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Prenatal Exposure to Medroxyprogesterone Acetate

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Abstract: Depo-Provera®; Depot-Medroxyprogesterone Acetate (DMPA) is one of the most used contraceptives and it is currently used by more than 30 million women in most of developed and developing countries. This study was designed to investigate the prenatal effect of DMPA in rats. Eighty pregnant rats (*Sprague-Dawley*) were assigned into eight groups, daily injected with DMPA doses (Vehicle 0; 2.7; 5.4 and 10.8 mg/day) on gestation day 6 (GD6) through (GD15 or 20) then sacrificed to evaluate the intrauterine growth retardation. Results indicated that DMPA induced reduction in numbers of implantation sites, live embryos and increased the mortality and resorption rate as well as decrease the embryonic crown rump and body weight. Also, DMPA induced decrease in male anogenital distance and skewed the sex ratio toward females and induced severe skeletal anomalies. In conclusion, these findings support the claims that DMPA, while being a progestational agent, is also embryo-toxic and teratogenic.

Key words: Depo-Provera® · Rat embryo · Skeletal system · Malformations · Contraception

INTRODUCTION

Unintended pregnancies in low-and middle-income countries remain a serious obstetric problem. Although their impact on individual women or couples varies, such pregnancies have been linked to a number of social and health problems, such as poor birth outcomes due to inadequate access to effective maternity services and the hazards of illegal and unsafe abortion [1,2]. Globally, it is estimated that about 210 million recognizable conceptions occur annually. Despite the widespread use of highly effective contraceptive methods in France and Denmark (82% of contraceptive users use a medical contraceptive), one in every three pregnancies is reported to be unplanned. Of these pregnancies, 65% occur among women who were using contraceptives at the time they got pregnant [3, 4]. While, in USA about three million pregnancies in the United States (48%) were unintended in 1994. Some 53% of these occurred among women who were using contraceptives [5]. Since, unintended pregnancies and associated birth defects are attracting national attention as public health problems that are once again on the rise specially with using hormonal contraceptives. DMPA is a long-acting, injectible progesterone derivative contraceptive that is currently used by more than 30 million women in both developed and developing countries. DMPA exert its effect by inhibit the LH surge, corpus luteum formation and maturation of the endometrial thinning. Unplanned pregnancies recorded among Canadian women although they have access to a wide range of contraceptive options [6]. Like many pharmacologically active agents, sex hormones may adversely affect the fetal development at any stage [7]. However, despite its overall splendid record of reliability and its low ratio of number of motivations per period of protection, the use of DMPA has raised some concerns. Initially, these arose from the recognition progestin-induced genital defects of male fetuses that led the FDA (Food and Drug Administration) to indicate that it prudent to avoid the use of these drugs during the first trimester of pregnancy [8]. Of equal or greater concern has been the accumulation of non-genitourinary congenital defects in infants whose mothers received progestins during pregnancy. These include meningomyelocele or hydrocephalus [9], teratology of Fallot [10, 11] and adrenocortical carcinoma [12, 13]. It was found that among delivered pregnancies, DMPA use was associated with decreases in fetal weight, increases in the rates of cleft palate and urinary tract abnormalities. Exencephaly and heart abnormalities were also significantly more frequent, in babies from women who have used hormonal contraception. Also, increases in ectopic pregnancy rate

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and fetal anomalies due to contraceptive failures were recorded among women using DMPA [14]. Several toxicological studies has been published concerning the safety of DMAP on experimental animals during pregnancy at high doses reached (3, 30, 100, 300, 1000 mg/kg/day) to mice, rat and rabbits [15]. While, other animals studies on the results of administering DMPA on pregnancy day 2 returned sporadic increases in dead and resorbed fetuses, a decrease in fetal weight and an increase in the rates of cleft palate and malformed or abnormally developed fetuses [16,17]. A single dose of DMPA was intramuscularly administered to 12 time-mated pregnant monkeys causes selective embryotoxicity in both male and female fetuses [18]. DMPA was shown to exert an embryotoxic effect inducing resorption, embryo weights/litter reduction, reductions in humeral and femoral diaphyseal length in proportion to a reduction in overall growth [19].

A Canadian study on contraception was carried out during 2006 and it revealed that 50% of sexually active women in Canada using DMPA as hormonal contraceptive [20] and this method was nearly 100% effective with perfect use; however, typical failure rates in the range of 3 to 9% was recorded [21,22] and this reflects the fact that adherence with daily, weekly, monthly, or even tri-monthly regimens is a problem. Generally, women commonly fail to take hormonal contraception as directed. Extensive epidemiological studies were performed to assess the possible risks of birth defects in women who have used hormonal contraception. It was found that among delivered pregnancies DMPA use was associated with decreases in fetal weight, increases in the rates of cleft palate and urinary tract abnormalities. Exencephaly and heart abnormalities were also significantly more frequent, but this increase was not dose-dependent [14]. DMPA use may adversely affect bone mineral density. However, racial differences and the reversibility of these changes are poorly understood [23, 24].

Many studies carried out on DMPA users found that Chinese women who used DMPA over a period of 3 years, had a retardation in bone loss with time, possibly due to the effect of progesterone in decreasing bone turnover [25,26]. Also, DMPA injectable contraception may decrease bone density and increase the risk for osteoporosis in later life [27,28]. Bone mineral density (BMD) was significantly decreased in the femoral neck and lumbar spine adolescents using DMPA for one to two years, the study team attributed the demineralization to hypoestrogenation [29]. Also, statistically significant decrease in BMD between DMPA users and controls at 6 months, 12 months, 18 months and 24 months [30]. To evaluate the action of progestins and estrogen on bone metabolism in early menopausal women, one hundred thirty-two menopausal women were randomized into a 2-year clinical trial [31]. They concluded that estrogen remains the primary bone active agent in hormone therapy, while progestins have significantly less activity because it decreased and induced resorption of bone formation markers.The purpose of this study was to estimate the influence of DMPA on the development of skeletal system of rat embryos during postimplantation period. In light of this evidence and reports that in Egypt more than 3.1% of women using DMPA and other contraceptives are pregnant at the time of injection, according to the Demographic and Health Survey (DHS) data from the most recent survey in every country that collected calendar data on contraceptive failure since 2002-2008 to calculate the unintended with and without contraceptive failure on women aged between 15-49 years [32,33]. So, it's important to test for the effects of DMPA on the fetus under analogous circumstances. It's well known that DMPA is not prescribed during pregnancy; although some women are receive DMPA during early pregnancy, due to DMPA failure and due to sustained effect of DMPA even after the discontinuation. So, this study designed to investigate the prenatal effect of DMPA in rats and evaluating the development of the skeletal system bones which is one of the classical model systems that have been used for studying vertebrate morphogenesis.

MATERIALS AND METHODS

Animals and Mating: This study was carried out using sexually mature male and female rats Sprague-Dawley rats, 2 months old, from central farm for experimental animals of Vaccera, Giza, Egypt.They were housed in 12-hrs dark and 12-hrs light and fed a standard rodent pellet diet to acclimate for two weeks then the proestrous female cohabited (1:1) nightly with male. On the next morning the presence of a vaginal semen plug enabled the designation of day zero of pregnancy [34,35].

Drug: Depo-Provera was received from one of the family planning private clinics in Cairo. It also sold in Egypt for the contraception use in sterile Depot-aqueous solution for intramuscular injection as used in this study is manufactured by The Upjohn Company (Kalamazoo, Michigan, U.S.A.)

Experimental Groups: Eighty pregnant females were divided into two sections of 40 rats. Both sections of 40 rats were divided into four groups (10 pregnant rats / group), the first group was considered as the control group and the last three groups were treated groups. These animals were intramuscularly injected on gestation day six (GD6) with DMPA doses (Vehicle 0; 2.7; 5.4 and 10.8 mg/rat/day) through (GD15 or 20). These doses were converted from human dose 150 mg, two-and three folds to rat's dose by using multiplication factors for dose conversion between different species[36].

Maternal and Embryo-Fetal Study: This maternal and embryo-fetal study was conducted in accordance with the U.S. Environmental Protection Agency TSCA (Toxic Substances Control Act) Test Guidelines [34]. All animals were observed twice daily for mortality and toxicological effects. Body weight and feed consumption values were recorded on GD 6, 15 and 20. Post exposure observations were performed approximately a half hour after exposure. On GD 15 and 20, all female rats were euthanized via carbon dioxide inhalation and a cesarean section and a macroscopic postmortem examination were performed on each. During the macroscopic postmortem examination, uteri, ovaries, liver, kidneys, adrenal, spleen, lungs and heart weights were recorded. Uterine implantation data including number of live, dead and resorbed fetuses, number of corpora lutea were recorded. Fetuses were removed from the uterus, weighed, their gender was determined and they were examined for external malformations, weighed and measured for crown rump length as well as anogenital distance then divided into two groups the first group was fixed in Bouin's solution. While, second group were placed in acetone to dissolve the body fats, then transferred to potassium hydroxide (1%) to clear the skeletons and alizarin red S to stain the bones. After staining, the fetuses were examined under a dissecting microscope for skeletal anomalies [37-39]. Implantation index, live fetuses index dead fetuses index and resorption index were calculated [40].

Statistical Analysis: The statistical analysis of the obtained data was done and the analysis was revised by SPSS 12 for windows (2003).The Student's "t"-distribution were adopted for assessment of significant changes occurring between the groups [41].

RESULTS

Maternal Body and Organ Weights: Body weights were recorded during 6th up to 15th or 20th days of gestation and comparison among the control and DMPA exposure groups were carried out. The results showed that DMPA induced body weight gain in all treated groups and this increase in the body weight reached maximally (~ + 32.77 %) with dose dependant and statistically significant ($P \leq 0.01$) manner (Tables 1).

Table 1: Effect of DMPA on body and organ weights (gm) the data expressed as relative weight of pregnant female rat organs (g/100gBW) at both 15th and 20th days of gestation

	Gestation Days (6GD to GD 15)				Gestation Days (6GD to GD 20)			
Groups	GI	GII	GIII	GIV	GV	GVI	GVII	GVIII
Dose	Control	2.7 mg / day	5.4mg / day	10.8 mg/day	Control	2.7 mg / day	5.4mg / day	10.8 mg/day
Body Weight	212.30 ± 1.34	236.29* ± 1.10	254.29* + 2.10	265.10* ± 1.12	215.10 ± 1.24	239.19* ± 2.30	264.49* + 2.40	285.60* ± 2.22
%	--	+ 11.30 %	+ 19.77 %	+ 24.87 %	--	+ 11.19 %	+ 22.96 %	+ 32.77 %
Liver	4.14 ± 0.22	4.46* ± 0.13	4.83* ± 0.41	5.22* ± 0.41	4.20 ± 0.12	4.55* ± 0.11	4.88* ± 0.21	5.52* ± 0.14
%	--	+ 7.72 %	+ 16.66 %	+ 26.08 %	--	+ 8.33 %	+ 16.19 %	+ 31.42 %
Ovary	0.04 ± 0.01	0.06* ± 0.01	0.07* ± 0.01	0.07* ± 0.02	0.05 ± 0.01	0.06* ± 0.01	0.07* ± 0.01	0.07* ± 0.02
%	--	+ 50.00 %	+ 75.00 %	+ 75%	--	+ 16.66 %	+ 40.00 %	+ 40.00%
Uterus	0.23 ± 0.02	0.29* ± 0.03	0.33* ± 0.12	0.38* ± 0.11	0.22 ± 0.02	0.33* ± 0.03	0.38* ± 0.12	0.43* ± 0.11
%	--	+ 26.08 %	+ 43.47 %	+ 65.21%	--	+ 50.00 %	+ 72.72 %	+ 95.45 %
Kidneys	0.89 ± 0.04	0.93* ± 0.04	0.98* ± 0.04	1.22* ± 0.04	0.87 ± 0.03	0.96* ± 0.04	1.02* ± 0.03	1. 45* ± 0.04
%	--	+ 4.49 %	+ 10.11 %	+ 37.07 %	--	+ 10.34 %	+ 17.24 %	+ 66.66 %
Adrenal	0.04 ± 0.01	0.06* ± 0.01	0.07* ± 0.01	0.07* ± 0.02	0.04 ± 0.01	0.06* ± 0.01	0.07* ± 0.01	0.07* ± 0.02
%	--	+ 50.00 %	+ 75.00 %	+ 75%	--	+ 50.00 %	+ 75.00 %	+ 75%
Spleen	0.54 ± 0.01	0.64* ± 0.03	0.75* ± 0.04	0.82* ± 0.03	0.54 ± 0.01	0.64* ± 0.03	0.75* ± 0.04	0.82* ± 0.03
%	--	+ 15.62 %	+ 38.88 %	+ 51.85 %	--	+ 18.51%	+ 21.00 %	+ 51.85 %
Lungs	0.74 ± 0.01	0.83* ± 0.02	0.89* ± 0.01	0.92* ± 0.01	0.72 ± 0.01	0.81* ± 0.02	0.93* ± 0.01	0.98* ± 0.01
%	--	+ 12.16 %	+ 20.27 %	+ 25.00 %	--	+ 12.50 %	+ 29.16 %	+ 36.11 %
Heart	0.54 ± 0.02	0.61* ± 0.02	0.65* ± 0.03	0.69* ± 0.01	0.53 ± 0.02	0.64* ± 0.02	0.68* ± 0.03	0.76* ± 0.01
%	--	+ 12.96 %	+ 20.37 %	+ 27.77 %	--	+ 20.45 %	+ 28.30 %	+ 43.39 %

Data expressed as mean ± SD. and SD = Standard Deviation. % = Percentage of change from control. * = Significant. (+ /-) = Increased / Decreased from control.

Table 2: Embryotoxicity data for rat embryos exposed to DMPA doses in Utero during gestation day 6 up to both 15th and 20th days of gestation

	Gestation Days (6GD to GD 15)				Gestation Days (6GD to GD 20)			
	GI	GII	GIII	GIV	GV	GVI	GVII	GVIII
Groups								
Dose	Control	2.7 mg / day	5.4mg / day	10.8 mg/day	Control	2.7 mg / day	5.4mg / day	10.8 mg/day
Implantation Sites	9.90 ± 1.03	7.70* ± 1.41	6.91* ± 1.10	6.10* ± 0.87	9.90 ± 1.37	8.41* ± 1.42	7.30* ± 0.82	7.41* ± 0.96
% of Change	--	-22.22 %	-30.20 %	-38.38 %	--	-15.05 %	-26.26 %	-25.15 %
Corpora Lutea	10.20 ± 0.91	8.00* ± 1.33	7.10* ± 1.19	6.50* ± 1.35	10.10 ± 1.10	8.60* ± 1.26	7.50* ± 0.70	7.38* ± 0.96
% of Change	--	-21.56 %	-30.39 %	-36.27 %	--	-14.85 %	-25.74 %	-26.93 %
Implantation Index	99.08 %	96.25 %	97.18 %	93.84 %	98.01 %	97.67 %	97.33 %	97.35 %
Live Fetuses	9.73 ± 1.19	6.20* ± 0.78	4.90* ± 0.87	3.60* ± 0.21	9.79 ± 1.37	6.57* ± 1.34	4.85* ± 0.56	4.15* ± 0.31
% of Change	--	-36.27 %	-49.64 %	-63.00 %	--	-32.89 %	-50.45 %	-57.60 %
Index of Live Fetuses	97.97 %	80.51 %	71.01 %	59.01 %	96.96 %	78.57 %	67.12 %	55.40 %
Dead Fetuses	0.11 ± 0.31	1.20* ± 0.63	1.50* ± 0.37	2.00* ± 0.94	0.13 ± 0.53	1.20* ± 0.42	1.50* ± 0.52	2.12* ± 0.41
Death Index	1.11 %	11.72 %	18.94 %	22.54 %	1.05 %	11.85 %	17.75 %	24.50 %
Resorbed Fetuses	--	0.31 ± 0.48	0.50 ± 0.25	0.75 ± 0.47	--	0.61 ± 0.15	0.91 ± 0.13	1.30 ± 0.48
Resorption Index	--	4.02 %	7.23 %	12.29 %	--	7.25 %	12.46 %	17.45 %

Data expressed as mean ± SD. and SD = Standard Deviation. % = Percentage of change from control. * = Significant. (+ /-) = Increased / Decreased from control.

Table 3: Fetal dimensions and Sex ratio data for rat fetuses exposed to DMPA doses in Utero during gestation days 6 up to 15th and 20th

	Gestation Days (6GD to GD 15)				Gestation Days (6GD to GD 20)			
	GI	GII	GIII	GIV	GV	GVI	GVII	GVIII
Groups								
Dose	Control	2.7 mg / day	5.4mg / day	10.8 mg/day	Control	2.7 mg / day	5.4mg / day	10.8 mg/day
Crown-rump (cm)	2.57 ± 0.11	2.26* ± 0.05	1.95* ± 0.13	1.71* ± 0.07	3.85 ± 0.05	3.43* ± 0.08	2.96* ± 0.14	2.26* ± 0.09
% of Change	--	-12.06 %	-24.12 %	-33.46 %	--	-10.90 %	-23.11 %	-41.30 %
Body Weight (gm)	2.63 ± 0.11	2.22* ± 0.09	1.96* ± 0. 15	1.77* ± 0.07	3.96 ± 0.25	3.54* ± 0.12	3.23* ± 0.10	2.00* ± 0.17
% of Change	--	-15.58 %	-25.47 %	-32.69 %	--	-10.61 %	-18.43 %	-49.49 %
Sex Ratio (M/F)	62/58	33/42	26/32	20/23	59/61	31/44	27/32	22/27
% of Males	51.66 %	44.00 %	44.83 %	46.51 %	49.16 %	41.33 %	45.76 %	44.89 %
% of Females	48.34 %	56.00 %	55.17 %	53.49 %	50.84 %	58.67 %	54.24 %	55.11 %
Male Anogenital Distance (mm)	2.76 ± 0.05	2.35* ± 0.07	1.93* ± 0.10	1.59* ± 0.08	3.14 ± 0.05	2.55* ± 0.05	1.99* ± 0.11	1.63* ± 0.09
% of Change	--	-14.85 %	-30.07 %	-42.39 %	--	-18.78 %	-36.62 %	-48.08 %

Data expressed as mean ± SD. and SD = Standard Deviation. % = Percentage of change from control. * = Significant. (+ /-) = Increased / Decreased from control.

Table 4: Skull, clavicle and scapula bones ossification in rat embryos exposed to DMPA

		Stage 15th day of Gestation (%)			Stage 20th day of Gestation (%)		
	Skeletal System Part	GII	GIII	GIV	GVI	GVII	GVIII
Skull	Complete Ossification	71.83	53.29	46.39	64.57	58.93	43.71
	Incomplete Ossification	28.17	46.71	53.61	35.43	41.07	56.29
Clavicle	Complete Ossification	25.39	17.12	0.00	32.91	29.82	0.00
	Incomplete Ossification	74.61	82.88	100	67.09	70.18	100
Scapula	Complete Ossification	78.11	55.46	41.32	75.74	62.19	41.62
	Incomplete Ossification	21.89	44.54	58.68	24.26	37.81	58.38

DMPA doses from six up to 15th or 20th days of gestation induced significant increase in the liver (~ + 31.42 %), ovaries (~ + 75.00 %), uteri (~ + 95.45 %), kidneys (~ + 66.66 %), adrenal (~ + 75.00 %), spleen (~ + 51.85 %), lungs (~ + 36.11 %) and heart weights (~ + 43.39 %) of pregnant rats. These changes were dose dependant and statistically significant ($P \leq 0.01$). (Table 1).

In Utero-Fetal Exposure Effect: DMPA treatment induced decrease in the implantation sites (~-30.58 %), implantation index (93.84 % vs. 99.08 and 98.01% for the control GI and GIV) and Corpora lutea (~-36.27 %) as well as reduction in number of live fetuses reached maximally (~-63.00%). Also, the index of live fetuses showed reduction reached maximally (~-55.40 % vs. 97.97% and 96.96 % for the control GI and GIV) as shown in Tables 3 and 4. DMPA doses induced intrauterine death which reached (death index ~ + 24.50 % vs. 1.11 and 1.05 % in control GI and GIV). Referring to fetal resorption induced by DMPA doses it was found that DMPA induced fetal resorption reached maximally (~ + 17.45 %) these changes were statistically highly significant ($P \leq 0.01$) as shown in Tables 2 and 3.

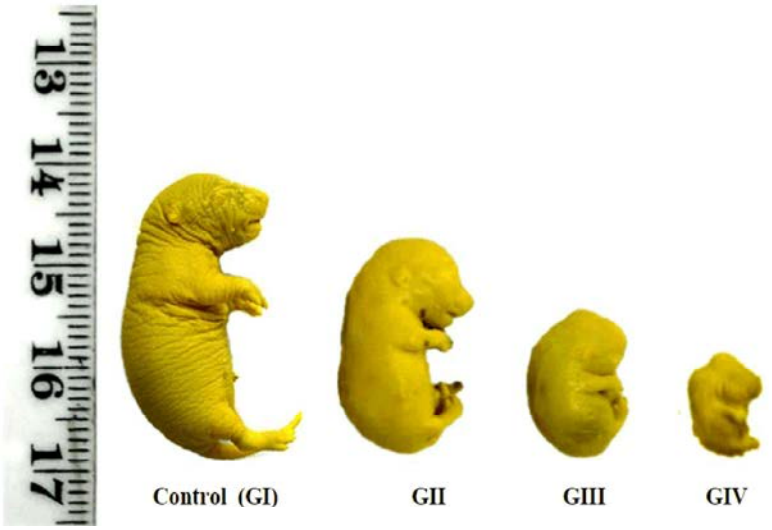

Fig. 1: Showing Crown - Rump Length of Rat Embryos at 15th days of gestation

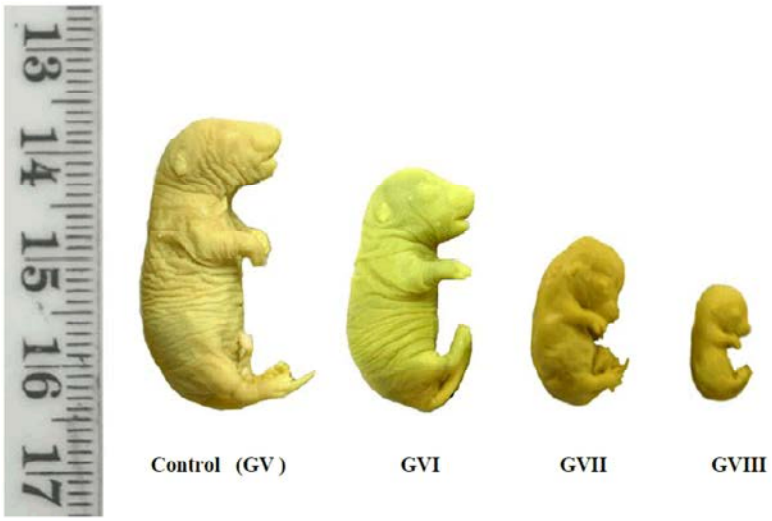

Fig. 2: Showing Crown - Rump Length of Rat Embryos at 20th days of gestation

By evaluating the teratogenic potential of DMPA several fetuses were observed with reduction in both crown rump and body weight which reached ~-33.46 and ~-49.49 %, respectively. Also, DMPA induced decrease in male anogenital distance (~-48.08 %) and skewed the sex ratio (% of female ~ 55.11%) toward females. (Tables 2 and 3; Figures 1 and 2). All these changes were statistically highly significant ($P \leq 0.01$).

Assessment of Bone Calcification and Anomalies: Following alizarin red S staining, various skeletal bones were seen and by examining these bones using dissecting microscope the obtained data revealed that DMPA doses during pregnancy induced reduction in the ossification of the skull bones, and these reductions were dose and time dependant. Maximum percentage of reduction in ossification was recorded among the fetuses obtained from GIV and GVIII the most affected bones were nasal, temporal, parietal and occipital. While, ossification of mandibles, maxilla and frontal bones were moderately affected. Also, gradual reduction in ossification of clavicle and scapula were observed and recorded as seen in table4 and in plate 1.

The present work DMPA induced several abnormalities of ribs of rat embryos represented by missing of some ribs (maximum ~ 26.39 %), shortness of ribs (maximum ~ 28.69 %) and rib fusion (maximum ~ 24.26 %).

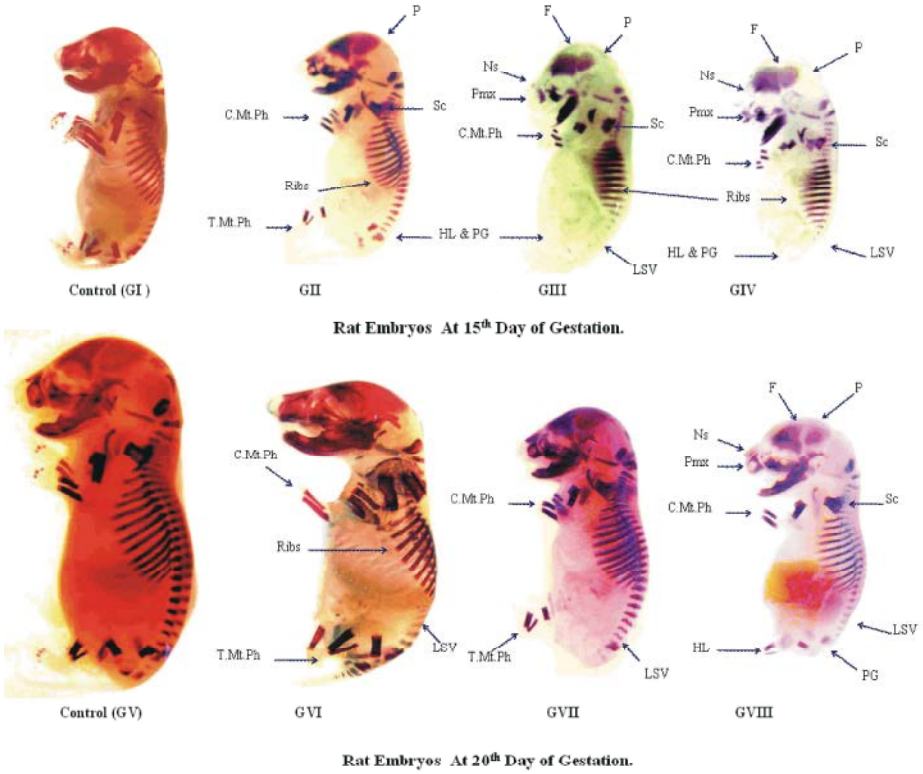

Plate 1: Effect of DMPA doses on the ossification of the skeletal system of rat embryos.
Where: GII&GVI; GIII&GVII; GIV&GVIII are treated with DMPA doses (2.7; 5.4 and 10.8 mg/rat/day); P: Parietal; Sc: Scapula; HL&PG: hind limb and pelvic girdle; T.Mt.Ph: traso-metatarsal and phalanges; F: frontal; Ns: nasal; Pmx: premaxilla; C.Mt.Ph: carpo-metacarpales and phalanges; LSV; lumbo-sacral vertebrae; PG: pelvic girdle.

Table 5: Percentage of abnormalities and ossifications in ribs, vertebrae and sternebrea bones in embryos exposed to DMPA doses

		Stage 15th day of Gestation (%)			Stage 20th day of Gestation (%)		
	Skeletal System Part	GII	GIII	GIV	GVI	GVII	GVIII
Ribs	Normal	23.82	24.18	22.34	24.88	24.96	23.62
	Missed	25.29	26.11	26.39	24.19	25.23	25.68
	Short	25.67	27.33	28.69	26.67	27.83	28.33
	Fused	25.22	22.38	22.58	24.26	21.98	22.37
Sternebrae	Missed	25.19	29.72	33.35	27.86	30.29	31.18
	Fused	18.29	38.78	42.25	38.36	45.83	47.39
	Normal	56.52	31.50	24.40	33.78	23.88	21.43
Vertebral Centra	Fused	12.29	25.51	31.12	16.63	32.28	43.16
	Missed	45.68	51.14	54.47	41.16	31.92	36.62
	Normal	42.03	23.35	14.41	42.21	35.80	20.22
Fore Limb	Humerus	53.62	31.16	18.23	57.18	39.32	27.16
	Incomplete Ossification	46.38	68.84	81.77	42.82	60.68	72.84
	Ulna	47.19	22.64	16.14	49.38	41.67	32.39
	Incomplete Ossification	52.81	77.36	83.86	50.62	58.33	67.61
	Radius	39.52	29.11	11.36	41.61	40.29	26.57
	Incomplete Ossification	60.48	70.89	88.64	58.39	59.71	73.43
Hind Limb	Femur	41.93	18.61	0.00	43.36	0.00	11.26
	Incomplete Ossification	58.07	81.39	100	56.64	100	88.74
	Tibia	46.21	41.29	0.00	41.69	32.14	18.29
	Incomplete Ossification	53.79	58.71	100	58.31	67.86	81.71
	Fibula	44.64	39.62	0.00	42.86	31.62	16.35
	Incomplete Ossification	55.36	60.38	100	57.14	68.38	83.65

Vertebral centra of embryos from all treated groups, were found to be less ossified than those in control as seen in Table 5 and plate 2. Some of cervical, thoracic, lumbar and caudal vertebrae were missed. The most common abnormalities were manifested in missing of central discs (maximum ~ 54.47 %) as well as fused (maximum ~ 43.16 %) of vertebral column.

Bones of Sternebrae were observed completely ossified in control groups at both 15th and 20th days of gestation. While, examination of embryos from treated rats with DMPA revealed that, the percentage of fused sternebrae reached (maximum ~ 47.39 %) the missed sternebrae its percentage in the treated groups reached (maximum ~ 33.35 %).

The bones of both fore-and hind limbs were completely ossified in control rat embryos. Fore limb bones examined in embryos from DMPA treated groups showed reduction in ossification of Humerus (maximum ~ 81.77 %), ulna (maximum ~ 83.86 %) and radius (maximum ~ 88.64 %). While, these doses induced sever incomplete ossification of femur, tibia and fibula. DMPA doses induced incomplete ossification in the proximal bones in both fore-and hind limbs as carpals, metacarpals, fore phalanges, tarsal, metatarsal and hind phalanges. In addition, all bones of the treated fetuses showed retardation in their length and size when compared with those of control as shown in Table 5 and plate 1.

DISCUSSION

Injectable hormonal contraception with long-acting steroidal preparations has become an important method of family planning methods. The most intensively studied and widely used formulation is depot Medroxyprogesterone acetate, a long-acting progestogens, now marketed as a contraceptive in more than 130 developed and developing countries and used by some 40 million women. Depo-Provera is the most widely used long-term reversible contraceptive in the US and is used throughout the world. Apparent contraceptive failures with Depo-Provera may be due to administration when the woman is already pregnant or to other factors [14].

Endogenous natural progestins are essential for the initiation and maintenance of pregnancy in the rat. With regard to progesterone, growth and development of the embryo and fetus are unaffected over a wide range of progesterone concentrations in the maternal plasma. While, exogenous progestins and their derivatives induced fetal malformations [42].

In this study, DMPA induced significant increase in the body weight and several body organs weight. Although there have been subjective reports that most hormonal contraceptives are associated with little or no effect on body weight [43]. Some studies have failed to find that DMPA is associated with significant weight gain [44]. But there are a lot of scientific reports published concerning the deleterious health consequences of overweight and obesity during DMPA administration and some women attribute their weight gain to such use [33]. Also, it was noted that the product labeling for DMPA notes a tendency for women to gain weight during DMPA use: an average of 5.4 pounds by 1 year of use, 8.1 pounds by 2 years, 13.8 pounds by 4 years and 16.5 pounds by 6 years [45]. Our results were in a greement with other previous studies [46,47] who reported body weight gain in the female rats treated with DMPA (2.7 mg/rat or 5.4 mg/rat) for ten and fifteen days. Also, similar observations were reported by many authors[48-60]. Concerning the reasons of why DMPA use leads to weight increase?. Because of its anabolic effects and fluid retention [61,62] and this increase could depend on modifications on the hypothalamic appetite control center associated with the use of DMPA[63]. However, another study attributed that weight increase depends on fat deposition, higher appetite and dietary ingestion [64]. However, these results conflict with the study of Pelkman *et al*. [65] who reported that DMPA did not cause weight gain in young women over a 3-month administration period.

In the present study, DMPA doses induced reduction in numbers of implantation sites, number of corpora lutea and number of live embryos. It also, induced increase in mortality and resorption rate among embryos at both (GD15th and 20th) as well as decrease in embryonic crown rump and body weight. Also, DMPA induced decrease in male anogenital distance and skewed the sex ratio toward females. DMPA exerts its effects by freely diffusing into the nucleus of target cells where it binds to the progesterone receptor, affecting transcription of selected genes and ultimately resulting in protein synthesis. Progesterone receptors have a very narrow tissue distribution, being found primarily in the female reproductive tract [66, 67]. So, DMPA inhibits the secretion of gonadotrophin which, in turn, prevents follicular maturation and ovulation and results in endometrial thinning and transforms proliferative endometrium into secretory endometrium. The contraceptive action of DMPA is believed to result from inhibition of ovulation, thickening of cervical

mucus, creation of an atrophic endometrium and premature luteolysis [67-69]. Also, reduction of number of corpora lutea considered another factor for reduction of embryos; whereas corpora lutea secret endogenous progestins for caring pregnancy [70].

Our study attributes these embryonic malformations to the role of DMPA which may be induced alteration in steroidal hormones and conjugated with serum lipids, which may inhibit gene expression of certain proteins, which leads to foetal anomalies. Similar results were recorded and discussed by many authors examining DMPA for embryotoxicity as [16-19, 71-77].

Several reports discussed different modes of action by which steroid hormones may induce embryonic malformation, one of these suggested that steroid hormone or its active metabolites may act directly on the embryos or may be stored in the embryo and remain inactive until a steroid metabolism is established. Then start to exert its influence on the target organs on days of high specific sensitivity. Also, steroid hormone may be act indirectly on the preimplantation embryo by disturbing oviduct fluid, affect the endometrial or the development of the decidua [16, 17].

The molecular mechanism by which steroid hormones exert their biological effects includes induction of a profound increase in RNA and protein synthesis. Steroid hormones enter most cells by passive diffusion whereas they bind to specific cytosolic and nuclear receptors. Once bound, the receptor undergoes a conformational change converting the receptor complex from an inactivated form into an activated form. The activated receptor complex has a high affinity for specific nuclear sequences referred to as steroid response elements, which have characteristics of classic enhancer elements. Binding of the steroid-receptor complex typically results in gene activation (i.e., gene transcription) and increased mRNA synthesis. mRNA is then translated on cytoplasmic ribosomes thereby increasing the expression of hormone responsive proteins, which in turn alters cell function, growth, differentiation then cell death or impair the embryos and resulted in malformation or anomalies. [78-80]. So, the reductions occurred in the numbers of still live rat embryo, crown rump, embryo body weight and increase in the resorbed, subcutaneous hematoma as well as died embryo may attributed to the previous reasons. Proteins, such as uteroferrin and retinol-binding protein (RBP), secreted by the uterus in swine constitute one mechanism by which vitamins and minerals are transported to the developing conceptus [81]. Uterine protein secretion is primarily controlled by mechanism(s). An alternative mechanism may be the duration that the uterus is exposed to progesterone which leads to increase in the uterine protein secretion and may induce embryonic loss and possibly increased uterine capacity via a decrease in the developmental rate of the conceptuses [82-85]. Thus, the induced serious effects to the developed embryos may be also, referred to the increases in the secretion of uterine protein which may impair the embryos.

Administration of very high amounts of exogenous progesterone during early pregnancy could have deleterious effects on neural tube closure [86] which support our results. Anogenital distance is the length between the anus and the genital tubercle. In rodents and primates, the anogenital distance is greater for males than females, the increased growth of this region occurs in response to testosterone [40]. In this study, reduction in anogenital distance recorded in male rat embryos exposed to DMPA doses *In Utero,* which supported by many studies [87-90] who reported alteration and permanent genital defect in the genitalia of mouse and rat embryos for mothers treated with progesterone on GD12-16. However, one contradicted study [91] showed that no association between first-trimester exposure to sex hormones generally and external genital malformations.

Fetuses obtained from DMPA treated pregnant rats in the present study showed several skeletal defects and this deficiency in the mineralization of the skeletal system of the fetuses referred to DMPA doses [18,19, 72-77, 92,93]. DMPA induced skeletal calcium mass defects in the treated rats due to decrease in the intestinal calcium absorption and alteration in Ca^{+} metabolism which leads to significant reduction in bone calcification (decreased bone Ca^{+} accretion and resorption rates). The increases in endogenous fecal Ca excretion explain the decrease in bone calcification and hence reflect the reasons of skeletal calcium mass defects [30,94,95]. In dogs, steroid hormones modulates cholecalciferol metabolism with moderate effects on intestinal mineral absorption and specific effects on bone formation [96]. Exogenous progesterone during early pregnancy could induce deleterious skeletal malformations effects and affect neural tube closure [86]. Finally, due to antagonistic effect between DMPA and estrogen which resulted in deficiency in serum estrogen levels, which will lead to bone mineral density loss [97].

In conclusion, the results of this study revealed DMPA induced several teratogenic effects in rat embryos. So, the present investigation advising the woman use Depo-Provera to make sure that she is not pregnant.

These findings shed more light on the effects of DMPA and support the claims that this hormone, while being a progestational agent, is also embryo-toxic and teratogenic.

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