EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) IMMUNOLOCALIZATION IN THE SIALOADENECTOMIZED RAT OVARIES

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ABSTRACT

Intra-ovarian regulation of follicular maturation is modulate by various factors. Among these, Epidermal growth factor (EGF) is vital factor. Epidermal growth factor (EGF) have potent mitogenic effects on granulosa and theca cells.

The aim of present study was to investigate the effects of sialoadenectomy on the distrubution of immunoreactive epidermal growth factor receptor (EGFR) in the rat ovaries.

Twenty Adult female Wistar albino rats were divided into two groups, a control and one experimental groups. The experimental groups of rats were subjected to sialoadenectomy in order to create EGF deficiency. After 60 days of sialoadenectomy (EGF deficiency) and control group rats were killed by pentobarbital and their ovaries removed. The sections were stained with APAAP immunohistochemical staining for evaluation using a light microscope.

A statistically significant reduced body weight was noted in the experimental groups of rats when compared to the control group. This marked EGFR immunoreactivity was maintained in control rat ovaries, whereas it deacreased in sialoadenectomized rat ovaries.

In conclusion, the present study indicated that there is paralel relationship between ovary follicule cells EGFR immunolocalization and EGF deficiency.

Introduction

Intra-ovarian regulation of follicular maturation is modulated by various factors. Among these, growth factors are important local actors.

Epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-á) have potent mitogenic effects on granulosa and theca cells. However, their effects on steroidogenesis by these cells is controversial, and there is limited information regarding their effects on luteal cell steroidogenesis.

EGF is a mitogenic polypeptide hormone which in vivo stimulates ectodermal (epithelia) and endodermal cell growth and in vitro growth of epithelial cell and fibro-

blasts (1). EGF is formed in salivary glands, kidney tubules and intestinal tract and occurs in nanogram guantities in plasma. EGF is a small, 53 amino acid, single chain polypeptide that is found at highest concentrations in salivary glands. (2,3), exert diverse effects in numerous tissues, including those of the ovary (4,5).

The submandibulary gland in mouse is a rich source of epidermal growth factor and there is at least ten times as much EGF in the submandibulary glands of male mice than those female mice. After sialoadenectomy, plasma EGF decreased rapidly and was undetectable by 3 weeks, indicating that the submandibular gland is a major sourse of circulating EGF (6,7,8).

Growths factors, acting via paracrine and/or outocrine mechanisms, are blieved to modulate the effects of pituitary gonadotropins on ovarian follicular growth and differentiation (9).

EGF deficiency by sialoadenectomy has reported to cause abortions and intrauterin growth retardation in mice (10,11), suggesting EGF plays a role in female reproduction function.

We examined the immunohistochemical localization of epidermal growth factor receptor (EGFR) in the sialoadenectomized rat ovaries.

Materials and Methods

Animals

Twenty adult female Wistar albino rats, 180-200 days old and 225-250 g in weight were obtained from the Department of Medical Science Application and Research Centre of Dicle University (**DÜSAM**) - Diyarbakýr. They were housed in invidual cages in temparature-controlled enviroment (22° C) with a 12 : 12 h light-dark cycle. All rats were fed standard pellet food and ad libitum tap water, which were performed according to criteria outlined i the ''Guide for the Care and Use of Laboratory Animals'' prepared by the National Academy of Sciences and published by the National Institues of Health.

The rats were randomly diveded into following groups:

The first group of rats were not subjected to sialoadenectomy (control group) (n: 10).

The second group of rats were anesthetized with an intramuscular injection of Ketamine HCl (50mg/kg) and xylazine (10 mg/kg). To remove the salivary glans, a 15 mm incision was made below mandible, bilaterally submandibular glands removed (n: 10).

After 60 days of sialoadenectomy (EGF deficiency) and control group rats were killed by pentobarbital and their ovaries removed. The ovaries were fixed in Bouin's solution, parafin embeded and sectioned 4-5 m m thick.

Immunohistochemistry

In the present study, monoclonal anti –EGFR primary antibody (Sigma, Clone No: 29.1) AP conjugated goat anti-rat IgG secondary antibody (Sigma), monoclonal mouse APAAP complex (Sigma) and fast red TR/Naphtol (Sigma) enzyme-substrate complex were used for immunocytochemical staining. All the staining procedures were done in humidified chambers, and PBS buffer was used between all staining steps.

All 4-5m m thick parafin sections were briefly cleared, then rehydrated in decreasing concentrations of ethanol and put into 0.1 % tripsin for proteolysis. In order to avoid undesired background staining, put into 20% goat serum in PBS for 20 minutes. Monoclonal anti-EGFR primary antibody (dilution: 1/200) was applied to the sections for 3 hours at 37 °C in a humidified staining chamber. Sections were then incubated in anti-mouse IgG secondary antibody (Lab Vision, dilution: 1/1000) for 1 hour s, and they were put into the APAAP complex for

an hour. Following this step, sections were incubated in the fast red/TR naphtol mixture until the specific regions were stained red, and then the sections were either briefly put into Mayer's hematoxylene in order to visualize the nuclei, or were not subjected to counterstainig. Sections were mounted with a glycerol-PBS mixture (1:1 glycerol: PBS). The control staining of some sections was performed without the primary antibody, and no EGFR immunostaining was observed in these sections.

Statiscal Analysis

Statistical validation (body weight) of significant sialoadenectomy effects was accomplished using the Mann-Whitney U test. Whole parameters were compared between sialoadenectomy groups and control groups (123).

Results and Discussion

A- Body weight

Mean body weights are presented in **Fig.1.** Initial weights of control and sialoadenectomized animals are essentially identical on the day of surgery. However, by the time the preference studies were initiated, control animals gained significantly more weight than sialoadenectomy rats and maintained this differential until the end of the studies (day 60).

A statistically significant reduced body weight was noted in the experimental groups of rats when compared to the control group ($P < 0.001$).

B- : Immunohistochemical Changes :

The aim of present study was to investigate the effects of sialoadenectomy on the distrubution of immunoreactive Epidermal Growth Factor Receptor (EGFR) in the rat ovaries.

There was considerable changes in immunoreactivity among section evenwithin groups, the difference in the stainining EGF-R between control and sialoadenectomy groups was clear (**Table 1**).

The control staining of some sections was performed without the primary antibody, and no EGFR immunostaining was observed in these sections (**Fig. 2a, 2b**).

Immunohistochemistry of EGFR in the control groups showed strongly marked staining in theca cells, garanulosa cells and corpus luteum (**Fig. 3a, 3b**).

TABLE

Immunohistochemical localisation of EGF-R in the sialoadenectomized and control rat ovaries

	Region	
Control groups	Corpus luteum	++
	Theca cells	++
	Granulosa cells	++
Sialoadenectomy groups	Corpus luteum	+
	Theca cells	±
	Granulosa cells	+
++: marked staining	**+:** moderate staining	**±:** slight staining

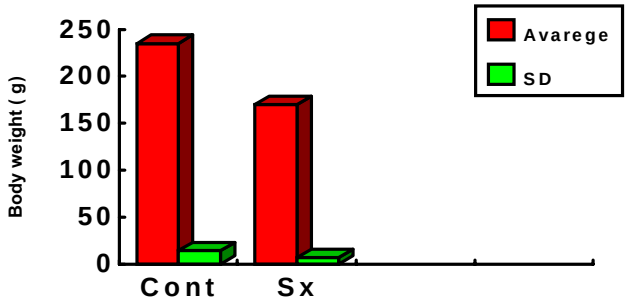

Fig. 1. Body weight of control and sialoadenectomized rats in the end of experimental period.

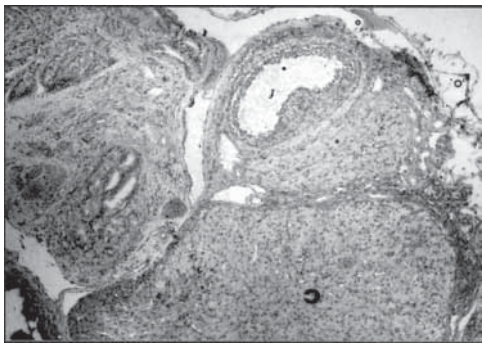

Fig. 2a. Histological appearance of control groups. The control staining was performed without the primary antibody. Corpus luteum (c), Follicule (f), immunogens show no positive staining, original magnification X10).

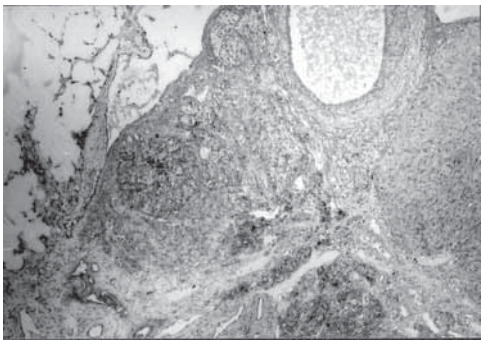

Fig. 2b. Histological appearance of subject to sialoadenectımy. The control staining was performed without the primary antibody. Immunogens show no positive staining (Original magnification X 10).

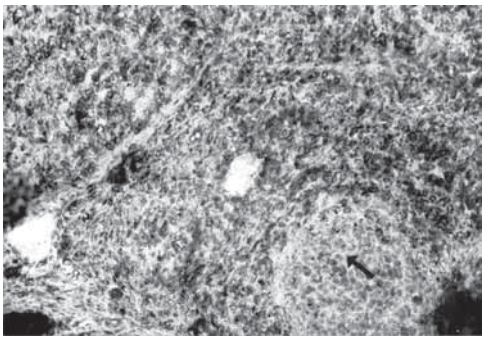

Fig. 3a. Immunohistochemical localisation of EGFR in the control rat ovary. Immunostaining was performed using secondary antibodies (Polycolonal). EGFR marked staining in corpus luteum (arrow) (Original magnification X 40)

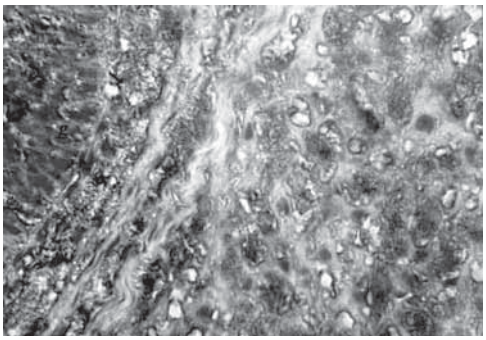

Fig. 3b. Immunohistochemical localisation of EGFR in the control rat ovary. Immunostaining was performed using secondary antibodies (Polycolonal). EGFR marked staining in corpus luteum, theca cells (arrow) and granulosa cells (g) (Original magnification X 100).

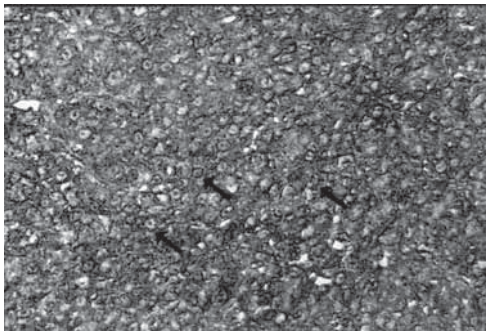

Fig. 4a. Immunohistochemical localisation of EGFR in the sialoadenectomized rat ovary. Immunostaining was performed using secondary antibodies (Polycolonal). EGFR show slight staining in corpus luteum (arrows)(Original magnification X40).

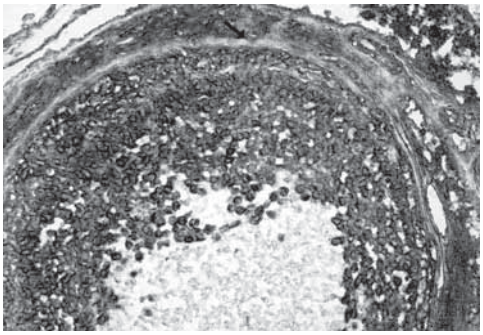

Fig. 4b Immunohistochemical localisation of EGFR in the sialoadenectomized rat ovary. Immunostaining was performed using secondary antibodies (Polycolonal). EGFR show slight staining in granulosa cells (g), but theca cells (arrow) were moderate staining (Original magnification X40).

We obviously observed in sialoadenectomized rat ovaries significantly decreased EGFR numbers.

In the sialoadenectomized rat corpus luteum showed moderate staining with EGFR (**Fig. 4a**), although slight staining was seen granulosa cells, theca cells showed moderate staining (**Fig. 4b**).

Epidermal growth factor (EGF) has been shown to have a positive effect during oocyte in vitro maturation in several species.

One possibility is that EGF acts locally in a paracrine or autocrine fashion (13). Alternatively, we hypothesize that the physiological role of EGF may depend on target tissue changes in the expression of EGF-R, which may be modulated by endocrine influences.To experimental study this hypothesis furter, we have used a sialoadenectomized rat model to evaluate, under immunohistochemical changes in EGF-R expression.

In mamalian species, evidence suggests that the number of EGF-Rs change during follicular differentiation (14). During luteinizing hormone (LH) / follicle stimulating hormone (FSH) induced differentiation of rat and porcine granulosa cells EGF-R numbers increase (15). Receptors were present on the hierrarcy of follicles of chicken but it was not possible to demostrate any clear differences in the receptor expression with follicular maturity (16).

EGFR immunoreactivitity was considered, strongly marked staining was seen in the theca cells and granulasa cells of control rat ovary (**Fig. 3a,b**), whereas slight staining was seen granulosa cells of sialoadenectomized rat ovary (**Fig. 4a**). These findigs of present study are important.

A statistically significant reduced body weight was noted in rats of the experimental group when compared to those in the control group. Therefore, these results mentioned here are also supported by Nanda and Catalinotto (17 *)*.

The presence of epidermal growth factor receptors (EGF-R) and the ligands epidermal growth factor/transforming growth factor-alpha (EGF/TGF alpha) have been re-

ported in mammalian ovaries where they are implicated in folliculogenesis and steroidogenesis. Evidence is presented to show that authentic EGF/TGF alpha receptors are expressed by the avian granulosa cells (19).

After sialoadenectomy, plasma EGF decreased rapidly and was undetectable by 3 weeks, indicating that the submandibular gland is a major source of circulating EGF (6, 7, 8). EGF deficiency was affected ovaries. We obviously observed slight staining of EGFR and significantly decreased EGFR numbers in sialoadenectomized rat ovaries. These findings of present study indicate that EGF deficiency has immunostaining effects on ovaries.

Lozernzo and Liu (20 | observed the increase of oocyte nuclear maturation rate when using EGF in culture media and the inhibition of maturation by tyrphostin A-47, suggests a physiological role for EGF in the regulation of equine oocyte maturation. These findings were accordance with our study.

In conclusion, the present study indicated that there is paralel relationship between ovary follicule cells EGFR immunolocalisation and EGF deficiency.

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