Website: www.aijournals.com/journals/aanat/ **DOI: 10.21276/aanat.2016.2.1.5**

ORIGINAL ARTICLE ISSN: 2455-5274

Effects of Methotrexate on Ovary: An Experimental Study on Albino Rat

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Date of Submission: *20-10-2015*
Date of Acceptance: *06-06-2016*
Date of Publishing: *23-06-2016*

ABSTRACT

Background: Methotrexate is an anti-cancer drug but carries toxic effects on genital system and the study was conducted to find out the exact mechanism involved and to search for remedy. **Methods:** Twelve female albino rats 6 experimental and 6 control received methotrexate. [in former] and normal saline. [in latter], 1 mg/kg, intraperitoneally for 6 weeks. **Results:** H/E stained sections from ovary of experimental rat showed poorly developed Graafian follicles with loss of ova. There were follicular spaces in these rats with albuminous fluid and inflammatory cells. Granulosa lutein cells also showed degeneration due to inflammation. Cells were found to be smaller, shrunken, and irregular with vacuolated lighter colour cytoplasm. **Conclusion:** The drug is safer to be used in those patients who have completed their family.

Keywords: *Methotrexate, Ovary, Female Rat, Histopathology.*

INTRODUCTION

Methotrexate was developed for the treatment of cancer in 1988. The drug is employed not only in the treatment of various kind of neoplasm instead additionally used for treatment of psoriasis, acute lymphoblastic leukaemia as well as myeloblastic leukaemia.[1] Recently methotrexate is used for the treatment of juvenile rheumatoid arthritis which increases the chance of toxicity in patients. In women, methotrexate is also used in medical management of ectopic pregnancy.[2] Methotrexate is toxic to bone marrow, liver, lung, kidney, gut[3], and gonads.[4-6] Main reason behind scientists concern to explore methotrexate is not only its toxic effects on genital system but also to find out the exact mechanism involved and remedy.

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Methotrexate is known to affect egg production in female.[4] Impact of methotrexate on oocyte yield was reported by Mc Laren et al.[7] showing a decline, a fact of great significance as far as fertility was concerned. Sensitivity of oocyte to methotrexate toxicity prior to and after LH surge was an interesting report in an experimental study.[8] The former was found to be relatively resistant. The purpose of present experiment is to explore detailed histopathological alterations ovary after injecting therapeutic dose of methotrexate in female rats.

MATERIALS AND METHODS

Twelve female rats of 190 to 200 g were divided into experimental and control groups i.e. of 6 animals each. Animals received standard pellet laboratory diet (Lipton India Limited) and water ad-libitum. Control rats received injection of normal saline, 1 ml, intraperitoneally, weekly for 6 weeks. Experimental rats received methotrexate, 1 mg per kg intraperitoneal injection, weekly for same duration. Nembutal was injected 30 mg per kg, intraperitoneally to anaesthetize the rats. Karnovsky's fixative was transfused through left ventricle to fix the ovary. A midline incision from xiphoid process to pubic symphysis was made in female rats to open abdominal and pelvic cavities. Uterus was exposed and the attachments of fallopian

tubes in its lateral walls near fundus were found out [Figure 1]. Fallopian tubes were traced up to infundibulum which guided the location of ovaries [Figure 2]. Tissue of 2-3 mm size was obtained from ovary with the help of sharp knife. Thin sections of ovary were obtained by using wax embedding technique. Sections were stained with hematoxylin (for 10 minutes) and eosin (for 2 minutes). DPX was used for permanent mounting.

RESULTS

In control group in female rats, we observed that the ovarian cortex contains developing follicles in all the stages of development [Figure 3]. The first part of oogenesis starts in the germinal epithelium, which gives rise to the development of ovarian follicles. Primordial follicle is made of oocyte surrounded by a single layer of follicular cells [Figure 3]. Primary follicle shows enlarged oocyte surrounded by multiple layers of cuboidal granulosa cells and a thin membrane like zona pellucida between oocyte and granulosa cells [Figure 3].

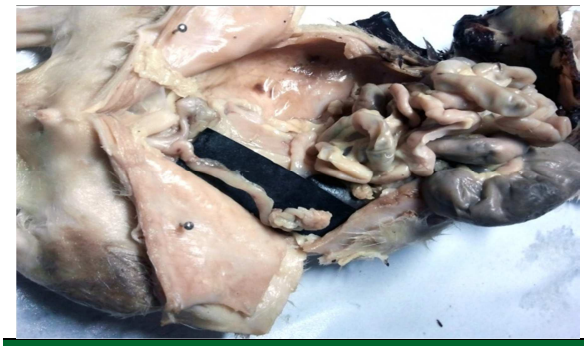

Figure 1: Dissection showing the fallopian tube and ovary of the left side in female albino rat.

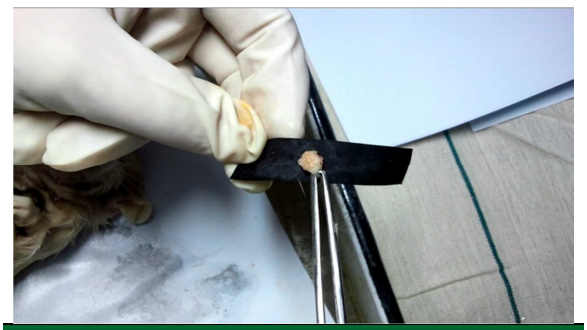

Figure 2: Left ovary of female albino rat.

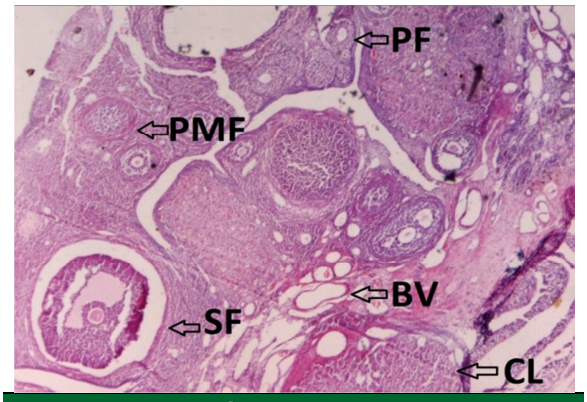

Figure 3: Ovary, Control case 3, H&E× 10.
Cortex shows various stages of developing follicle. Primordial follicle. [PF], primary follicle. [PMF], secondary follicle. [SF] & a portion of corpus luteum. [CL] and blood vessel. [BV] in medulla.

Secondary follicle shows fluid filled spaces, which fuse to form follicular antrum [Figure 3]. Mature graafian follicle shows secondary oocyte eccentrically placed [Figure 4]. Fluid filled cavity forms follicular antrum and zona granulosa is seen as evenly thick layer of cells at the periphery. Cumulus oophorus is a mass of granulosa cells around oocyte [Figure 4]. Corona radiata attaches to zona granulosa. Fibrous theca cells surround the follicle forming the outer most layer [Figure 4]. Corpus luteum is made of undulating cords of polyhedral eosinophilic lutein cells with central round nuclei-*9, small nucleolus, highly eosinophilic granular cytoplasm [Figure 5]. In experimental group in the female rats, the cortex of ovary shows the following findings. There are poorly developed Graafian follicles with loss of ova [Figure 6]. Irregular follicular antral spaces contain albuminous fluid and inflammatory cells are seen as macrophages, monocytes and lymphocytes [Figure 7].

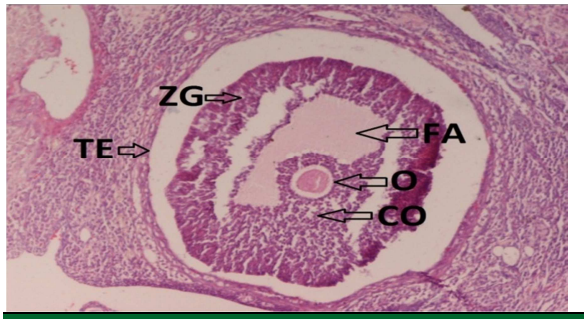

Figure 4: Ovary, Control case 3, H&E× 10.
Mature graafian follicle showing sec. oocyte. [O] eccentrically placed. Fluid filled cavities forming follicular antrum. [FA] Zona granulosa. [ZG] is multiple cell layer at periphery. Cumulus oophorus. [CO] surrounds oocyte. Spindle of theca cells. [TE] at periphery.

There is degeneration of granulosa lutein cells due to inflammation, cells are smaller, shrunken, irregular with vacuolated lighter colour cytoplasm [Figure 8]. Corpora lutea show infiltration by inflammatory cells such as lymphocytes, monocytes, macrophages and also mature lipocytes [Figure 8].

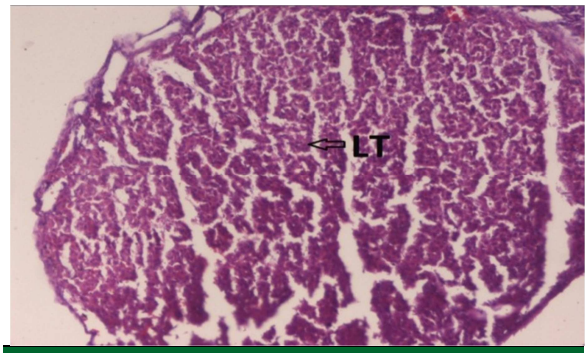

Figure 5: Ovary, Control case 4, H&E× 10.
Corpus luteum shows sheets of polyhedral lutein cells. [LT] with highly eosinophilic granular cytoplasm separated by fat cells.

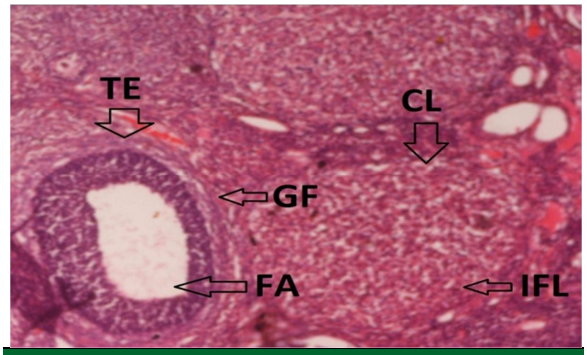

Figure 6: Ovary, Exp. case 1 , H&E× 10.
Poorly developed graafian follicle. [GF] with loss of ovum. Prominent theca external. [TE] & irregular granulosa cell layer, follicular antrum. [FA] Small corpora lutea .[CL] infiltrated by inflammatory cells. [IFL], lipocytes & brown hemosiderin pigment.

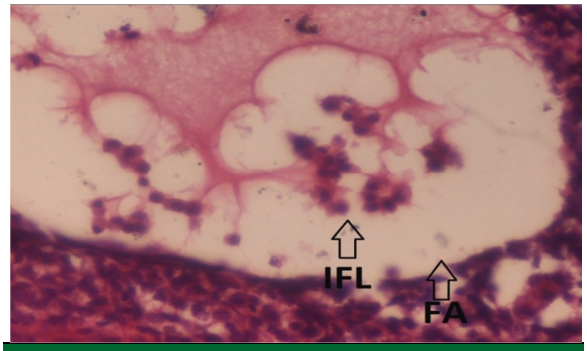

Figure 7: Ovary, Exp. case 3, H&E× 40.
Follicular antrum. [FA] shows albuminous fluid & macrophage, monocyte, lymphocyte as inflammatory infiltrate .[IFL].

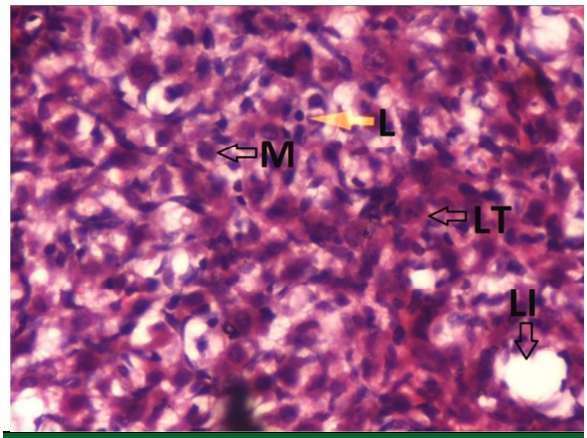

Figure 8: Ovary, Exp. case 4, H&E× 40.
Corpus luteum shows infiltration by lymphocyte. [L], macrophage.[M] & mature lipocytes.[LI]Degeneration of granulose lutein cells. [LT] due to inflammation, cells are smaller irregular with vacuolated lighter colour cytoplasm.

DISCUSSION

In their first experiment, Mizoguchi and Dukelow (1981) noticed inhibition of fertilization in hamster oocytes treated with methotrexate. They found that oocytes were relatively resistant to methotrexate prior to the LH surge but sensitive to the compound after this event. Methotrexate caused defective ova (degenerated or with aberration of chromosomal numbers) at higher dose. This could explain lowered fertility in hamsters treated with methotrexate.[8] In the present experiment, we observed degeneration and defective follicle after methotrexate injection in rat ovary. Our findings were in partial agreement with above experiment.

Cheleb and Majeed (2009) did their experiment on rat using intramuscular injection of methotrexate. In ovary, they showed absence of corpora lutea and reduced Graafian follicles.[6] In the present experiment we studied effects on ovary of rats with intraperitoneal injection .We also observed poorly developed Graafian follicle and abnormal corpus luteum. These findings are in agreement with above experiment.

Gol et al. (2009) studied influence of high dose methotrexate therapy on the primordial follicles of the mouse ovary. They found that primordial follicles were significantly reduced in experimental group.[9] Abnormal follicles were also observed in methotrexate intoxicated rats in our experiment. Uyar et al. (2013) studied effect of single dose methotrexate on ovarian reserve in women with ectopic pregnancy. They found no difference in ovarian reserve in control and experimental groups and concluded that single dose

methotrexate had no effect on ovarian reserve in women with ectopic pregnancy. In the present experiment we studied histopathological changes on ovary after methotrexate injection and observed abnormal Graafian follicle with loss of ovum and abnormal corpus luteum with infiltration by inflammatory cells.[10]

Aforementioned changes can be explained by studying the mechanism of action of methotrexate, which competitively inhibits dihydrofolate reductase. Folic acid is needed for DNA synthesis. Methotrexate acts specifically during RNA and DNA synthesis thus it is cytotoxic during S phase of the cell cycle. It thus inhibits growth and proliferation the cells. Thus, its cytotoxicity probably results in its side effects on many organs of the body including ovary.

CONCLUSION

The drug is safer to be used in those patients who have completed their family.

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How to cite this article: Battan G, Tandon R, Vasenwala SM, Faruqi NA. Effects of Methotrexate on Ovary: An Experimental Study on Albino Rat. Acad. Anat. Int. 2016;2(1):23-7.

Source of Support: Nil, **Conflict of Interest:** None declared.