European Journal of Obstetrics & Gynecology and Reproductive Biology 188 (2015) 56–60

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Investigation of the effects of unilateral total salpingectomy on ovarian proliferating cell nuclear antigen and follicular reserve: experimental study

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ARTICLE INFO

Article history:
Received 15 June 2014
Received in revised form 12 February 2015
Accepted 19 February 2015

Keywords:
Salpingectomy
Ovarian reserve
PCNA
Apoptosis
Malondialdehyde

ABSTRACT

Objective: We aimed to investigate the effects of unilateral total salpingectomy procedure on ovarian follicular reserve, apoptosis, and proliferating cell nuclear antigen (PCNA) staining in this study.
Study design: Fourteen female Wistar Albino rats of 12 weeks were randomly divided into two groups. Abdomen midline incision was conducted under general anesthesia.

Group 1(G1) ($n = 7$): Group in which only the abdomen was opened and closed, Group 2(G2) ($n = 7$): Group that underwent right total salpingectomy.

After 1 month, abdomens of all rats were opened. Ovaries were macroscopically evaluated. Right ovarian tissue was quickly removed, fixed in 10% formaldehyde, and paraffin blocks were prepared.' The existence of fibrosis was identified with the usage of light microscope. Follicles were microscopically classified and counted. The prevalence of cytoplasmic immune staining and TUNEL staining was scored semi-quantitatively.
Statistical analysis: : SPSS 17.0 software was used for the statistical analysis of data. First, Kruskal–Wallis variance analysis was conducted, and then Mann–Whitney *U* test was utilized for inter-group dual comparisons for parameters found as $p < 0.05$.
Results: While the number of CL was found out dramatically high, secondary follicle count was found out to be significantly low in G2. Also in G2, although the number of atretic follicle and fibrosis were found out significantly increased, and the score of the angiogenesis was found to be significantly decreased in CL. When compared PCNA immunoreactivity in granulosa cells with the control group, there was a significant decrease in G2. When compared the malondialdehyde (MDA) immunoreactivity with G1 a significant increase was established in G2. Apoptosis score of ovarian follicles in granulosa cells was significantly higher in G2.
Conclusions: In this experimental study, the decrease in the ovarian reserve and PCNA staning of granulosa cells, an increase in apoptosis, fibrosis and the number of atretic follicles in unilateral total salpingectomy operation were analyzed in rats. We found out significantly higher MDA staining rates in G2 in comparison to in G1. According to the study, the unilateral total salpingectomy procedure can damage to the same side ovarian tissue by means of the ischemia and reperfusion injury at the ovarian tissue.

Introduction

Salpingectomy is a kind of surgical procedure which is also used frequently for illnesses damaging ovarian artery blood support such as ectopic pregnancy, hydrosalphinx, and pyosalpinx [1,2].

The effects of salpingectomy procedure on ovary are under debate. Since, while Dar et al. [3] did not establish any negative effects on ovarian tissue in cases that were conducted laparoscopic salpingectomy due to ectopic pregnancy. Chan et al. [4] suggested that laparoscopic unilateral salpingectomy had negative impact on ovarian function in ectopic pregnancy cases.

It was reported that tubal surgical procedures have negative effects on ovarian blood circulation and ovarian reserve due to the damage inflicted on ovarian blood vessels in humans and rats

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http://dx.doi.org/10.1016/j.ejogrb.2015.02.028
0301-2115/

[4–6]. In addition, some authors suggest that uterine and tubal lymphatics may also be damaged by tubal surgery [7,8]. Pepler et al. [9] established in two separate studies that both uterine artery ligation and hysterectomy disrupted ovarian blood circulation and thus negatively affected ovulation in rats. Zackrisson, et al. [10] demonstrated in their experimental study that both ovarian artery ligation and uterine artery ligation had negative impacts on ovarian blood circulation and ovarian functions.

Anti-proliferating Cell Nuclear Antigen (PCNA) expression is an essential regulator of cell cycle. It appears as an indicator in follicular growth. In some cases, it leads as the primary indication of granulosa cell expansion and oocyte growth [11]. PCNA was also suggested to be a key regulator throughout the ovarian follicle development [12].

Objective

We aimed to investigate the effects of unilateral total salpingectomy procedure on ovarian follicular reserve, apoptosis, and PCNA staining in this study.

Materials and methods

Animals

Twenty eight adult Wistar Albino rats with regular estrus cycle, weighing 200–240 g, and 14 weeks of age were kept in 12-h light (08:00–20:00) and 12-h dark photoperiod, at a fixed room temperature of 21–23 °C, and fed with standard pellet and city water. Permission for this study was granted by the Ethics Committee of the Medical Faculty of Firat University. This experimental study was conducted in Experimental Animals Laboratory of Firat University.

Experimental design

Oral feeding of rats was stopped 18 h before the experiment; only water was allowed. In order to provide rats with anesthesia, Ketamine (Ketalar, Eczacıbaşı Warner-Lambert, Istanbul, Turkey) 60 mg/kg and Xylazine (Rompun, Bayer, Istanbul, Turkey) 7 mg/kg were administered intramuscularly to left anterior foot muscle. Rats were placed on their backs on the operation table; antisepsis was provided by washing the surgical area with 10% povidone iodine solution, and abdomen midline incision was conducted.

Rats were randomly divided into two prospective groups.

Group 1 ($n = 7$): Group in which only the abdomen was opened and closed, Group 2 ($n = 7$): Group that underwent right total salpingectomy.

Abdominal layers were closed in a continuous manner with 3/0 silk suture. After 1 month, abdomens of all rats were opened at estrus phase with transverse subcostal incision under anesthesia. Ovaries were macroscopically evaluated. The presence of cyst and the dimensions of the existing cysts were measured in mm and recorded.

Histological preparation of tissues and evaluation of fibrosis

Right ovarian tissue was quickly removed, fixed in 10% formaldehyde, and paraffin blocks were prepared for histological and immunohistochemical examinations. Sections of 5 μm taken from paraffin blocks were stained with Masson's trichrome stain. Under light microscopy, fibrosis was evaluated by the same histologist and semi-quantitatively scored. An ordinal scale was created for remission in angiogenesis and presence of fibrosis in CL (no = 0p, with = 1p, too much = 2p) created.

Microscopic classification for follicular development

Method by Mazaud et al. [13] was utilized. In the light microscopic examination of ovary sections belonging to all groups, follicle classification was carried out according to characteristics noted below.

Primordial follicle: Oocyte was surrounded either partially or completely by granulose progenitor cell. *Primary follicle*: Follicle in which a single layer of cubic granulose cells was observed around oocytes, Antral (secondary) follicles: Follicle in which oocyte was covered with more than two layers of granulosa cells and in which antrum formation commenced, *Tertiary* (*Graafian*) *follicle:* Follicle that possesses a single and big space (antrum), in which a decreasing number of granulosa cells surround an antrum full of follicular fluid, and that oocyte surrounded by some granulosa cells (cumulus cells). *Atretic antral*: Degenerated oocyte along with pyknosis in granulosa cells, low number of pyknotic granulosa cells, and hypertrophic thec interna.

For each rat, the total number of atretic follicles was calculated [14].

Immunohistochemical examination

Sections of 5–6 mm thickness obtained from paraffin blocks were taken to glass slides with polylysine. Deparaffinized tissues were passed through graded alcohol series and boiled in citrate buffer solution at pH6 in microwave oven (750 W) for 7 + 5 min for antigen retrieval. In order to prevent surface staining, tissues were incubated with primary antibodies (Monoclonal Mouse Anti-Proliferating Cell Nuclear Antigen, M0879, Dako, Baltimore, MD, USA, Anti-ab6463 antibody, Malondialdehyde Abcam, Cambridge, UK) for 60 min following treating with Ultra V Block (TA-125-UB, the Lab Vision Corporation, USA) solutions. After the application of primary antibodies, tissues were incubated with secondary antibodies (30 min) (biotinized anti-mouse/rabbit IgG, Diagnostic BioSystems, KP 50A, Pleasanton, USA), Streptavidin Alkaline Phosphatase (30 min) (TS-060-AP, the Lab Vision Corporation, USA) and Fast Red Substrate System (TA-125-AF, the Lab Vision Corporation, USA). Tissues that were conducted a contrasting staining with Mayer's haematoxylin were treated with PBS (Phosphate Buffered Saline) and distilled water, and then, closed with the appropriate shutdown solution. The preparations were examined and evaluated under Olympus BX50 microscope and photographed.

Extensity of the staining was taken as the basis when evaluating immunohistochemical staining. The extensity of cytoplasmic immune staining was semi-quantitatively scored from 0 to +3 points (0: none, 1: mild, +2: medium, +3: severe).

TUNEL staining

Sections in 5–6 mm thickness obtained from paraffin blocks were taken to glass slides with polylysine. In accordance with the manufacturer's instructions, cells undergoing apoptosis were established by using ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon, cat no: S7101, USA). Tissues deparaffinized with Xylene were treated with graded alcohol and washed with phosphate buffered saline (PBS). Incubated with 0.05% proteinase K, tissues were then incubated for 5 min with 3% hydrogen peroxide in order to prevent endogenous peroxidase activity. After washing with PBS, tissues were incubated with 6-min Equilibration Buffer and again incubated at 37 °C in a humid environment with study solution (70% μl Reaction Buffer + 30% TdT Enzyme) for 60 min. Maintained in Stop/Wash Buffer for 10 min, tissues were then treated with Anti-Digoxigenin Peroxidase for 30 min. Apoptotic cells were imaged with Diaminobenzidine