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Effect of Sialoadenectomy on the development of male reproductive organs in mice (Mus musculus).

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Abstract: Submandibular gland is rich source of epidermal growth factor (EGF). To investigate the role of salivary EGF on the development of male reproductive system we have performed the sialoadenectomy (removal of submandibular glands) of the 20 days old male mice and this sialoadenectomised mice was sacrificed on 45, 60 and 90th day. The testis, seminal vesicle, prostate gland, caput and cauda epididymis were dissected out for protein estimation and histological studies. It was found that the there was significant loss in the weight of these organs, protein content in the sialoadenectomised mice as compared to control. The histological structures of these organs were also changed in the sialoadenectomised mice.

Keyword: Sialoadenectomy, Histology, weight, Protein

INTRODUCTION :

The submandibular gland is rich source of epidermal growth factor (Barka,1980).Epidermal growth factor (EGF) was originally isolated from mouse submandibular glands as a concomitant of nerve growth factor and since then it has been recognized by its ability to stimulate precocious incisor eruption and eyelid opening in newborn mice (Cohen, 1962). A large body of evidences indicates that EGF is synthesized, stored and secreted by granular convoluted tubule (GCTs) cells of submandibular gland and also by the cells of sublingual glands. EGF was first demonstrable in scattered GCT cells at 20th day in male and 30th days of age in female mice (Gresik and Barka, 1978). EGF is single polypeptide chain having aspargine at the NH2- terminus, arginine at the COOH – terminus and a pool of 53 amino acids having molecular weight of 6000 Daltons. EGF levels vary during development and in response to several hormones - testosterone, thyroxin and growth hormone (GH) regulate the concentration of EGF in the submandibular gland of rat (Hiramatsu et al., 1994).

Endogenous EGF is likely to exert a profound physiological role in number of epithelial tissues in adult organisms.EGF has been considered an autocrine factor acting in the regulation of early placental growth (Mauro et al., 1995), in the formation of mesenchymal cells and in the morphogenesis of many organs, including teeth and brain, the male reproductive tract, the gastrointestinal tract and in cardiac differentiation (Thesleff et al., 1995, Kato et al., 1995, Gupta, 1996, Goldman et al, 1996). Many studies have revealed that the salivary glands are closely related to the testis. EGF secreted mainly from the salivary glands which modulates the spermatogenesis and thus acts as an autocrine and / or paracrine factor. So it is hypothesized that an axis might exist between the testis and salivary gland (Yan et al., l998). Radhakrishnan et al., (1992) showed that testis is infact is a source of EGF. Leydig cells are the principle source of EGF in the testis. On attainment of sexual maturity, the germ cells, primary spermatocytes and round spermatids form EGF with onset of spermatogenesis (Yan et al., 1998). The presence of EGF in the testis is important for epididymal physiology (Fawcett, 1979). In Vitro, studies have indicated that EGF regulates testosterone production by an established MA-10 Leydig cell line (Lloyd and Ascoli, 1983, Ascoli et al., 1987) as well as by normal Leydig cells isolated from different species (Welsh et al, 1982, Sordoillet, 1991). These effects raise the distinct possibility that the EGF has some effect on male reproduction and to participate in androgen synthesis (Verhoeven and Cailleau, 1986; Sordoillet et al., 1991). Suarez-Quian et al., (1994) showed that sialoadenectomy of adult C3H male mice made them infertile.

The practice of sialoadenectomy in rodents has been a useful tool in studying the role of salivary EGF. In rats, removal of submaxilary glands greatly reduced the rate of 3H-thymidine incorporation into gastric mucosa (Tepperman et al., 1989) and delayed the healing of gastric ulcers (Skov et al., 1986).

According to Naz and Kaplan (1993), EGF has either no effect or an inhibitory/negative on human sperm capacitation and /or acrosome reaction, especially at higher concentration. The blood plasma EGF concentration in infertile men was significantly lower as compared with fertile men and the sperm count, sperm motility, sperm viability also lower in infertile men as compared with fertile men (Adekunle et al., 2000). In the present investigation it was decided to study the maternal role of salivary EGF (both) on the development and differentiation of testicular elements and spermatogenesis and functions of testis.

2. Material and Methods.

a) Material

Male and female albino mice *(Mus musculus*) were used for the present investigation. The breeding pairs of mice were obtained from Hindustan Antibiotic Ltd. Pune. The mice were allowed to breed in the air-conditioned animal house in the department. The mice were housed in aluminum cages having dimension of 10 x 8 x 12 inches in group of 3 to 4 with rice husk bed. They were fed with Amrut Rat/Mice feed, which was obtained from Pranav Agro Industries Ltd. Sangli and water was given *ad libitum.*

b) Sialoadenectomy:

Sialoadenectomy is the removal of submandibular glands from both sides. For the study of effect of sialoadenectomy on the development and functions of male reproductive system, male mice of 20 days old juvenile male mice weighing 9 gms to 12 gms were used for sialoadanectomy. They were fasted overnight before operation. The sialoadanectomy of 20 days old male mice was carried out between 9.00 am. to 10.00 am.

The operated male mice were maintained in animal house with proper care for 45 days, 60 days, and 90 days. The controls were sham operated.

b) Methods:

1) Weight of tissue:

The control and sialoadenectomised male mice were sacrificed by cervical dislocation at 45 days, 60 days and 90 days. The testis, seminal vesicle, prostate gland, caput epididymis, cauda epididymis were removed and weighed immediately and the observations were recorded.

2) Histology (Gurr, 1962)

Mice were starved for overnight before they were sacrificed by cervical dislocation. The organs of male reproductive system namely testis, seminal vesicle, prostate gland, caput epididymis and cauda epididymis were dissected out and fixed in 10% Neutral Buffered Formalin for 24 hrs. at 4^0C. The tissues were washed under running tap water for 12 hrs. dehydrated through alcohol grades, cleared in xylene and embedded in paraffin wax (58 to 60^0c). The sections were cut at a thickness of 7 micron on rotator microtome (Spencer type). The sections were mounted on albuminized glass slides.

Some of the sections were stained with haematoxylin-Eosin (HE) a general staining method described by (Gurr, 1962).

3) Estimation of Protein (Lowry et al., 1951)

Homogenization of the tissues was carried out using refrigerated glass mortar for instaneous freezing and gradual thawing with chilled distilled water. The perfectly uniform homogenates were centrifuged at 10ºC at 5000 rpm for 10 minutes. The supernatant was used for estimation of protein. The protein content of testis, seminal vesicle, prostate gland, caput and cauda epididymis were estimated by the method of Lowery et al., 1951.

Results:

1) Effect of sialoadenectomy on the weight of male reproductive in mice (Table no.1)

The weight of testis of 45 days old sham operated mice was75±1.2003, 95.2±2.1213 and 110± 1102.4495 respectively. There was significant decrease in the protein content in the testis of sialoadenectomised mice of above age groups.
The weight of seminal vesicle in 45 days old sham operated mice was 28±1.5811.

The weight of seminal vesicle, sialoadenectomised and 24+2.1213. In 60 days old sham operated mice and 90 days old sham operated mice, the weight of seminal vesicle was 32±1.7320, 38±2.000 respectively. Similar significant ($P<0.01$) difference in seminal weight was found in sialoadenectomised (5:7, 9:11)

The weight of prostate gland of 45, 60 and 90 days old sham operated mice was 14±1-000, 18±1.5811, 22±2.000 respectively. The significant difference ($P<0.001$) in prostate gland weight was observed in sialoadenectomised mice.

The weight of caput epididymis of 45, 60 and 90 days old sham operated mice was 9±1.5811, 13±2.2360. 15±2.5455 respectively. The significant ($P<0.001$) difference in the weight was found in the caput epididymis of sialoadenectomised mice of above age groups as compared to control mice.

The weights of cauda epididymis of 45 days old sham operated mice were 5±2.000,7±1.4142,11±1.000respectively. In cauda epididymis of sialoadenectomised mice, the same trend was observed like that of other organs.

2) Effect of sialodenectomy on protein concentration (mg/gm) of male reproductive organs in mice.

The protein concentration in testis of 45, 60 and 90 days old control mice was 79 ± 4.183395 ± 7.0710 and 118 ± 4.1833 respectively. In sialoadenectomised mice, it was 55 ± 6.1237, 70 ± 5.7008 and 99 ± 6.5192 respectively. The protein content was decreased significantly ($P < 0.001$) as compared to normal.

In 45 days, 60 days and 90 days old control mice, the protein concentration in seminal vesicle was 55 ± 4.1833, 83 ± 5.7003 and 93 ± 4.4721 respectively. There was loss in the increase of protein content in seminal vesicle of sialoadenectomised mice of above age groups. As compared to control mice of respective age groups, there was significant ($P<0.001$) decrease in protein content in seminal vesicle of sialoadenectomised mice.

The protein content in prostate gland of 45days, 60 days and 90 days old control mice 41 ± 4.1833, 66 ± 4.1833 and 71 ± 4.1833 respectively. As shown in table no. 2, there was significant difference/loss protein content in prostate gland of sialoadenectomised mice of all age groups.

In 45 days, 60 days and 90days old control mice, the protein concentration in caput epididymis was 55 ± 5.0000, 73 ± 6.7082, 92 ± 5.7008 respectively. The protein concentration in caput epididymis was reduced significantly reduced in sialoadenectomised mice of same age groups as compared to control mice. The same trend was observed in the loss of protein content in cauda epididymis.

3) Effect of sialoadenectomyon the histology of male reproductive organs.

In control mice (Fig.1) the testis showed normal histological structure consisting of semniferous tubules, interstial cells (Leydig cells). The epithelium consists of Sertoli cells and spermatogonia cells from which all of the spermatozoa derived through different stages like spermatogonia primary spermatocyte secondary spermatocyte spermatids spermatozoa. I n sialoadenectomised mice (Fig.2) the histological observations revealed that the significant reduction in the number of sperm. This reduced number of sperms was thought to result in the reduction in the number of early spermatid, but increase in number of spermatogonia round cells and / or primary spermatocytes.

The reduction in the number of epididymal spermatozoa was also accompanied by the significant reduction in the size as well as lumen of seminiferous tubules.

In control mice (Fig.3) the seminal vesicle showed the generalized normal histological structure. The mucosa is folded, forming numerous irregular chambers or crypts. The epithelium is composed of cuboidal or columnar cells and irregular shaped basal cells. The lamina propria is rich in elastic fibers and forms continuous layer around the vesicle. The histological structure of the seminal vesicle of sialoadenectomised mice, showed the changes in the epithelium. The epithelial cells become slightly small and compact.

The fig. No.5 shows the histological structure of prostate gland of normal mice. A vascular capsule of fibro elastic tissue containing numerous smooth muscle fibres in its inner layer surrounds the gland. From the capsule, broad septa penetrate into the interior to form the abundant stroma which separates the scattered tubules or alveoli. The epithelium shows different glands and alveoli.

In sialoadenectomised mice (fig.6) the histological structure of prostate gland showed changes like the epithelial cells becomes smaller in size and also the gland also become more compact and reduced in the size.

The fig.7, 9shows histological structure of the caput and cauda epididymis is same. The epithelium of epididymis consists of two types of cells namely narrow, tall columnar cells and rounded basal cell. The columnar cells bear nonmotile processes called stereocillia. The basal cells are numerous. In sialoadenectomised mice fig. 10, 11 the histological structure of caput epididymis showed certain changes like decrease in the size of lumen and reduced number of sperms. The epithelium shows small, narrow compact columnar cells, the basal cells reduced in size.

Discussion:

In the present investigation, we have studied the effect of sialoadenectomy on the weight of organs, histology and protein content in the testis, seminal vesicle, prostate gland, caput epididymis and cauda epididymis. The weight of above organs was markedly decreased in the sialoadenectomised male mice. Li et al., (1983) reported that there was 12 to 13 % reduction in the body weight in the sialoadenectomised mice. Peerhentupa et al., (1984) reported the lower body mass in sialoadenectomised mice. Similar reports were shown by Liu et al., (1994) and Rey and Wakasugi (1995) in the sialoadenectomised mice.Tsutsumi et al., (1986) also showed significant decrease in the testis weight of sialoadenectomised mice where as Russel et al., (1990) showed non-significant decrease in testis weight in sialoadenectomised mice. Pillai and Walvekar (2002) have shown the decrease in the weight of testis and epididymis. (Liu et al., (1992) reported the decrease in weight of seminal vesicle and prostate gland in sialoadenectomised mice.EGF playing an important role in maintaining the integrity of the seminal vesicle and prostate gland in mouse (Sonis et al., 1992). The membranes of seminal vesicle and prostate gland contained binding sites for EGF.From the above consistent evidences, it becomes apparent that the salivary secretion containing EGF may be having a role in the growth of body and organs. As EGF is potent mitogen, stimulation, proliferation and differentiation and growth of epidermal and mesodermal tissue, might be important factor in modulating organogenesis and function of the reproductive organs.

We have also studied the effect of salivary EGF of male mice on the histological structure of testis, seminal vesicle, prostate gland, caput epididymis and cauda epididymis.

The results of our histological studies are in agreement with those of Reys and Wakasugi (1995) and Liu et al., (1994) and Russel et al., (1990). Reys and Wakasugi (1995) studied the histological structure of testis of sialoadenectomised mice and revealed that the significant reduction in the number of epididymal spermatozoa in the seminiferous tubules. They also observed the reduced size of seminiferous tubules; decrease in the number of early spermatids in the testis of sialoadenectomised mice which was consistent with our observations. Similarly Liu et al., (1994) showed decrease in the number of sperms in the testis of sialoadenectomised mice.Suarez-Quian et al., (1994) suggested that the fall in plasma EGF concentrations after sialoadenectomy in adult male mice leads to dimilution in spermatogonial mitotic activity in the basal compartment of seminiferous epithelium. In addition, EGF induces differentiation of type A spermatogonia in vitro (Haneji et al., 1998). Salivary EGF directly stimulates spermatogonial proliferation (spermatogenesis) and its production by the

submandibular gland is under the influence of testosterone (Bunny et al., 1972). However, the removal of the submandibular gland does not result in the complete cessation of spermatogenesis. The possible explanation for this could be EGF may be producted by other organs (Bunny et al., 1972) and other gamete factors of testicular origin affect mitotic activity in the seminiferous tubules (Bellve and Zheng, 1989).

The protein content was decreased significantly in testis, seminal vesicles, prostate gland, caput epididymis and cauda epididymis of sialoadenectomised mice as compared to control. Epidermal growth factot receptor (EGFR) has been shown to be present in the Leydig cells and Sertoli cells peritubular cells, interstitial cells and cells of epididymis of adult mouse testis (Suarez-Quian and Niklinski, 1990; Suarez-Quian et al., 1994), in the epididyrnis and vas deferens of non-human primates as well as in the prostate glands of rats (Traish and Wotiz, 1987). Pillai and Walvekar (2002) showed non-significant decrease in protein content in epididymis and testis of sublingualectomised mice but significant decrease was in sialoadenectomised mice. Bodare and Pillai (2007) showed nonsignificant decrease in protein content of testis, epidiymis, seminal vesicle, prostate gland of the offsprings of sublingualectomised mother. But significant decrease in the protein content of the above organs in the offsprings of sialoadenectomised and salivariadenectomised mother. Thus from all above evidences it can be concluded that the salivary EGF might be playing role in the development of male reproductive organs in mice.

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Table No.1
Effect of sialoadenectomy on the weight of male reproductive in mice.

Sr. No.	Organs	Age	Animal Control	Sialoadenectomised	't' value	Statistical Significance
1	Testis	45 60 90	75±1.5003 95.2±2.1213 110±2.4495	71±2.000 88±1.8708 105±0.6583	3.5776 5.5366 3.7796	P<0.01 P<0.01 P<0.01
2	Seminal vesicle	45 60 90	28±1.5811 32±1.7320 38±2.0000	24±2.1213 25±1.7386 34±2.1213	3.3809 4.8365 3.0678	P<0.01 P<0.01 P<0.01
3	Prostate gland	45 60 90	14±1.0000 18±1.5811 22±2.0000	11±1.0000 14±1.2247 17±1.5811	4.7436 4.4736 4.3854	P<0.01 P<0.01 P<0.01
4	Caput epididymis	45 60 90	9±1.5811 13±2.2360 15±2.5475	6±1.0000 9±1.5811 11±1.4242	5.5865 3.2661 2.3836	P<0.01 P<0.01 P<0.01
5	Cauda epididymis	45 60 90	5±2.0000 7±1.4142 11±1.0000	3±1.2247 4±0.7071 8±1.3228	2.8868 3.8740 4.0458	P<0.01 P<0.01 P<0.01

Table No.2
Effect of sialodenectomy on protein concentration (mg/gm) of male reproductive organs in mice.

Sr. No.	Organs	Age	Animal Control	Sialoadenectomised	't' value	Statistical Significance
1	Testis	45 60 90	79±4.1833 95±7.0710 118±5.7008	55±6.1237 70±5.7008 99±6.5192	7.2362 5.6884 4.9057	P<0.01 P<0.01 P<0.01
2	Seminal vesicle	45 60 90	55±4.1833 83±5.7003 93±4.4721	31±3.5355 67±4.4721 76±5.0000	9.7381 4.9379 5.6672	P<0.01 P<0.01 P<0.01
3	Prostate gland	45 60 90	41±4.1833 66±4.1833 71±4.1833	28±5.7008 48±4.4721 58±4.1833	4.1113 6.5727 4.9134	P<0.01 P<0.01 P<0.01
4	Caput epididymis	45 60 90	55±5.0000 73±6.7082 92±5.7008	41±4.1833 53±5.7008 77±5.7008	4.8555 5.0860 4.1602	P<0.01 P<0.01 P<0.01
5	Cauda epididymis	45 60 90	41±4.1833 66±4.1833 71±5.7008	28±4.1833 48±4.1833 58±8094	4.5354 6.4252 3.0219	P<0.01 P<0.01 P<0.01

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PLATE-1