THE EFFECT OF SIALOADENECTOMY AND EPIDERMAL GROWTH FACTOR ON TESTES

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ABSTRACT
It is aimed to determine the ultrastructural changes of testis that were formed in the testis of pupy rats which were born from the mother rats with sialoadenectomy. In our investigation, 30 adult female Sprague-Dawley rats which had been weighted 250-350 gr were used. The female rats were divided into three equal groups of control (Sham), Sialoadenectomy (Sx) and Sialoadenectomy + Epidermal Growth Factor (Sx + EGF).

The rats in the control group were mated without any process had been made. The rats in the group of Sx were mated after sialoadenectomy and waiting for three weeks in order to decrease of the plasma EGF level. After the sialoadenectomy it had been waited for three weeks and then the rats in the group of Sx + EGF were mated. On days 16-19 of pregnancy, a total of 50 microgram (µg) EGF was given to the animals in the same group with orogastric tub as 12.5 µg daily to each animal. The fetuses in each three group born following pregnancy were kept to grow up until the 28th day. After 28 days the testes of male infant rats that had born from three groups were fixed in buffered gluteraldehyde of 2.5% and following this process the specimens were processed with routine histologic procedures and then were examined with TEM.

Seminifer tubules and Leydig cells of the rats in the control group were normal. The basal membranes of seminifer tubules were thick and large intercellular spaces was established between the spermatogonia in the group of Sx. It was observed that Leydig cells were small and there was a dilatation between the cistemas of SER.

The basal membran of seminifer tubul in the Sx + EGF group was thick contrast to group of control however there was no intercellular space between the germ cells. While Leydig cells were found peritubuler localization and viewed normally, however a evident meiotic division was observed in spermatogonia.

In conclusion, exogen EGF had beneficial effect in the reversibility of the structural changes at the testes of the pupy rats depending on the absence of maternal EGF.

Introduction
The submandibular salivary gland in the mouse is a rich source of growth factors such as epidermal growth factor (EGF) and β nerve growth factor (pNGF). Epidermal growth factor is a 6000 dalton, 53 amino acid polypeptide (1) and is known to elicit a wide variety of cellular responses including membrane morphological changes,activation of various trasport responses and changes in cellular metabolism in addition to stimulating mitogenesis.
A series of recent observations suggests that growth factors and, in particular EGF, may play an important role in the male reproductive system (2-4). Several groups of investigators have demonstrated EGF receptors in the germ cells undergoing spermatogenesis, Sertoli cells, peritubular cells and interstitial cells (5-9).

A report by Tsutsumi et al. examined the effect of sialoadenectomy or removal of the submandibular gland on selected testis and epididymal parameters (10). Sialoadenectomy caused plasma epidermal growth factor (EGF) to reach undetectable levels by three weeks epididymal sperm decreased by 55%, spermatids in the testis decreased 40-55%. Replacement of EGF to mice restored these parameters to normal levels. The authors suggest that EGF might play a role in sperm production by influencing meiosis (11). Recently Suarez-Quian et al. (1989) localized EGF receptor in rat and monkey testis within Sertoli cells (8). There is considerable responses of Sertoli cells (see Suarez-Quian et al., 1989). EGF may also exert an inhibitory effect on Leydig cells (12).

In 1986, Tsutsumi et al. reported that sialoadenectomy (Sx) in male mice caused peripheral EGF levels to become undetectable and decreased sperm count by 45%. This effect could be both prevented and reversed by exogenous EGF administration (10).

**Materials and Methods**

**Animals**
Eight-week-old sexually mature female Sprague-Dawley rats were obtained from Health Medical Application and Research Center of Dicle University (Diyarbakir, Turkey). The animals were housed at 22°C with a 14 hour light/0 hour dark daily cycle and received free access to food and water. They were divided into three groups initially (1) normal controls, (2) sialoadenectomized (Sx), (3) Sialoadenectomy +Epidermal Growth Factor (Sx+EGF) treated.

**Surgical Procedure**
Sialoadenectomy was performed through a transverse neck incision under ketamine (30 mg/kg body weight, intramuscularly) and Xylazine (8 mg/kg body weight, intramuscularly) anesthesia. Following a three week period of recovery, the rats were mated. On days 16-19 of pregnancy, a total of 50 microgram (μg) EGF was given to the animal in Sx+EGF group with orogastric tub as 12.5 ug daily to each animal. The male fetuses born following pregnancy were kept to grow up until the 28th day. Male puppy rat of 28 days in all groups were sacrificed, and their testes were removed.

**Histological Procedure**
Tissue was prepared for Electron Microscopie examination. Testes were perfused-fixed with 4% glutaraldehyde in phosphate buffer solution (PBS) post-fixed in 1% Osmium tetraoxide, dehydrated through a graded series of alcohols, and embedded in araldite. One-um sections were cut from the araldite embedded blocks and stained with 1% toluidine blue for light microscopic examination semi-thin sections of the testis eight hundreds angstrom thick sections were stained with uranyl acetate and lead citrate and examined with Jeol 1100 electron microscop.

**Results and Discussion**

a) **Light Microscopy**
The sections of the control groups were in the normal appearance (Fig. 1). Testis sections from Sx group revealed that spermatogonia were separated from their basement membranes, with separations in the intercellular spaces. Leydig cells were found to be atrophic (Fig. 2). Higher magnification of the testis sections from Sx+EGF group, meiotic division of spermatogonia
were evident, Leydig cells were in normal appearance with peritubular location.

b) Electron Microscopy

In the testis sections from control groups, seminiferous tubular basement membrane was in normal thickness. Tubular structures, intercellular spaces (Fig. 4a), and Leydig cells were in normal appearance (Fig. 4b). In the sections of testis from Sx group, thickening in the seminiferous tubular basement membrane and wide spaces between spermatogonia were observed (Fig. 5a). Leydig cells were small and cisterna of smooth endoplasmic reticulum was dilated (Fig. 5b). To the testis sections of Sx +EGF group, seminiferous tubular basement membrane was thicker than those of in the control group, however, intercellular spaces between germ cells was not evident (Fig. 6a). Leydig cells were in normal appearance and in peritubular location (Fig. 6b).
Fig. 4a. Electron micrograph of testis from control groups all structures show in the normal appearance (Lead citrate-Uranyl acetate stained, x 3000.)

Fig. 4b. Electron micrograph of testis from control group. Leydig cells were seen in normal appearance (Lead citrate-Uranyl acetate, x 3000).

Fig. 5a. Electron micrograph of testis from Sx group. Note thickening in seminiferous basal lamina (double arrow) and wide spaces(*) between spermatogonia (Lead citrate-Uranyl acetate, x 3000).

Fig. 5b. Electron micrograph of testis from Sx group. Note Leydig cells were small and cistema of smooth endoplasmic reticulum (arrow) was dilated (Lead citrate-Uranyl acetate, x 4400).

Fig. 6a. Electron micrograph of testis from Sx+EGF group. Seminiferous tubule basement membrane (arrows) is seen thicker than those of in the control group however, intercellular spaces between germ cells was not evident (Lead citrate-Uranyl acetate, x 3000).

Fig. 6b. Electron micrograph of testis from Sx+EGF group. Note Leydig cells are seen in normal appearance and in peritubular location (Lead citrate-Uranyl acetate, x 3000).
Discussion
We have examined the effect of Sx on spermatogenesis and androgen production in 8-week-old Sprague-Dawley rats. Our experiments confirmed earlier observations about the effects of Sx on spermatogenesis (10). In addition, we made the following observations:
Sialoadenectomy resulted in an increase thickness of basal membran of seminiferous tubul. Sialoadenectomy caused dilatation the intercellular of spermatogonia. Also Leydig cells were atrophic and smoothe endoplasmic reticulum (SER) sistemae was dilate. Treatment with EOF reversed the decrease these results (Fig.6).
Tsutsumi et al. (10) reported that it took 3 to 4 weeks for the sperm count to decline maximally and another 3 to 4 weeks for complete correction of the defect with EGF administration. Therefore, our protocol also adopted a 4-week period of Sialoadenectomy and EGF replacement before the animals were sacrificed. Quantitative analysis of spermatogenesis revealed that Sialoadenectomy resulted in a decline in the number of preleptotene spermatocytes, thereby causing a decline in the number of pachytene spermatocytes, spermatids and spermatozoa.
The results of our histologic studies are in disagreement with those of Tsutsumi et al.(10) who reported that Sialoadenectomy increased the number of primary spermatocytes but decreased the of round spermatids, thereby suggesting that EGF unpaired the meiotic division.
In our study, the animals were 8 weeks old at the of surgery and had just reached or were reaching puberty, while in the study by Tsutsumi et al.(10) they were already mature at the time of surgery. This is the first report to demonstrate the effects of Sx and EGF replacement on androgen levels in peripubertal animals in vivo and to suggest that EGF may play an important regulation role in testosterone biosynthesis during the transition from the prepubertal to the pubertal stage, in vitro studies on the effect of EGF on testosterone production have shown both inhibitory and stimulatory effects. The results of our studies indicate a definite role for salivary gland EGF in testicular function. However, recent studies have shown that the testis, like the salivary glands and kidneys, is capable of synthesizing EGF precursor (EGFp), an integral membrane protein of Mr 140,000 (13).

REFERENCES