Morphometric and histopathologic evaluation of the effects of cloprostenol and equine chorionic gonadotropin administration on the reproductive organs of female rats

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Received 10.10.2018

Accepted 04.12.2018

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Summary

The aim of this study was to determine the short-, mid- and long-term effects of cloprostenol (a synthetic analogue of prostoglandin F_{2α}, PG) and equine chorionic gonadotropin (E) administration on reproductive organs (uterine tissue and ovaries) in female rats. Three different groups, PG, E, and control (C), were created, as well as six subgroups of the PG and E groups. After the treatment procedure, reproductive organs were removed surgically 7, 14, and 21 days after the last injection. Morphometric and histopathological changes in tissues were evaluated. It was shown that PG and E had a moderate proliferative effect on epithelial cells and endometrial glands, especially in the mid-term. It was also observed that, regardless of the time of application, some pathological changes can result from hormone administration.

Keywords: cloprostenol, equine chorionic gonadotropin, estrus synchronization, rat

The use of reproductive hormones in animals is frequent in postpartum disorders and infertility, as well as in biotechnology and management of reproduction. In particular, the continuity of breeding of economically valuable animals and special breeds could be ensured through the administration of exogenous hormones. It is recommended that prostaglandin F_{2α} (PGF_{2α}), alone or in combination with a hormone that stimulates follicle development, be used as a luteolytic agent to increase reproductive efficiency and the pregnancy rate. However, this is generally costly and may not produce the expected effect in some cases (11, 27).

Cloprostenol is a potent PGF_{2α} analogue. Therefore, reproduction may be controlled by the lysis of the corpus luteum and by inducing a subsequent follicular

phase with ovulation (27). Equine chorionic gonadotropin, which is a gonadotropic hormone produced in the chorion of pregnant mares, is widely used to obtain a large number of oocytes by the induction of superovulation in many animals (13, 18-21, 25). In mice, eCG and cloprostenol can promote the secretion of FSH and LH (27). However, there is a large variation in response to hormone stimulation even within species (6). Only limited information is currently available about the effects of PG (cloprostenol) and E (equine chorionic gonadotropin) on the development of the reproductive organs in rats.

The aim of the present study was to evaluate morphometrically and histopathologically the effects of the short-, mid-, and long-term PGF_{2α} and eCG administration on the reproductive organs of female rats.

Material and methods

Animals. Forty-two adult female Sprague-Dawley rats weighing 200-250 g were obtained from the Experimental Animal Center at Gazi University (Ankara, Turkey) and quarantined for two weeks. All rats were maintained in accordance with the directions of the Guide for the Care and Use of Laboratory Animals. The present study was approved by the Experimental Animal Ethics Committee of Gazi University (G.Ü.ET-15.076). The animals were housed in polysulfone cages at 21-24°C and 40-45% humidity under light-controlled conditions (12 h light/12 h dark) at the Laboratory of Animal Breeding and Experimental Research Center, Faculty of Pharmacy, Gazi University, and given ad libitum access to food and tap water during the experimental period.

Experimental procedure. The rats were randomly divided into three groups: (i) control group (C, $n = 6$), (ii) cloprostenol group (PG, $n = 18$), and (iii) equine chorionic gonadotropin group (E, $n = 18$). The PG and E groups were randomly subdivided into three subgroups each: PG7 ($n = 6$), PG14 ($n = 6$), PG21 ($n = 6$), E7 ($n = 6$), E14 ($n = 6$), and E21 ($n = 6$). The rats in the C group were intraperitoneally administered saline injection (0.6 ml/rat) on days 0 and 4. The PG7, PG14, and PG21 subgroups were intraperitoneally given 200 µg/kg cloprostenol acetate (PGF Veyx® Forte, Veyx-Pharma GmbH, Schwarzenborn, Germany) on days 0 and 4 (5, 27). The E-7, E-14, and E-21 subgroups were intraperitoneally injected with 30 IU eCG (Folligon® 5×1000 IU, Intervet, Netherlands) on days 0 and 4 (10).

Determination of estrus cycle phases of rats. Vaginal smear samples were taken to determine the estrus cycle before and during the experiment. The samples stained with Giemsa were analyzed under a light microscope. The phases of the estrus cycle were identified as *proestrus* (oval nucleated epithelial cells), *estrus* (irregular-shaped cornified squamous epithelial cells), *metestrus* (fragmented, cornified epithelial cells and smaller darker stained leukocytes), or *diestrus* (nucleated epithelial cells, leukocytes predominate) (7).

Termination of the experimental phase. The rats were sacrificed on day 7 in the subgroups PG7 and E7, on day 14 in PG14 and E14, and on day 21 in PG21 and E21. The longitudinal and transverse diameters of the uterine horns were measured with a micrometer, and uterine tissue volumes were calculated. Both ovaries and uterine tissue of each rat were removed and weighed together on a precision scale. After that, the ovaries were also weighed separately.

Histopathological evaluation. The uterine horns and ovaries were fixed in 10% buffered formalin for 48 hours. The specimens were embedded in paraffin, sectioned at 5 µm, and stained with haematoxylin-eosin. The sections were evaluated under a light microscope (Nikon® Eclipse E600, Japan). Endometrial and myometrial structures in uterine sections were assessed, and their lengths were measured. Furthermore, the numbers of preantral follicles, antral follicles, and luteal cells in every ovary were recorded.

Statistical analysis. The length of uterine layers in the experimental groups and the days were analyzed for statistical significance by Student's t-test and one-way analysis

of variance (ANOVA). The significance of differences in follicle counts in the right and left ovaries during preantral, antral, and luteal periods were performed with Kruskal-Wallis and Mann Whitney U tests. All results were expressed as mean $\pm$ SEM. Differences were considered statistically significant at $p < 0.05$.

Results and discussion

Estrus synchronization is important in enhancing reproductive management, especially in farm animals. The goal of synchronization programs is to achieve estrus onset irrespective of the stage of the reproductive cycle at the initiation of treatment without adversely affecting pregnancy rates after breeding (12). The exogenous hormones most commonly used to manipulate the reproductive function are PGF₂ α , progesterone, GnRH, and eCG (3). All studies on synchronization protocols and hormone administration focus on follicular waves, ovarian functions, hormonal profiles, and adverse effects in the form of ovarian and oocyte degeneration (1, 4, 11, 29). The effects of different synchronization protocols and administration of different hormones have been investigated for decades. However, there is lack of information about the effects of certain hormones on the histopathology of the reproductive tract. In the present study, morphometric and histopathological changes in the rat's reproductive tract related to PGF₂ α and eCG were examined.

The reproductive data for rats synchronized by E and PG are summarized in Table 1. An increase in the combined weight of uterine and ovarian tissues and in the weight of ovaries alone was observed in the E7 and PG7 groups. The weight gain was statistically different from that in other groups. These differences were statistically insignificant in the E14 and PG14 groups. The longitudinal diameters of the left and right uterine horns on day 14 in the E and PG groups were the highest. The longitudinal diameters of uterine horns were statistically different in the E14 group compared with those in the control, E7, and E21 groups. These diameters were also statistically different on day 14 compared with those in the control and PG7 groups. On the other hand, the transversal diameter of uterine horns did not show statistically significant differences. In the reproductive cycle of female rats, the histological appearance of the reproductive tract changes continuously throughout the estrus cycle. Follicular cysts were seen in the ovaries of two animals from the E7 group (Fig. 1) and one animal from the E21 and PG7 groups each. The appearance of ovarian follicles may not be sufficient for the determination of the phases of the estrus cycle, because of the shortness of the cycle. Moreover, the use of corpus luteum morphology to determine the stage of the cycle may not be sufficient either. Therefore, the vagina, cervix, and uterine sections should be evaluated in addition to ovarian section. Besides, certain physiological changes occur in

Tab. 1. Changes in weights and diameters of uterine and ovarian tissues 7, 14, 21 days post-administration

Parameters		Groups	Mean $\pm$ SEM				P value (by days)
			Control (n = 6)	7 th Day (n = 6)	14 th Day (n = 6)	21 st Day (n = 6)	
Weight gain of uterine + ovarian tissues (g)		E	1.10 $\pm$ 0.14 ^a	1.90 $\pm$ 0.09 ^b	1.32 $\pm$ 0.10 ^a	1.25 $\pm$ 0.12 ^a	P < 0.001
		PG		1.41 $\pm$ 0.08 ^b	0.97 $\pm$ 0.05 ^a	1.01 $\pm$ 0.09 ^a	P = 0.015
		P value (by groups)		P = 0.002	P = 0.009	P = 0.153	
Weight gain of ovarian tissues (g)		E	0.82 $\pm$ 0.12 ^a	1.37 $\pm$ 0.05 ^b	0.89 $\pm$ 0.09 ^a	0.80 $\pm$ 0.12 ^a	P = 0.002
		PG		1.12 $\pm$ 0.08 ^b	0.72 $\pm$ 0.05 ^b	0.78 $\pm$ 0.08 ^b	P = 0.021
		P value (by groups)		P = 0.029	P = 0.143	P = 0.890	
Longitudinal diameter of uterine horns (mm)	Right	E	5.17 $\pm$ 0.25 ^a	5.42 $\pm$ 0.44 ^a	6.67 $\pm$ 0.25 ^b	5.67 $\pm$ 0.17 ^a	P = 0.009
		PG		5.38 $\pm$ 0.38 ^a	7.00 $\pm$ 0.37 ^b	6.42 $\pm$ 0.30 ^b	P = 0.002
		P value (by groups)		P = 0.955	P = 0.467	P = 0.054	
	Left	E	5.42 $\pm$ 0.20 ^a	5.17 $\pm$ 0.31 ^a	6.50 $\pm$ 0.13 ^b	5.00 $\pm$ 0.13 ^a	P < 0.001
		PG		5.25 $\pm$ 0.46 ^a	6.67 $\pm$ 0.31 ^b	6.17 $\pm$ 0.28 ^b	P = 0.020
		P value (by groups)		P = 0.883	P = 0.628	P = 0.004	
Transversal diameter of uterine horns (mm)	Right	E	0.22 $\pm$ 0.03 ^a	0.33 $\pm$ 0.03 ^b	0.25 $\pm$ 0.02 ^{ab}	0.18 $\pm$ 0.05 ^a	P = 0.037
		PG		0.27 $\pm$ 0.05 ^a	0.20 $\pm$ 0.03 ^a	0.20 $\pm$ 0.03 ^a	P = 0.486
		P value (by groups)		P = 0.290	P = 0.174	P = 0.765	
	Left	E	0.22 $\pm$ 0.03 ^a	0.35 $\pm$ 0.02 ^a	0.23 $\pm$ 0.02 ^a	0.20 $\pm$ 0.07 ^a	P = 0.065
		PG		0.30 $\pm$ 0.04 ^a	0.20 $\pm$ 0.04 ^a	0.18 $\pm$ 0.05 ^a	P = 0.248
		P value (by groups)		P = 0.341	P = 0.515	P = 0.845	

Explanation: ^{a, b} – groups with different letters in the same line are statistically different

the uterine glands, epithelium, and stromal layer of endometrial tissue during the estrus cycle. A small inflammatory cell infiltration can be observed in the rat's uterus during the proestrus stage, and leukocyte infiltration during the estrus stage (28, 30).

In this study, the histopathological analysis of uterine sections showed mild mononuclear inflammatory cell infiltration in 3 animals in the control group. It was thought that there were leukocyte infiltrations due to the stage of the estrus cycle. This finding was confirmed

by vaginal smear samples and it is in agreement with the studies mentioned above. The E7, E14, and E21 groups showed moderate hyperplasia in uterine surface epithelium and glands. One animal from the E7 group and two animals from the E21 group had extravascular erythrocytes, a limited number of focal mononuclear cell infiltrations, and some hemosiderin-laden macrophages in endometrial propria (Fig. 2). One animal from the E14 group had papillary hyperplasia foci in endometrial epithelium and some spilled epithelial

Fig. 1. E-7 group, 6th animal, follicular cyst in ovary. $\times$ 4 magnification

Fig. 2. Group E-7, 2nd animal; extravasated erythrocytes, hemosiderin-laden macrophages (arrows), and papillary epithelial hyperplasia in uterine endometrium, $\times$ 20 magnification

Fig. 3. E14 group, 4th animal, papillary hyperplasia foci in endometrial epithelium, spilled epithelial cells and neutrophilic inflammatory cells. $\times 10$ magnification

Fig. 4. E-21 group, 3rd animal, narrowing of the uterine lumen with moderate gland hyperplasia (arrows) in the endometrium, $\times 10$ magnification

cells with neutrophilic inflammatory cells within the lumen (Fig. 3). In three animals from group E21, the uterine lumen was remarkably narrowed due to hyperplasia in uterine glands and surface epithelium (Fig. 4). In three animals from the PG7 group, mild hyperplasia was observed in both endometrial epithelium and glands, whereas in the other groups in which $\text{PGF}_2\alpha$ was administered, mild hyperplasia was observed only in the endometrial glands. Two animals in the PG21 group had mild neutrophilic cell infiltration in both the endometrium and the lumen. It was determined that the length of the epithelium, endometrium, and myometrium did not change either in the experimental groups or in the experimental period (Tab. 2).

Prostaglandins, especially $\text{PGF}_2\alpha$, have been acknowledged as critical molecules in regulating the physiology and pathology of the reproductive tract. $\text{PGF}_2\alpha$ regulates the life-span of the corpus luteum (CL) and is called a luteolytic hormone. It also affects myometrial contractility and parturition (12, 23, 24). In the human uterus, $\text{PGF}_2\alpha$ activates the phospholipase C pathway and plays a role in epithelial cell function dur-

ing the proliferative phase of the menstrual cycle (16). Another role of this hormone in endometrial epithelial cells is regulating the ionic composition of the luminal fluid, including Na and K concentrations, and the fluid volume in the uterine lumen (26). In the present study, $\text{PGF}_2\alpha$ was administered to rats irrespective of luteal tissue present in the ovary on the day of administration. The effect of $\text{PGF}_2\alpha$ on the architecture of the reproductive tract was examined. In the PG groups, there was hyperplasia in both uterine glands and epithelial layers. The uterine + ovary weight was significantly higher in the PG7 group than in the control group, but it was similar to that in the E7 group. These results show that $\text{PGF}_2\alpha$ played a proliferative role in epithelial cells and endometrial glands, especially in the PG7 group, and this finding is in accordance with those of Milne and Jabbour (16). An edematous appearance of uterine horns was detected, especially in the rats from the PG7 group. It was therefore concluded that $\text{PGF}_2\alpha$ caused an alteration in the ion concentration of uterine fluid and an increase in luminal fluid, as earlier observed by Vetter and O'Grady (26).

Tab. 2. Length of uterine layers by experimental group and day

Length (μm)	Group	Mean $\pm$ SEM				P values (by days)
		Control (n = 6)	7 th Day (n = 6)	14 th Day (n = 6)	21 st Day (n = 6)	
Epithelium	E	14.37 $\pm$ 1.41	11.54 $\pm$ 1.30	15.56 $\pm$ 0.85	20.91 $\pm$ 4.23	P = 0.069
	PG		16.37 $\pm$ 2.58	17.98 $\pm$ 1.64	20.93 $\pm$ 3.68	P = 0.304
	P values (by groups)		P = 0.126	P = 0.219	P = 0.996	
Endometrium	E	450.87 $\pm$ 83.67	384.85 $\pm$ 16.29	388.18 $\pm$ 41.25	438.48 $\pm$ 40.41	P = 0.730
	PG		459.50 $\pm$ 81.16	466.98 $\pm$ 95.54	484.73 $\pm$ 58.45	P = 0.993
	P values (by groups)		P = 0.388	P = 0.466	P = 0.520	
Myometrium	E	332.40 $\pm$ 27.09	435.28 $\pm$ 40.00	380.95 $\pm$ 12.78	390.95 $\pm$ 38.74	P = 0.183
	PG		405.83 $\pm$ 24.87	360.72 $\pm$ 34.71	385.52 $\pm$ 25.84	P = 0.317
	P values (by groups)		P = 0.625	P = 0.596	P = 0.914	

Explanation: as in Tab. 1

Tab. 3. Number of follicles and luteal structure by experimental group and day

Number of follicles and luteal structures		Group	Median (25%-75% quartile)				P values (by days)
			Control (n = 6)	7 th Day (n = 6)	14 th Day (n = 6)	21 st Day (n = 6)	
Preantral follicles	Left	E	7.5 (4-10) ^a	4 (1.75-4.25) ^b	8 (5.5-10.75) ^a	6 (3.75-6) ^{ab}	0.018
		PG		10.5 (6.5-12.25)	7 (5.75-7.75)	10 (5-10.5)	0.436
		P values (by group)		0.002	0.589	0.589	
	Right	E	5.5 (4.75-7.25) ^a	4 (2.75-5.75) ^b	7.5 (4.75-10.25) ^a	9.5 (7-11.25) ^a	0.015
		PG		11(7.5-12.5) ^b	10.5 (7.25-12.25) ^b	6 (4.5-8) ^a	0.019
		P values (by group)		0.004	0.179	0.180	
Antral follicles	Left	E	7.5 (4-9.25)	4.5 (3.5-6.25)	7.5 (4.5-8.25)	5 (4.5-6.5)	0.256
		PG		7 (5.75-11.5)	5.5 (3.5-10)	7 (4.5-8.5)	0.758
		P values (by group)		0.041	0.818	0.818	
	Right	E	6 (3-7.5) ^a	6 (4-10.25)	7 (5.5-9.75)	8 (7-9.25)	0.395
		PG		9 (6.5-10.5) ^{ab}	10 (7.75-11) ^b	6 (3.5-6) ^a	0.024
		P values (by group)		0.309	0.309	0.310	
Luteal structures	Left	E	4.5 (3-7) ^a	12 (9.75-17) ^b	13 (11.5-16.5) ^b	9 (7.5-10.5) ^{ab}	0.001
		PG		7 (5.75-13.5)	3.5 (1.75-4.5)	4 (2-6.5)	0.038
		P values (by group)		0.132	0.002	0.002	
	Right	E	4.5 (2.75-6) ^a	21.5 (12-25.25) ^b	14 (10.25-20.5) ^b	12 (10.25-15.25) ^b	0.003
		PG		10.5 (5.5-13.25)	4 (2.75-6)	5 (4-5.5)	0.054
		P values (by group)		0.041	0.002	0.002	

Explanation: ^{a, b} – groups with different letters in the same line are statistically different at $p \leq 0.05$

Follicle counts were expressed in median with percentages of 25%-75%. According to the results, especially in the luteal phase, the number of follicles in both right and left ovaries in the experimental groups showed statistically significant changes. It was seen that follicle numbers in the PG group were lower than those in group E on all days. Follicle counts were different between groups during preantral and antral periods on day 7 (Tab. 3).

The usage of eCG in farm animals, rodents, and other species have been studied for a long time, especially to promote the superovulation response of ovaries (2, 9, 14, 27). However, there are some undesirable effects of eCG on ovaries, such as unovulatory and cystic follicles or reduced oocyte and embryo quality, and studies carried out so far revealed only the endocrine profile (15, 17, 22). In the present study, follicular cysts were observed in some rats in the E7 and E21 groups after eCG administration, similar to the above-mentioned studies. In addition, some pathological changes, such as extravasated erythrocytes and hemosiderin pigments, were detected in the uterine tissue of one rat in the E7 group. Hyperplasia in uterine glands and intraluminal fluid were observed in many rats in all eCG groups. Yuan et al. report that eCG may encourage uterine growth (31). Dezhkam and Sadrkhanlou state that the growth of uterine tissue following eCG administration may be associated with the rising estrogen concentration due to the large number of follicles in the ovaries (8). Our findings, such as an increased ovarian follicle

number and uterine hyperplasia and growth, were in accordance with those of Yuan et al. and Dezhkam and Sadrkhanlou (8, 31).

Yuan and Foley suggest that a few generations of corpora lutea from previous ovulatory cycles may be present in the ovary, and each corpus luteum persists for twelve or fourteen days in rats and mice (30). eCG has a half-life of 40 hours, and its action continues for up to 10 days in circulation (9). In the present study, the administration of eCG increased the number of follicles, and the number of corpora lutea was significantly higher than that in the control or PG groups. It was seen that the number of corpora lutea in the E7, E14 and E21 groups remained high for up to 21 days after eCG administration. These results are consistent with previous studies (9, 30) indicating that eCG exerts its effect on ovaries for up to 10 days, and corpora lutea may persist in rats during consecutive cycles.

In conclusion, there are numerous scientific studies and practices regarding estrus synchronization, superovulation, and fertility promotion by means of PGF₂ α and eCG in different animal species. However, despite all these hormonal applications, pregnancy rates are similar to those for natural mating. The results of this study demonstrate that, in addition to ovarian and endocrine changes, more attention should be devoted to the effects of these hormones on the genital tract. Therefore, further studies on farm animals are required to improve their fertility.

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