THE STUDY OF EFFECT OF EPIDERMAL GROWTH FACTOR (EGF) IN TESTES GERM CELL UNDER LIGHT MICROSCOPE IN NEWBORN MICE

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SUMMARY : Epidermal Growth Factor (EGF) is a polipeptide of 53 aminoacid and a potent mitogen in many tissues. It is reported that EGF stimulate mitosis and meiosis in sparmatogenesis. There are no investigations about its effect on testes. In this study EGF was applied to the newborn male mice from the first day of birth for 10 days. The testes were taken out on the 10^{th}, 20^{th}, 30^{th} and 45^{th} days following birth. The most definite difference between the study and the control group was detected on the 30^{th} day. As a result it was shown that EGF had been effective in early development of the germ cell.

Key Words: *Epidermal Growth Factor, Testes.*

INTRODUCTION

Epidermal growth factor (EGF) is a polipeptide of 53 aminoacid that is widely known to act as a mitogen as well as a differentiation factor for a wide variety of cell types (7).

EGF was isolated from mouse submandibular gland by Dr. Stanley Cohen. The human form of EGF was first isolated from urine and was initially called urogastron (3, 4).

EGF has been detected in a variety of human tissues and fluids. It is a potent mitogen in many tissues. It increases ion uptake, glycolysis, RNA and DNA synthesis in the cell. It is reported that EGF stimulate mitosis and meiosis in spermatogenesis. There are no investigations about the effect of EGF on testes morphology. For this purpose, we investigated the effect of EGF on the testes from newborn to puberty period of mice by light microscopy.

MATERIALS AND METHODS

In this study 30 Balb/c mice were used. The puberty period is between 29-49 days in mice. We formed mice groups on 10^{th}, 20^{th}, 30^{th} and 45^{th} days.

We gave 0.04 ml EGF and serum physiologic solution subcutaneosly to newborn mice each day for 10 days starting form birth and used study and control groups.

At 10^{th}, 20^{th}, 30^{th} and 45^{th} days mice were decapitated and testes samples were taken out.

The samples were fixed in Bouin solution for 12 hours (2,5,6). Samples were then prepared for light microscopic procedure and they were embedded in wax, sectioned at 4,5μ by Reichert-Jung microtome and stained with Hematoxylene and Eosine.

RESULTS

In the control group of the 10th day a part from spermatogonia, germ cells and Leydig cells were not seen clearly (Fig. 1). However in the study group of the 10th day after EGF injection for at 10th day, spermatogonium, spermatocyte I, spermatocyte II were visualized. Lumen was seen in seminiferous tubules. Leydig cells were present around the blood-vessels (Fig. 2).

In the control group of the 20th day, spermatogonium, spermatocyte I and a few spermatocyte II were observed (Fig. 3). In the 20th study group spermatogonium, spermatocyte I, spermatocyte II were seen in seminiferious tubules (Fig. 4).

In this study the most definite difference between the study and control groups was detected in the groups formed on 30th day. In the control group, spermatogonium spermatocyte I, spermatocyte II were observed but spermatide and spermium could not be differentiated (Fig. 5). In the study group

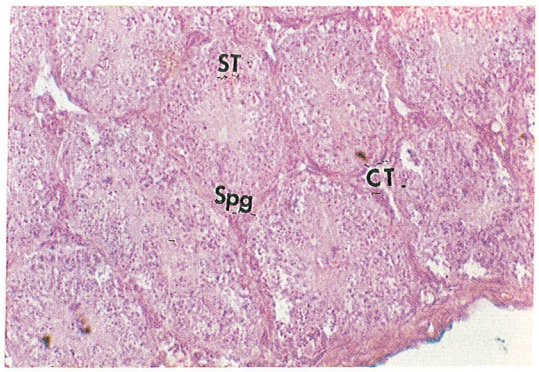

Fig - 1 : Seminiferous tubule (ST) in 10 days control group. Spermatogonium (Spg), Interstitial connective tissue (CT). Hematoxylene-Eosine X 200.

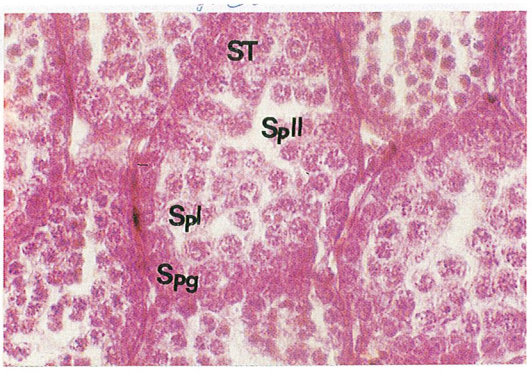

Fig - 3 : Seminiferous tubule (ST) in 20 days control group spermatogonium (Spg), spermatocyte I and spermatocyte II (SpII). Hematoxylene-Eosine X 400.

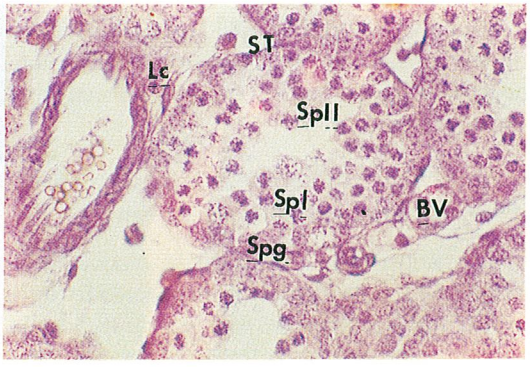

Fig - 2 : Seminiferous tubule (ST) in the 10th day study group. Spermatogonium (Spg), spermatocyte I (SpI) and spermatocyte II (SpII) were observed. Leydig cells (Lc) were seen around blood vessels (BV) Hematoxylene-Eosine X 400.

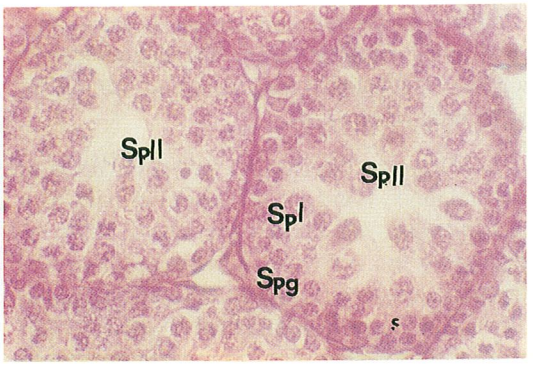

Fig - 4 : Seminiferous tubule (ST) in the 20th day group after EGF injection for 10 days. Spermatogonium (Spg), spermatocyte I (SpI) and spermatocyte II (SpII). Hematoxylene-Eosine X 400.

spermatogonium, spermatocyte I and II as well as spermatide and mature spermium were observed in the walls of the seminiferous tubules. In this group, adult testis structures were observed (Fig. 6).

On the other hand, on the 45th day, spermatocyte I, spermatocyte II, spermatide and spermium were seen in the control group (Fig. 7).

In the study group all of the germ cells on tubuli wall were observed (Fig. 8).

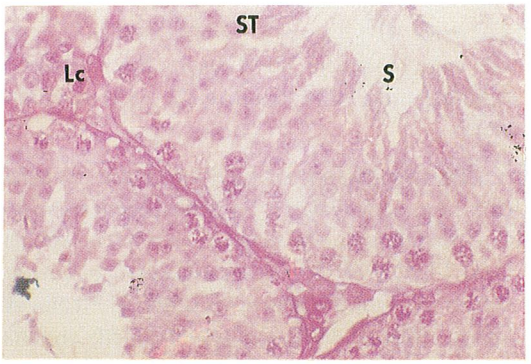

Fig - 7 : Seminiferous tubule (ST) in 45 days control group Leydig cells (Lc) and spermium (S) were seen. Hematoxylene-Eosine X 400

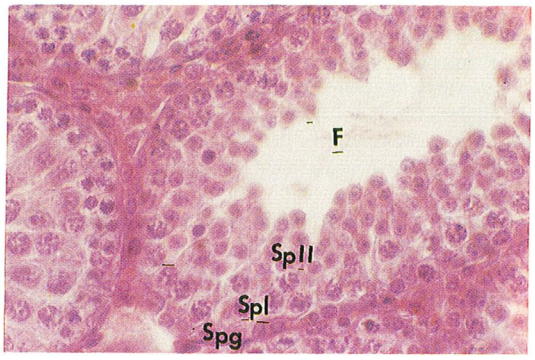

Fig - 5 : Seminiferous tubule in 30 days control group. Spermatogonium (Spg), spermatocyte I (SpI) and spermatocyte II (SpII) were observed. Fibrin like substance (F) was seen in the lumen. Hematoxylene-Eosine X 400

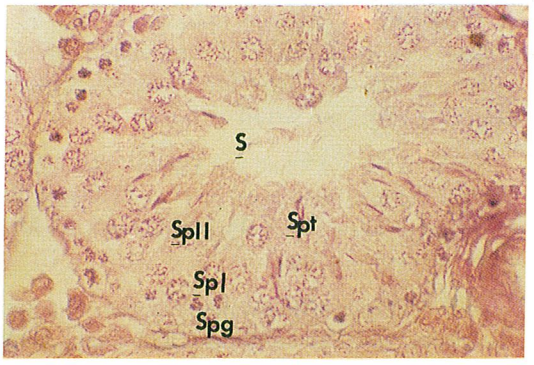

Fig - 8 : Seminiferous tubule (ST) in 45th day group after EGF injection for 10 days spermium (S) were seen in lumen. Spermatocyte I (SpI), spermatocyte II (SpII) spermatid (Spt), spermium (S). Hematoxylene-Eosine X 400.

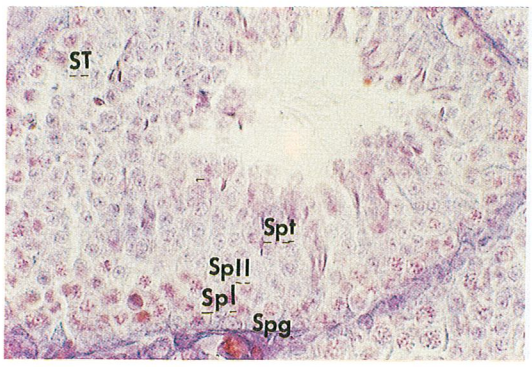

Fig - 6 : All the type of germ cells were developed in 30th day study group. Spermatogonium, spermatocyte I (SpI), spermatocyte II (SpII), spermatide (Spt) and spermium (S) were present. Hematoxylene-Eosine X 400.

DISCUSSION

It has been reported that EGF is produced in reproductive organs. However, its physiological function is not clear (1).

Puberty in mice is between 29-49 days after birth (11).

The begining of spermatogenesis is approximately on the 34.5th day, the mitotic phase takes about 8 days, the meiotic phase approximately 13 days, and spermiogenesis about 13.5 days. Our results are also consistent with the view that EGF plays a role in male reproductive function by stimulating the

meiotic phase of spermatogenesis (10).

In this study, spermatogonia, the germ cell tubule, Leydig and Sertoli cells were not found differentiated in the 10^{th} day control group. On the other hand in the 10^{th} day study group Sertoli and Leydig cells were observed. These results show that EGF stimulate meiotic division in the early development.

EGF regulate spermatogenesis but the mechanism of action of EGF on spermatogenesis is not clear. It is thought that there are two basic explanations. First, EGF is a potent mitogen in various cells. It may stimulate meiosis of the spermatocyte directly. Second, EGF may also indirectly stimulate spermatogenesis by acting on the Sertoli cells and Leydig cells. There are many studies supporting this hypothesis (9).

Suarez Quian and et al. supporting this hypothesis showed that localization of EGF in mouse testes were in Leydig cells and Sertoli cells (12) and EGF receptors were found on these cells.

On the other hand many studies report that EGF receptors are located near the seminiferous tubules in rat testes and they effect directly the development of Leydig cells and Sertoli cells (8).

In this study we found that seminiferous tubules reached the adult structural level at 30 days in the study group while it took 45 days to reach adulthood in the control group.

We conclude that the EGF exerts its action especially on spermatogenesis and brings about earlier development.

REFERENCES

1. Byers SW, Hadley MA, Djakiew D, Dym M : Growth and characterization of polarized monolayers of epididymal epitelial cells and sertoli cells in dual environment culture chambers. J Androl 1986; 7 : 59-68.
2. Byyny RL, Orth DN, Cohenn S : Epidermal growth factor : Effects of androgens and androgenergic Agents. Endocrinology 1974; 95 : 776-785.
3. Carpenter G, Cohenn S : Epidermal growth factor. Ann Rev Biochem 1979; 48 : 193-216.
4. Carpenter G : Receptors for epidermal growth factor and other polypeptide mitogens. Ann Rev Biochem 1987; 56 : 881-914.
5. Disbrey DB, Racic HJ : Histological laboratory methods. Edinburg and London : E and S Livingstore 1970; 93-95.
6. Elson DS, Browne CA : Identification of epidermal growth factor-like activity in human male reproductive tissues and fluids. J Clin Endoctrinol Metab 1984; 58 : 589-594.
7. Erbaş D : Epirdermal growth factor. Gazi Tıp Dergisi. 1990; 1 : 30-34.
8. Gridley FM : Laboratuar el kitabı. Ankara : Örnek Matbaası 1954; 32-33.
9. Holmes DS, Sportts G, Simith G : Rat sertoli cells secrete a growth factor (EGF) binding to its receptor. The Journal of Biology Chemistry 1986; 261 : 4076-4080.
10. Radhak Rishnan Bi Oke-BO : Characterization of epidermal growth factor in mouse testes. Endocrinology 1992; 131 (6) : 3091-3099.
11. Rough R : The mouse its repreduction at development. Publishing Burget Company Mineapolice 1969; 227.
12. Suarez Quian AC, Dae M, Onada M : Epidermal growth factor receptor localization in monkey testes 1989; 41 : 921-932.

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