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RESEARCH ARTICLE

Effect of Exposure to Progesterone During Intrauterine Period on Reproductive System of Female Mouse Morphological and Biochemical Study

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Abstract

Background:progesterone used as a therapeutic agent in human pregnancy. The sufficient evidence to hypothesize that progesterone may have teratogeniceffect on the reproductive system of female offspring.

Objectives: This study was established to explore the individual impact of different doses of progesterone during intrauterine period on reproductive system of female mouse, and on some reproductive parameters.

Materials and Methods:ninety adult female mice (8-10 weeks) and weight (25-28) gm divided into three groups. Control group (G1) was treated daily (IM) injection with sesame seed oilfrom 1-14 day of pregnancy, and other two groups that treated with different doses of progesterone G2 (0.4mg/kg. B.wt/day), G3 (0.8mg/kg. B.wt/day), daily (IM) injection of progesterone from 1-14 day of pregnancy then selecting of Female offspring and sacrificed at age zero day, one month, two month. Parameter analysis involvedanogenital distance , body and ovary weights, , length of epithelial cells, number and diameter of follicles (Primordial , Primary , Secondary and Mature antral) , corpus luteum and using histological section by (motic image plus) and hormonal assay including progesterone and Estrogen.

Results: The results of this study demonstrated that at one month, two months after birth, there was a highly significant increase $P< 0.01$ in theanogenital distance . The statistical analysisshowed no statistical significant difference in the body and ovary weights , at one month after birth, The statistical analysisshowed highly statistically significant decreased($P< 0.01$) in both G2 and G3 in the number of follicles . At one,two months after birth, there was a highly significant decreased ($P< 0.01$) in both G2 and G3 in the number ofcorpora lutea. At age two month, there was a highly significant decreased ($P< 0.01$) in both G2 and G3 in the height of epithelial cell layer.There was a highly significant decreased ($P< 0.01$) in both G2 and G3 in the diameter of follicles (Primordial , Primary , Secondary and Mature antral) follicles . There was a highly significant decreased ($P< 0.01$) in both G2 and G3 in Estrogen and Progesterone levelof female offspring when compared with the control group G1.

Conclusion: The results showed higher doses of progesterone for long period in pregnancy can impact some reproductive parameters of female offspring mice.

Keywords:Progesterone, corpora lutea, Estrogen,anogenital distance

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Introduction:

Progestagen use as a therapeutic agent in human pregnancy. progesteronewas classified in Group 2B, possibly carcinogenic to humans, based on sufficient evidence in experimental animals(1).

Antenatal administration of progesterone reduces the risk of preterm birth before 37 weeks (2), as well as the risk of a newborn being born with a birth weight of less than 2500 g. Progesterone also appears to reduce the risk of preterm delivery in women with a short cervix, but population-wide screening of pregnant women to identify those with a short cervix cannot be recommended until this finding is confirmed(3) (4).

Premature female infants receiving this hormonal treatment demonstrated increased uterine growth and changes in vaginal cytology, indicating that hormonal exposure alters neonatal physiology, cardiovascular malformations, and other birth defects In response to the reports of virilization of female fetuses from pregnancies treated with progestagens(5).However, there are doubts concerning the teratogenic capacity of progesterone specifically in relation to non-genital congenital lesions, prolonged use of OCPs may cause the fetus to develop abnormally, causing problems with the heart(6) ,esophagus, kidney, anus and limbs and in utero exposure to hormonally- active chemicals increase in the incidence of male reproductive tract abnormalities, like cryptorchidism(failure of the testes to descend into the scrotum) and hypospadias(urethral opening along the shaft of the penis(7).

Therefore, the objectives of this study are Detection the effect of using different doses of progesterone by pregnant female mice on the gonadal development of the female offspring and detect the changes histologically and morphometriclly.

Materials and Methods:

Ninety healthy mature female mice (8-10 weeks age), were divided equally into threegroups : two experimental(G1,G2) and control group according to the level of the dose. When females were placed with males the estrous cycle of most females would restart within three days. Both mating and ovulation typically, but not invariably, occur during estrus, which generally occur approximately six hours after the onset of the dark period and last 12-14 hours.

Females were examined daily for a vaginal plug and the finding of a plug was considered as the first day of pregnancy. Pregnant females were then removed into separate cages(8).

Two doses of Progesterone were used: 0.4 and 0.8 mg/kg animal BWfrom day 1 to day 14 of pregnancy.

The drug was administered by IM daily injections. While the control groupanimals received daily IM injections of sesame seed oil from day 1 to day 14 of pregnancy.

Newborn female mice were distinguished from males by noting the greater anogenital distance and larger genital papilla in the male. Newborn females were then divided into 3 groups: 1-animals sacrificed at the age of zero day (N=20) ,2- animals sacrificed at the age of one month (N=20),3- animals sacrificed at the age of two months (N=30).

The anogenital distance were of the female offspring and this can be easily distinguished by external examination of the length between the anus and genital papilla(9). This was done using a ruler and a dissecting microscope.The reproductive organs including the ovary, oviduct and uterus were taken out and cleaned from the surrounding adipose and connective tissues then transferred to a plastic Petri dish containing normal saline. The ovaries and oviducts were separated carefully without any damage (10)and were blotted dry from normal saline using filter paper and weighed with electric precision balance (11).The weight of the right ovary was recorded immediately after the dissection. The ovaries, and uterus were then fixed in formalin (10%) for 24 hours and processed for paraffin histology sections.

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Histological preparation for light microscope was achieved according to the following procedure (12):

Every section was photographed and the degree of follicle development was assessed counting only the follicles in which the nucleus of the oocyte was visible(13). . Because each ovary yielded about 140 sections, we counted about 50 sections per ovary(14). For each follicle, the mean of two diameters, one perpendicular on the other, was measured using TV-Based -computer assisted microscope with Morphometry(figure 3.8) .The actual measurements were done using the image analyzer software, Motic Image. Measurement was from the outermost to the innermost layers of granulosa cells(15).Progesterone and E2 were measured in individual serum samples from two month-old animals using mouse Elisa kits. Mice were anesthetized, and blood was collected by cardiac puncture.

Results:

Daily administration of either 0.4 mg/kg BW or 0.8 mg/kg BW of progesterone to pregnant mice from day one to day fourteen of gestation caused the following changes in their female offspring at day zero, one month and two months after birth.

A-Morphological changes

1-The anogenital distance changes:

Just after birth, at day zero, there was a non significant increase in G2 and a significant increase ($P<0.05$) in G3 in the anogenital distance of female offspring when compared with the control group G1. At one month after birth, there was a highly significant increase $P< 0.01$ in both G2 and G3 in the anogenital distance in comparison with the control G1. At two months after birth, there was a highly significant increase ($P< 0.01$) in both G2 and G3 in the anogenital distance in comparison with the control G1(Table1)(Figure1).

2-Weight changes

Just after birth, at day zero, there was no significant differencein G2 and G3 in the weight of female offspring when compared with the control group G1. At one month after birth, there was a no significant difference in both G2 and G3 in the weight in comparison with the control G1. At two months after birth, there was a no significant difference in both G2 and G3 in the weight in comparison with the control G1(Table 2) (Figure 2).

3-Ovarian weight changes

Just after birth, at day zero, there was no statistical significant differencein both G2 and G3 in the ovarian weight of female offspring when compared with the control group G1. At one month after birth, there was a no significant difference in both G2 and G3 in the ovarian weight in comparison with the control G1. At two months after birth, there was a no significant difference in both G2 and G3 in the ovarian weight in comparison with the control G1(Table 3) (Figure 3).

B-Histological changes:

1- Number of follicles

Just after birth, at day zero, the statistical analysisshowed no significant differencein both G2 and G3 in the number of follicles of female offspring when compared with the control group G1. At one month after birth, there was a highly significant decrease($P<0.01$) in both G2 and G3 in the number of follicles in comparison with the control G1. At two months after birth, there was a no significant difference in both G2 and G3 in the number of follicles in comparison with the control G1(Table 4) (Figure 4).

2- Number of corpora lutea

At one month after birth, there was a highly significant decrease ($P<0.01$) in both G2 and G3 in the number ofcorpora lutea of female offspring when compared with the control group G1. At two months after birth, there was a highly significant decrease($P<0.01$) in both G2 and G3 in the number of corpora luteaof female offspring when compared with the control group G1(Table 5) (Figure 5).

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3-Lumenal epithelium height of uterus

At age two months, there was a highly significant decrease (P< 0.01) in both G2 and G3 in the height of epithelial cell layerof the uterus of female offspring when compared with the control group G1(Table 6) (Figure 6).

4-Diameter of follicles and corpora lutea of female offspring two months old

There was a highly significant decrease (P< 0.01) in both G2 and G3 in the diameter of primordial follicles of female offspring when compared with the control group G1. There was a highly significant decrease (P< 0.01) in both G2 and G3 in the diameter of primary follicles of female offspring when compared with the control group G1.There was a highly significant decrease (P< 0.01) in both G2 and G3 in the diameter secondary of follicles of female offspring when compared with the control group G1.

There was a highly significant decrease (P< 0.01) in both G2 and G3 in the diameter of antral of follicles of female offspring when compared with the control group G1. There was no significant difference in both G2 and G3 in the diameter of corpora lutea in comparison with the control G1(Table 7) (Figure 7).

C-Hormonal assay

At the age of two months, there was a highly significant decrease (P< 0.01) in both G2 and G3 in estrogen level of female offspring when compared with the control group G1. And, there was a highly significant decrease (P<0.01) in both G2 and G3 in progesterone level of female offspring when compared with the control group G1(Table 8) (Figure 8).

Table (1):Anogenital distance

Parameter	Group 1 (control)				Group 2 (0.4 mg/kgBW)				Group3 (0.8 mg/kgBW)			
	N	Mean	SD	SE	N	Mean	SD	SE	N	Mean	SD	SE
Day zero	21	0.495	0.059	0.013	21	0.800	0.055	0.012	21	2.286	3.185	0.695
One month	21	2.022	0.063	0.014	21	3.999	0.084	0.018	21	5.005	0.074	0.016
Two months	21	3.991	0.054	0.012	21	5.991	0.054	0.012	21	7.000	0.089	0.020

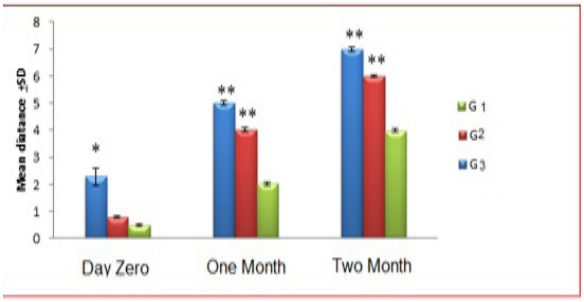

Figure 1: Anogenital distance means ± SD. G1 control, G2 0.4mg/kgBW progesterone, G3 0.8mg/kgBW progesterone.
NS= no statistical significant difference.
*=Statistically significant difference (P<0.05).
** =Highly statistically significant difference (P< 0.01).

Table(2):Weight changes

Parameter	G1 (control)				G 2 (0.4 mg/kgBW)				G 3 (0.8 mg/kgBW)			
	N	Mean	SD	SE	N	Mean	SD	SE	N	Mean	SD	SE
Animal weight day zero	20	1.091	0.336	0.075	20	1.188	0.208	0.046	20	1.250	0.243	0.054
Animal weight one month	20	10.495	1.430	0.320	20	10.635	1.054	0.236	20	10.215	1.601	0.358
Animal weight two month	25	26.360	1.523	0.305	25	26.256	1.676	0.335	25	25.980	1.712	0.342

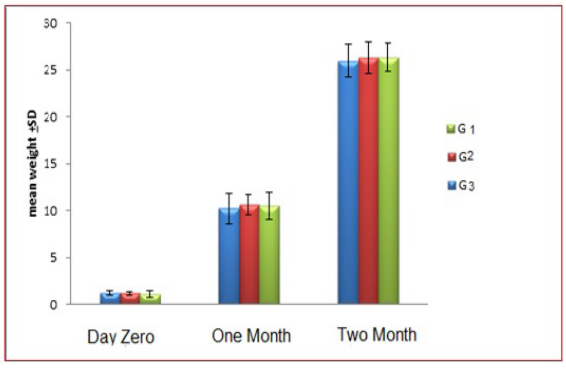

Figure 2: Weight changes means ± SD. G1 control, G2 0.4mg/kgBW progesterone, G3 0.8mg/kgBW progesterone.
NS= no statistical significant difference.
*=Statistically significant difference (P<0.05).
** =Highly statistically significant difference(P< 0.01).

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Table (3):Ovarian weight changes

Parameter	G1 (control)				G 2 (0.4 mg/kgBW)				G3 (0.8 mg/kgBW)			
	N	Mean	SD	SE	N	Mean	SD	SE	N	Mean	SD	SE
Ovarian weight Day zero	19	0.013	0.001	0.000	19	0.013	0.001	0.000	19	0.013	0.001	0.000
Ovarian weight one month	19	0.026	0.002	0.000	19	0.027	0.002	0.000	19	0.027	0.002	0.000
Ovarian weight two month	29	0.038	0.000	0.000	29	0.038	0.001	0.000	29	0.038	0.001	0.000

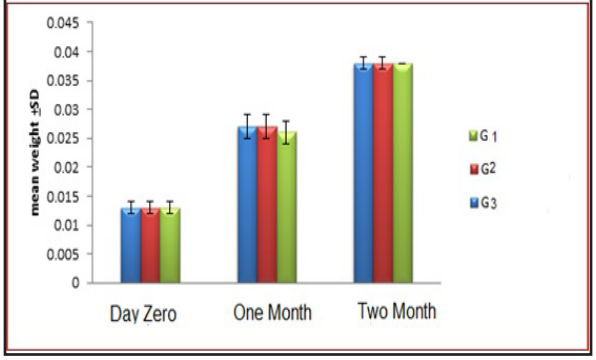


Figure (3): Ovarian weight changes means ± SD. G1 control, G2 0.4mg/kgBW progesterone, G3 0.8mg/kgBW progesterone.
NS= no statistical significant difference.
*=Statistically significant difference (P<0.05).
**=Highly statistically significant difference (P< 0.01).

Table (4) :number of follicles

Parameter	G1 (control)				G 2 (0.4 mg/kgBW)				G3 (0.8 mg/kgBW)			
	N	Mean	SD	SE	N	Mean	SD	SE	N	Mean	SD	SE
Number of follicles zero day	21	5533.300	109.615	23.920	21	6071.200	8283.278	1807.560	21	4045.500	24.091	5.257
Number of follicles one month	21	4046.400	47.409	10.346	21	3174.500	282.518	61.650	21	2357.600	20.346	4.440
Number of follicles two month	21	1897.800	49.921	10.894	21	1405.500	51.596	11.259	21	1821.400	2657.961	575.650

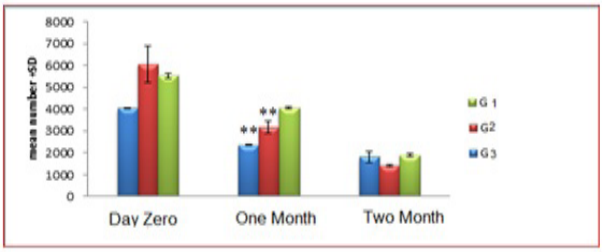
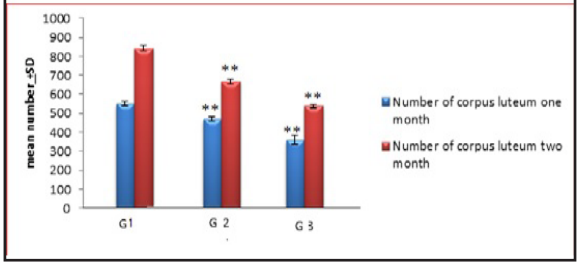


Figure 4: Number of follicles means ± SD. G1 control, G2 0.4mg/kgBW progesterone, G3 0.8mg/kgBW progesterone.
NS= no statistical significant difference.
*=Statistically significant difference (P<0.05).
**=Highly statistically significant difference (P< 0.01).

Table (5):Number ofcorpora lutea

Parameter	G1 (control)				G 2 (0.4 mg/kgBW)				G 3 (0.8 mg/kgBW)			
	N	Mean	SD	SE	N	Mean	SD	SE	N	Mean	SD	SE
Number of corpus luteum one month	18	550.150	13.563	3.197	18	469.720	13.856	3.261	18	371.160	21.844	5.149
Number of corpus luteum two month	18	842.650	16.360	3.856	18	666.900	10.759	2.536	18	549.240	9.088	2.142



Figure(5): Number of corpora lutea, means ± SD. G1 control, G2 0.4mg/kgBW progesterone, G3 0.8mg/kgBW progesterone.
NS= no statistical significant difference.
*=Statistically significant difference (P<0.05).
** =Highly statistically significant difference (P< 0.01).

Table (6):Lumenal epithelium height of uterus

Parameter	G1 (control)				G 2 (0.4 mg/kgBW)				G3 (0.8 mg/kgBW)			
	N	Mean	SD	SE	N	Mean	SD	SE	N	Mean	SD	SE
height of epithelial cell layer	19	156.580	13.749	3.154	19	138.950	5.104	1.171	19	138.950	5.104	1.171

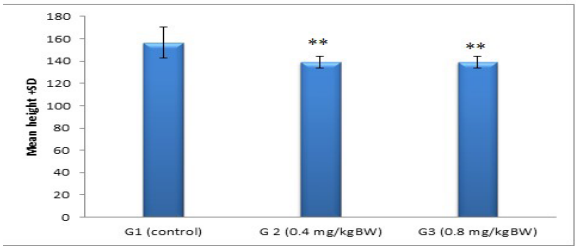


Figure (6):Lumenal epithelium height of uterus
NS= no statistical significant difference.
*=Statistically significant difference (P<0.05).
** =Highly statistically significant difference (P< 0.01).

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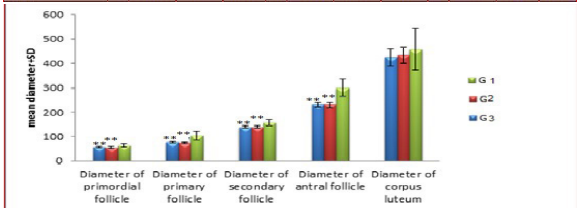
Table (7): Changes in the diameter offollicles

Parameter	G1 (control)				G 2 (0.4 mg/kgBW)				G 3 (0.8 mg/kgBW)			
	N	Mean	SD	SE	N	Mean	SD	SE	N	Mean	SD	SE
Diameter of primordial follicle	19	64.737	5.394	1.237	19	55.790	4.171	0.957	19	57.105	3.843	0.882
Diameter of primary follicle	19	103.260	18.064	4.144	19	74.684	3.181	0.730	19	75.158	3.202	0.735
Diameter of secondary follicle	19	156.580	13.749	3.154	19	138.950	5.104	1.171	19	138.950	5.104	1.171
Diameter of antral follicle	19	299.530	35.414	8.125	19	229.740	11.488	2.636	19	230.680	9.604	2.203
Diameter of corpus luteum	19	457.370	83.017	19.228	19	433.160	33.175	7.611	19	424.470	35.154	8.065



Figure(7): Changes in the diameter offollicles means ± SD. G1 control, G2 0.4mg/kgBW progesterone, G3 0.8mg/kgBW progesterone. NS= no statistical significant difference. *=Statistically significant difference (P<0.05). ** =Highly statistically significant difference (P< 0.01).

Table (8): Changes in hormonal levels (progesterone and estrogen)

Parameter	G1 (control)				G 2 (0.4 mg/kgBW)				G3 (0.8 mg/kgBW)			
	N	Mean	SD	SE	N	Mean	SD	SE	N	Mean	SD	SE
Estrogen	30	178.930	5.166	0.943	30	97.200	3.388	0.618	30	42.333	3.377	0.617
Progesteron	30	16.133	0.287	0.052	30	18.117	0.210	0.038	30	17.153	0.185	0.034

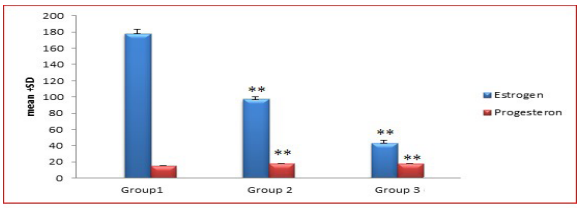


Figure (8): Changes in hormonal levels (progesterone and estrogen) means ± SD. G1 control, G2 0.4mg/kgBW progesterone, G3 0.8mg/kgBW progesterone.

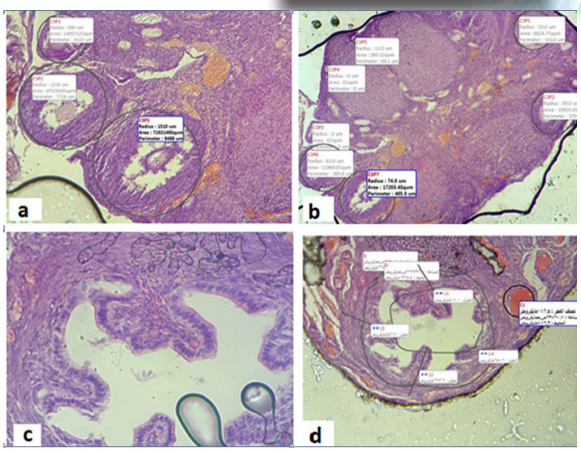


Figure (9): Paraffin sections of 2 month old female offspring, showing measurements for morphometry. a: ovary, control, X 40. b: ovary, progesterone 0.4mg/ kg BW, X 40. c: uterine tube, progesterone 0.8mg/kg BW, X 100. d: uterine tube, control, X 40.

Discussion:

Environmental and pharmaceutical compounds differentially impact the organs individually and/or the system in its entirety in a time- and dose-dependent way(16). The age of an animal at the time of exposure to an endocrine disruptor has considerable importance in termining the extent of detrimental effects detected in the adult, because age is inversely related to the magnitude of effects on development and organization of aspecific tissue(17),(18). We used the anogenital distance to detect the virilizing effect of progesterone on female offspring when the drug was given to their mothers during pregnancy. Our results proved the virilizing effect of progesterone by showing that when the drug was given at 0.8mg/kg BW there was a significant increase in the anogenital distance at zero day, and when given at 0.4mg/kgBW and at 0.8mg/kgBW there was a highly significant increase in the anogenital distance one month and two months after birth.

Progesterone has been shown to inhibit 5 α-reductase, another important enzyme in steroid hormone metabolism (19).

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Inhibition of 5α-reductase resulted in decreased conversion of testosterone to DHT, leading to increased testosterone (20), which caused masculinization of the internal and external genitalia.(21)

An analysis of the human reports and comparable experimental data suggest that the fetal effects of these agents are not analogous to their effects on the end organs in the postnatal animal. . In a second study that

Our results showed no significant difference in weight of female offspring in both G2 and G3 at zero, one month and two months when compared with the control group G1, nor was there change in weights of ovaries in treated groups. In human and mice, cholesterol and steroid transfer was shown to affect correct regulation of fetal growth and development(22),(23) and mouse fetuses were shown to be dependent on transfer of maternal cholesterol throughout gestation for normal development, much more than the human(24)(25).

Concerning the number of ovarian follicles, we found no significant difference in progesterone group at day zero but at one month there was a highly significant decrease (P< 0.01) in both G2 and G3 in comparison with the control G1.

It has recently been revealed that a female-specific molecular program was in progress in embryonic female gonads as early as E11.5 (26).The ovary was relatively insensitive to gonadotropins during the first week of postnatal life and maintenance of follicular growth depended on continuous exposure to gonadotropin (27).

Two months after birth, there was a no significant difference in both G2 and G3 in the number of follicles in comparison with the control G1. In support for this proposal recent reports have suggested that progesterone might play a role in the ovulatory process by regulating ovarian proteolytic enzymes involved in the digestion of the collagenous connective tissue of the follicular wall thereby resulting in follicular rupture and release of the oocyte (28).

Our results showed that the number of CL were highly significantly decreased (P<0.01) after the administration of 0.4mg/ kg.B.wt/ day or double dose of progesterone at age one or two months.

CL is a transitory ovarian structure that arises from the ovulated follicle, and its main function being the secretion of progesterone, which is needed for blastocyst implantation and pregnancy maintenance (29). Inappropriate exposure of the female reproductive tract to steroids during developmentally critical periods can disrupt the organizational events necessary for growth and differentiation, leading to altered adult phenotypes, and decrease reproductive efficiency (30),(31).

At age two months, there was a highly significant decrease (P< 0.01) in both G2 and G3 in the height of epithelial cell layer of the uterus of female offspring when compared with the control group G1, together with a highly significant decrease (P< 0.01) in both G2 and G3 in Estrogene level of female offspring when compared with the control group G1, as shown by our results. The decrease of estrogen level might be the cause for the change in the morphology of epithelial cell layer, since it has been found that estrogen strongly increased proliferative activity in uterine tissues (32), (33) and changed the type of luminal and glandular epithelia and the morphology of epithelial cells (34).

There was a highly significant decrease (P< 0.01) in both G2 and G3 in the diameter of primordial, primary and secondary antral follicles of female offspring when compared with the control group G1. The cause for this change in diameter may be due to the role played by progesterone on signal transduction pathway (35).

The endometrium is the site of embryo implantation and its preparation for embryo reception is dependent on the estrogen and progesterone. These hormones cause some molecular and cellular events during uterine receptivity and the balance between them is important for cyclical changes of endometrium (36).

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There was a highly significant decrease (P< 0.01) in both G2 and G3 in the diameter of primordial, primary, secondary antral follicles of female offspring when compared with the control group G1.

progesterone can play a role in signal transduction pathways (37).

Our results showed a highly significant decrease (P< 0.01) in both G2 and G3 in estrogen levels of female offspring when compared with the control group G1. This decrease in estrogen level may be due to the decrease in follicles size shown in our results, and since a direct relationship between follicle size and the concentration of estradiol has already been found (38).

Our results showed a highly significant decrease (P< 0.01) in both G2 and G3 in progesterone level in female offspring when compared with the control group G1.

This may be due to the decrease in number of corpora lutea shown in our results, since the development of the corpus luteum was accompanied by an increase in the level of the steroidogenic enzyme P450scc that converted cholesterol to pregnenolone which was in turn converted to progesterone(39).

Conclusion: The results showed higher doses of progesterone for long period in pregnancy can impact some reproductive parameters of female offspring mice.d as a transcription

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