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Evaluating the Abortifacient Capacity of Depo-Provera on Pregnant Wistar Rats

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ABSTRACT

Depot medroxyprogesterone acetate (DMPA) is a long acting reversible hormonal contraceptive that is injected every three months. It is marketed under the brand name Depo-Provera and it is believed to have an abortifacient property. The aim therefore was to evaluate the abortifacient capacity of Depo-Provera on Pregnant rats. In the study design, twelve pregnant sexually mature adult female rats with an average weight of 150g were used, they were divided into three groups of four rats; A, B and C respectively. Group A served as the control group. Group B served as the high dose group (200ml/kg), Group C served as the low dose group (100ml/kg). Results showed that all the rats from group A at the end of gestation, gave birth to normal and healthy liters. No mortality was recorded. Histologically, the uterus showed normal endometrial lining with intact epithelial arrangement. The myometrium appeared normal with diverse arrangement of muscle fibers in longitudinal and circular layers. In groups B and C, there was bleeding or spots from the vagina, blood stains around the eyes and mouth were observed, also fetal mortality was recorded with subsequent mortality of the pregnant rats. The epithelial lining of the uterus lost its columnar orientation with distortion of the cilia, thus making it less ciliated. There was also a reduction in mucosal fold. In conclusion, Depo-Provera could be said to be fetotoxic and harmful to pregnant rats because its inability to induce abortion in the pregnant rats led to the eventual death of the rats. These findings suggest that Depo-Provera should not be considered as a conventional abortion therapy in any medical condition where abortion is advised or in societies where abortion is legalized.

Key words: Depo-Provera, effect, fetus, uterus, wistar rats, fetotoxicity.

INTRODUCTION

Medroxyprogesterone acetate (MPA), also known as depot medroxyprogesterone acetate (DMPA) in injectable form and marketed under the brand name Depo-Provera, is a long acting reversible hormonal contraceptive birth control medication.[1][2] It is available both alone as a progestin only contraceptive, and in combination with an estrogen.[3][4] It is also believed to have an abortifacient property.[5] MPA is also commonly used in the form of DMPA as a long-acting progestogen-only injectable contraceptive to prevent pregnancy in women.[6][7] It is administered via intramuscular or subcutaneous injection and forms a long-lasting depot, from which it is slowly released over a period of several months. It takes about a week to take effect if administered after the first five days of the period cycle, and is effective immediately if administered during the first five days of the period cycle. Estimates of first-year failure rates are about 0.3%.[8] Common side effects include menstrual disturbances such as absence of periods, abdominal pain, and headaches.[2] More serious side effects include bone loss, blood clots, allergic reactions, and liver problems.[2]

The use of Depo-Provera as contraceptive is common among Indian women but it is not without varied side effects. The results of a study carried out by Mukherjea and colleagues, indicated that depot formulation of medroxyprogesterone acetate (Depo-Provera) at a dose of 150 mg at 90-day intervals appears to be acceptable to Indian women.[9] They revealed that some of the Indian women experienced irregular and excessive menstrual bleeding which diminished with long-term use of the contraceptive and a high percentage of them became amenorrheic but they also observed that there was tendency for the endometrium of some of the women to become atrophic with the prolongation of the therapy. [9] Fraser and Dennerstein in 1994 also reviewed the experience of Australian women treated with depot medroxyprogesterone acetate (DMPA). [10] It was a detailed retrospective review of clinical data on 363 women treated with DMPA for more than 20 years. They revealed that no pregnancy occurred within three months of an injection of DMPA and that a median delay in the return of fertility in those wishing to conceive immediately after withdrawal of the DMPA was 9.2 months. They further noted that the common side effects

include menstrual disturbance and superficial dyspareunia or reduced libido which occurred in 8% of their studied population. Their findings confirm that Depo-Provera is a very effective and acceptable contraceptive method although with tolerable side effects.

Depo-Provera contraceptive injection when administered at the recommended dose to women every 3 months inhibits the secretion of gonadotrophin which, in turn, prevents follicular maturation and ovulation and results in endometrial thinning. [11] [12] Contrary to what was observed after oral administration, in women given DMPA, suppression of ovarian function was paralleled by that of the endometrium, which showed either suppression or atrophy, for as long as 16 weeks after the last injection and in some cases irregular endometrial growth patterns may be present up to 33 weeks after injection. [13]

Depo provera has also been shown to affect memory in female Wistar rats. [14] This was demonstrated by Olanrewaju and colleagues. Their study showed that not only natural progesterone, but also the synthetic progestin MPA, is detrimental to learning and memory, by possibly modulating the GABAergic system in cognitive brain regions, in actively reproducing rats. [14] They asserted that despite Depo Provera being effective in modulating hormonal interaction to prevent conception in actively reproducing females, learning and memory depression could be one of its adverse effects.

Amidst these, literatures are dearth on the outcome of effect of Depo Provera on pregnant women who might want to extol the presumed abortifacient property of Depo Provera to remove unwanted pregnancies on in those who unknowingly had a shot of Depo Povera injection before confirming their pregnancy status. Therefore, the aim of this study was to evaluate the abortifacient capacity of Depo-Provera on Pregnant Wistar Rats.

MATERIALS AND METHODS

Research Design: The study design was experimental which studied the effect of Depo-Provera on developing fetus and histology of uterus of Wistar rats. The study made use of a total number of 12 pregnant Wistar rats divided into 3 groups; A, B and C. Ethical approval to carry out the study was granted by the Faculty of Medical Sciences Ethical Committee, Cross River University of Technology, Okuku Campus, Nigeria.

Animal Grouping: The animals were grouped into 3 groups of four pregnant rats each as follows:
Group A served as the control group. They received normal Saline
Group B served as the high dose group. They received 200ml/kg shot of Depo Provera
Group C served as the low dose group. They received 100ml/kg shot of Depo Provera

Animal Care: Sexually mature adult female Wistar rats with average weight of 150g were obtained from the Animal House of Cross River University of Technology, Okuku Campus, Cross River State, Nigeria. The animals were made to acclimatize for a period of two weeks before the commencement of the experiment. The animals were kept in clean plastic cages with saw dust bedding under standard laboratory conditions (natural day/night cycle, ambient temperature) and were fed with lab. pelleted food and water given ad libitum with weights taken daily with an electronic weighing scale calibrated in grams. All animals were handled in strict accordance with the National Institute of Health guidelines for the care and use of laboratory animals.

Cycle Determination: After two weeks of acclimatization, vaginal smears from the female rats were viewed under the light microscope to determine the females that would be receptive to the males during mating. Vaginal secretion was collected in the morning between 8.00AM and 10.00AM with a plastic pipette filled with 10µl of normal saline by inserting the tip into the vagina of the rat but not deeply. Vaginal fluid was then placed on the glass slide and observed under the light microscope (x 10 magnification) according to the method of Marcondes et al.[17] The proestrus stage, the period prior to sexual receptivity, was microscopically monitored for follicular growth, and at estrus proper, the animals were mated. Vaginal smears of the mated animals were assessed for the presence of sperm plug. The first day to see the sperm plug was taken as the day one of pregnancy.

Dose Determination: The experimental doses (high and low) used in the study were derived from the works of Olanrewaju et al. [14]

Drug Administration: Administration was in high and low doses given intramuscularly according to study design. For the high dose, 200ml/kg was administered while for the low dose, 100ml/kg was administered intramuscularly using the muscles of the thigh once weekly for a period of three weeks. Depo-Provera procured was Pfizer brand. The ampoule was shaken vigorously for about 10 seconds to allow for uniform concentration.

Sacrifice: All the Pregnant rats that were administered low and high doses of Depo-Provera died before the end of their gestation period. Specifically, 2 rats from the high dose group died 2 days after second administration while the other rats from this group died on the second and third day following the last administration. Meanwhile all rats from group C, low dose group died within one week following last administration but on different days. Only animals from group A (Control) reached their gestation period and were sacrificed using chloroform inhalation method. After physical

examination, the uterus was harvested from each dead rat and from the sacrificed animals and fixed in a labeled bottle with 10% formal alcohol for 48 hours and later moved into 70% alcohol until ready for tissue processing. The tissue was processed by routine tissue processing procedure and stained using H & E histological staining procedure.

Note: Since all the rats in the treatment groups did not die at the same time and day, upon observation of death of any rat, the dead rat was removed from the cage and the abdomen was opened up to removed dead fetus which already had offensive odor with darkish brown coloration.

RESULTS

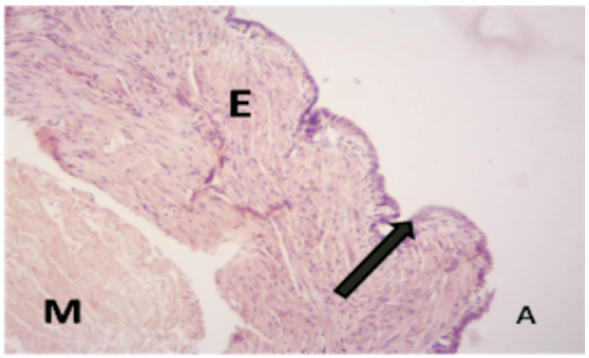
General observation--Control (Group A): After a careful observation and monitoring, it was noticed that all the rats in group A fed well throughout their gestation period. At the end of gestation, each rat gave birth to normal and healthy liters. No mortality was recorded.

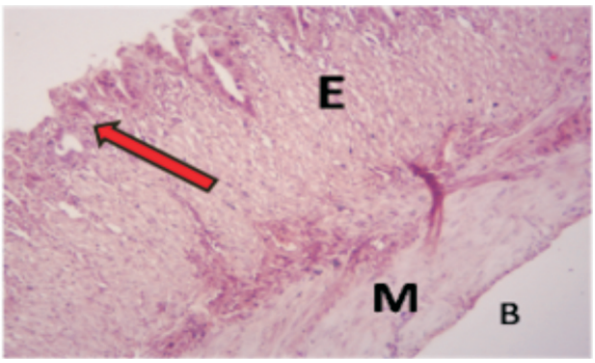
High Dose (Group B): Rats in high dose group showed bleeding or spots from the vagina, blood stains around the eye and mouth and death of fetus were recorded in all Group B rats with subsequent death of the pregnant rats. Two rats from this group died 2 days after second administration, whereas others died second and third day after last administration.

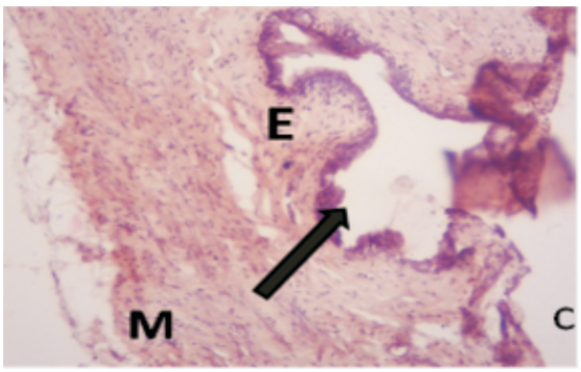
Low Dose (Group C): Similarly, bleeding or spots from the vagina, little blood stains around the eye and mouth were observed in rats from this group. Death of pregnant rats with fetus was also recorded. Rats in this group died after last administration. All the Pregnant rats in this group died before term.

Histological results--Control (Group A): Uterus showed endometrial lining with intact epithelial arrangement. Also, the myometrium appeared normal with diverse arrangement of muscle fibers in longitudinal and circular layers for the control group.

Treated Group B: In group B, high dose pregnant rats, the epithelial lining of the uterus lost its columnar orientation, and there was distortion of cilia. There was also reduction in mucosal fold.







In the present study, pregnant female rats treated with Depo-Provera presented unpredictable bleeding or spotting from the vagina. This agrees with earlier report that Depo-Provera caused unpredictable bleeding or spotting from the vagina some of which lasted for 1-7 days, or for more than 15 days in some cases. [19] Norplant contraceptive users have also experience irregular, unpredictable uterine bleeding. [20] [21] The pathophysiology of the irregular uterine bleeding is currently still vague but it has been reported that the extent of uterine bleeding, even though it can be persistent and prolonged, is usually not clinically significant. [22]

A proper evaluation of the effects that synthetic progestogens, administered alone, have on the endometrium must take into account factors like the duration and timing of treatment; A good example of the importance of timing as reported by Xing et al., showed that progesterone, administered from cycle day 2 to day 6, does not influence endometrial appearance on day 6, whereas, if it is delivered from day 7 to day 11, the endometrial appearance on day 11 is significantly altered. It seems therefore that the endometrium can only respond to progesterone when a sufficient number of specific receptors have been induced by the oestrogen priming. [23] In the current study the administration of Depo Provera was in high and low doses given intramuscularly once weekly for a period of three weeks. For the high dose group B, 200ml/kg was administered while for the low dose group C, 100ml/kg was administered. It was observed that death of the Pregnant rats and their fetuses occurred earlier in the high dose group whereas in the low dose group the rats survived up to the third administration of the Depo Provera which thus shows that the amount of dose administered is also a key factor in evaluating the effect of contraceptives given before pregnancy was confirmed. It has been reported that early, high-dose in utero exposures to Depo-Provera may affect fetal growth. [24] Gray and Pardthaisong study in Chiang Mai, northern Thailand also reported that the children exposed in utero to Depo-Provera had higher neonatal and infant mortality rates. [25] Thus, it is obvious that fetuses from pregnancies after Depo-Provera injection may be at increased risk for death base on the amount of the Depo Provera that was administered.

Histologically the current study revealed that the uterus of the control Group A showed endometrial lining with intact epithelial arrangement, similarly, the myometrium appeared normal with diverse arrangement of muscle fibers in longitudinal and circular layers (Figure 1A). Low dose pregnant rats exhibited slightly similar histological arrangement to the control group but with end disorientation of lamina propria (Figure 1C). The epithelial lining of uterus of the high dose pregnant animals lost its columnar orientation and distortion of cilia as well as reduction in mucosal fold (Figure 1B). It has been reported that in women given Depo Provera, suppression of ovarian function is followed by either suppression or atrophy of the endometrium and in some cases irregular endometrial growth patterns may be present up to 33 weeks after injection.[13] Hagenfeldt and Landgren also studied endometrial histology under the influence of progesterone and found that, with time, there was a suppression of the proliferative activity and changes leading to poorly developed glands, and ultimately to an atrophic endometrium. [26] Exogenous steroids have also been known to disrupt the normal tightly controlled relationship between the growth of endothelial cells and the capillaries and the glandular and cellular components of the endometrium. Hickey and colleagues demonstrated changes in components apparently important for the structural integrity of the endothelial cell basement membrane. [27] Histological appearance of the endometrium of a pre-menopausal woman treated with daily doses of 200 mg of medroxyprogesterone orally for 30 days also presented an endometrium that has an atrophic epithelium, lying over a thick, diffuse decidualized stroma. [28] Histologically it has been shown in the current study that Depo Provera produces effects that are dose dependent on the endometrium of the Wistar rats Uterus.

CONCLUSION

In conclusion, there is no doubt that Depo-Provera is fetotoxic and harmful to the pregnant rats at the dosage administered. The findings also suggest that the drug might have adverse effect on the uterus if high doses are administered. The death of the pregnant rats confirms the abortifacient and fetotoxicity property of Depo Provera however it did not induce expulsion of the dead fetuses in the pregnant rats. The inability to expel the fetuses may have led to the eventual deaths of the rats as the dead fetuses became toxic to the health of the pregnant rats. It is therefore necessary to always ascertain if a woman is pregnant before giving her Depo Provera injection since in utero exposures to Depo-Provera may affect the fetus. These findings suggest that Depo-Provera should not be considered as a conventional abortion therapy in any medical condition where abortion is advised or in societies where abortion is legalized.

CONFLICT OF INTERESTS

Authors have declared that no conflict of interests exists.

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