Effect of Prenatal Exposure to Progesterone on Dams and Fetus Health in Albino Rats

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Abstract

This study investigated the effect of progesterone on the pregnant dams health and their offsprings teratology. Eighteen cyclic female rats were used in the study. They introduced to males in the proestrus stage for mating. The pregnant rats were divided into two groups; control group (n=9) and progesterone-treated group, which given progesterone 10 mg/kg starting from the first gestation day (GD1) till 19th day of gestation (GD19). The body weight gain of pregnant dams was monitored. The blood biochemical parameters as serum activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and Gamma-glutamyl transferase (GGT) as well as the levels of urea and creatinine for dams at GD19. Hepatic malondialdehyde (MDA) and reduced glutathione (GSH) were determined. Serum interleukin-6 (IL-6) and tumor necrosis factor- alpha (TNF-α) were monitored for dams at GD19. The fetal weight, cervical vertebral length (CVL) and placental weight were recorded. Fetal stain to the skeleton by Alizarin Red S stains was done. Finally histopathological changes for maternal as well as fetal liver and kidney. The treatment with progesterone resulted in significant ($P<0.05$) increase in all blood biochemical parameters than control. Hepatic MDA was significantly ($P<0.05$) increased however, GSH was reduced ($P<0.05$) in progesterone-treated group than control. Serum IL-6 was also increased ($P<0.05$) in progesterone-treated dams than control while TNF-α was non significantly altered. Fetal weight and CVL was significantly ($P<0.05$) reduced beside the presence of some skeletal malformations in progesterone-treated group while placental weight was non significantly altered. The liver and kidney showed various degrees of retrogressive changes in progesterone-treated group. Progesterone adversely affected mothers' health and their foeti.

Keywords: progesterone, rat fetus, biochemical parameters, oxidative stress

Introduction

Progesterone (pregn- 4- ene-3, 20-dione; abbreviated as P4) is an endogenous steroid hormone involved in the menstrual cycle, pregnancy, and embryogenesis of humans and other species. It belongs to a group of steroid hormones called the progestogens and is the major progestogen in the body ***(King and Brucker, 2010)***. P4 exerts its action in target tissues by genomic and non-genomic mechanisms ***(Brunette and Leclerc, 2001)***. It modulates the nuclear progesterone receptor (nPR) ***(King and Brucker, 2010)*** and membrane progesterone receptors (mPRs) that has been recently discovered ***(Thomas and Pang, 2012)***. Usually, P4 nuclear receptors are up regulated by estrogen ***(Ing and Belen Tornesi, 1997).***

Threatened miscarriage in early pregnancy is treated by progesterone in many countries around the world even though there does not seem to be any high-quality studies on the efficacy and safety of this treatment. Few randomized, controlled trials show that women who received progesterone were statistically significantly less likely to have recurrent miscarriages before 34 weeks, to have an infant with a birth weight of 2.5 kg or lower, or to have an infant diagnosed with intraventricular hemorrhage. However, the usage of progesterone therapy during pregnancy is mandatory in case of threatened abortion, miscarriage and assisted reproductive technologies ***(Golub et al., 2006).***

There are few studied concerning the effect of progesterone on mothers' health and fetal development. Therefore, the current study aims to investigate the effect of progesterone as a source of progesterone in a dose relevant to human exposure on dams' liver and kidney as well as fetal teratology.

Materials and Methods

Animals and mating procedures

Twenty adult females and 4 males, weighing approximately 210-250 g, were purchased from Laboratory Animal House of Faculty of Sciences, Suez Canal University, Egypt. Animals were kept in a standard plastic cage (three rats per cage). They were housed under standard condition maintained at room temperature and under natural daylight rhythm. Animals were kept to acclimatize for 10 days before the onset of the experiment. They were given standard rodent diet *ad libitum* as well as water supply.

The estrous cycle was checked for regulatory by daily vaginal smear for 2 successive cycles according to procedures of ***Allen (1922)***. Vaginal smears were taken from twenty female rats (n = 20) once daily at 7:00 am. The smears were dried on flame and methylene blue (Sigma Chemical Co., USA) stained for 2 minutes. The stained smears were examined under a microscope to determine the phases of the estrous cycle. Females with 2 successive

regular cycles (n=18) were used for this study, while irregular ones (n=2) were excluded. The regular cyclic females in proestrus were kept with males for overnight in the ratio (3:1). Mating was confirmed by the presence of sperm(s) in the vaginal smears ***(Piesta et al., 2009)***.

Experimental design

Eighteen pregnant females were split into 2 groups (9 animals per group), Group (I) serves as control; injected with olive oil starting from the first day of gestation. Group (II) injected intramacular with 10 mg/Kg body weight progesterone 50% (MARCYRL CO., Germany) in olive oil starting from the first day of gestation. The drug and vehicle were administered intramuscular daily till 19th day of gestation (GD19).

Dams body weight gain

Mothers body weight of control and treated were recorded at zero day of gestation and at GD19. The body weight gain of mothers was obtained by subtracting the final weight of dams from the initial weight of the same mother.

Blood and tissue sampling

Blood samples were collected using the orbital sinus technique from retro venous plexus via capillary tube from pregnant dams in plain tubes. The blood sampling was performed under the effect of light diethyl ether anesthesia. After the collection of blood samples, rats were euthanized by an overdose of diethyl ether. The sera were separated from plain tubes at 3000 rpm for 15 min after blood clotting. The liver and kidney of each rat were excised, washed by the saline solution to remove blood, dried by blotting between filter papers, and divided into two parts. The first part (0.5 gm. of tissue) was perfused with cold 2 ml of 0.15 M KCl (Liver homogenate and kidney homogenate); liver and kidney homogenates were used for the determination of reduced glutathione (GSH) and lipid peroxidation content. The second part of the dam's liver and kidney tissue as well as Liver and kidney of foeti were preserved in 10% formalin solution for histopathological examination.

Serum biochemical parameters

Serum activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and Gamma-glutamyltransferase (GGT) as well as the levels of urea and creatinine were measured using commercially available kits provided by DIACHEM Ltd., Hungary.

Hepatic malondialdehyde (MDA) and reduced glutathione (GSH

Lipid peroxidation content in liver homogenate was assessed spectrophotometrically using TBA-MDA assay ***(Hodges et al., 1999).*** Reduced glutathione hepatic content was measured by the method of ***Beutler (1963).***

Serum interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α)

Serum IL-6 and TNF-α were measured using commercially

available ELISA kits provided by Kamiya Biomedical Co. (Cat.No. KT-19418, USA) and Immuno-Biological Laboratories Co., Ltd. (Code no. 27194, Japan), respectively. The procedures were performed according to enclosed pamphlets.

Morphometry and placental weight

After aeuthanization of the pregnant mothers at GD19, the gravid uteri were carefully examined. The foeti and their placenta were excised from gravid uteri. The placental weight and fetal weights were recorded. Also, the fetal crown- vertebral length (CVL) was estimated via caliber as the distance from Fontanella to the root of tail (Amal et al., 2014). The foeti were carefully examined for any morphological malformations.

Skeletal preparation

Foeti of each group randomly divided into two parts, first part fixed in 10% formalin solution for 48 hrs. The second part was put in absolute ethyl alcohol. Foeti of both groups were detached from their skin and eviscerated then, preserved in absolute ethyl alcohol for 4 days. Finally, they were stained using Alizarin Red S stain (Astron Chemicals Co., India) according to ***Green (1952).*** This technique was used for the treatment of the specimens with a relatively weak solution of potassium hydroxide (KOH) which makes the muscles transparent and helps to limit the stains to bone ***(Staples, 1964).*** After staining accomplishment, the stained foeti were transferred to ascending series of glycerol and 2% aqueous KOH solution after which they were preserved in 100% glycerin. The stained preparations were carefully examined under the dissecting binocular microscope to study the different parts of the axial and appendicular skeleton. Photographs were taken for a skeletal system of control and maternally treated fetuses and the abnormalities were recorded.

Histopathology

Formalin-fixed liver and kidneys of both dams and foeti were dehydrated in alcohol gradient then embedded in paraffin wax. Several 5-µm sections from each sample were cut then stained with hematoxylin and eosin (H&E) for histopathological examination ***(Carleton et al., 1980)***.

Statistical analysis

The data presented as mean ±SE. Student T-test was applied for the statistical analysis for the present data of both groups. The result was considered to be significant when *P* is less than 0.05 (SPSS software, version 16.0; SPSS Inc., Chicago, IL, USA).

Results

Dams body weight gain

The dams' body weight gain exhibited significant ($P<0.05$) elevation in progesterone 10 mg/kg treated group than control (Fig. 1).

Serum biochemical parameters

The serum activities of of ALT, ALP, ADH and GGT as well as the levels of urea, and creatinine exhibited significant ($P<0.05$)

elevation in Progesterone 10 mg/kg treated mothers (Table 1).

Hepatic and renal malondialdehyde (MDA) and reduced glutathione GSH

Table 1, demonstrated that progesterone 10 mg/kg treated group exhibited significant ($P<0.05$) up-regulated MDA level than the control group in both liver and kidney homogenate. Meanwhile, GSH activity revealed significant ($P<0.05$) decline in progesterone 10mg/kg group than control in both liver and kidney homogenate.

Serum interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α)

The level of IL-6 exhibited significant ($P<0.05$) increment in progesterone- treated group than control animals. The level of TNF-α showed a non-significant difference between the control group and progesterone- treated rats.

Morphometry and placental weight

The gravid uteri of Progesterone 10 mg/kg group showed unequal distribution of foeti in the 2 horns (Fig. 2). The fetal weight and CVL were significantly ($P<0.05$) lower in progesterone 10mg/kg group than control. The placental weight exhibited non-significant alterations between the two groups (Table 2).

Skeletal preparation

Skeletal preparation showed marked incomplete ossification in appendicular skeleton and axial skeleton. The parietal bone, occipital condyles and supraoccipital bone were faintly stained with Alizarin Red S stain in progesterone-treated fetus when compared to normal control (Fig. 3&4). Also, the absence of metacarpal and metatarsal bones were evident as well as coccygeal vertebrae (Fig. 3) in progesterone treated fetus when compared to normal rats.

Histopathology

Liver of pregnant rat at GD19 of control group displayed normal architecture of hepatic tissue with normal hepatocytes and central vein. Progesterone- treated pregnant rats showed cloudy swelling of hepatocytes, fibrosis, congested central vein and lymphatic infiltration. The control fetus displayed normal hepatic tissue, central veins, and sinusoids. The fetus liver maternally treated with progesterone, exhibited congested sinusoids (Fig. 5).

The kidney of the pregnant rat at GD19 day displayed normal architecture of renal tissue with normal glomeruli and renal tubules in control pregnant dams. Pregnant dam treated with progesterone, showed cloudy swelling of renal tubules epithelial tissue, degenerated glomeruli, hemorrhage. Control fetus kidney displayed normal renal tissue with normal glomeruli and RT. Fetal kidneys maternally treated with progesterone exhibited atrophied glomeruli beside the presence of necrotic renal tubules (Fig. 6).

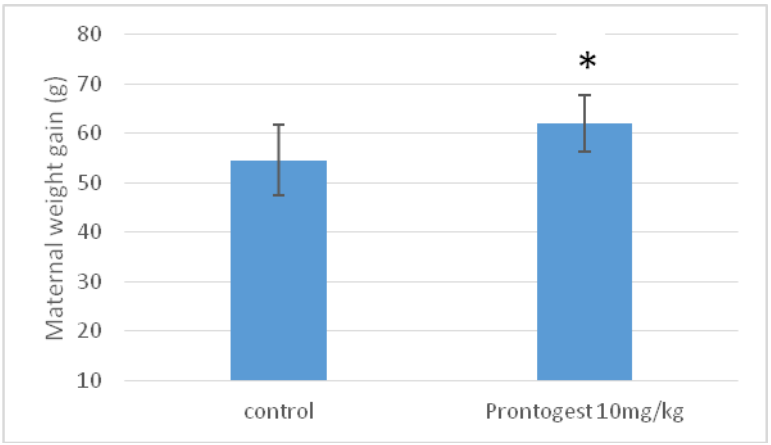

Fig. 1: Effect of progesterone 10 mg/kg on maternal body weight.
Data presented as mean ±SE.
*Means sigficant difference at P<0.05 as compared to control.

Table (1): Effect of progesterone administration to pregnant albino rats on liver enzymes, renal biomarkers, hepatic and renal GSH and MDA

Parameter/ group	***Control***	***Progesterone 10mg/kg***
ALT(U/L)	25.00 ± 0.57	28.66 ± 0.66*
ALP (U/L)	21.00 ± 0.57	22.66 ± 0.33*
LDH (U/L)	362.66 ± 3.28	389.66 ± 3.48*
GGT (U/L)	15.66±0.33	17.00 ± 0.57*
Urea (mg/dL)	17.66 ± 0.34	19.66 ± 0.43*
Creatinine (mg/dL)	1.00 ± 0.01	1.09 ± 0.07
IL-6 (pg/mL)	10.00 ± 0.00	11.66 ± 0.33*
TNF-α (pg/mL)	5.36 ± 0.38	6.33 ± 0.40
Liver MDA (nmole/g tissue)	0.16 ± 0.001	0.19 ± 0.0006*
Liver GSH (nmole/g tissue)	40.59 ± 0.38	33.61 ±0.44*
Kidney MDA (nmole/g tissue)	0.14 ±0.005	0.16 ±0.004*
Kidney GSH (nmole/g tissue)	41.49 ± 0.97	33.26 ± 0.68 *

Data presented as mean ±SE.
*Means sigficant difference at P<0.05 as compared to control using student T-test.

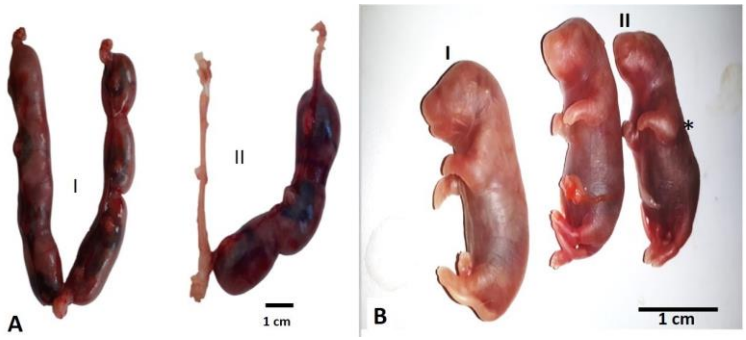

Fig. 2 : (A) gravid uterus of control dams (I) is showing equal distribution of foeti in two horns. The gravid uterus of progesterone (10 mg/kg) group (II) is showing unequal distribution of foeti in the two horns. (B) photo of control rat fetus (I) showing normal size compared to progesterone- treated group (II) showing reduced size and club hand (*)

Table (2):*Effect of progesterone administration to pregnant albino rats on fetal weight, CVL and placental weight*

Parameter/ group	***Control***	***Progesterone 10mg/kg***
Fetal weight (g)	3.95 ± 0.04	3.18 ± 0.05*
Fetal CVL (Cm)	3.99 ± 0.01	3.10 ± 0.02*
Placental weight (g)	0.57 ±0.013	0.57 ±0.013

Data presented as mean ±SE.
*Means sigficant difference at P<0.05 as compared to control using student T-test.

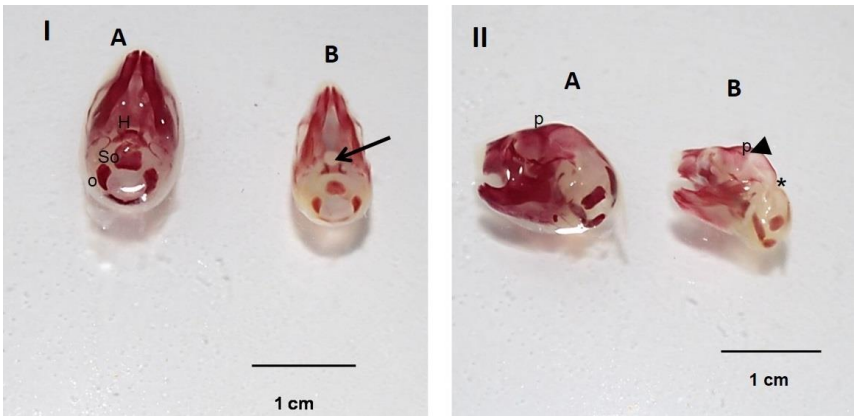

Fig. 3: (I) Ventral view photograph of fetus skull at GD 19 showing the low bone density of hyoid bone (arrow), occipital condyles and supraoccipital bone (star) in progesterone treated fetus (B) in comparison to normal control (A). (II) Lateral view photograph of parietal bone (p) is faintly stained as well as wide fontanella (star) due to incomplete ossification in progeterone treated fetus (B) in comparison to normal control (A)

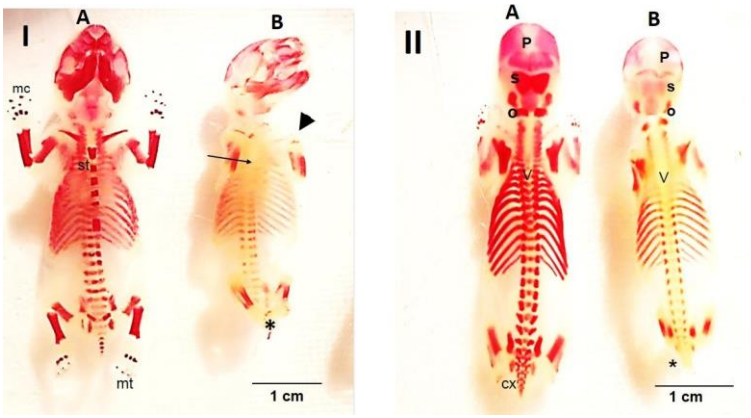

Fig. 4: (I) Ventral view photograph of whole mount fetus at GD 19 showing the absence of sternebrae (st) (arrow), metacarpal bones (mc) (arrowhead) and metatarsal (mt) (star) in progesterone-treated fetus (B) in comparison to normal control (A).
(II) Dorsal view photograph showing faint ossification of parietal bone (p), occipital (o), supraoccipital bone (s) and vertebrae (v) in progesterone-treated fetus (B) in comparison to normal control (A). Also, coccygeal vertebrae (cx) ossifications were absent (star) in progesterone-treated fetus (B) in comparison to normal control (A).

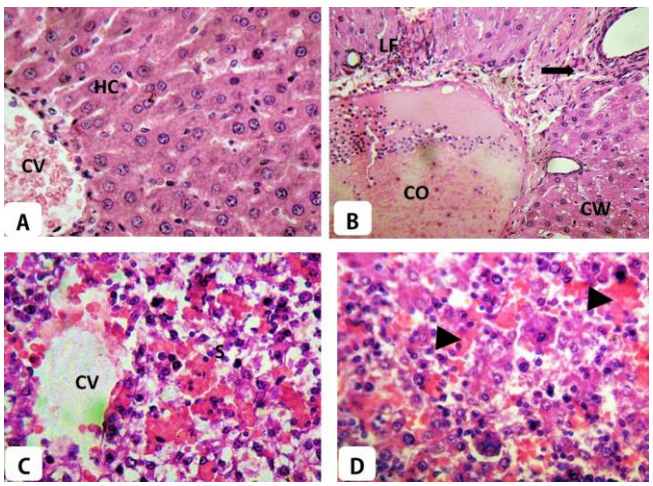

Fig. 5: Liver section of the pregnant rat at GD19 and its fetus: (A) control pregnant rat displayed normal architecture of hepatic tissue with normal hepatocytes (HC) ; (CV) central vein. (B) pregnant rat liver treated with progesterone 10 mg/kg, showed cloudy swelling of hepatocytes (CW), fibrosis (arrow), congested central vein (CO) and lymphatic infiltration (LF). (C) control fetus displayed normal hepatic tissue (CV) central vein; (S)

sinusoids. (D) fetus liver maternally treated with progesterone 10 mg/kg, exhibited congested sinusoids (head arrows). (magnification, 400X)

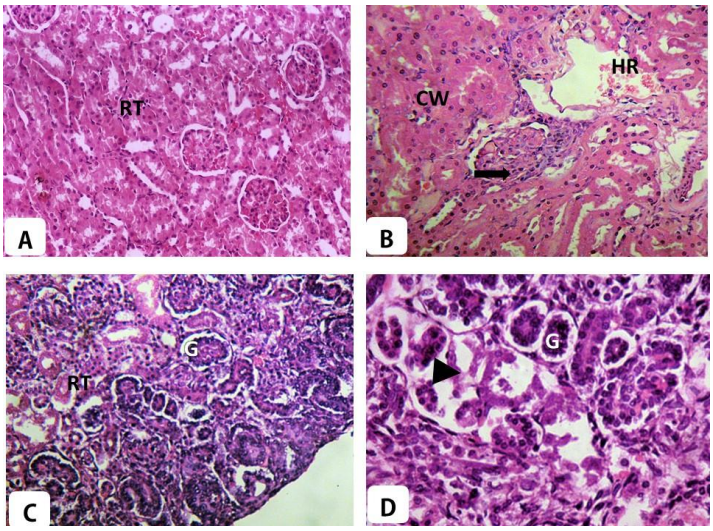

Fig. 6: Kidney section of the pregnant rat at GD19 and its fetus: (A) control pregnant dam displayed normal architecture of renal tissue with normal glomerulus (G); renal tubules (RT). (B) pregnant dam treated with progesterone 10 mg/kg, showed cloudy swelling of renal tubules epithelial tissue (CW), degenerated glomerulus (arrow), hemorrhage (HR). (C) control fetus kidney displayed normal renal tissue (normal glomerulus (G); renal tubules (RT). (D) fetus kidney maternally treated with progesterone 10 mg/kg exhibited atrophied glomerulus (G) besides necrotic renal tubules (head arrows). (magnification, 200X)

Discussion

The maternal exposure to progesterone to treat miscarriage and abortion and its adverse effect on maternal health and fetal teratology is not fully discussed or studied. The present research aimed to study the use of progesterone on a dose equivalent to human exposure on maternal health and fetal teratology.

The dams exposure to progesterone significantly increased the body weight gain, these results were in agreement with ***Hervey and Hervey (1967)*** while contradicted with those reported by ***Bruce and Bartholomeusz (1976).*** These results may be attributed to progesterone influencing effect and body lipid promoting action ***(Wiegratz and Kuhl, 2006).***

Current results revealed a significant increment in activity of ALT in progesterone- treated rats at GD19 than control. These results coincide with ***Toyoda et al. (2012)*** who demonstrated that pretreatment with progesterone induced a significant

($p<0.05$) increase in plasma ALT. However, ***Rahman and Wendon (2002)*** found that progesterone couldn't alter ALT activity or reduced it than normal value respectively. The presence of ALT in the plasma here denoting that there were deleterious effects on hepatocytes that lead to their damage and liberation of such enzyme to plasma ***(Ramaiah, 2007).***

Serum ALP was significantly ($p<0.05$) elevated in progesterone-treated dams than control at GD19. These results were in harmony with ***Naz et al. (2016)*** and ***Suzuki et al. (1980)***. The increased serum ALP activity could be attributed to the hepatocytes function where the ALP is produced in case of biliary pressure. Moreover, it is closely related to the disturbances in hepatic secretory activities ***(Giannini et al., 2005; Kumar et al., 2008)*** as well as the hepatic damage condition.

Serum LDH enzyme was significantly ($p<0.05$) elevated in progesterone treaed animals. These results were in accordance with Mizoguchi et al. (2010). LDH could be increased under the condition of lipid peroxidation and oxidative stress and thus leading to increased lactic acid and their salts ***(Jovanovic et al., 2010).*** For the hepatic injury, LDH could be considered less specific than ALT ***(Gitlin and Serio, 1992)***. The results of LDH augmented the present results concerning elevated the levels of lipid peroxidation and reduced GSH as well as the elevated activities of ALT, ALP and GGT.

The present results revealed a significant ($p<0.05$) increase in hepatic and renal MDA content in progesterone-treated dams at the dose 10 mg/kg than control. These results indicated that progesterone is a casual factor for elevated lipid peroxidation to the membrane of both hepatocytes and renal cells. The elevation of lipid peroxidation suggested that progesterone is injurious for both liver and kidney of pregnant dams that subsequently induced free radicals and oxidative stress ***(Steller et al., 2018)***. These results illustrated the observed retrogressive changes in hepatic and renal histopathology. The same trend was found in the current study where GSH was significantly decreased in both liver and kidney homogenate of progesterone-treated dams than control. These results indicated that there is the exhaustion of the antioxidant reserve represented by GSH that confirmed the previous finding of elevated MDA. This may be due to the active metabolism of progesterone to highly reactive compounds and radical that could indicate lipid peroxidation and oxidative stress. ***Fedotcheva et al. (2012)*** stated that among the targets of the steroid hormones are mitochondria, which as the main source of reactive oxygen species (ROS) in the cell play a central role in the development of various pathologies. In contrast to the previous findings

Sainz et al. (2000) reported no effect of progesterone on liver MDA.

Serum urea was increased in progesterone-treated dams than those of control. These results were similar to those obtained by ***Matsuo et al. (1986)*** and ***Cheung and Lafayette (2013)***. However, the serum creatinine value showed non-significant increment in progesterone treated dams and control ones. These results seemed to be in close similarity with those of Abbassi-Ghanavati et al. (2009). The elevation in serum urea level which is the end product of protein metabolism which is excreted by kidneys

into urine. This indicated that there was impairment in the kidney function ***(Gowda et al., 2010).***

IL-6 was significantly increased in progesterone-treated dams than those of control. IL-6 is considered one of the inflammatory cytokines that denoting an inflammatory or degenerative condition in either liver ***(Schmidt-Arras and Rose-John, 2016)*** or kidney ***(Fielding et al., 2014).*** It is promoted via ROS can lead to deleterious effects on several processes and disruptions of different cellular components. Among these negative outcomes, the ROS can modulate cytokines expression and activation of redox-sensitive transcription factors AP-1, P53and NF- Kappa B that proceed a cascade of apoptosis mediation ***(Yoshida et al., 1999).***

The present study cleared the adverse effects of maternal progesterone supplementation on the fetal weight and CVL than those of control. These results were parallel to the previous study of ***Seegmiller et al. (1983)*** while disagreed with those of ***Bakry (2013).*** These results confirmed the adverse effect of progesterone on fetal development and growth however placental weight is not altered. This may be attributed to the ability of progesterone to provoke lipid peroxidation and increase ROS production which subsequently causes DNA damage and so could produce mutations that alter fetal growth and development ***(Hundal et al., 1997; Schwarz et al., 2009).*** This data was manifested by the observed renal degeneration and hepatic deteriorations which were observed in the fetal histological sections.

Skeletal defects of foeti prenatally treated with progesterone were observed as delaying in ossification in bones as well as impairment in cartilage formation. These results coincided to ***Lammer and Cordero (1986)*** and ***Bakry (2013).*** Present data revealed that the prenatal treatment with progesterone can alter bone mineralization and cartilage formation leading to malformations on the skeleton. Moreover, the elevated maternal levels of serum alkaline phosphatase were suggestive for bone catabolic status that could be inferred on fetuses where progesterone had an affinity to cross the placental barrier

to developing fetus ***(Kumar and Magon, 2012)***.

In conclusion, progesterone treatment 10 mg/kg for pregnant dams seemed to have adverse consequences on hepatic and renal health of pregnant dams. These adverse consequences manifested by increased MDA and decreased GSH that mediated serum proinflammatory cytokine IL-6 which mediated their cell apoptotic effect. The apoptotic effect of progesterone on hepatic and renal tissues of the current study could be sensed by increased their serum enzymes activities as well as deviated histopathological observation that denoting their failure to accomplish their physiological function in the proper way. Also, adverse effects on fetal skeleton and size as well as kidney and liver were noticed.

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الملخص العربى

تأثير التعرض قبل الولادة للبروجسترون على صحة الامهات و الاجنة في الجرذان البيضاء

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يهدف البحث الحالي الى دراسه تأثير البروجسترون على صحة الامهات الحوامل و اجنتها. حيث تم استخدام ١٨ من اناث الجرذان منتظمة الشبق في هذه الدراسة. وقدموا للذكور في مرحلة ا ما قبل الشبق للتزاوج. تم تقسيم الفئران الحوامل إلى مجموعتين. مجموعة ضابطةو عددها ٩ جرذان ومجموعة معالجة بالبروجسترون و عددها ٩ جرذان والتي أعطت 10 ملغ / كجم البروجسترون ابتداء من يوم الحمل الأول حتى اليوم ال ١٩ من الحمل. تم رصد معدل زيادة وزن جسم الامهات الحوامل. الي جانب قياس الدلالات البيوكيميائية في مصل الدم مثل نشاط الانزيمات التالية: أمين الالانين الناقل(ALT) ، الفوسفاتيز القلوي (ALP)، (LDH)، جاما الجلوتاميل الناقل (GGT) ، كذلك مستوى اليوريا والكرياتينين للامهات الحوامل في اليوم التاسع عشر من الحمل. تم تعيين مستوى الدهون المؤكسدة و الجلوتاثيون المختزل (GSH) فى الكبد. تمت قياس إنترلوكين-٦ وعامل نخر الورم - ألفا فى مصل دم الامهات فى اليوم ١٩ من الحمل. تم تسجيل وزن الجنين ، وطول العمود الفقري العنقي ووزن المشيمة. تم إجراء فحص للهيكل العظمي للاجنة. وأخيرا التغيرات النسيجية المرضية لكبد و كلى كل من الام و الاجنة. اظهرت النتائج الحالية ان علاج الامهات الحوامل بهرمون البروجسترون الي وجود زيادة ذات دلاله معنوية لجميع الدلالات الكيميائية فى مصل الدم بالمقارنة بالمجموعة الضابطة . ارتفع معدل اكسدة الدهون في نسيج الكبد بينما انخفض مستوى الجلوتاثيون المختزل في المجموعة المعالجة بهرمون البروجسترون بالمقارنة بالمجموعة الضابطة. ازداد مستوى انترلوكين-٦ بمصل دم الامهات المعالجة سلفًا بهرمون البروجسترون بينما لم يتم يتغير مستوى عامل نخر الورم - ألفا TNF-α بشكل ملحوظ عن المجموعة الضابطة. انخفضت اوزان الاجنة وطول العمود الفقري العنقي انخفاضا معنويا الى جانب وجود بعض التشوهات الهيكلية في المجموعة التي تمت معالجة امهاتها بهرمون البروجسترون بينما لم يتم تغيير وزن المشيمة بين المحموعتين. أظهر الفحص النسيجي للكبد والكلى درجات مختلفة من التغيرات التراجعية في المجموعة التي تمت معالجتها بهرمون البروجسترون. مما سبق يمكن استنتاج أن المعالجة بهرمون البروجسترون يؤثر تأثيرا سلبيا على الامهات الحوامل و اجنتها.