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PROGESTERONE ADMINISTRATION DURING PREGNANCY IS EMBRYO-TOXIC.

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ABSTRACT

Background & objectives: Progesterone is a therapeutic agent in human pregnancy but its effects on the developing embryo and fetus have long been a concern. We aimed to find any changes in the morphology of the reproductive system and in blood levels of estrogen and progesterone of females mice exposed to progesterone during intrauterine life. **Methods:** Ninety adult pregnant mice were divided into three groups and injected daily with two different doses of progesterone for fourteen days: G1, control group: sesame seed oil; G2: progesterone at a dose of 0.4 mg / kg body weight (BW) / day and G3: progesterone at a dose of 0.8 mg /kg BW / day. New borne females were sacrificed at zero day, one month and two months of age. Morphology and morphometry of reproductive system were studied and hormonal assay was performed. **Results:** At one and two months after birth, there was a highly significant increase ($P < 0.01$) in the anogenital distance and a highly significant decrease in the number of corpora lutea. At one month after birth there was a highly significant decrease in the number of ovarian follicles. At the age of two months, there was a highly significant decrease in the height of epithelial cell layer and a highly significant decrease in the diameter of ovarian follicles. There was a highly significant decrease in estrogen and progesterone levels. All changes were in both G2 and G3. **Interpretation & conclusions:** The administration of progesterone during pregnancy has adverse effects on certain aspects of the reproductive system of female embryos indicating that progesterone is embryo toxic.

KEYWORDS: Anogenital distance, morphometry of ovarian follicles, progesterone teratogenicity.

INTRODUCTION

Progesterone is essential for maintenance of pregnancy in women and is responsible for the development of a healthy endometrium that is necessary for pregnancy.[1] The antenatal administration of progesterone reduced the risk of preterm birth before the thirty seventh week.[2],[3] Effects on the developing embryo and fetus due to maternal progestagen exposure during pregnancy have long been a concern in human medicine. End points that have been investigated in human and animal studies included altered genitalia in both the male and female fetus, cardiovascular malformations, changes in vaginal cytology, esophagus, kidney, anus and limbs.[4] In utero exposure to hormonally-active chemicals increased 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).[5]

In this work we aimed to explore the effect of progesterone at different doses on the reproductive system and on blood levels of estrogen and progesterone of mice females when administered to their mothers during intrauterine life.

MATERIAL AND METHODS

Ninety healthy mature female mice (BALB/C strain) were used. They were obtained from the animal house of the High Institute of Infertility Diagnosis And Assistant Reproductive Technology (HIID & ART) / Al-Nahrain University, Baghdad, Iraq. The animals were used according to the general guidelines of laboratory animals (Iraqi general health law, experimental protocol section, 1981) and according to the protocol of the Laboratory Animal Center of Baghdad University, Iraq. The experimental protocol was approved by the HIID & ART / Al-Nahrain University, Baghdad, Iraq, in 2/10/2012. In the animal house, the temperature was controlled between 22-24^0 C and 12 hours light and 12 hours darkness. The animals were housed in plastic cages; 3mice per cage, with wire grid covers measuring 28 x 15x14 cm supported on ventilated racks.

Two doses of progesterone were used: 0.4 and 0.8mg / kg animal body weight (BW) at daily intramuscular injections[6] from day one to the day fourteen of pregnancy. Progesterone solution was prepared by dissolving progesterone vial (50mg) in sesame seed oil.[7] The female mice were divided into three groups: group one (G1), control group, was injected with

sesame seed oil, group 2(G2) was injected with 0.4 mg / kg animal BW of progesterone and group 3 (G3) was injected with 0.8 mg / kg animal BW of progesterone. Newborn females were then divided into 3 groups: Animals sacrificed at the age of zero day (n: 20), animals sacrificed at the age of one month (n: 20) and animals sacrificed at the age of two months (n: 30).

Measurement of the anogenital distance

To find the masculinizing effect of progesterone we measured the anogenital distance of the newborn females.[8] This was done using a ruler and a dissecting- microscope.

Tissue processing for histology

The weight of the right ovary was recorded immediately after dissection. The ovary and uterus were then fixed in 10% formalin, tissue processed, embedded in paraffin and stained with hematoxyllin and eosin for histological examination as described before [(9), (10).]

Morphometry

Every section was photographed and follicle development was assessed by counting only the follicles in which the nucleus of the oocyte was visible[11] and by counting the total number of follicles per ovary. For each follicle, the mean of two diameters, one perpendicular on the other was measured. The height of epithelial cell layer in the endometrium was measured by using software Motic image plus.

Morphometric analysis

For morphometric analysis, images were captured using TV-Based -computer assisted microscope with morphometry and 10X and 4X objectives. The actual measurements were done using the image analyzer software, Motic Image, after accurate calibration using a stage micrometer.

Hormonal assay

Estrogen and progesterone assay was performed by ELISA, based on the principle of a solid phaseenzyme-linked immunosorbent assay.[12], [13]

Statistical analysis

Data were analyzed using Microsoft Office Excel (2007). Numeric variables were expressed as mean, standard deviation (SD) and standard error (SE). Nominal data were expressed as numbers. Independent sample t-test was used to compare means of two groups.[14]

Differences between groups were considered highly significant at ($P < 0.01$), significant at ($P < 0.05$) or non- significant at ($P > 0.05$).

RESULTS

At one month and two months after birth there was a highly significant increase ($P < 0.01$) in the anogenital distance for both G2 and G3 in comparison with the control G1 (Table 1).

Table1: The anogenital distance.

Parameter	G 1 (control)				G 2 (0.4 mg / kg BW)				G 3 (0.8 mg / kg BW)			
	N	Mean mm	SD	SE	N	Mean mm	SD	SE	N	Mean mm	SD	SE
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

At one month after birth, there was a highly significant decrease ($P < 0.01$) in the number of follicles for both G2 and G3 in comparison with the control G1 (Table 2).

Table 2: Number of follicles at one month after birth.

Parameter	G1 (control)				G 2 (0.4 mg / kg BW)				G3 (0.8 mg / kg BW)			
	N	Mean	SD	SE	N	Mean	SD	SE	N	Mean	SD	SE
Number of follicles	21	4046.400	47.409	10.346	21	3174.500	282.518	61.650	21	2357.600	20.346	4.440

At one and two months after birth, there was a highly significant decrease ($P < 0.01$) in the number of corpora lutea for both G2 and G3 in comparison with the control G1 (Table 3).

Table 3: Number of corpora lutea

Parameter	G1 (control)				G 2 (0.4 mg / kg BW)				G 3 (0.8 mg / kg BW)			
	N	Mean	SD	SE	N	Mean	SD	SE	N	Mean	SD	SE
Number of corpora lutea at one month	18	550.150	13.563	3.197	18	469.720	13.836	3.261	18	371.160	21.844	5.149
Number of corpora lutea at two months	18	842.650	16.360	3.856	18	666.900	10.759	2.536	18	549.240	9.088	2.142

At the age of two months, there was a highly significant decrease ($P < 0.01$) in the height of epithelial cell layer of the endometrium for both G2 and G3 in comparison with the control G1 (Table 4).

Table 4: Lumenal epithelial height in endometrium.

Parameter	G1 (control)				G 2 (0.4 mg / kg BW)				G3 (0.8 mg / kg BW)			
	N	Mean µm	SD	SE	N	Mean µm	SD	SE	N	Mean µm	SD	SE
Height of epithelial cells	19	156.580	13.749	3.154	19	138.950	5.104	1.171	19	138.950	5.104	1.171

At the age of two months, there was a highly significant decrease ($P < 0.01$) in the diameter of primordial, primary, secondary and mature antral follicles for both G2 and G3 in comparison with the control G1 (Table 5).

Table 5: Diameter of follicles.

Parameter	G1 (control)				G 2 (0.4 mg / kg BW)				G 3 (0.8 mg / kg BW)			
	N	Mean µm	SD	SE	N	Mean µm	SD	SE	N	Mean µm	SD	SE
Diameter of follicles												
Primordial	19	64.737	5.394	1.237	19	55.790	4.171	0.957	19	57.105	3.843	0.882
Primary	19	103.260	18.064	4.144	19	74.684	3.181	0.730	19	75.158	3.202	0.735
Secondary	19	156.580	13.749	3.154	19	138.950	5.104	1.171	19	138.950	5.104	1.171
Antral	19	299.530	35.414	8.125	19	229.740	11.488	2.636	19	230.680	9.604	2.203
Corpus luteum	19	457.370	83.817	19.229	19	433.160	33.175	7.611	19	424.470	35.154	8.065

There was a highly significant decrease ($P < 0.01$) in estrogen and progesterone levels for both G2 and G3 (Table 6).

Table 6: Serum estrogen (pg/ml) and progesterone (ng/ml) levels.

Parameter	G1 (control)				G 2 (0.4 mg / kg BW)				G3 (0.8 mg /kg BW)			
	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
Progesterone	30	16.133	0.287	0.052	30	18.117	0.210	0.038	30	17.153	0.185	0.034

DISCUSSION

It has been shown that in animals, the anogenital distance at birth reflected androgen levels during pregnancy and predicted adult anogenital distance.[8],[15] We used the anogenital distance to detect the virilizing effect of progesterone on newborn females when the drug was given to their mothers during pregnancy. Our results proved that progesterone had a virilizing effect by showing that when the drug was given at 0.8 mg / kg BW there was a significant increase in the anogenital distance at zero day, and when given at 0.4 mg /kg BW and at 0.8

mg /kg BW there was a highly significant increase in the anogenital distance at one month and two months after birth.

Progesterone has been shown to inhibit 5α-reductase, an important enzyme in steroid hormone metabolism.[16] Inhibition of 5α-reductase resulted in decreased conversion of testosterone to DHT, leading to increased testosterone[17], which caused masculinization of the internal and external genitalia.[18] Therefore, since drugs of this type may induce mild masculinization of the external genitalia of the female fetus, as well as hypospadias in the male fetus, it is wise to avoid using the drug during the first trimester of pregnancy.

Gonadal differenation is critical for the development of sexual phenotype, however, the function of most genes and molecular mechanisms regulating gonadal differentiation and development of the female reproductive tract remain largely not clarified[18], and in contrast to testicular differentiation very little is known about the molecular regulation of embryonic ovarian development. 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 embryonic day 11.5.[19] The ovary was relatively insensitive to gonadotropins during the first week of postnatal life and maintenance of follicular growth depended on continuous exposure to gonadotropins.[20] Granulosa cells have a prolonged but finite lifespan in the female. Once a follicle begins growing and granulosa cells proliferate, most of the cells die through apoptosis. Less than one third of follicles and granulosa cells will contribute to the ovulatory pool and subsequently develop into luteal cells after ovulation.[21] Toxicants that specifically target the oocyte can have an irreversible negative impact on fertility and cause premature ovarian failure and aging with consequences of secondary diseases associated with reproductive aging, including cancers.

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. Progesterone induces apoptosis, disintegration of the cell membrane and upregulates the P53 gene - a tumor suppressing gene.

It is also noteworthy to mention how synthetic progestins such as medroxyprogesterone acetate (Provera) or norethindrone occupy the progesterone receptor site and inhibit the

binding of endogenous progesterone to the receptor. Synthetic progestins do not activate the P53 gene and also prevents the production of the body's own progesterone. This will cause chemically induced progesterone deficiency which is similar to natural progesterone deficiency.[22], [23]

Our results show that at the age of two months, there was a highly significant decrease in the height of epithelial cell layer of the uterus and in estrogen level ($P < 0.01$) in both G2 and G3. The decrease of estrogen level might be the cause for the change in the morphology of epithelial cell layer[24], [25] and changed the type of luminal and glandular epithelia and the morphology of epithelial cells.[26]

The decrease in diameter of primordial, primary and secondary antral follicles of new borne female mice may be due to the role played by progesterone on signal transduction pathway.[24] It has been found that a membrane progesterone receptor (mPR) activates signal transduction pathways in selected cell types, namely granulosa cells, thymocytes and others.[25] In addition, the decrease in estrogen levels shown by our results may be due to the decrease in follicles size, since a direct relationship between follicle size and the concentration of estradiol has already been found.[27]

The decrease in progesterone level in our results may be due to the decrease in number of corpora lutea, 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.[28], [29]

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