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Comparative Embryotoxic Effects of Two Injectable Contraceptives, DMPA and Mesocept, in Rats

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Abstract

Injectable contraception has been available for over 50 years and is currently used by some 35 million women worldwide. Depo-Provera®; Depot-Medroxyprogesterone acetate (DMPA) and Mesocept® are used as contraceptive agents in Egypt. Possible contraceptive effects on embryos remain controversial. The aim of this study was to assess the effects of the two injectable contraceptives on embryos of rats. Sixty pregnant rats (Sprague-Dawley) were assigned into ten groups, these animals were intramuscularly injected on the sixth day of gestation (GD6) with single dose of DMPA or Mesosept (Vehicle 0; 2.7; 5.4; 0.99 and1.98 mg/rat). Animals were sacrificed on day 15 or 19 of gestation and the embryos examined. Results showed that Both DMPA and Mesocept caused embryonic toxicity manifested in malformations and in a reduction of the number of surviving embryos, increased the mortality and resorption rate as well as causing a decrease in the embryonic crown rump ($p < 0.05$). In addition, there was a widespread developmental malformation of the skeletal system. These findings shed more light on the effects of DMPA and Mesocept, and support the claims that these hormonal contraceptive are embryo-toxic and have teratogenic effect.

Keywords: Depo-Provera®, Mesocept®, Rat embryo, Malformations, Skeletal abnormalities, Contraception

1. Introduction

Hormonal and non-hormonal contraception is widely used, even in many of the world's least-developed countries (1). Steady progress in contraception research has been achieved over the past 50 years (2). At the world level, 63% of women in the reproductive age group are reported to be using contraception, for a total of 716 million women worldwide (2). Novel products such as new implants, contraceptive vaginal rings, transdermal patches and newer combinations of oral contraceptives have recently been introduced in family planning programs (2). Since its introduction in the 1960s, hormonal contraception has been increasingly accessible and widely used for both planning and limiting births in developing countries (2). However, about 10% of all women of reproductive age use combined hormonal contraception and that number is still increasing (3).

Currently, 35 million women worldwide use injectable hormonal contraception to prevent pregnancy, twice as many as a decade ago (4). Nevertheless, unintended pregnancies in both the developing and developed countries remain a serious obstetric problem (2,5). The leading factors that contribute to unintended pregnancies include “an unmet need for family planning” or “contraceptive failure”. Nearly 50% of unintended pregnancies occur in contraceptive users; however, only 10% of such pregnancies result from true method failure. (2,6-8). Unintended pregnancies and associated birth defects are attracting national attention as public health problems that are once again on the rise

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specially with using hormonal contraceptives (6). DMPA is the fifth most commonly used

contraceptive method worldwide (9). The available progestin-only injectables included in the current methods of contraception are norethisterone enanthate (NET-EN) and depot medroxyprogesterone acetate or Depo-Provera® which were approved by the FDA in 1992 (10). However, Mesocept is one of combined injectable contraceptives (CICs), delivers 50 mg NET-EN and 5 mg estradiol valerate in oily solution (11).

DMPA and Mesocept are synthetic estrogen and progesterone which exert their effect by freely diffusing into the nucleus of target cells where they bind 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 (12). So, Contraceptive injection has multiple mechanisms of action providing contraception (13). Progestins may prevent ovulation by suppressing LH secretion (13). In addition, progestins may impede the transport of sperm through the cervical canal by thickening cervical mucus (14). Progestins also may inhibit implantation by causing alteration or transformation of the endometrial lining or alter the transformation of sperm or ovum within the fallopian tubes (13,14). So, the FDA (Food and Drug Administration) indicated that it prudent to avoid the use of these drugs during the first trimester of pregnancy (5,15).

Several toxicological studies has been published concerning the safety of DMAP on experimental animals during pregnancy at high doses (3, 30, 100, 300, 1000 mg/kg/day) to mice, rats and rabbits (16). While other animal studies administering DMPA on day 2 pregnancy showed increase 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 including teratogenicity and fetotoxicity (17-19). However, other animal studies have shown that high doses of progestogens can cause masculinisation of the female fetus (19).

Extensive epidemiological studies were performed to assess the possible risks of birth defects in women who have used hormonal contraception (20). It was found that among delivered pregnancies, DMPA use was associated with decrease in fetal weight, increase in the rate of cleft palate and urinary tract abnormalities (20). Exencephaly and heart abnormalities were also significantly more frequent, but this increase was not dose-dependent (20). Also, increases in ectopic pregnancy rate and fetal anomalies were recorded among women using DMPA (20).

Many studies carried out on DMPA users found that Chinese women who used DMPA over a period of 3 years, had retardation in bone loss with time, possibly due to the effect of progesterone in decreasing bone turnover (21). Also, injectable contraception may decrease bone density and increase the risk for osteoporosis in later life (5). One hundred thirty-two menopausal women were randomized into a 2-year clinical trial to evaluate the effect of both estrogen and progesterone on bone metabolism. The study showed that estrogen remains the primary bone active agent in hormone therapy, while progestins have significantly less activity (5). The present study aimed to shed more light, evaluate and compare the reproductive toxicity induced by two injectable contraceptives: 3-monthly Injectable DMPA (Depo-Provera-progesterone-only) and the one-monthly Injectable combined contraceptive (Mesocept) in female pregnant rats and their embryos during different developmental stages.

2. Materials and Methods

Animals and Mating: Eight to ten weeks-old sexually mature male and female rats *(Sprague-Dawley)* of an average body weight of 140-180 gm were used the present study. Rats were normal, healthy and obtained from the Animal House Department of the Nile Company for Pharmaceutical and Chemical Industry, Egypt. These animals were housed under regular periods of dark and light (12-hours dark and 12-hours light) at 20-25°C. Animals were marked and housed 2 per cage, and fed on a standard rodent pellet diet during the periods of the experiments. Then at the proestrous phase, mature males were caged overnight with virgin females in a ratio of one male to two females per cage. In the next morning, the presence of the vaginal plug was considered or designated as the first day of pregnancy (22-24). The pregnant females were removed from the mating cages and re-housed in groups, where they remained undisturbed until the morning of the 6th day of gestation under the same conditions mentioned previously.

Drugs: (1) Depo-provera® DMPA; the drug is sold in Egypt for contraception use in sterile Depot-aqueous solution for intramuscular injection. DMPA is progestin-only and manufactured by The Upjohn Company (Kalamazoo, Michigan, U.S.A.). (2) Mesocept®; was received from one of combined injectable contraceptives (CICs) of the family planning private clinics in Cairo. The drug is solution

in 1 ml bottle contains 50 mg norethisterone enanthate and 5 mg estradiol valerate (11). Mesocept is manufactured by Chemical Industries Development CID (11). Both DMPA and Mesocept were received

from one of the family planning clinic in Al Hussien Hospital in Cairo.

Experimental Groups: Sixty pregnant rats were divided into two main groups (A&B) of 30 pregnant rats each. Each group was divided into five subgroups of 6 pregnant rats/group, the first subgroups (A0) and (B0) were considered as the control groups, while the other eight subgroups (A1, A2, A3, A4 and B1, B2, B3, B4) were assigned as treated groups. These animals were intramuscularly injected on day six of gestation (GD6) with DMPA or Mesosept doses (Vehicle 0; 2.7; 5.4; 0.99 and 1.98 mg/rat) through (GD15 or 19). These doses were converted from human dose to rat dose using multiplication factors for dose conversion between different species as indicated by Paget and Barnes (1964) (25).

Teratogenic Investigation: All animals were weighed and observed daily to estimate the changes in the behavior and in the body weight during the experiment. The pregnant rats were sacrificed by cervical dislocation and the gravid uteri were dissected out. Foetuses were removed from the uterus, weighed and examined for the live, dead and resorbed foetus, and the numbers were recorded (26,27).

Foetuses from all pregnant rats were divided into two subgroups. foetuses in the first subgroup were fixed in Bouin's solution for a period of 2 days before assessment for external malformations and measuring of the crown-rump length. Foetuses were then photographed with standard scale (28).

Foetuses in the second subgroup were placed in 10% formalin for skeletal system examination.

Skeletal Examination: Foetuses were fixed in 10% formalin for 7 days, then cleared in 2% KOH for three days, and stained with Alizarin red S dissolved in 2% KOH for two days. Then clearing of the skeleton was carried out by passing the foetuses through different concentrations of glycerin and 2% KOH. Then fetuses were examined under a dissecting microscope for the skeletal anomalies. Finally, foetuses were stored in pure glycerin (29,30).

Statistical Analysis: The statistical analysis for the obtained data was done using SPSS 13 for windows 2007, and *p* values of less than 0.05 were considered significant. The Student's "t"-distribution were adopted for assessment of significant changes occurring between the groups (31).

3. Results

Effects of DMPA and Mesocept on utero fetal parameters:

Examining the gravid uteri and counting implantation sites, survived, dead and resorbed embryos after in utero exposure to both DMPA and Mesocept showed sever effect in both stages of the experiment. This decrease in implantation site were maximum - 43% and - 60%, in survived embryos were -46.9% and -100 %, while number of dead embryos reached three folds more than normal mortality rate. Referring to fetal resorption, DMPA did not induce fetal resorption at these dose levels. While Mesocept doses induced fetal resorption reached -100% at the highest dose level. These increases and decreases were statistically significant ($P \leq 0.01$) when compared to the control (Tables 1, 2 plate 1).

Measuring the crown rump length of survived embryos exposed to DMPA and Mesocept doses indicated a decrease in the crown rump values of the survived embryos, reaching -43.8% and - 38.5% respectively. These changes were statistically significant ($P \leq 0.01$). Also, the body weight of fetuses showed great loss reaching - 71.9% and - 73.7% in DMPA and Mesocept doses, respectively. These decreases were statistically significant ($P \leq 0.01$) as shown in tables 1 and in figures 2&3.

Assessment of fetal Bone Ossification and skeletal anomalies

During the different developmental period in either day 15 or day 19 of gestation, the obtained data revealed that DMPA and Mesosept doses during pregnancy induced reduction in the ossification of bone, fore limbs, ribs vertebral centra and the hind limbs and these reductions were dose and time dependant. Complete ossification of the different parts of the skull (nasal, frontal, parietal, interparital, supraoccipital and basiocciptal as well as premaxilla, maxilla and mandibles), was also observed. Maximum percentage of reduction in ossification was recorded and tabulated in table 3 and plates 2, 3.

The present work revealed normal rib patterns and complete ossification of the ribs in control fetuses as represented in table 3 and figure 5 (A&B). In contrast to these results, abnormalities were induced by DMPA and Mesocept in rat fetuses showing irregular shape of ribs in all cases, missing ribs 80 % and incomplete ossification of ribs maximum 100 % in groups A1, A2, B1, and B2, respectively, and in table 3, plate 6 A1, A2, B1, & B2. While, in groups A3 and B3 with Mesocept showed less side effects on the fore limbs and seen in table 3 as figure 5 (A3&B3).

Vertebral centra of embryos from all treated groups were found to be less ossified than those of the control groups as seen in figure 5 and table 3. Some of cervical, thoracic, lumber vertebrae were missed in fetuses in groups A1 and A2. The data showed that administration of DMPA to the pregnant rats on day 15 of gestation induced abnormalities in most fetuses from the treated groups. These abnormalities are manifested in incomplete ossification, missed central discs, delayed ossification as well as scoliosis of the vertebral column.

In addition, as developmental progress on day 19 of gestation, the same abnormalities were observed, with sever congenital abnormalities in the vertebral column such as scoliosis and missing central discs in groups B1, and B2 respectively, as seen in figure 5 (B1&B2) and table 3. On the other hand, groups A3 and B3 treated by Mesocept showed less side effects on the vertebral centra as shown in table 3 and figure 5 (A3&B3).

Table 1. Effect of DMPA and Mesocept on rat embryos exposed to different doses on 15 and 19 days of gestation.

Groups	Gestation Days (6 GD to GD 15)					Gestation Days (6 GD to GD 19)				
	Control A	DMPA		Mesocept		Control B	DMPA		Mesocept	
Dose (mg/rats)		2.7 mg A_1	5.4 mg A_2	0.99 mg A_3	1.98 mg A_4		2.7 mg B_1	5.4 mg B_2	0.99 mg B_3	1.98 mg B_4
Implantation sites	10 +0.5	6.8^{**}+0.3	5.7^{**}+0.6	5.8^{**}+0.8	5.2^{**}+0.6	9.5+0.6	5.8^{**}+0.6	6.8^{**}+0.5	3.8^{**}+0.7	4.7^{**}+0.3
% of Change	----	- 32%	-43%	- 42%	-48 %	----	- 38.9%	-28.4%	-60%	-50.5 %
Survived	9.8 +0.3	6.5^{**}+0.4	5.2^{**}+0.3	4^{**}+0.4	-----	9.3+0.5	5.5^{**}+0.4	6.2^{**}+0.6	8^{**}+0.3	----
% of Change	----	- 33.7%	- 46.9%	-59.2%	-100 %	----	- 40.9%	-33.3%	-91.4%	-100 %
Dead	1 +0.4	$2^{N.S}$+0.6	3^{*}+0.7	4^{**}+0.7	-----	1+0.4	$2^{N.S}$+0.6	4**+0.5	4**+0.6	----
% of Change	----	+100%	+200%	+300%	-100 %	----	+100%	+300%	+300%	-100 %
Crown rump	0.03+ 1.6	0.04+**1	0.05+**0.9	0.05+**1	-----	3.9+0.03	0.08+**2.5	0.08+**2.4	0.08+**2.9	----
% of Change	----	- 37.5%	- 43.8%	-37.5%	-100 %	----	- 35.9%	-38.5%	-25.7%	-100 %
Body weight	0.57+0.03	0.01+**0.16	0.01+**0.16	0.01+**0.15	-----	3.1+0.08	0.08+**2.4	0.1+**1.8	0.09+**1.9	----
% of Change	----	71.9 %	-71.9 %	-73.7 %	-100 %	----	-22.6 %	-41.9 %	-38.7 %	-100 %

Data expressed as mean ± standard error. % = Percentage of change from control. ** = ≤0.01. * = ≤0.05. N.s. = Non significant. (+ /-) = Increased / Decreased from control.

Table 2. Effect of DMPA and Mesocept on implantation sites and percentage of survived, dead and resorbed rat embryos exposed to different doses on day 15 and 19 of gestation.

Groups	Gestation Days (6 GD to GD 15)					Gestation Days (6 GD to GD 19)				
	Control A	DMPA		Mesocept		Control B	DMPA		Mesocept	
Dose (mg/rat)		2.7 mg A1	5.4 mg A2	0.99 mg A3	1.98 mg A4		2.7 mg B1	5.4 mg B2	0.99 mg B3	1.98 mg B4
Implantation sites	60	41	34	35	31	57	35	41	23	28
Live (%)	98.3	95.1	91.2	68.6	----	98.2	94.3	90.2	21.7	----
Dead (%)	1.7	4.9	8.8	11.4	----	1.8	5.7	9.8	17.4	----
Resorbed (%)	----	----	----	20	100	----	----	----	60.9	100

The data represented as percentage (%).

Table 3. Teratogenic effect of DMPA and Mesocept on the skeletal system of the rat embryos.

Skeletal Parts	Skeletal malformation	Day 6 to day 15 of gestation (%)				Day 6 to day 19 of gestation (%)			
		DMPA		Mesocept		DMPA		Mesocept	
		2.7 mg A_1	5.4 mg A_2	0.99 mg A_3	1.98 mg A_4	2.7 mg B_1	5.4 mg B_2	0.9 mg B_3	1.98 mg B_4
Skull	**Incomplete ossification**	80	100	10	----	80	81.8	15	----
Ribs	**Irregular shape**	30	----	5	----	100	54.5	5	----
	Missed	20	80	10	----	80	18.2	10	----
	Incomplete ossification	50	100	5	----	100	63.6	10	----
Vertebral centra	**Missed**	30	100	----	----	40	45.5	-----	----
	Scoliosis	50	----	10	----	40	36.4	15	----
	Normal	20	----	90	----	20	18.1	85	----
Fore limbs	**Incomplete ossification**	100	100	----	----	----	91.9	10	----
Hind limbs	**Incomplete ossification**	100	100	----	----	20	91.9	----	----

The data represented as percentage (%).

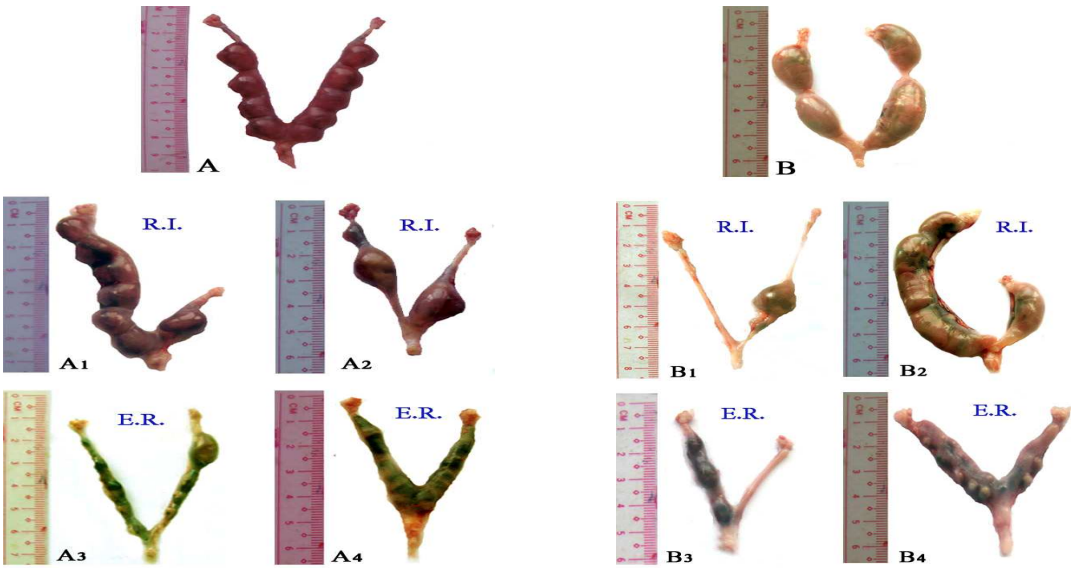

Plate 1. Uteri obtained from control, DMPA and Mesocept treated pregnant rats on 15 or 19 day of gestation, showing equal distribution of embryo in control A&B and unequal distribution of embryo in the two horns in (A1, A2) and (B1, B2) and resorbed of embryos in (A3, A4) and (B3, B4). Where: R.I. = reduction of implantation sites and E.R. = abnormal early resorbed embryos.

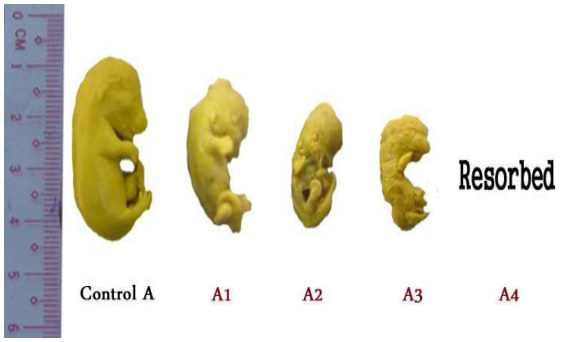

Figure 2. Photomicrographic picture of fetuses of control, DMPA and Mesocept treated rats on day 1 of gestation, showing abnormalities in the crown rump and loss in body weight in groups A1, A2, A3 and resorbed in group A4.

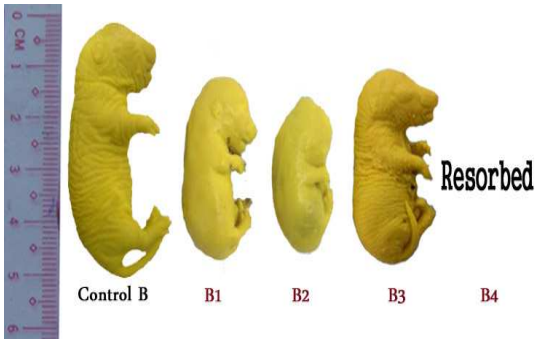

Figure 3. Photomicrographic picture of fetuses of control, DMPA and Mesocept treated rats on day 19 of gestation, showing abnormalities in the crown rump and loss in body weight in groups B1, B2, B3 and resorbed in group B4.

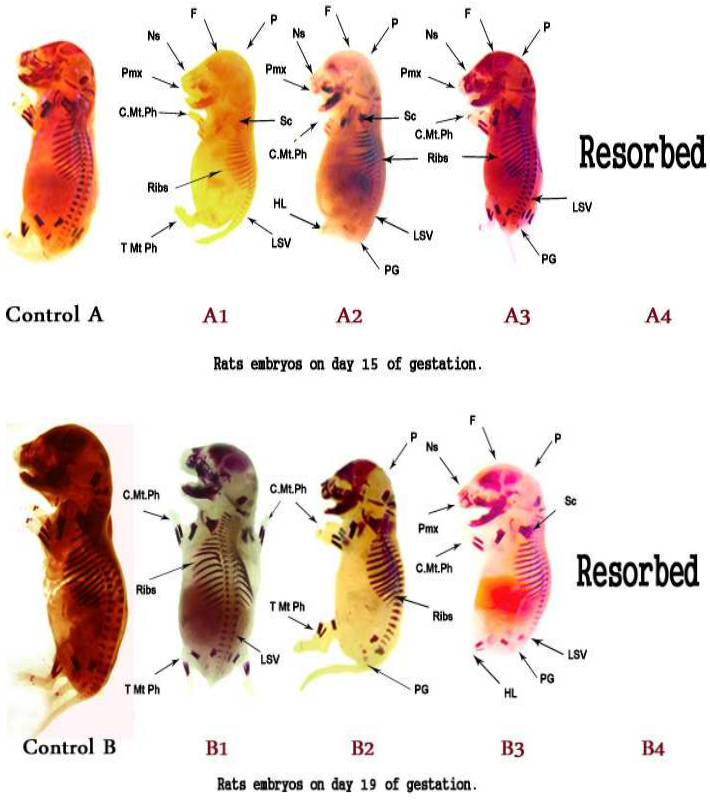

Plate 2. Photomicrographic picture of the ossification of the skeletal system of rat embryos on control, DMPA and Mesocept treated rats on 15 and 19 days of gestation. Where: A1, A2, A3, A4 and B1, B2, B3, B4 treated groups with DMPA and Mesosept doses (Vehicle 0; 2.7; 5.4; 0.99 and 1.98 mg/rat) through (GD15 or 19); 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.

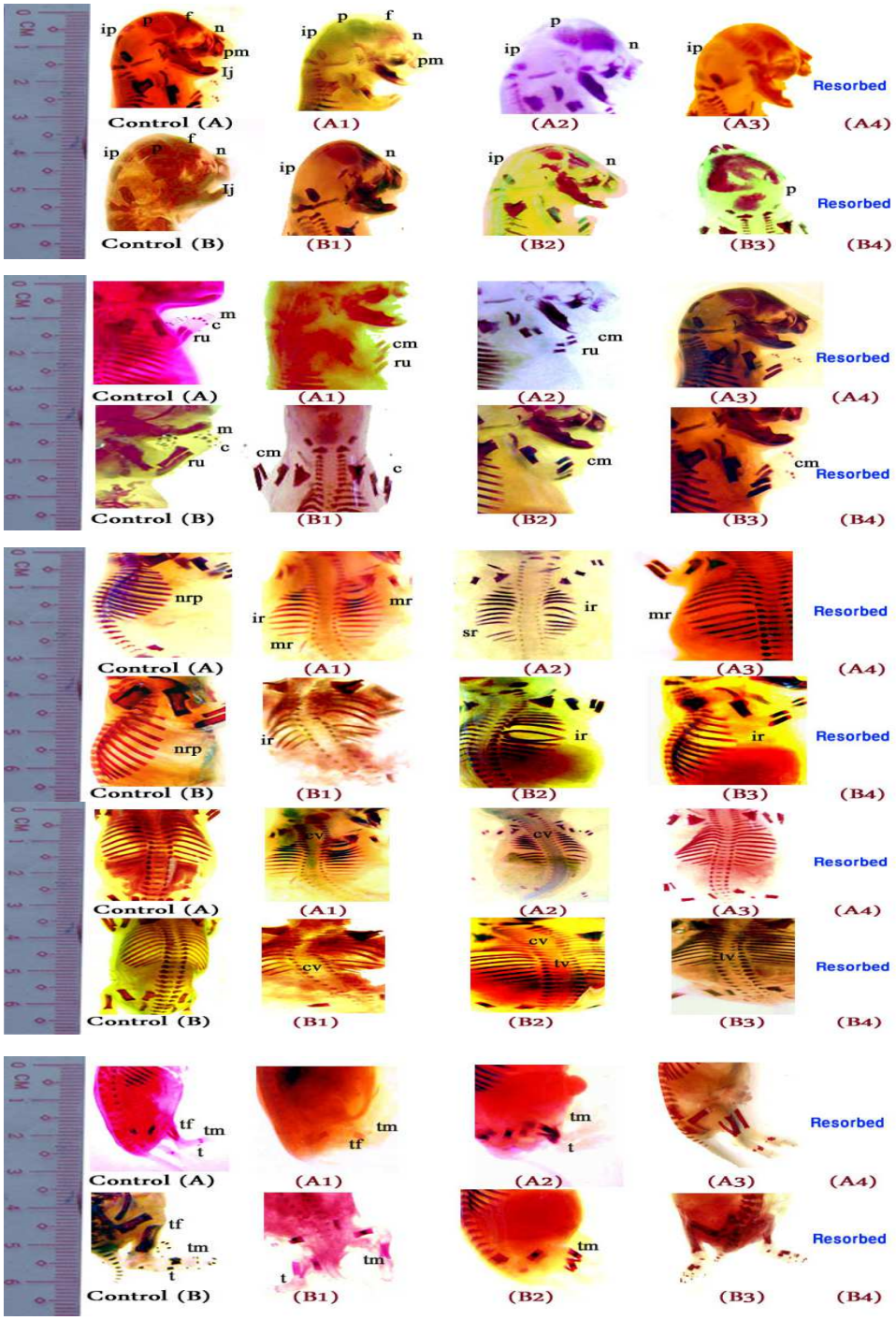

Plate 3. Representative of skeletal abnormalities (Ossification of the skull, fore limbs, ribs vertebral centra and the hind limbs) induced by DMPA and Mesocept in fetuses of female rats on 15 and 19 day of gestation; Where: A, B control on 15 and 19 days of gestation. A1, A2, A3, A4 and B1, B2, B3, B4 treated groups with DMPA and Mesosept doses (Vehicle 0; 2.7; 5.4; 0.99 and 1.98 mg/rat) through (GD15 or 19); P: parietal, Ip: interparital, F: frontal, n: nasal, pm: premaxilla, Ij: lower jaws, m: metacarpals, c: Carpals, ru: radio-ulna, mr: missed ribs, ir: irregular, vc: vertebral central, tv: thoracic vertebral and t: tarsals, tm: metatarsal, tf: tibio-fibula.

4. Discussion

In this study, rat's embryos were exposed in utero to doses of two injectable contraceptives, DMPA and Mesocept during different stages of pregnancy. The doses of both injectable contraceptive exert there effects which were manifested in uterine dismorphology, post-implantation death, resorption of embryos, as well as diminished growth. These results are similar to that observed by (Mugahed, 2005) (32). However, (Taylor, 1986) suggested that experimental animals may destroy their malformed offspring. Female mice may cannibalize newly delivered abnormal pups (33). As a result of that, the practice among teratologists is to deliver the term foetuses by cesarean section one day before normal parturition is expected. This practice not only allows recovering malformed foetuses but also allows examination of dead and resorbing foetuses in situ (33). DMPA and Mesocept are synthetic estrogen and progesterone which exert their effect by freely diffusing into the nucleus of target cells where they bind 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 (12). Our results were in agreement with other previous studies (12-14,32,33).

Our study attributes these anomalies to DMPA and Mesocept, which may induce alteration in steroidal hormones and their conjugation, may inhibit gene expression of certain proteins that leads to fetal anomalies. Similar results were recorded by many authors examining DMPA and Mesocept for embryotoxicity (5,34-36).

Alteration in steroidal hormones and conjugation with serum lipids, which may inhibit gene expression of certain proteins leading to foetal anomalies has also been reported (17,18,34,37-44). Several reports suggest an association between intrauterine exposure to progestational drugs in the first trimester of pregnancy and genital abnormalities in male and female fetuses. Some of these drugs induce mild virilization of the external genitalia of the female fetus, and increased association of hypospadias in the male fetus (45-47). Also, the modes of action responsible for these embryotoxic and teratogenic effects may be caused by the hormone itself or its active metabolites, which act directly on the embryos or may affect the endometrium and the development of the deciduas (48,56). Steroid hormones may be stored in the embryo and remain inactive until a steroid metabolism is established (48,56). Development of the pre-implantation embryo may be disturbed indirectly through influences on oviduct fluid. Long acting progestins may induce a delayed teratogenic action; the hormones or their active metabolites may remain in the dam as well as in the embryo and affect target organs on days of high specific sensitivity (48,56).

Some studies in women suggested that, any teratogen administered in the pre-embryonic phase (day 0–14) of pregnancy will have an ‘all or nothing’ effect, leading either to the death of the conceptus or causing no harm at all (36). In the embryonic phase (3–8 week’s gestation) the main organ systems and body features are formed and teratogens may cause gross malformations. During the fetal phase (9 weeks to delivery), when organs grow and develop functionally, drugs can cause growth retardation, structural abnormalities and organ dysfunction. The development of the central nervous system (CNS) and the cardiovascular system are of particular importance to the timing of psychotropic drug exposure. Malformations in these two organ systems are of particular concern when a child is exposed in utero to the mood stabilizers lithium, valproate and carbamazepine (46).

Mesocept contains Estrogens which have been identified as mitotic inhibitors (49). It was suggested that the structural specificity is related to mitotic inhibition. Estrogens are mitotic poisons, which at high concentration, cause metaphase arrest, abnormal cell division and chromosome aberrations (49). Earlier work showed that administration of pharmacological doses of estrogens caused high levels of embryonic mortality, although when administered later in gestation, estrogens had less effect on survival but retarded fetal growth (48,50). Estrogens are converted to catechol estrogens and these, or the resulting quinones, interact with DNA, or else through redox cycling produce oxidative damage to DNA and hence cause mutations (46,51). So, extension of the pharmacology and clinical use of synthetic estrogens and progestins alone as DMPA or in combination as (Mesocept) can prevent reproduction when given at the appropriate time and dose (52). The mechanism for their contraceptive effects is complex, but involves interruption of tubular transport of the ova, as well as hormone-induced changes to the uterine lining which prevents suitable implantation and subsequent development of the fertilized ovum. Therefore, the reduction in the implantation sites and the other abnormalities observed in the present study could be a subsequent effect of the contraceptives mechanism of action. Data collected from rodents and nonhuman primates show that the administration of contraceptives during

pregnancy is embryolethal (48). Our results were in agreement with other previous studies (5,48,45-52). Also the reductions occurred in the numbers of still live rat embryo, crown rump, embryo body weight and increase in the resorbed, as well as dead embryo may be 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. Uterine protein secretion is primarily controlled by hormonal mechanisms (34,5). An alternative mechanism may be the duration that the uterus is exposed to progesterone which leads to increase in the uterine protein secretion and induce embryonal loss, possibly via a decrease in the developmental rate of the conceptuses (34,5). Similar results were reported by (Waldum *el al.,* 1998) who reported that the lipid soluble steroid hormones affect the growth of their target cells by interaction with hormone responsive elements in the nucleus, affecting the regulation of gene expression (53). Furthermore, administration of certain injectable contraceptives to female rodents during early pregnancy resulted in reduced fertility, implantation failure, postimplantation loss and teratogenicity (5,34,54,55). The role of progesterone during the endometrial cycle is critical to the health and function of the endometrium. The critical events leading to uterine receptivity depend extensively on the timely and adequate action of progesterone, acting through specific progesterone receptors (PR) molecules (56). Down-regulation of estrogen receptors (ER) and up regulation of other proteins may be critical for the complex pattern of gene expression that fosters the nascent embryo and the attachment reaction. Disturbances in progesterone action are apparent in a variety of clinical diagnoses (56).

Skeletal defects of fetuses are manifested in incomplete ossification of most skull bones, irregular and missed ribs, absence and Scoliosis of vertebral centrae as well as incomplete ossification of both fore and hind limbs (5,34,57-60). The obtained data indicated deficiency in the mineralization of the skeletal system of the foetuses which are related to the treatment. These results are in agreement with those reported by Bakry, 2005, Wieck and Gregoine, 2006, Freeman and Shulman, 2010, Ling *et al.,* 2009, Bakry *et al.,* 2010; Harel *et al.,* 2010 and Rahman and Berenson, 2010 (34,36,57,58,5,59,60). On the other hand, Mesocept showed fewer side effects of skeletal defects on foetuses because it contains estrogen. These results are in agreement with those reported by Bartholomeusz *et al. 1999* (50). Loss of bone mineral density is not a concern with combined hormonal methods, including combined injectables. Compared to progestin-only injectable contraceptives, combined injectables lead to fewer progestin-related side-effects, given that the relative dosing is lower, and that the progestin is combined with estrogen. Combined injectable contraceptive has less effect on bone mineral density (61), due to the ontogeny of steroid receptors in the developing embryo to in the role of steroid-receptor interactions in limb development, particularly the process of endochondral ossification (5,34,41).

DMPA induced skeletal calcium mass defects in the treated rats. This may cause 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 reflect the skeletal calcium mass defects (62,59). The present work suggested that this drug interacted with Ca+ ions and induced alteration in Ca+ metabolism which resulted in various skeletal malformations manifested by incomplete ossification of most skull bones, irregular and missed ribs, absence and scoliosis of vertebral centrae as well as incomplete ossification of both fore and hind limbs (63,59). These finding are in agreement with the results of Tryfonidou *et al.,* 2003 and Harel *et al.,* 2010 (63,59). DMPA may induce an inhibition of endochondral bone growth of rat embryos at both stages of the study, decreases serum estrogen levels and intestinal Ca absorption which can lead to bone calcification loss of rat embryos, and may slow endochondral bone formation and undifferentiating chondrocytes which resulted in poor skeletal mineralization of rat embryos, which is in agreement with earlier reports (64,65). The injactable contraceptives effect on users also causes osteoporosis (66-71). When bone mineral density is reduced, bones become fragile and more likely to break. Typical fragility fractures occur in the spine, rib, hip and wrist. Bone health may be influenced by many factors, including pregnancy, lactation and use of hormonal contraceptives. Hypoestrogenemia is one of the most important causes of bone loss in women. Most women who use DMPA have hypoestrogenism. Estrogen deficiency is associated with bone loss in users of DMPA and may have plasma estradiol levels close to menopausal values (58,72). The present study suggested that DMPA may reduce the alkaline phosphatase activity due to its binding activity to serum proteins, and this may result in incomplete ossification of rat embryo and impair liver function (73). Mesocept has central and peripheral mechanisms, primarily on ovulation

inhibition and alteration of the cervical mucus. In addition, Mesocept causes morphological and enzymatic changes in the endometrium which have the effect of rendering nidation difficult. The contraceptive reliability of the depot injection of Mesocept is similar to that achieved by daily ingestion of progestogens-estrogen combined preparations (11).

5. Conclusion

The results of the present study revealed that both DMPA and Mesocept induced harmful and several teratogenic effects in rat embryos. Evidence from the studies reviewed strongly suggests that a woman who uses DMPA or Mesocept should make sure that she is not pregnant. Therefore, care must be taken when the millions of women around the world decide to use these injectable contraceptives for birth control.

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