

Original Article

Histological Changes in Ovary Treated with Dexamethasone and Cefotaxime Sodium

Omairi, S^{1*}, Alyodawi, K¹, Al Qaisi, T²

1. Department of Anatomy and Biology, College of Medicine, University of Wasit, Al Kut, Iraq

2. Department of Pathology and Forensic Medicine, College of Medicine, University of Wasit, Al Kut, Iraq

Received 17 January 2022; Accepted 1 April 2022

Corresponding Author: ssalman@uowasit.edu.iq

Abstract

Dexamethasone (DEX), which is a corticosteroid hormone (glucocorticoid), has been used to treat different conditions, such as immune system disorders, certain skin and eye disorders, as well as breathing problems. Cefotaxime sodium, also called Claforan, is synthesized from a naturally occurring material (semisynthetic). It is a broad-spectrum cephalosporin antibiotic that could be utilized for parenteral administration. The present study aimed to investigate histological changes occurring in the tissues and cells of the rats' ovary (primordial, primary, secondary, antral, and mature follicle) treated with Cefotaxime sodium, as well as DEX, and evaluate the impacts of these medications on animals' fertility. In total, 40 female adult Wistar rats were divided into four groups (n=10). The control group received 0.5 ml/kg of distilled water daily for five days as a placebo. The second group was injected with 0.5 mg/kg of DEX daily for five days. The same amount of Claforan (0.5 mg/kg) was injected into the third group daily for five days, and the fourth group received 0.5 mg/kg of both Claforan and DEX daily for five days. Afterward, the ovaries were prepared for histological examination. The ImageJ image analysis system was used to detect morphometric parameters and calculate the area of these organs. The findings of the present study showed that the DEX and Claforan brought changes to the ovarian area and the number of follicles. The ovarian area significantly increased ($P<0.007$) in the DEX-treated group (mean \pm SEM=7.3 \pm 0.5 mm²), compared to the control group (mean \pm SEM=4.6 \pm 0.20 mm²). However, DEX was found to decrease body weight. Furthermore, the ovarian area significantly increased in the Claforan-treated group (mean \pm SEM=8.6 \pm 0.6 mm²); however, their body weight significantly decreased ($P<0.008$), in comparison with the control and DEX-treated groups. The combination treatment (i.e., DEX + Cefotaxime sodium) significantly increased ($P<0.009$) the area of ovaries even more, compared to single treatments (mean \pm SEM=9.6 \pm 0.4 mm²). Overall, both DEX and Claforan brought histological changes to ovaries. However, the effect of DEX on ovaries was less than that of Claforan. The concurrent administration of both medications was found to have more significant effects on rats' ovaries.

Keywords: Reproductive physiology, Oogonia, atresia

1. Introduction

The development of the female reproductive system is a complex process. The Wolffian, mesonephros, and Müllerian ducts differentiate in different manure to form the uterus and vagina (1, 2). During the ovarian development in mammals, oogonia enter the mitotic division and increase in number. While in the meiosis division, it develops into oocytes, which then enter the

first meiotic prophase, followed by leptotene, pachytene, zygotene, and diplotene. In the diplotene stage, somatic cells surround the oocytes and close off on the follicles. In this stage, the proliferation capability of the oocyte closely diminishes when entering the meiosis division. During the postnatal life, the primordial follicles are reduced progressively through folliculogenesis, atresia, or ovulation. The female

reproductive efficacy has been determined based on the ovarian quality, which is defined by the number of follicles and the factors regulating folliculogenesis (3).

It has been shown that the hypothalamic-pituitary-adrenal (HPA) axis is mainly responsible for the activation of nerve cells in the paraventricular nucleus of the hypothalamus to produce both the corticotropin-releasing hormone and arginine vasopressin. This action will profoundly contribute to the production and secretion of the adrenocorticotrophic hormone (ACTH) by stimulating adenohypophysis (anterior pituitary gland). The ACTH plays a vital role in the secretion and synthesis of adrenal androgens and glucocorticoids (4). It has been observed that the functions of the hypothalamic-gonadal (HAG) axis are mainly mediated by the HPA axis, which induces the maturation of the reproductive organs. Interestingly, the pathway of the HAG axis profoundly promotes the reproductive system through signaling endocrine. It induces Gonadotropin hormone-releasing hormone (GnRH) secretion, which then prompts pituitary gland cells (gonadotroph cells) to synthesize the luteinizing hormone (LH), and follicle-stimulating hormone (FSH), which regulate the steroid hormone production and the ovulation process (5).

The LH and FSH regulate the functioning of female reproductive organs and ovaries. The FSH of growing follicles is responsible for stimulating the proliferation of granulosa cells and the production of estradiol. Moreover, peptide hormones, which are essential for the feedback regulation of FSH, are also produced by growing follicles.

Small follicles are considered responsible for the production of Inhibin B and its high levels during the early follicular phase, while the dominant follicles cause the rising levels of Inhibin A in the later follicular phases. Notably, the remaining reproductive lifespan could be majorly detected depending on the number of follicles, which is defined as the ovarian reserve. Moreover, when follicles start growing, they produce anti-müllerian hormone, which dramatically reduces in

the antral follicle (follicle which contains a fluid cavity and becomes FSH-dependent) (6).

Several previously published studies have reported that dexamethasone (DEX), a synthetic glucocorticoid, has a lower mineralocorticoid activity. It is considered an anti-inflammatory medication that is 30–40 times as potent as hydrocortisone and up to 16 times as strong as prednisolone. Its half-life is about 3 h, while its action duration may be longer. It is bound to plasma proteins at much lower levels than other glucocorticoids (7). Glucocorticoids have a wide range of therapeutic uses for conditions, such as asthma, allergy, infection, as well as autoimmune diseases, including glomerulonephritis, rheumatoid arthritis, sclerosis, Systemic Lupus Erythematosus Gland failure, joint pain, and lymphoid cancers (8).

The DEX is bound to its receptors in the cytoplasm by passing through the cell membrane and entering the cell nucleus through a drug-receptor complex. Through binding with specific areas of the DNA, this complex leads to the stimulation of the Messenger RNA transcription and subsequently, the development of enzymes that are ultimately responsible for the systematic effects of corticosteroids. In the same line of thought, DEX applies its anti-inflammatory effects by preventing the accumulation of inflammatory cells in the inflammation zone, the inhibition of phagocytosis, and the diffusion of enzymes responsible for the inflammation and inhibition of the production and dissemination of chemical mediators relevant to inflammation (8).

On the other hand, it has been revealed that Claforan (Cefotaxime sodium) is a semisynthetic antibiotic with a wide spectrum of activity against Gram-positive and negative bacteria. It has been widely used in the treatment of serious and life-threatening infections, such as pneumonia, gonorrhea, brain abscess, endocarditis, preoperative prophylaxis, and typhoid fever (9).

A series of experiments have shown that hypersensitivity reactions, including skin rashes, urticaria, eosinophilia, and anaphylaxis, are the

most common contrary effects associated with Cefotaxime sodium and other cephalosporins. Other side effects may include diarrhea, vomiting, nausea, pseudomembranous colitis, and transient elevation in liver enzymes (10, 11).

In order to protect female reproductive systems, it is essential to avoid unnecessary exposure to medications that affect oogenesis proliferation and survival, as well as oocytes or follicular formation. Notably, the use of DEX in the period of oogenesis (germinative cell) proliferation might lead to the inhibition of the mitotic division of germinative cells, thereby reducing the overall number of oocytes. On the other hand, the use of Cefotaxime sodium causes tissue irritation, which could result in tissue damage (12). Therefore, the current study was designed to investigate histological changes occurring in the tissues and cells of the rats' ovaries (primordial, primary, secondary, antral, and mature follicle) treated with Cefotaxime sodium, as well as DEX, and evaluate the impacts of these medications on animals' fertility.

2. Materials and Methods

2.1. Study Design

In total, 40 female adult Wistar rats were divided into four groups (n=10). Group 1 (control group) received an Intramuscular (IM) 0.5 mg/kg of normal saline as a placebo for five days. Group 2 was injected with an IM DEX at a dose of 0.5 mg/kg for five days. Group 3 received an IM Claforan at a dose of 0.5 mg/kg for five days. Group 4 was injected with an IM mixture of Claforan and DEX at a dose of 0.5 mg/kg for five days. After the experimental procedure, all animals were euthanized by an overdose injection of Ketamine and Xylazine (König S.A., Avellaneda, Argentina); then, they were dissected and their ovaries were removed for histological evaluations. The histological process was performed in the laboratory of the College of Medicine (University of Wasit, Al Kut, Iraq).

2.2. General Histological Preparations

The specimens were processed as follows:

2.2.1. Fixation

All the specimens were fixed in 10% neutral buffered formalin (BDH) for 24 h.

2.2.2. Dehydration, Clearing, and Embedding

Specimens were passed through a graded series of ethanol alcohols (BDH) (70% for 24 h, 90% for 9 h, and 99% for 2-3 h). Xylene (Merck, USA) was used for a 15-30-min clearing till the specimens became transparent. Afterward, the specimens were transferred to a bath of melted paraffin. Paraffin was used as the Paraplast tissue embedding media, and the samples were placed in an oven (65°C) for about 2 h. Melted paraffin was poured into the metal mold (in an L-shape), and the sample was put in a way that allowed sectioning in a sagittal section. Thereafter, each block was cut at 5 μ by Reichert –Jung 3030 – mot Biocut Microtome (Reichert Technologies) and stained with Harris Hematoxylin and Eosin (H & E stain).

2.2.3. Morphometric Analysis

Microscopes contain a TV-Based computer utilized for image capturing at amplification powers of 10 and 40. Following an accurate calibration using a stage micrometer, the ImageJ analyzer software was used for measurement. This procedure was conducted to determine the area of ovaries and the number of follicles.

2.3. Statistical Analysis

Data were analyzed using the SPSS statistical software (version 19.0, LED Technology, USA) and Microsoft Office Excel 2013. Numeric data were expressed as mean \pm SEM. The One-Way ANOVA was used to compare the results of the control and treated groups. Differences were considered statistically significant when *P*-value was less than 0.05.

3. Results

Light microscopic evaluations revealed that histological sections in the ovaries obtained from the control group were normal. Additionally, numerous ovarian follicles in different stages were detected through the microscopic examination in the control group (Figure 1A).

The recorded data of the present study showed no changes in histological sections from the ovaries treated with DEX (group 2); however, there was an increase in the number of follicles (Figure 1B), in comparison with the control group. Moreover, histological sections of the ovaries treated with Claforan (group 3) showed an increase in the size of the ovary more than in group 2, while the number of follicles decreased (Figure 1C). The ovaries from the fourth group (treated with a mixture of DEX and Claforan) showed a significant increase in size (area) and a dramatic decrease in the number of follicles, compared to groups 2 and 3

(Figure 1D). Table 1 showed a statistically significant increase ($P<0.05$) in the area of ovaries treated with a mixture of DEX and Claforan. The values related to the reproductive parameters are given in table 1. The recorded data regarding the measurement of the ovarian surface area showed statistically significant ($P<0.05$) differences between the control group and Group 2 (0.007), Group 3 (0.008), as well as Group 4 (0.009). The mean \pm SEM of the body weight of animals treated with DEX and Claforan, at doses of 1 mg in five days, was significantly higher ($P<0.05$) than that of the control group (Table 2).

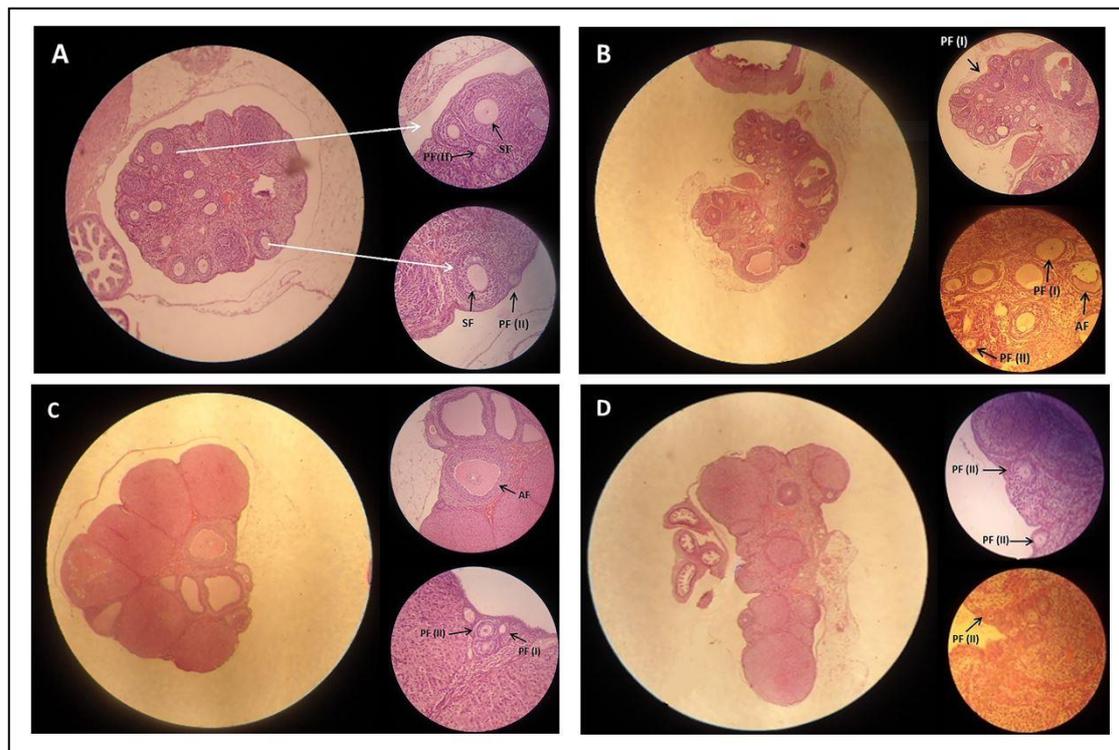


Figure 1. Histological sections of ovaries stained with hematoxylin and eosin were tested under a microscope (4 \times). (A) The control group shows a normal section of ovaries, compared to sections from the treated group. (B) The group treated with DEX shows an increase in the number of follicles and the ovarian area. Sections from group (C) treated with Claforan show a decrease in the number of follicles and an increase in the area of ovaries; however, less than that of group B. Group (D) shows a marked increase in the area of ovaries but a decrease in the number of follicles. Notice: Primordial follicle (PF(I)), the primary follicle (PF(II)), Antral Follicle (AF), and Secondary Follicle (SF).

Table 1. Area measurement of the ovary in the control group and groups treated with Dexamethasone, Claforan, and a mixture of both). Results are shown as mean±SEM

Group		mean±SEM Area mm ²	P-value (ANOVA) Lysergide LSD Area mm ²	P<0.05*	P-value for the control vs. the treated group (ANOVA) Area mm ²
Dexamethasone	Control group	7.3±0.5	0.007		
Claforan	ovary area	8.6±0.6	0.008		0.001
Mix (Dexamethasone+Claforan)	4.6±0.20 mm ²	9.6±0.4	0.009	P<0.05*	

Table 2. Mean±SEM decrease in body weight

Group		mean±SEM body weight (gm)	P-value (ANOVA) LSD body weight (gm)	P<0.05*	P-value for the control vs the treated group (ANOVA) Body weight (gm)
Dexamethasone	Control group	106.25±7.41	0.06		
Claforan	122.5±1.443 body	94.58±6.28	0.07		0.025
MIX (Dexamethasone+Claforan)	weight (gm)	90.66±5.23	0.08	P<0.05*	

4. Discussion

Observations of the present study have shown a number of ovarian histological changes following DEX and Claforan IM injection. Compared to the control group, the treated groups showed that DEX induces the size of ovaries and the number of ovarian follicles, which is concomitant with body weight reduction. However, the Claforan-treated group also experienced an increase in the ovarian size and a reduction in the number of follicles, accompanied by a decrease in body weight. Furthermore, animals treated with a mixture of DEX and Cefotaxime sodium showed an increase in the ovarian area, with a fewer number of follicles.

Previous studies illustrated that the administration of DEX significantly decreased body weight (13). According to Amar, Shama (14) and De Vos, Saladin (15), the metabolism of lipids and carbohydrates could be affected by DEX. Moreover, DEX could induce the sensation of satiety and promote energy expenditure, because it promotes adipose tissues for more leptin secretion. This can be a suitable interpretation of weight loss for subjects treated with DEX for a long time (14, 15). In contrast, Maciel, Chamberlain (16) revealed no impact of DEX injection on body weight. In the same line of thought, DEX would profoundly affect the estradiol action through its direct influence on the pituitary gland. Moreover, FSH-stimulated

follicular steroid production could be induced by DEX. The interaction of DEX with follicular progesterone production and its inhibition by the transforming growth factor- β (TGF- β) indicate that glucocorticoid and the TGF- β interaction are essential for the granulosa cell differentiation as follicle matures through pre-antral stages (17).

Several studies have shown that different anatomical locations, such as the hypothalamus, the anterior pituitary gland, and the ovary, represent the main impacts of DEX. Furthermore, the administration of synthetic glucocorticoids can inhibit the GnRH-stimulated release of LH and FSH from the pituitary by the reduction of hypothalamic GnRH release (18). On the other hand, ovarian steroidogenesis is strongly affected by glucocorticoids. Therefore, the effect of glucocorticoids on the target tissues of gender steroid production could be considered another mechanism of the HPA axis to influence reproductive function (19). Additionally, several studies have shown that a low dose of DEX would improve ovarian responsiveness at the initiation of ovulation cycles (16).

According to de Figueiredo Moraes, Teixeira (20), 10 days of treatment with DEX had no impact on rats' fertility, while extending the period to 15 or more days would result in high maternal mortality. As a result,

both the dose and duration of DEX administration are important factors in the efficiency of its action.

Another investigation has demonstrated that DEX increased the systemic glucose and insulin concentrations, decreased plasma concentrations of insulin-like growth factor-I and II without affecting insulin-like growth factor binding protein levels, did not affect the growth rate of dominant follicles, decreased plasma progesterone concentrations without affecting gonadotropin levels, and had no effect on plasma leptin concentrations (21).

Authors' Contribution

Study concept and design: S. M.

Omairi, S1 *, Alyodawi, K1, Al Qaisi, T

Acquisition of data: S. M.

Analysis and interpretation of data: S. M.

Drafting of the manuscript: K. A.

Critical revision of the manuscript for important intellectual content: K. A.

Statistical analysis: T. A.

Administrative, technical, and material support: T. A.

Ethics

All the study design and experimental procedures had been approved by the Ethics Committee at the Department of Anatomy and Biology, College of Medicine, University of Wasit, Al Kut, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

- Mukherjee S, Sanghamita R, Bhartiya N. Ovarian maldescent: a case report and discussion. *Int J Reprod Contracept Obstet Gynecol.* 2020;9(5):2164-7.
- Wylie L. *Essential Anatomy & Physiology in Maternity Care*: Elsevier Health Sciences; 2005.
- Myers M, Britt KL, Wreford NGM, Ebling FJ, Kerr JB. Methods for quantifying follicular numbers within the mouse ovary. *Reproduction.* 2004;127(5):569-80.
- Anderson R, Themmen A, -Qahtani AA, Groome N, Cameron D. The effects of chemotherapy and long-term gonadotrophin suppression on the ovarian reserve in premenopausal women with breast cancer. *Hum Reprod.* 2006;21(10):2583-92.
- Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids—new mechanisms for old drugs. *N Engl J Med.* 2005;353(16):1711-23.
- Ploner C, Schmidt S, Presul E, Renner K, Schröcksnadel K, Rainer J, et al. Glucocorticoid-induced apoptosis and glucocorticoid resistance in acute lymphoblastic leukemia. *J Steroid Biochem Mol Biol.* 2005;93(2-5):153-60.
- Gupta B. Role of dexamethasone in peri-operative anesthesia management: A review of literature. *Anesthesiol Open J.* 2017;2.
- Grad I, Picard D. The glucocorticoid responses are shaped by molecular chaperones. *Mol Cell Endocrinol.* 2007;275(1-2):2-12.
- Van Zanten A, Oudijk M, Nohlmans- Paulssen M, Van der Meer Y, Girbes A, Polderman K. Continuous vs. intermittent cefotaxime administration in patients with chronic obstructive pulmonary disease and respiratory tract infections: pharmacokinetics/pharmacodynamics, bacterial susceptibility and clinical efficacy. *Br J Clin Pharmacol.* 2007;63(1):100-9.
- Fahmy MA, Diab KA. In vivo genotoxicity studies of cefotaxime. *Cytologia.* 2009;74(4):417-25.
- Rivkin AM. Hepatocellular enzyme elevations in a patient receiving ceftriaxone. *Am J Health-Syst Pharm.* 2005;62(19):2006-10.
- Peker E, Cagan E, Dogan M. Ceftriaxone-induced toxic hepatitis. *World J Gastroenterol.* 2009;15(21):2669.
- Eason JM, Dodd SL, Powers SK. Use of Anabolic Steroids to Attenuate the Effects Glucocorticoids on the Rat Diaphragm. *Physic Ther.* 2003;83(1):29-36.
- Amar M, Shama IA, Enaia A, Hind A, Hager A. Effects of various levels of oral doses dexamethasone (Al-nagma) abused as cosmetic by Sudanese women on Wistar rats. *J Med Sci.* 2013;13(6):432.
- De Vos P, Saladin R, Auwerx J, Staels B. Induction of ob Gene Expression by Corticosteroids Is Accompanied by Body Weight Loss and Reduced Food Intake (*). *J Biol Chem.* 1995;270(27):15958-61.
- Maciel S, Chamberlain C, Wettemann R, Spicer L. Dexamethasone influences endocrine and ovarian function in dairy cattle. *J Dairy Sci.* 2001;84(9):1998-2009.

17. Roy SK, Wang J, Yang P. Dexamethasone inhibits transforming growth factor- β receptor (T β R) messenger RNA expression in hamster preantral follicles: Possible association with NF-YA. *Biol Reprod.* 2003;68(6):2180-8.
18. Lv F, Wan Y, Chen Y, Pei L, Luo D, Fan G, et al. Prenatal dexamethasone exposure induced ovarian developmental toxicity and transgenerational effect in rat offspring. *Endocrinology.* 2018;159(3):1401-15.
19. Lee VH, Lee LT, Chow BK. Gonadotropin-releasing hormone: regulation of the GnRH gene. *FEBS J.* 2008;275(22):5458-78.
20. de Figueiredo Moraes E, Teixeira VW, Teixeira ÁAC, da Silva WE, Batista APC, de Lemos AJJM, et al. Effect of the treatment with dexamethasone, for 10 and 15 days, on the fertility in induced rats to polycystic ovaries, by constant illumination. *Int J Morphol.* 2008;26(3):659-63.
21. Tayade PM, Jagtap SA, Borde S, Chandrasekar N, Joshi A. Effect of *Psoralea corylifolia* on dexamethasone-induced insulin resistance in mice. *J King Saud Univ Sci.* 2012;24(3):251-5.