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THE ROLE OF SUBLINGUAL AND SUBMANDIBULAR GLANDS IN THE TESTICULAR FUNCTIONS IN MICE *(MUSMUSCULUS).*

BODARE, R. D.,[1] PILLAI, M. M.[2]

[1]P. G. Department of Zoology, S. G. M. College, Karad(M.S.) India 415 124;

[2]Department of Biotechnology, KIT, Kolhapur 416 004 (M.S.) India 416004.

E. mail: bodare_62@rediffmail.com

ABSTRACT:

In the present investigation, the effect of salivariadenectomy on the testis of mice with respect to various parameters like histology, spermatogenesis and fertility test was studied. For this purpose, pregnant female mice on the 10th day of gestation were subjected to sublingualectomy sialoadenectomy and salivariadenectomy. The 10 days old male offsprings of of above operated mother were again subjected tosublingualectomy sialoadenectomy and salivariadenectomy. The control groups were sham operated. They were sacrificed at the age of 45, 60 and 90 days. The study report showed that histological structure of testis was changed, spermatogenesis was adversely affected and reduction in the fertility rate was observed in the all operated groups as compared to control groups.

Key words: Sublingulectomy, Sialoadenectomy, Salivariadenectomy, fertility, Spermatogenesis,

INTRODUCTION:

Spermatogenesis is a complex process consisting ofsequential and highly organized steps of germ cell proliferationand differentiation, resulting in the generation offunctional spermatozoa, in the testis [1]. A wide range ofhormones and growth factors regulate these processes inan endocrine manner mainly through Sertoli cells, a kindof somatic cells interacting directly with germ cells in thetestis, eventually [2].

The epidermal growth factor (EGF) is best characterized and most extensively studied factor that occurs in the submandibular gland [3]. A large body of evidences indicates that EGF

is synthesized, stored and secreted by granular convoluted tubules (GCTs) cells of submandibular gland and also by the cells of sublingual glands. Epidermal growth factor is a growth factor that plays an important role in the regulation of cell growth, proliferation and differentiation [4]. A series of recent observations suggest that the growth factors and in particular epidermal growth factor may play an important role in the reproductive system [5- 19]. Many studies have revealed that the salivary glands are closely related to the testis. EGF secreted mainly from the salivary glands modulated the spermatogenesis and thus acts as an autocrine and/ or paracrine factor. Furthermore, testosterone could increase the EGF expression [20] and change the amylase activity in salivary gland. The EGF mRNA level found fall after male mice were castrated [21]. So it is hypothesized that an axis might exist betweenthe testis and salivary gland [22]. On attainment of sexual maturity, the germ cells, primary spermatocytes and round spermatids form EGF with onset of spermatogenesis [23].

In vitro, studies have indicated that EGF regulates testosterone production by an established MA-10 Leydig cell line [24,25] as well as by normal Leydig cells isolated from different species [26,27]. EGF is also shown to stimulate transferring and ABP production by isolated Sertoli cells. These effects raise the distinct possibility that EGF has some effect on male reproduction and to participate in androgen synthesis [28,29] and stimulation of sperm capacitation [7]. Several other investigators have demonstrated EGF receptors in the sperm cells undergoing spermatogenesis, Sertoli cells. EGFR is also present on the spermatozoa suggesting that EGF might also influence fertility showing its direct effect on spermatozoa. This was proved by Furuya et al. [5] who found that EGF could stimulate sperm capacitation by activation of tyrosine kinase of EGFR on spermatozoa. The binding of EGF to its receptors initiates many biological effects on the testis such as modulating Leydig cell proliferation, spermatogenesis and Sertoli cell activity [28]. EGF and EGFR have been immunolocalized during the testicular postnatal development and during the seminiferous epithelium cycle with in the different germ cell types [29].

The practice of sialoadenoctomy in rodent has been useful in studying the role of salivary EGF. In earlier study, role of sublingual EGF was totally neglected. In the present investigation, it was decided to study the role of EGF of submandibular and sublingual glands on the development and differentiation of testicular elements and spermatogenesis and functions of testis.

MATERIAL AND METHODS:

Male albino mice *Mus musculus* were used for the present investigation. Breeding pairs of mice were obtained from Hindusthan Antibiotics Ltd., Pune. They were allowed to breed in the animal house. They were housed in aluminum cages in groups of 3 to 4 and were supplied with Amrut Rat/Mouse feed (Pranav Agro Industries) and water *ad libitum.*

Salivariadenectomy includes removal of both salivary glands i.e. sublingual and submandibular glands .Sublingualectomy includes removal of sublingual glands and sialoadenectomy means the removal of submadibular glands. The pregnant female mice on the 10^{th} day of their gestation period were underwent sublingualectomy, sialoadenectomy and salivariadenectomy. The 10 day old male offspring of all operated females were again underwent subligualectomy, sialoadenectomy salivariadenectomy. Operated mice were maintained separately in animal house with proper care. Controls were sham operated. After 60 days these operated male offspring were sacrificed by cervical dislocation and testis was dissected out for further experimentation.

Histology[29]: Testis were dissected out and fixed in 10 % neutral buffered formalin for 24 hrs. at 4 ^{o}C. The tissue was washed under running tap water, dehydrated through alcohol grades and cleared in xylene. The paraffin sections were cut at thickness of 7 micron on rotatory microtome (Spencer type). The slides were stained by using Hematoxylene - Eosin technique [28].

Quantitation of spermatogenesis [30]: The histological slides of testis, prepared by Hematoxylene - Eosin technique, were used for quantitation of spermatogenesis. The number of germ cells in each seminiferous tubules were estimated by determining the number of nuclei of each cell type. The numbers of spermatogonia, primary spermatocytes, secondary spermatocytes and round spermatids were counted under light microscope. The number of elongated sperms was not counted.

Fertility Test [31]: Fertility test was carried out in male of 45 days, 60 days and 90 days old mice along with its operated mice. To carry out the fertility test, the virgin females of 3 months old were caged with males. The next morning, the vaginal smears were observed under the microscope for the presence of spermatozoa. If spermatozoa were present in the smear, the males were considered positive. If spermatozoa were absent, the males were placed with another set of females and the test was repeated.

Sperm count [32]: Sperm count in the animal was determined by improved Neubauer haemocytometer method, which is considered as standard for sperm counting. A single

epididymis was dissected out, weighed accurately and minced in 2 ml of 0.9% saline. The sample was used for sperm count epididymis.

RESULTS:

The weight of testis control mice was 110 ± 2.4495 respectively. There was non significant difference in the weight of testis of subligualectomised mice compared to control mice. However, a significant difference was observed in the sialoadenectomisedand salivariadenocetomised mice as compared to control. The histological structure of testis of control mice showed normal histological structure consisting of seminiferous tubules, interstitial cells. The epithelium consists of Sertoli cells and spermatogenic cells which gives rise to primary, secondary speramatocytes, spermatids spermatozoa (Fig. 1). The testis of sublingualectomised mice showed slight alterations (Fig.2). In sialoadenoctomised mice the histological observations revealed that the significant reduction of the number of sperm (Fig. 3). The testis of salivariadenoctemoised mice showed the highly significant reduction in the number of sperms and the lumen of seminiferous tubules become decreased (Fig. 4).

The results of epididymal sperm content is shown in Table.2. There was a slight significant difference in the sperm count in the testis of sublingualectomised mice, but it was highly significant in the testis of sialoadenectomised and salivariadenectomised mice as compared to control. The striking observation was that salivariadenectomised mice testis showed much reduction in the sperm count as compared to all above groups.

The results of quantitative analysis of spermatogenesis are shown in Table 3. The striking results are seen in primary and secondary speramatocytes. The control mice, the number of spermatogonia, primary speramatocytes, secondary speramatocytes and spermatids were 0.73 x 106, 0.91 x 106, 2.47 x 106, 4.54 x 106 respectively. There was significant decrease in the secondary speramatocytes and spermatids in the testis of sublingualectomised mice and highly significant decrease in the secondary speramatocytes and spermatids in the testis of sialoadenoctemosied and salivariadenoctomised mice.The results of fertility test showed in Table 4. In 90 days old control mice the number of fertile males was 9/10 and the litter size produced by control male was 12.2 ± 0.8367 the number fertile males, fertility rate and litter size was decreased significantly in the all operated mice of the above age groups.

DISCUSSION:

We have examined the effect of salivariadenectomy on the various parameters of testis of mice with different age groups. The weight of testis was significantly decreased in the sialoadenoctemosied and salivariadenoctomised mice as compared to the sublingualubectomised and control mice. Tsutsumi et al. [8], Liu et al. [12], Reys and Wakasugi [13] reported the same results in sialoadenoctemosied mice. The protein and lipid concentration was decreased in sublingualectomised, sialoadenoct- emosied and salivariadenoctomised mice. Pillai and Walvekar [15] showed non significant decrease in protein content in the epididymis and testis of sublingualectomised mice, but significant decrease in sialoadenoctomised mice. The histological structure of testis and the sperm count in the epididymis was markedly decreased in sublingualubectomised, sialoadenoctemosied and salivariadenoctomised male mice at different age intervals. The sperm contents increased with respect to increase in age of mice. Comparatively less decrease in sperm count was found in sublingualubectomised then other two operated mice. Our experimental results were consistent with the work of many Scientist. According Tsutsumi et al. [8] the epididymal sperm count was decreased by 45 to 55% in sialoadenoctomised mice, the decrease was in spermatids and spermatozoa. According to our investigation the decrease in sperm count was 33 to 45% , which was almost similar to Tsutsumi. Russelet. al. [10] reported that there was 14 to 15 % reduction in epididymal sperm count which was not to the degree reported by Tsutsumi. Russelet. al. [10] was unable to confirm the magnitude of results of Tsutsumi due to the differences and sensitivities of methodologies utilized. Pillai and Walvekar1[4] showed 15 % decrease in sperm count in sialoadenoctomised mice which was very similar to the results of Russel et al. [10] Pillai and Walvekar [15] showed 6 % decrease in the sperm count in sublingualubectomised mice. Similar results were shown by Liu et al. [12] Reys and Wakasugi [13] and Leng and Fang [14] in sialoadenoctomised mice.

Above investigation showed the possibility of sublingual and submandibular gland in controlling spermatogenesis. This suggests that there exist submandibular gland-testis axis in the control of spermatogenesis. There is long history in literature in support of this hypothesis that a relationship between salivary glands and generation of organs [34-36] . There was direct evidence that sialoadenectomy significantly led to testicular involution in group of immature rats [36] . In guinea pigs, Suzuki showed that sialoadenectomy significantly impaired spermatogenesis. Further, Weis and Bucker [36] demonstrated that ligation alone of the submandibular gland duct lead to degeneration of germinal epithelium in over 50 to 90% of the seminiferous tubules.

The quantitative analysis of spermatogenesis showed marked decrease in the number of spermatids and spermatozoa in the testis of all operated mice. Liu et. al. [12] and Tsutsumiet. al. [8] also reported that after sialoadenoctomy the number of primary spermatocytes was increased but the number of spermatids and spermatozoa were significantly decreased. The administration of exogenous EGF to sialoadenoctomised mice restored the sperm content. According to Tsutsumiet. al. [8] the decrease in spermatids and spermatozoa was due to impaired meiosis by EGF. Liu et. al. [12] reported that the number of spermatogonia were not altered significantly, but the number of pre leptotene spermatocytes, pachytene spermatocytes and round spermatids were decreased.The statistical analysis showed sperm count, fertility rate, number of secondary speramatocytes, round spermatids were significantly reduced and this reduction was significantly higher in the salivariadenectomised mice than sublingualectomised and sialoadenectomised mice. The above results showed that the submandibular gland has drastic effects on the development of testis and spermatogenesis than removal of sublingual gland and removal of both the glands hjas more drastic effect on spermatogenesis. The lowering of sperm content may be due to lowering of salivary EGF. The fertility rate, litter size was also significantly decreased in sialoadenoctemosied and salivariadenoctomised mice. This result is also consistent with the results of Reys and Wakasugi. These results indicating the role of salivary EGF in the development of testis and regulation of spermatogenesis.

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Table 1: Effect of salivariadenectomy on the weight of testis in mice (Weight in mg).

Sr. No	Animals	Body weight	' t ' value	Statistical significance
1.	Control (5)	110 ± 2.4495		
2.	Subligualectomy (5)	108 ± 1.8708	1.4509(9:10)	P > 0.1
3	Sialoadenectomy (5)	105 ± 1.6583	3.7796(9:11)	P < 0.01
4	Salivariadenectomy (5)	104 ± 2.000	4.2424(9:12)	P < 0.001

Values are mean ± S.D.Numbers of paranthesis indicate number of animals

Table 2: Effect of salivariadenectomy on sperm count in epididymis in mice (sperm count X 10^6 / epididymis).

Sr. No	Animals	Sperm count	' t ' value	Statistical significance
1.	Control (5)	9.1223 ± 0.1425		
2.	Subligualectomy (5)	7.469± 0.4813	7.7674(9:10)	P > 0.1
3.	Sialoadenectomy (5)	5.085 ± 0.1750	40.8690 (9:11)	P < 0.001
4.	Salivariadenectomy (5)	4.157 ± 0.2056	44.8851(9:12)	P < 0.001

Values are mean ± S.D.Numbers of paranthesis indicate number of animals

Table 3: Effect of salivariadenectomy on the quantitative analysisof spermatogenesis in mice

Sr. No	Animals	Spermato-gonia (X 10^6/Testis)	Primary Spermatocytes (X 10^6/Testis)	Secondary Spermatocytes (X 10^6/Testis)	Round Spermatids (X 10^6/Testis)
1.	Control(5)	0.73	0.91	2.47	4.54
2.	Subligualectomy (5)	0.86	0.95	1.87	3.82
3.	Sialoadenectomy (5)	0.97	1.28	1.44	2.70
4.	Salivariadenectomy (5)	1.16	1.41	1.17	2.47

Values are mean ± S.D, SL- sublingualectomised, SM – sialoadectomised,

SL+ SM – salivariadenectomised, Numbers of paranthesis indicate number of animals

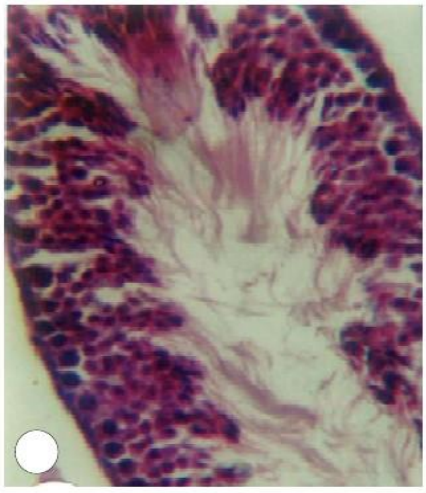
Table 4: Effect of salivariadenectomy on fertility in male mice.

Sr. No	Animals	Fertility (No. of fertile males /total no. of males)	Percentage of decrease in fertility	Litter size	't' value	Statistical Significance

1.	Control(5)	9/10	10%	12.2 ± 0.8367		
2.	Subligualectomy (5)	7/10	30%	10.6 ±0.5755 9.6	3.0649(9:10)	P< 0.01
3.	Sialoadenectomy (5)	6/10	40%	± 1.3416	3.6769(9:11)	P< 0.01
4.	Salivariadenectomy (5)	6/10	40%	8.8 ± 1.3038	4.9076(9:12)	P< 0.01

Values are mean ± S.D, SL- sublingualectomised, SM – sialoadectomised,

SL+ SM – salivariadenectomised, Numbers of paranthesis indicate number of animals



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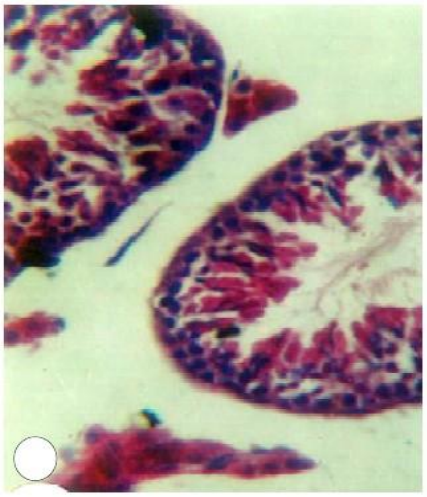
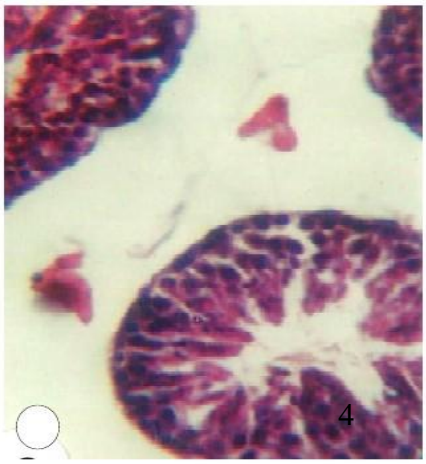


2

T.S. of Testis of control mice T.S of Testis of Sublingualectomised mice



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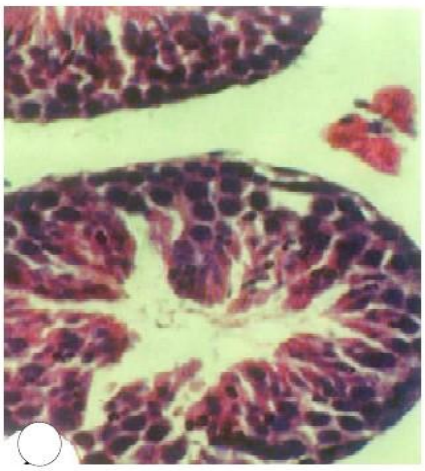

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T.S. of Testis of Sialoadecetomised mice T.S. of Testis of Salivariadenectomised mice