Effects of steroid hormones on uterine tissue remodeling of mouse menopause model

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ABSTRACT

Since the uterine is a sensitive tissue to steroid hormones, the aim of the present study was to investigate the effects of 17β-estradiol (E2) and progesterone (P4) alone or in combination on morphological and morphometrically parameters of ovariectomized mouse uterus.
Adult virgin female mice (8-10 weeks old)were ovariectomized and treated with E2, P4, E2 followed by P4 and the oil vehicle alone for 5-days period. Uterine tissue was removed, and processed for histology assessment. The total uterine diameter were significantly higher ($P < 0.05$) following E2 treatment and Maximum diameter of uterine lumen, myometrium and endometrium were recorded after this treatment regimen. Sequential treatment with oestradiol and then progesterone caused both mitotic activity and cell degeneration. P4 treatment induced signs of active secretion in the endometrium glands and symptoms of degeneration and cell death. Estradiol treatment induced growth of uterine tissue. Subsequent treatment with progesterone stimulated uterine tissues to reach maximum size and maturity which is necessary to modify the uterus in preparation for pregnancy.

Keywords: Estrogen; Progesterone; Histology; Mice; Uterine.

INTRODUCTION

The uterine is a dynamic tissue that undergoes cycles of proliferation, differentiation shedding (humans and primates) or apoptosis (rodent)which are affected by ovarian sex steroids; 17β-estradiol (E2) and progesterone (P4) during each reproductive cycle[1].
The E2 and P4 cause some molecular and cellular changes in uterine tissue [2] which are mediated by their receptors [3, 4]. Changes in the ratio of E2 to P4 during the oestrous cycle reflected in the patterns of uterine tissue such as cellular proliferation, angiogenesis, extracellular matrix remodelling, cell differentiation [1] and size [3-6]. E2 and P4 prepare the endometrium to establish successful implantation [7]. E2 stimulate epithelial (glandular and luminal) and stromal cell proliferation [8] and induced expression of E2 and P4 receptors [9].WhileP4 is essential for appropriate secretory transformation of the glands and stromal decidualization to facilitate implantation [10]. It has been shown that removal of endogenous E2 and P4 leads to thin atrophic endometrium with few glands, considerably reduced luminal epithelium, dense stroma and apoptosis of estrogen-sensitive cells [11]. Administration ofE2 results in regeneration of the endometrium with proliferation of epithelial and stromal cell, increase the number of glands and stromal edema [11]. Priming mice with E2 followed by P4 treatment induce decidualization which can be reversed by removal P4 [12].
Since the uterine is a sensitive tissue to steroid hormones the present study was undertaken to investigate effects of exogenous E2and P4 alone or in combination inovariectomizedmice on morphologic and morphometric parameters of uterus.

MATERIALS AND METHODS

Reagents

All reagents were purchased from Merck-Germany unless otherwise stated.

Animals and surgical procedures

In this experimental study, adult virgin female National Medical Research Institute (NMRI) mice(8-10 weeks old; n= 45) supplied from Iran Pasteur Institute were cared for and used according to the guide of laboratory animals of our university that fulfils and follows declaration of Helsinki as revised in Tokyo

2004. They were housed under controlled environmental conditions at 24 ± 2°C and relativehumidity of 44 ± 2% with a 12-hour dark/12 hour light cycle (light on at 7:00 AM) and provided with food and water *adlibitum* for at least one week before use. Animals were weighed before and after the experiment.Estrous cycle of animals were determined by evaluation of vaginal smears and those which were on diestrus phase selected for next step[13]. Anesthesia was induced by intraperitoneal injection ofxylazineand ketamine (10 and 100 mg/kg/BW respectively). When the animals ceased to react, they were placed on an operating board. The females were bilaterally ovariectomized by the paralumbal route[14].

Experimental Design

Two weeks after surgery, the mice were randomly distributed into five groups as follow: 1) Control: Ovariectomized mice without any treatment, 2) Sham: Ovariectomized mice treatedwith drug vehicle (40µl; Sigma Aldrich,St. Louis, MO) 3) E2: Ovariectomized mice treated with E2, (8 µg/kg/BW; Sigma-Aldrich,St. Louis, MO) 4) P4: Ovariectomized mice treated with P4, (50 µg/g/BW; Sigma Aldrich,St. Louis, MO) and 5)E2&P4: Ovariectomized micetreated with combination of E2 and P4, which were received E2 (8 µg/kg/BW) on the first day and P4 (50 µg/g/BW) from the second day to the fifth day [15].

Vehicle and route of hormone administration

In the present study, we used sesame oil, as a vehicle for steroid hormones. The vehicle (Sham; 40 µl) or a steroid hormone (40 µl) were injected subcutaneously using a 1 ml glass tuberculin syringe fitted with a 29-gauge needle. Animals were treated for 5 days.

Tissue preparation and histological techniques

The day after the last injection, sampling was carried out on the one-third of the uterinehorns, then were placed in fresh buffered formalin for fixation, washed and dehydrated in an ethanol series of ascending concentration, cleared in xylene, and embedded in paraffin wax.Afterwards, transverse cross-sections of 5 µm in thick were obtained, mounted on microscope slides, after dewaxing with xylene and hydration in an ethanol series of descending concentrations, were stained with hematoxylin and eosin as described previously (RUSSELL WESTWOOD , 2008).

Morphological and morphometrically evolutions

Five slides from different mice within each group and twodifferent fields of each slides were assessed by using a light microscope (Nikon, Japan). The evaluationcriteria on each slide were as follow: total thickness (µm) of the uterine wall, thickness of endometrium and myometrium (µm),thenumbers of stromal cells, endometrial glands, blood vessels, connective tissue and epithelium. For this purpose were evaluated.

Statistical analysis

All data were presented as mean ± SD. Statistical analysis was performed using SPSS-ver.16 software package for windows (SPSS Inc, Chicago, IL, USA). For each analysed variable, data were submitted to normality analysis (*Kolmogorov-Smirnov* test) and One-way analysis of variance (ANOVA) and Tukey's HSD Post Hoc test was used to compare the groups. A significance level of ($P<0.05$) was considered to indicate as statistical difference.

RESULTS

Comparison of mice weight, revealed no significant differences before and after treatment (Table1; $P>0.05$). Uterus of the control group was small and inactive and showed a slit like lumen.Also, compaction of stromal cells was seen.Eosinophilic cytoplasm and variety of nuclear morphology were detectedand. Dead cells were observed sporadically. Some glands with small diameter and the few number of blood vessels was observed in the stroma. Perimetrium layer and circular and longitudinal muscles layers of myometrium were thin. (Figure 2). There was no different change of histological criteria of uterine tissues in shame group as compared with the control group(Figure2). The histology properties of E2 treated group were as follow: The uterus lumenhad multiple folds and decreased space. Also, stromaedema and cellular compaction were observed. There was remarkable degeneration and necrosis of epithelial cells. Mitotic activity was very low.The musculature of the uterus was in several layers. Oblique muscles between the circular and longitudinal muscle layers can be clearly seen. Vasculogenesis of endometrium and myometrium were increased (Figure 2).

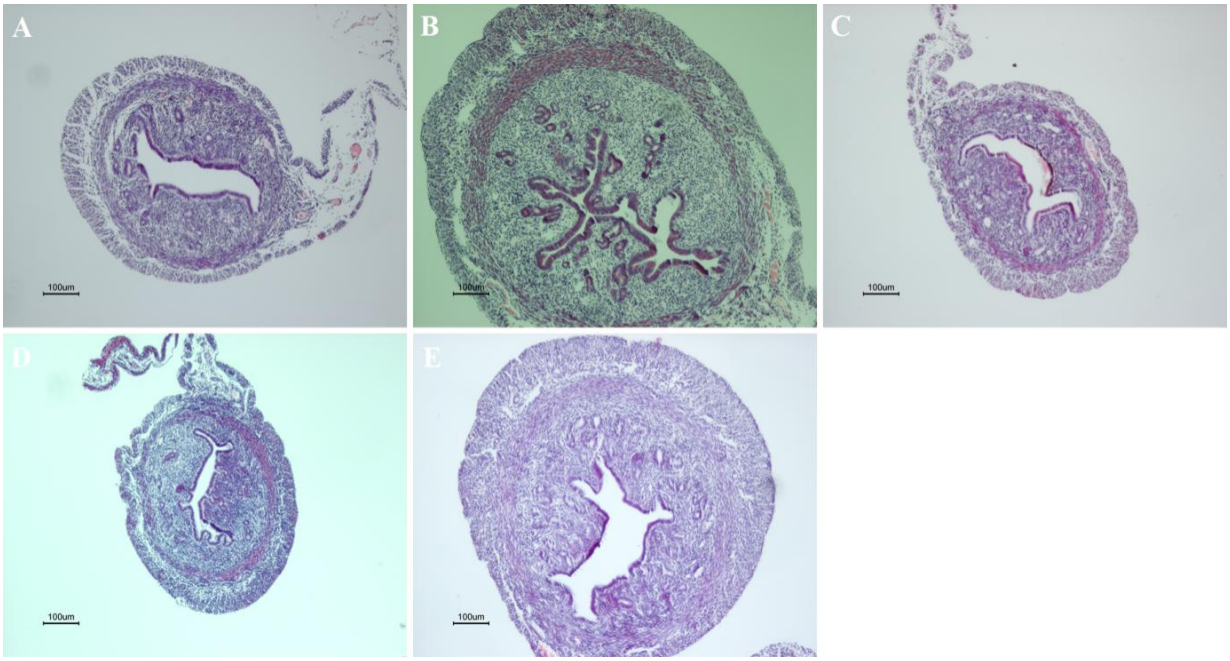

Figure 1: The Photomicrographs of whole uterine tissue in control (A), E2 (B), P4 (C), E2&P4 (D) and oil vehicle(E) treated groups to reveal lumen space and diameter of different part of uterine wall.

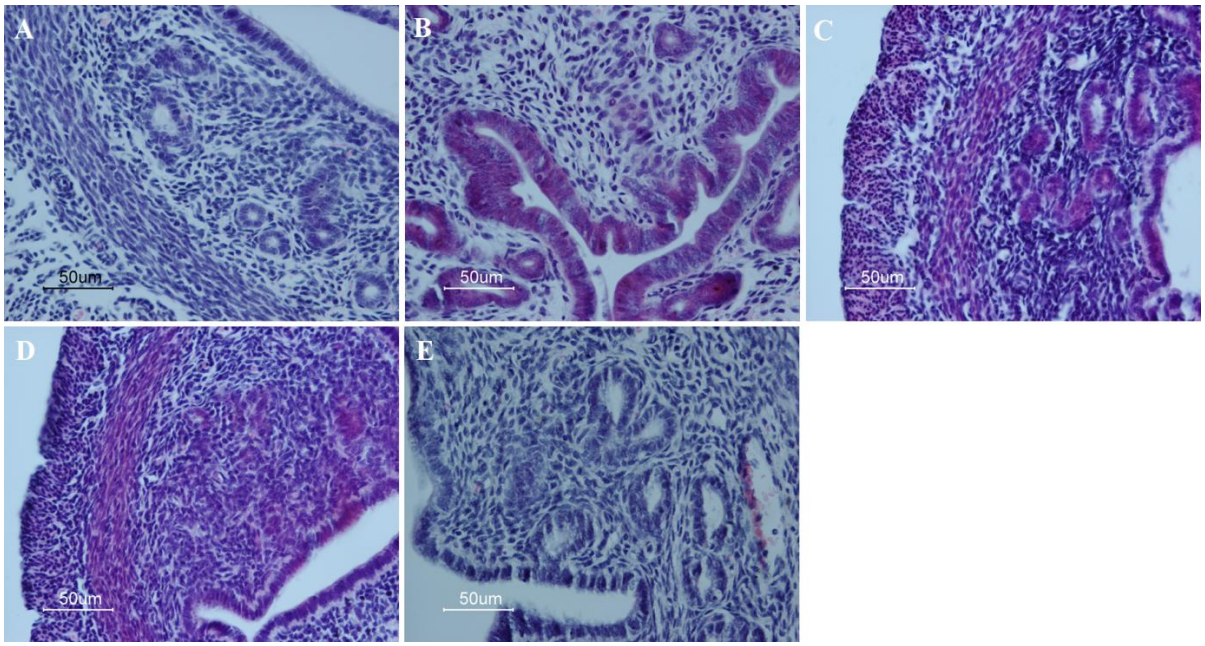

Figure 2: The photomicrographs of uterine tissue cross section in control (A), E2 (B),P4 (C), E2&P4 (D) and oil vehicle(E) treated groups

Table1: Body weights of mices in experimental groups

Group	**BW**(g)	**AW**(g)	**PW**(g)	**SW**(g)
Control	28.55±1.62	28.01±2.092	33.57±1.83^{ab}	33.57±1.83
E2	29.61±2.16	28.98±2.12	33.94±2.17^{a}	33.56±2.04
P4	28.06±2.06	27.19±1.97	32.19±2.06^{ab}	32.07±2.14
E2&P4	28.45±3.71	27.92±3.56	33.13±2.99^{ab}	33.98±2.86
Oil vehicle	29.83±2.09	29.57±1.76	30.88±2.01^{b}	32.38±2.89

Data are expressed as Mean±SD

E2:17β-Estradiol, P4: Progesterone, BW: body weight before of ovariectomized, AW: body weight after ovariectomized, PW: Pre-treatment body weightt ,SW: Post-treatment body weight (before Sacrifice).

Different superscripts in the same column reflect different levels of significant difference ($p<0.05$).

Table2. Diameter of whole and different layers of uterine tissue

Groups	**TD**(µm)	**RLP**(µm)	**LPL**(µm)	**RLE**(µm)	**LEL**(µm)	**RMP**(µm)	**LMP**(µm)	***No of stromal cells***
Control	682.8±51.4[a]	330.4±41.8[a]	276.0±27.9[a]	208.2±27.0[a]	176.5±23.6[a]	115.3±25.6[a]	108.5±21.2[ab]	333.4±47.8[a]
E2	1068.2±38.2[b]	470.9±56.4[b]	510.6±56.6[b]	320.7±59.4[b]	374.6±44.8[b]	151.3±17.3[b]	136.0±15.7[b]	269.7±45.5[b]
P4	626.7±83.4[a]	277.1±58.8[a]	257.3±33.7[a]	185.3±48.8[a]	155.0±28.0[a]	91.2±15.8[a]	102.6±21.5[a]	308.0±30.1[ab]
E2&P4	679.4±129.7[a]	283.9±53.8[a]	312.2±88.[7ac]	180.8±55.0[a]	197.8±81.9[a]	109.4±14.7[a]	115.0±23.8[ab]	423.1±8.7[c]
Oil vehicle	955.4±37.5[c]	425.6±90.5[b]	383.6±70.0[c]	282.3±91.0[ab]	223.8±64.3[a]	162.6±45.1[b]	135.3±27.0[b]	171.7±10.6[d]

Data are expressed as Mean±SD

E2:17β-Estradiol, P4:Progesterone, TD:Total Diameter, RLP:Right Lumen-Perimetrium, LPL: Left Lumen-Perimetrium, RLE: Right Lumen-Endometrum, LEL: Left Lumen-Endometrum, RMP: Right Myometrium-Perimetrium, LMP: Left Myometrium-Perimetrium.

Different superscripts in the same column reflect different levels of significant difference ($p<0.05$).

The P4 treated group histology evaluation showed, small, avascular slit-like lumen. The stromal cellular compaction were similar to control group. Also, there were eosinophilic stromal cells. Endometrial glands with signs of active secretion were seen. Symptoms of degeneration and cell death were observed irregularly. Myometrium layer was thin, the space between the circular and longitudinal layers is narrower and thickness of obliquely muscle layer was reduced (figure 2).Treatment with E2 followed by P4 induced both mitotic activity, cell degeneration andstromal cellular compactionin different layer of uterus whereas, nucleus of cells areeuchromatin and larger than other groups (figure 2).

The total uterine diameters of the E2 and sham groups were significantly increase in comparison to other groups (Table 2; $p < 0.05$). Whereas, there were no significant differences among total uterine diameter of the P4, E2&P4 and control groups.

The diameter of uterine lumen of E2 and sham groups were significantly greater than other groups ($p < 0.05$). The diameters of myometrium in the E2 and sham groups were significantly increase in comparison to other groups (Table 2; $p < 0.05$).

The mean numbers of stromal cells per sections of uterine tissue in all groups were counted and summarized in Table2.The assessment of mean numbers of stromal cells showed that the mean numbers of stromal cells of the E2 &P4 and sham groups was the highest and lowest respectively ($p<0.05$). There was no significant differenceamong the mean numbers of stromal cells of P4, control and E2 groups ($p>0.05$).

DISCUSSION

In the intact mouse the cellular alterations of uterus during the reproductive cycle are regulated by the levels of circulating ovarian sex steroidssuch as E2 and P4[6]. These steroids can affect uterine cell proliferation and tissue remodeling, which are essential for preparation of the uterus to successful implantation and sustainof embryonic development [3, 5]. It seems that the ovariectomized mouse model is appropriate for evaluation of the effect of the exogenous steroid hormones on uterus tissue.

The present study showed that steroid hormones replacement regimen in ovariectomized mouse model affect the uterine morphology and histologyso that, total uterine, endometrium and myometrium diameters in E2treated group was increased significantly than other groups. These results may be due to the effect of E2 on the increase of the number and size of the uterine cells.

It appears that E2 affects uterus initially in the short period (24 h after injection) through the induction the expression of anti-apoptotic genes. However, It has been demonstrated that E2 by increase uterine RNA content and protein lead to an increase in cell size in the long term (48 h after injection) [3]. Also E2, down-regulating the expression of pro-apoptotic genes and cell cycle inhibitors simultaneously, whereas, induce or activate the cell cycle stimulators [10] which in turn, regulates proliferation, growth and endometrial cell survival [16]. It has been shown that the major effect of E2 on uterine size is on tissue hypertrophy (cell size as reflected by RNA/ DNA and protein/ DNA ratios) rather than tissue hyperplasia (changes in DNA content) [3].

It has been shown that E2 affects endometrium with activation of cell genome by its receptor in a target cell nucleus [6], so that, stimulates proliferation of endometrial epithelial and stromal cells through up regulatingproduction and secretion of transforming growth factor α (TGFα), epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) [17]. Although, in the absence of the E2, the division rate of the endometrial cells (luminal and glandular epithelial cells) is very low [18]. It has been shown that effects of E2on uterine atrophied tissues of ovariectomized ewes was greater than normal uterine tissues during the estrous cycle [3].

Results of the present study demonstrate that P4 administration alone or in combination with E2 to ovariectomized mice had no effect on uterine diameter,whereas increased the cellularity of stroma. In this regard, it has been known that E2 induce proliferation of epithelium (luminal and glandular), while P4 reverse this effects directly [1]. It seems that P4 inhibits the proliferative effects of E2 on epithelial cells through induction of proteins secretion from stromal cells]19] which in turn lead to differentiatethe epithelial cells to prepare uterine for implantation [20]. It has been investigated that the anti-Proliferative effect of P4 on endometrial epithelium is mediated via the progesterone receptor-A isoform]21]. P4 changes uterine stromal cells [22], to stimulate predecidual changes around spiral arterioles below the luminal epithelium which in turn prime the uterus to respond to decidualizing stimuli [16]. On the other hand, E2 not only decrease P4 receptor levels in the luminal epithelium but also, increase P4 receptor levels in the stroma and myometrium [23]. Thus, it seems that in the E2&P4 combination treated group, E2 increased expression of P4 receptor in the uterine stroma which in turn provide ideal conditions for P4 to increase stromal cells proliferation [24]. In addition, the results of this study showed, treatment of ovariectomized mice with E2 leads to an increase in the number of small glands with no signs of secretion, while in the P4 treated group the number of enlarged glands with signs of active secretion were increased. It has been known that, E2 have main role in the mediation of gland formation however,the treatment of ovariectomized rat with E2 induce uterine epithelial hypertrophy[1, 15, 18]. Also, in the E2 treated group, diameter of blood vessels ofthe endometrium and myometrium were increased and more distributed than other groups. It has been investigated that E2 can enhance angiogenesis and vasculogenesis through increasing the number of endothelial progenitor cells [25]. On the other hand, P4 increase E2-induced angiogenesis via increase the expression of endothelial nitric oxide synthase [26].

It appears that P4 inhibits expression of E2 receptors of uterine vascular endothelium, which in turn decreases E2-induced angiogenesis [25].

This is in agreement with our results in E2&P4 treated and P4 alone groups which there was no increase of distribution of blood vessels. It seems that both E2 and P4 have paradoxical actions, so that under different conditions, both can enhance as well as inhibit endometrial angiogenesis [27].

CONCLUSIONS

In conclusion, evidence of the present study indicate that E2 induced growth of uterine tissue. While, subsequent treatment with P4 stimulate uterine tissues to reach maximum size and maturity which is necessary to modify the uterus for pregnancy preparation. Also, this detail provide reasonable and practical guidelines for histopathology studies of the effects of steroid hormone on uterine tissue.

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