithelial stages of the cycle were presented. Although there were slight degree of focal signs of degeneration. In the fourth case, the testicles were severely degenerated where the seminiferous tubules were lined either by Sertoli and dark spermatogonia or Sertoli, zygotene and spermatid giant cells (Fig. 8). The focal signs of degeneration were either:

1) Disturbed spermiogenesis and abnormal association of spermatid generation, rounded spermatid of stage 1, elongated spermatid of stages 3 and spermatid giant cells (Fig. 9).

2) Agregation of pycnotic spermatocytes were observed in a corner of some seminiferous tubules cross sections. Swelling and granulation of spermatocytes cytoplasm were observed.
3) Coaggulative necrosis of secondary spermatocytes had a sporadic character.
4) Sporadic formation of spermatid giant cells.
5) The advanced stages in some cross sections were nearly free from mature spermatids (Fig. 10).

The nuclei of some interstilial cells were swollen and vacoulated.

L.H. treatment normalized the diameter of seminiferous tubules under stress ($P/ 0.95$). The Sertoli cell number was normal. The number and Sertoli cell ratio showed that type A spermatogonia were decreased ($P./ 0.999$). In spite of the fact that in the group of stress without treatment type A spermatogonia increased significantly ($P/ 0.95$). On the contrary L.H.positively influnced type B spermatogonia. While stress decreased the number and ratio of type B spermatogonia. L.H. treatment increased the number and ratio of type B spermatogonia hihger than normal.

L.H. had normalized the total number of spermatogonia and it's Sertoli ratio. This fact is explained by the sum of action of stress and L. H. on A and B types. The decreasing effect of L.H. on normal type A had been antagonized probably by the compansatory increasing effect of type A and also the significant increase of type B by L.H. So L.H. probably act on division and transformation of type A to B. FRENCH et al. (1974) suggested the spermatogonia as one site of the action of androgens.

L.H. treatment increased the total number of spermatocytes and it's Sertoli ratio in the stressed group to above the normal level inspite of the fact that stress decreased the total number and ratio of spermatocytes. For the different types of meiotic prophase, the fact was not clear on lepotene. Although L.H. increased it in normal control group. It increases the number of zygotene in stressed treated group. Although

it's number did not change in stress without treatment. The enhancing effect was also encountered for the deplotene, diakinesis and the secondary spermatocytes. In conclusion L.H. hormone is necessary for the correction of the pathogenic effect of summer stress condition on the process of spermatocytogenesis through activation of meiosis.

L.H. treatment increased the total number of spermatids in the stressed group. Although the total number of spermatids was doubled than the stress without treatment but it was not corrected to the normal level. The Sertoli cell ratio also reflected the same character.

Type A spermatid highly increased in number and ratio (nearly four times) in stressed L.H. treated group. While type B was reduced to it's half quantity. L.H. increased the number and ratio of type C & D spermatids; double the stressed untreated group; while C had reached to the normal level. Type D did not return to normal.

Inspite of the fact that L.H. treatment completely corrected the process of spermatocytogenesis through activation of meiosis. The correcting effect was not complete on the process of spermiogenesis. The rapid differentiation concluded in both normal and stressed group do not complete the mature spermatids (Type D spermatid) to it's normal level. Probably here synergetic action of F.S.H. (FRENCH et al. 1974) is needed for stimulation of the Sertoli to produce androgen binding proteins necessary for the transformation and concentration of androgen on the seminiferous tubules.

REFERENCES

Catt,K., Tsuruhara,T.; Mendelson,C.; Ketelslegers,J.M.and Maria L.Dufau. (1974):Gonadotropin binding and activation of the interstitial cells of the testis. Edited by Maria L. Dufau and Anthony R. Means. Vol. I. Current Topics in molecular endocrinology. Plenum Press. New York and London.

Dorrington,J.H. and Fritz,I.B. (1973): Biochem. Biophys. Res. Comm. 54: 1425. Cited by Franch et al. (1974).

El-Sherry, M.I.; Sanaa, M. Nassar; El-Naggar, M.A. (1980): Experimental summer stress in rabbits. I. Qualification and quantification of the spermatogenic cell cycle in normal Baladi rabbit. Assiut Vet.Med.J.Vol. 7, No. 13&14.

El-Sherry,M.I.; El-Naggar,M.A.and Sanaa,M. Nassar (1980): Experimental summer stress in rabbits. II: The quantitative and qualitative pathogenesis of the spermatogenic cell cycle of summer stressed rabbit. Assiut Vet.Med.J.Vol. 7, No. 13&14.

French,S.;Mclean,W.S.;Smith A.A.;Donald,J. Tindall,Samnel C. Weddington, Peter Peter P., Madhobanada S., Walter E. Stumpf. Shihadeh N. Nayfeh, Hansson V;, Trygsted O. and Martin Ritzer E. (1974) Androgen transport and receptor mechanism in testis and epididymis. Hormone binding and target cell activation in the testis. Edited by Maria L. Dufau and Anthony R.Means. Vol. I in current Topics in Molecular Endocrinology.

Katangole,C.B. and Naftolin; Shart R.V.(1974):Seasonal variation in blood luteinizing horomone and testosteron levels in rams. J.of Endocrinology. 60. No. 1. 101:106.

Lincoln,G.A. (1976): Seasonal variation in episodic secretion of luteinizing hormone and testosterone in the ram. J. Endocr. 69, 213-226.

Rhyives,W.E. and Ewing L.T. (197 3): Testicular endocrine function in herford bulls exposed to high ambient temperature. Endo. 92. 509-515.

Sepetliev,D.(1968): Statistical methods for scientific medical research. Editor. Medicine Moscow 1968.

Vande Wiele,R.L. and Ferin,M. (1974): The controle of pulsatile gonadotrophin secretion. Chronobiological Aspect of endocrinology symposia Medica Hoechst. 9. 203-219.

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	±0.8	± 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 Leptotene	Spermatocytes Zygotene	Spermatocytes Pachytene	Spermatocytes diplotene diakinesis	Secondary Spermatocytes	Secondary Total Spermatocytes	Spermatids A	Spermatids B	Spermatids C	Spermatids D	Total Spermatids	Diameter of seminiferous 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.

Table 3: Average number of cells, their Sertoli ratio and diameter of seminiferous tubules in normal L.H. treated rabbits

Case Number	Sertoli	Spermatogonia Type A	Spermatogonia Type B	Total Spermatogonia	Spermatocytes Leptotene	Spermatocytes Zygotene	Spermatocytes Pachytene	Spermatocytes diplotene diakinesis	Secondary Spermatocytes	Total Spermatocytes	Spermatids A	Spermatids B	Spermatids C	Spermatids D	Total Spermatids	Diameter of semineferous tubules in/U
1	6.4	5.6	6.2	11.8	8.3	10.5	19.9	7.9	13.6	60.2	40.4	8.9	17.9	38.8	106	203
2	5.8	8.4	3.3	11.7	11.6	22.1	14.7	5.1	11.0	64.5	11.2	49.6	9.7	47.6	118.1	211
3	5.0	6.3	8.4	14.7	13.6	15.3	10.7	0	5.1	44.7	70.7	6.9	7.4	25.2	110.2	197
Mean	5.7	6.7	6.0	12.7	11.2	16.0	15.1	4.3	9.9	56.5	40.8	21.8	11.7	37.2	111.4	203.7
S.D.	0.6	1.2	2.1	1.4	2.2	4.8	3.8	3.3	3.6	8.5	24.8	19.7	4.5	9.2	5.0	5.7
S.E.	±0.1	±0.2	±0.4	±0.3	±0.4	±0.9	±0.7	±0.6	±0.7	±1.5	±4.4	±3.6	±0.8	±1.7	± 0.9	± 0.6
Sertoli ratio	-	1.2	1.1	2.2	2.0	2.8	2.6	0.8	1.7	9.9	7.2	3.8	2.1	6.2	19.5	-

S.D.: Standerd Deviation

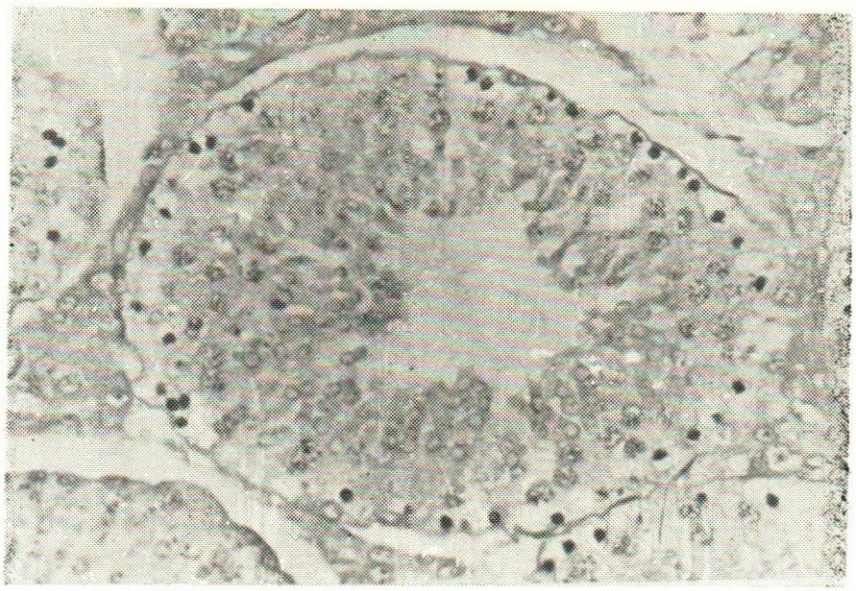
S.E.: Stander Error.

Table 4: Average nubmer of cells, their Sertoli ratio of seminiferous tubules in L.H. Stress treated rabbits.

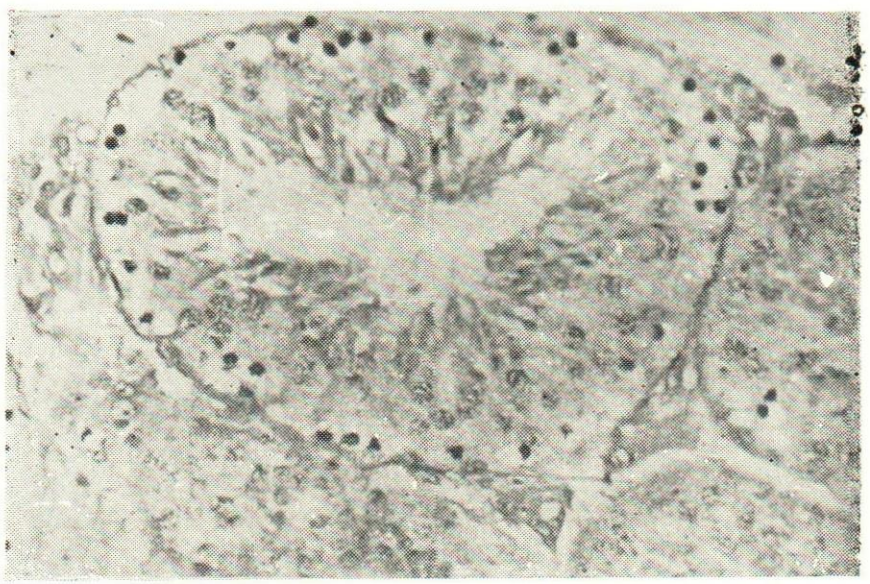
Case Number	Sertoli	Spermatogonia Type A	Spermatogonia Type B	Total Spermatogonia	Spermatocytes Leptotene	Spermatocytes Zygotene	Spermatocytes Pachytene	Spermatocytes diplotene diakinesis	Secondary Spermatocytes	Total Spermatocytes	Spermatids A	Spermatids B	Spermatids C	Spermatids D	Total Spermatids	Diameter of seminiferous tubules in/U
1	6.7	7.1	4.8	11.9	0	15.3	33.4	3.1	14.3	66.2	30.0	14.5	18.9	34.3	96.6	193.3
2	10.0	5.9	13.1	19.0	0.2	9.7	0	0	0	9.9	3.1	0	0	0	3.1	151.7
3	7.7	7.9	3.2	13.1	0	16.5	18.5	4.9	6.3	51.6	48.6	0	15.7	19.7	84.1	178.8
4	5.2	10.7	10.0	20.7	3.9	20.2	21.0	3.1	12.4	60.6	13.1	11.8	5.4	11.9	38.1	195.8
Mean	7.4	7.8	7.8	16.2	1.0	15.4	18.2	2.8	8.25	47.1	23.7	6.6	10	16.5	55.7	179.9
S.D.	1.7	1.7	4.0	3.7	1.6	3.8	11.9	1.8	5.6	22.0	17.3	6.6	7.6	12.5	37.5	17.7
S.E.	±0.3	±0.3	±0.6	±0.6	±0.3	±0.6	±1.9	±0.3	±0.9	±3.5	±2.7	±1.1	±1.2	±2.0	±5.7	1.6
Sertoli ratio	-	1.1	1.1	2.2	0.1	2.1	2.5	0.4	1.1	6.4	3.2	0.9	1.4	2.2	7.5	-

S.D.: Standerd Deviation

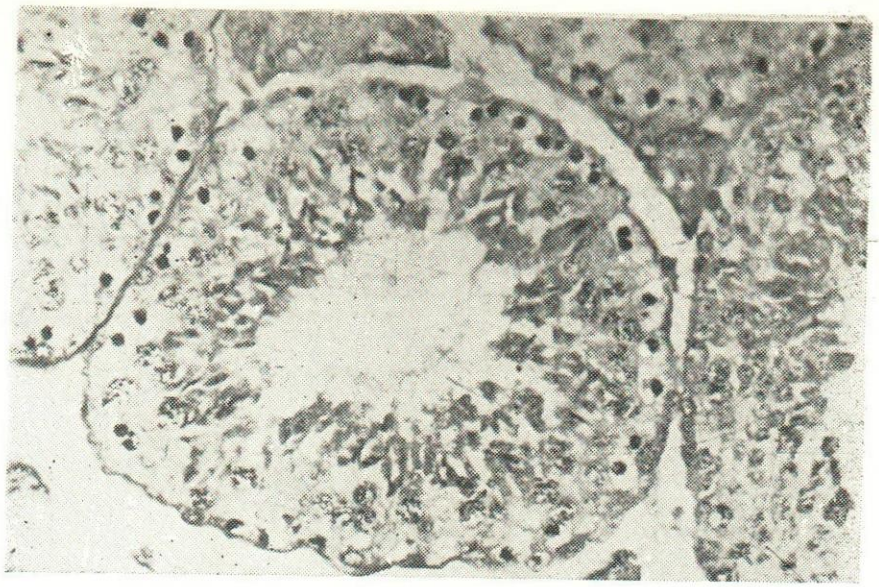
S.E.: STanderd Error.



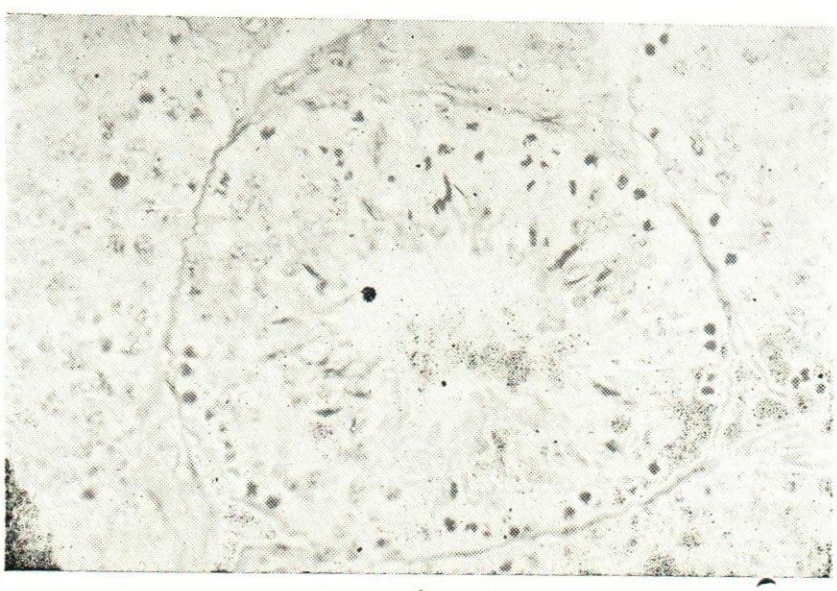
(Fig. 1) : Stage 1.
Elongated spermatids, beside rounded spermatids, Aggregation of the spermatid and spermatocytes nuclei. (H & E. 20 x 12.5)



(Fig 2) : Elongated spermatids of stage 3 in association with elongating spermatid of stage 2. Aggregation of spermatocyte nuclei. (H & E. 20 x 12.5).



(Fig. 3) : Stage 3 migration to form bundles was focally retarded. (H & E. 20 x 12.5).



(Fig. 4) : Stage 4 migration to form bundles were retarded. Focal aggregation of young spermatid nuclei due to focal failure of cytokinesis. (H & E. 20 x 12.5).