The effects of pituitary gland, HCG, and Testosterone on the testis and spermatogenesis of the adult male toad (Bufo viridis)

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Abstract :

Adult males of the toad Bufo viridis were injected with female pituitary glands, HCG and Testosterone, then testicular changes were histologically examined. pituitary glands and HCG treatment caused increase in the number of primary and secondary spermatogonia and decrease in the number of spermatocytes, spermatids and spermatozoa, but after injection of testosterone, the number of primary spermatogonia, spermatids, spermatozoa increased.

It therefor appears that HCG and pituitary gland have effects on the early stages of spermatogenesis while testosterone has stimulatory effect on the spermiation.

I Introduction:

A considerable number of studies have been performed on the regulationary mechanism of spermatogenesis in anurans, but the results reported have not always agreed in details. It is well known that the gonadal development of anurans larvae is greatly influenced by exogenous sex hormones. (Witschi, 1967). In Rana nigromaculata a sufficient amount of testosterone is needed for development of later stages of spermatogenesis and sperm preservation.

(Blair, 1946 - Iwasawa, 1985, 1986). Spermatogenetic activity has been found to resume in late spring when the concentration of androgens (testosterone plus dihydro testosterone) in the plasma is low, less than 5µg/ml (Licht, 1983 - Moore, 1980). Castration studies have provided further direct evidence for relationship berween pituitary and gonadal function in the bullfrog. After castration, there is typically a gradual rise in both FSH and LH which is detectable at about 4-7 days. These data indicate that gonadal secretions (steroids?) not only influence generaly pituitary secretion rate, but also the relative responsiveness of the two gonadotropins (Licht, 1985). On the other hand in anurans, there also exists a considerable literature on the effects of hypophysectomy on histological changes in testis (Lofts, 1974). Iwasawa found that in Rana nigromaculata hypophysectomy presents

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spermatogonial proliferation, but has no noticeable effect in the progress of spermatogenesis and the maintenance of spermatozoa (Iwasawa, 1976). Guha (et. al, 1978) demonstrated that gonadotropin independence during the process of spermatogenesis in the toad is acquired late in the secondary spermatogonial phase. These data show that the process of spermatogenesis and development of the testis are related to synchronous changes of plasma gonadotropins and androgens. To analyze the effect of exogenous gonadotropins and testosterone in Bufo viridis, the present work was undertaken.

II Materials and Methods:

All experiments were performed on the adult male toads of Bufo viridis collected from suburb of Tehran with body weight 27-30g and body length 6-7.5 cm. Room temperature was maintained at 20-25°C and photoperiod of 12L:12D suggested by Iwasawa was used (Iwasawa, 1984). pieces of sheep liver were offered to the animals every 3 day as food. we have done four experiments and in each experiment, groups of 3 toads were studied. The doses ot administered hormones to each toad are shown in tables 1,2,3. The pituitary glands used in the present study were obtained from adult female Bufo viridis and homogenized in the 0.64% NaCl solution (Humasan, 1972). The male toads received two female pituitary glands per day for three successive days. The human chorionic gonadotropin (HCG) was obtained from I.F.SERONO S.P.A. Company. This hormone was dissolved in the 0.64% NaCl for injection. Testosterone hormone was obtained from Aboreihan Company. The Testosterone concentration was reduced by dissolving the hormone in olive oil. 24 hour after injection of female pituitary gland or HCG, and three days after injection of testosterone, the testes of each toad were fixed in bouin's solution. Then embedded in paraffin wax. Serial sections were cut crossly with 6μm thickness and stained with Mayer's hematoxylin and eosin. The degree of histological changes was judged quantitatively in 15 cross sections of testes, diameter of seminiferous tubules were measured and the number of spermatogenic cells were counted. The results were tested statistically for significance by student t-test and analysis of variance.

III results:

Pituitary - treated group: After administration of 6 pituitary glands depigmentation was observed in the most part of the testis. Testes became enlarged and seminiferous tubules in these enlarged testes were expanded and became transparent, as they were clearly seen with naked eye. In histological studies, after administration of 2 pituitary glands, noticeable changes were not found in the number of the nest of spermatogenic cells, while in groups that were treated with 4 and 6 pituitary glands, the number of primary and secondary spermatogonia increased significantly (Figs 1,2,3). The number of spermatids and spermatozoa in the toads treated with 4 pituitary glands decreased (Table 1).

HCG-treated group: The size of left and right testes was not equal and generally one testis

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Table 1: Experimental procedure and results of t-test student diameter of seminiferous tubules and the number of spermatogenic cells after injection of HCG. (Mean ± SD)

Group	Diameter of seminiferous tubules (μ)	The number of spermatogenic cells				
		Primary spermatogonia	Secondary spermatogonia	Primary spermatocytes	spermatids	spermatozoa
Control	217.6±24.9	5.92±3.61	3.01±2.27	1.88±1.20	0.95±0.89	7.55±3.1
2Pituitary glands	228.1±22.16	3.81±0.84	2.50±0.92	1.33±0.83	0.66±0.31	7.03±1.69
P	0.1	0.05	0.1	0.1	0.1	0.1
Control	221.05±30.60	4.71±2.65	2.09±1.34	3.55±2	2.35±1.59	8.47±1.97
4Pituitary glands	254.5±50.99	8.22±2.02	2.94±2.38	3.61±2.18	1.32±3.98	6.68±1.87
P	0.05	0.01	0.1	0.1	0.01	0.05
Control	221.6±30.98	3.05±2.20	2.19±1.76	4.82±1.73	2.3±1.5	9.3±2.56
6Pituitary glands	263.8±36.02	5.23±1.40	4.94±1.58	5.25±0.99	2.27±1.30	8.29±2.89
P	0.01	0.01	0.001	0.1	0.1	0.1

Table 2: Experimental procedure and results of variance diameter of seminiferous tubules and the number of spermatogenic cells oafter injection of H C G (Mean ± SD).

Group	Diameter of seminiferous tubules (μ)	The number of spermatogenic cells				
		Primary spermatogonia	Secondary spermatogonia	Primary spermatocytes	spermatids	spermatozoa
Control	208.61±31.95	4.53±1.96	1.8±2.46	4.87±1.21	4.13±3.41	9.2±4.31
25 IU HCG	244.61±27.01	6.01±2.4	2.47±1.26	2.53±1.47	0.45±0.60	6.05±1.84
50 IU HCG	238.9±23.1	5.04±2.25	1.89±1.1	0.2±0.35	4.74±1.97	7.29±2.44
100 IU HCG	247.5±56.32	7.14±2.28	3.69±2.32	1.44±1.65	1.48±0.86	8.26±2.00
F	3.52	4.018	2.82	7.164	12.45	3.41
P	0.05	0.01	0.05	0.001	0.001	0.05
dr	27	1.63	1.8	2.06	1.64	2.06

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Table 3: Experimental procedure and results of analysis of variance diameter of seminiferous tubules and the number of spermatogenic cells after injection of testosterone.(Me an ± SI

Group	Diameter of seminiferous tubules (μ)	The number of spermatogenic cells				
		Primary spermatogonia	Secondary spermatogonia	Primary spermatocytes	spermatids	spermatozoa
Control	216.52±28.54	4.69±1.75	6.72±3.64	2.19±1.84	1.72±1.1	11.02±4.18
Testosterone(0.5) μg/BW/day	255.65±39.59	5.53±1.56	7.31±2.68	7.27±1.81	2.91±1.45	10.99±4.01
Testosterone (2.5)	257.49±31.62	5.73±1.62	5.1±1.37	6.1±2.51	4.35±1.38	11.48±3.62
Testosterone (5)	259.71±43.02	5.73±1.62	4.86±1.28	6.08±2.57	5.48±1.38	11.48±3.62
Testosterone (10)	245.45±21.03	6.51±1.30	1.99±1.39	3.55±1.75	5.31±1.50	17.11±2.31
F	4.22	2.82	12.5	14.52	17.19	8.66
P	0.05	0.05	0.001	0.001	0.001	0.001
dr	24.60	1.15	1.66	1.55	1.04	3.11

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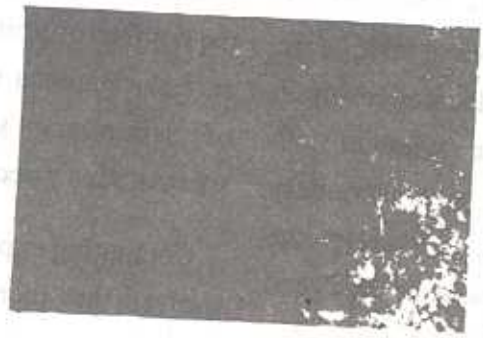

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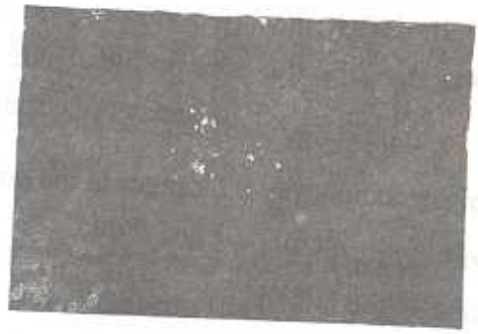

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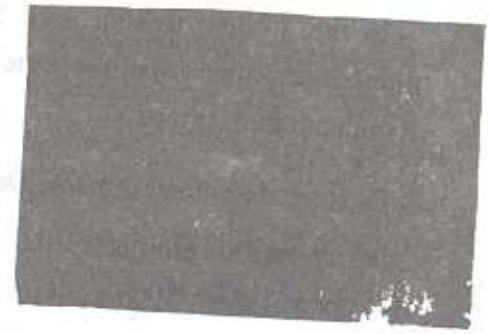

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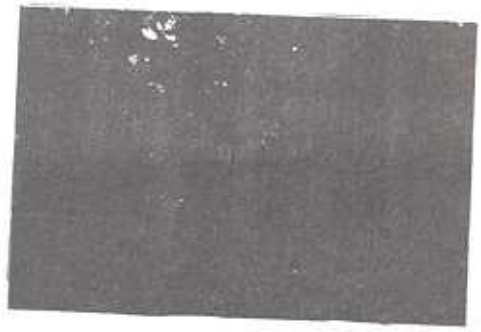
FIGS.1–6: Cross sections of testes. Magnification is the same in all photomicrographs. (1) in control, all of germ cells in spermatogenesis are seen. (2,3) Pitutary gland injection.Secondary spermatogonia, primary spermatocytes and spermatids are seen. (2) After 4 pituitary glands injection. (3) After 6 pituitary glands injection. (4,6) HCG - treated toads. Spermatogenesis is stimulated. (4). 25 IU HCG - treated toad. (5) 50 IU HCG - treated toads. (6) 100 IU HCG - treated toad.



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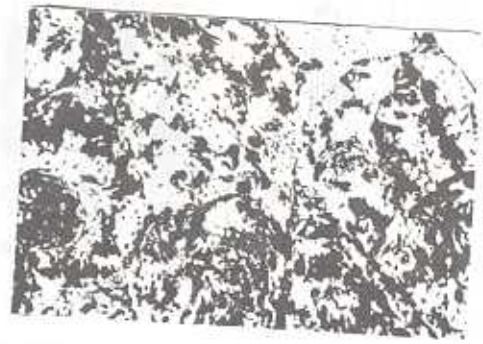

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FIGS.7,8: Cross sections of testes.Testosterone - treated toads. (7) After injetion of 5 μg / BWg / day testosterone, spermatocytes spermatids spermatozoa are seen. (8) After injetion of 10 μg / BWg / day testosterone, Numerous spermatozoa are seen.

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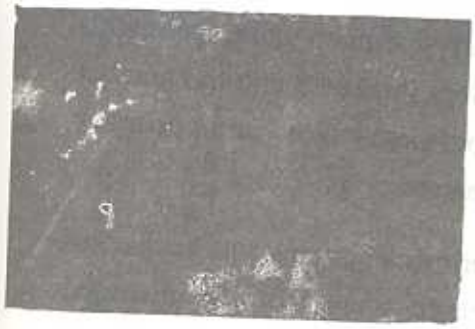

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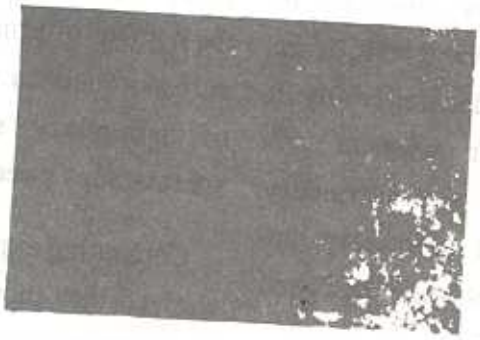

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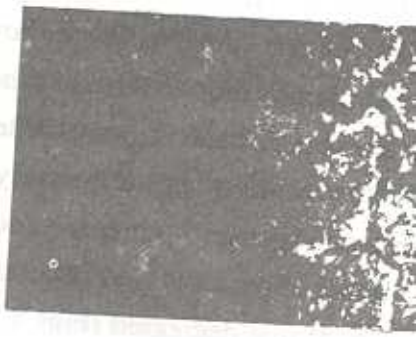

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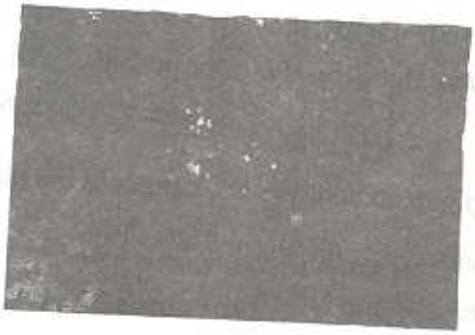

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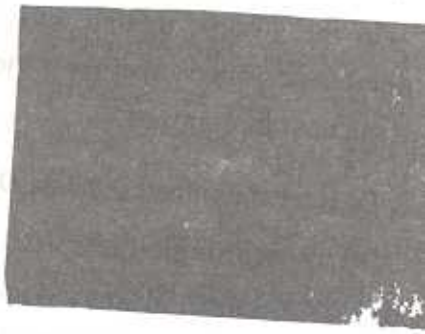

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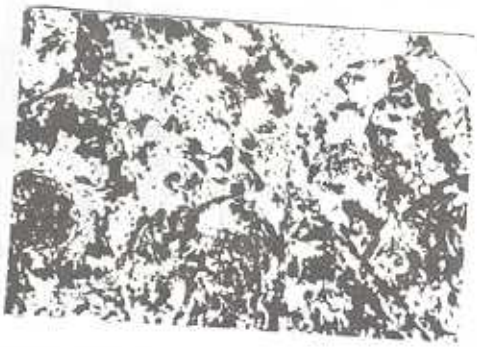

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enlarged (depigmentation was observed in enlarged one.) As it has been shown in table 2, the seminiferous tubules have expanded noticeably in 100IU HCG-treated group. After treatment with HCG, the number of primary and secondary spermatogonia increased and conversly the number of primary spermatocytes, spermatids and spermatozoa decreased (Figs.4,5,6).

In the groups, treated with 50 IU HCG, the number of primary spermatocyte was significantly less than other groups.

Testosterone-treated group: Depigmentation was clearly seen in groups treated with 2.5μg/gBW/day testosterone. Maximum increase in the lumen of seminiferous tubules was observed in the toads treated with 5μg/gBW/day testosterone. After administration of 10μg testosterone, a noticeable decrease in the tubular lumen relative to the other groups occurred. Comparing with control gr the number of primary spermatogonia, sperm and spermatozoa were increased and the nu of secondary spermatogonia and pri spermatocytes decreased (Figs, 7,8)

Comparing experimental groups with each showed that increased amount of adminis testosterone caused decrease in the num secondary spermatogonia, spermato spermatids and increase in the numb spermatozoa (Table 3).

Changes in the number of germ cells are sho Figs. 9-11.

In addition to microscopic change examined male sexual responsiveness occurrance of clasping behavior was used e of male sexual behavior. 24 hour after inj 4 pituitary glands, or 200 IU HCG

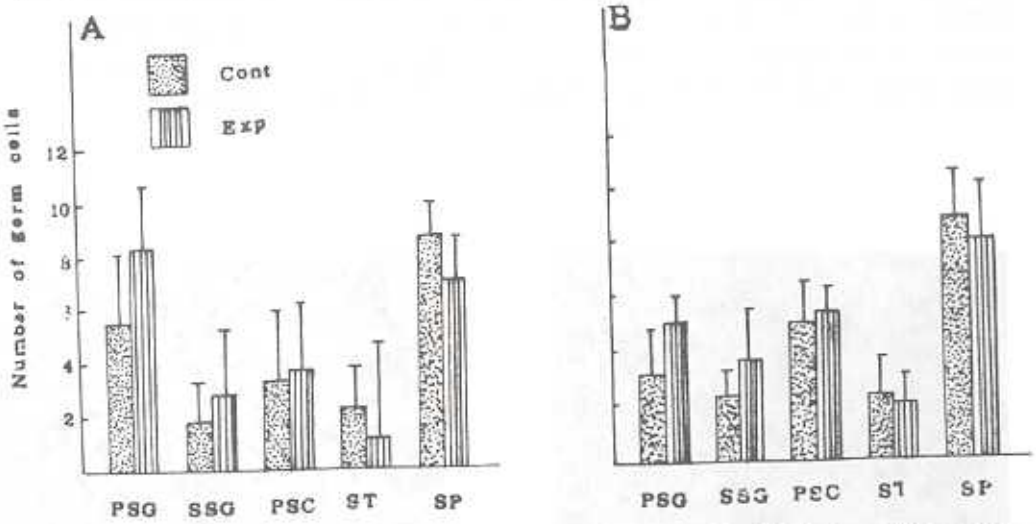

FIG.9. Histograms of changes in the number of germ cells following pituitary gland injection. (A) 4 pituitary g and (B) 6 pituitary glands. PSG, primary spermatogonium; SSG, Secondary spermatogonium; PSC, primary spermatocyte; ST, Spermarid; SP, spermatozoa .

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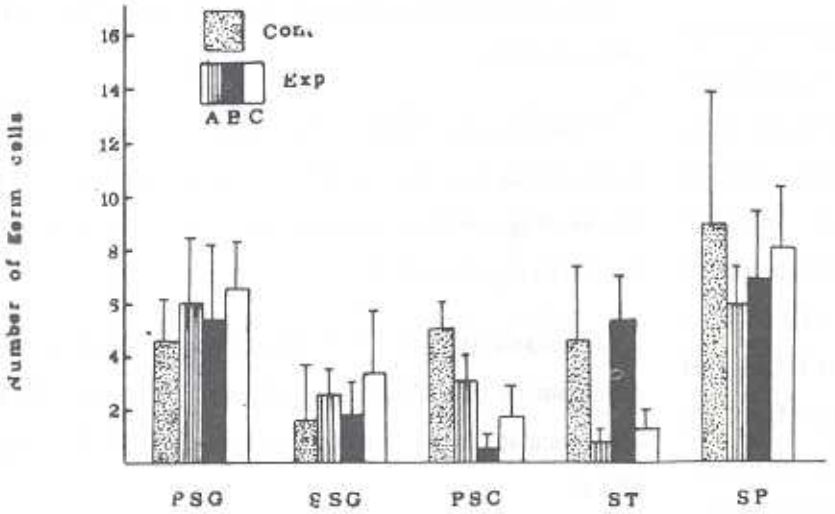

FIG.10. Histograms of changes in the number of germ cells following HCG injection. (A) 25 IU HCG. (B) 50 IU HCG (C) 100 IU HCG.

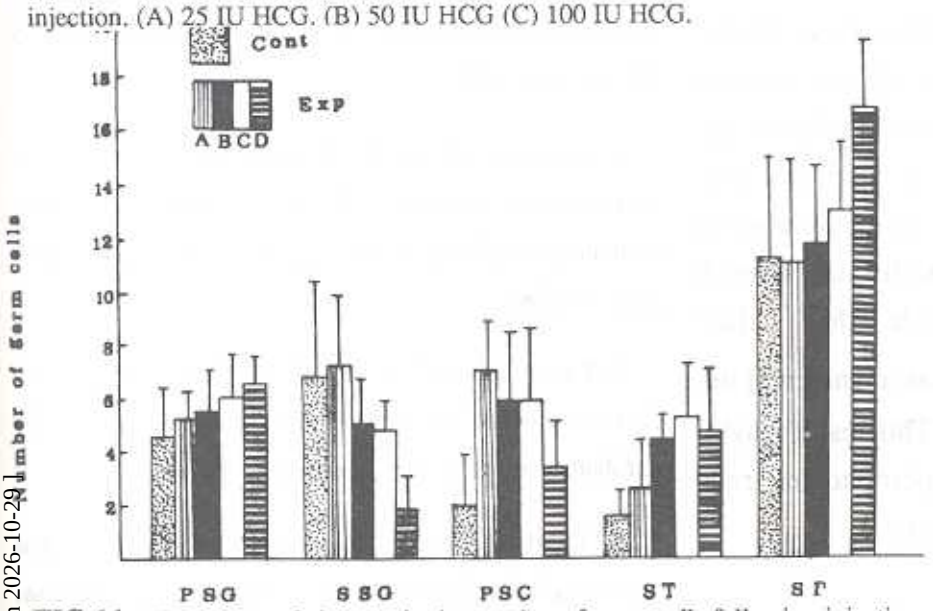

FIG.11. Histograms of changes in the number of germ cells following injection. (A) 0.5μg/BWg/day. (B) 2.5μg/BWg/day. (C) 5μg/BWg/day. (D) 10μg/BWg/day.

exhibited clasping behavior, also the male toads recieved daily 0.25mg testosterone accompaning 50 IU HCG for 4 days showed clasping, but testosterone injection alone for 12 days failed to restore courtship behavior.

IV Discussion

External observation in the testes of the toads treated with pituitary gland or HCG showed enlargement and depigmentation. It is concluded that depigmentation is related to expansion of the seminiferous tubules and disturbance of interstitial tissue. The results of histological studies reveal that after administration of the pituitary glands, the number of primary and secondary spermatogonia increase while the number of spermatids and spermatozoa do not change. We have seen mobile spermatozoa in the urine of the male toads in the end of each experimental period. Since the number of spermarids and spermatozoa did not change, in spite of increase in the number of primary and secondary spermatogonia, it can be concluded that spermatozoa are liberated from seminiferous tubules. Therefore it appears that administration of specific number of pituitary gland induces spermiation in addition to increase in the number of the primary and secondary spermatogonia. The results of HCG-treated group reveal that after

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treatment with HCG, not only the number of secondary spermatogonia increases, but also spermatogenic process is induced. Spermiation in 100 IU HCG-treated group is significant compared with the other HCG-treated groups. This finding suggests that different doses of HCG have different effects, a high dose of HCG, in addition to stimulation of spermatogonia proliferation, induces spermiation. Also with regard to chemical similarity between HCG and LH, probebly, HCG has a stimulatory effect on the testosterone secretion of the gonads. Presumably This effect may happen with higher dose of HCG. It seems that stimulation of spermiation after 100 IU HCG administration is related with stimulation of testosterone secretion. Since injection of special dose of testosterone alone causes increase the number of primary spermatogonia, spermatids or spermatozoa, therefore it seems that changes in the number of spermatogenic cells is relative to specific dose of testosterone. It is noticeable that, after administration of 10μg testosterone diameter of the seminiferous tubules decrease. This result maybe attributable to liberation of spermatozoa from seminiferous tubules in this group.

Acknowledgment

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