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Effect of Metronidazole drug in the Levels of some Pregnancy Hormones during Blastocyst Implantation in Rat Uterus

تاثير عقار الميترونيدازول في مستويات في بعض هرمونات الحمل خلال غرس الكيسة الاريمية في رحم الجرذ

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Abstract

This study aimed to determine the effect of metronidazole (MTZ) drug on the levels of progesterone and estrogen hormones during blastocyst implantation in the pregnant rat uterus during the implantation period. Understanding the roles of the variety of pregnancy hormones in uterine receptivity for implantation is essential to enhancing reproductive health and fertility in humans and domestic animals. Forty eight female rats of confirmed pregnancy have been used ,divided into two treated groups received oral dosage commercial drug, 9 mg / 200 gm of life body weight as treatment dose of drug, 18 mg as double the treatment dose and 27 mg as the triple treatment dose.

The results of this study revealed that taking treatment dose and double dose of the drug for 7 dpc group of pregnant rats cause decrease the level value of estrogen hormone in blood serum but this decrease didn't reach significant level $p>0.05$. It was significant $p<0.05$ at the triple dose, and it is significant $p<0.05$ inversely for 9 dpc period, It means that the estrogen hormone level was decreased when the drug dose was increased were there were significant decrease $p<0.05$ in the value of progesterone level of blood serum at treatment dose, double and triple dose of the drg. There was significant affect $p<0.05$ for the mean of progesterone level in blood serum of the treated animal.

Key wards : Implantation, Progesterone , Estrogen , Metronidazole.

الخلاصة

هدفت الدراسة الحالية إلى تبيان اثر عقار الميترونيدازول التجاري على معدلات قيم مستويات هرموني البروجيستيرون والاستروجين خلال غرس الأجنة في رحم الجرذ في اليومين السابع والتاسع من الحمل، حيث ان تقبل الرحم لانغراس الكيسة الاريمية في هذين اليومين يعتمد على هذين الهرمونين وفهم ادوارهما وغيرهما في تقبل الرحم لانغراس الكيسة الاريمية ضروري إلى تَحسين صحة الحوامل والخصوبة في البشر والحيوانات الأليفة. تم استخدام 48 جرذاً أنثى جرعت فمويا 9 ملغم من العقار / 200 غم من وزن الحيوان الحي و 18 ملغم و 27 ملغم يوميا ولمدة سبعة أيام وتسعة أيام ومن اليوم الأول من تأكيد الحمل. اجريت الدراسة في 2012 بوحدة ابحاث الرزازة وغربي الفرات - جامعة كربلاء.

أظهرت النتائج الاحصائية أن تجريع عقار الميترونيدازول بجرعة علاجية وضعفها أدى إلى انخفاض في مستوى هرمون الاستروجين في مصل دم الجرذان الحوامل الا انه لم يصل إلى مستوى المعنوية $p>0.05$ في حين أن تجريعها بثلاثة أضعاف الجرعة قد أدى إلى انخفاض معنوي $p<0.05$ في مستوى الهرمون. وعلاقة الارتباط r غير معنوية $p>0.05$ عكسية لفترة 7 أيام ومعنوية $p<0.05$ عكسية لفترة 9 أيام. وهذا يعني ان مستوى الاستروجين قد انخفض عند زيادة جرعة الميترونيدازول الى ثلاثة اضعاف الجرعة العلاجية . وان تجريع العقار بجرعة علاجية وضعفها وثلاثة اضعافها قد أدى إلى انخفاض معنوي $p<0.05$ في قيمة مستوى هرمون البروجيستيرون بمصل الدم وان علاقة الارتباط معنوية $p<0.05$ عكسية.

Introduction

Uterine receptivity to implantation is dependent on progesterone which is permissive to actions of interferon, chorionic gonadotropine (CG), prolactin and placental lactogen [(1,2,3,4 and 5)]. Implantation involves attachment of conceptus trophectoderm (Tr) of the developing conceptus (the embryo and its associated extra-embryonic membranes) to uterine luminal epithelium (LE) in a highly synchronized series of events requiring reciprocal secretory and physical interactions during

a restricted period known as the 'window of receptivity [6,7]. The 'window of receptivity to implantation' is established by actions of progesterone and, in some species, estrogen (E2) that regulate locally produced cytokines, growth factors, and others through autocrine and paracrine pathways[8]. A paradox is the role of progesterone to sequentially down-regulate expression of progesterone receptors (PGR) in uterine LE, as well as superficial glandular epithelia (sGE) and mid- to deep-glandular epithelia (GE) as a prerequisite for endometrial receptivity to implantation; however, PGR continue to be expressed in stromal and myometrial cells of the uterus. Subsequent effects of progesterone on PGR-negative uterine epithelia are likely mediated by stromal cell-derived growth factors [9].

Progesterone is a female hormone produced by the ovaries during release of a mature egg from an ovary (ovulation). Progesterone and estrogen together, these two hormones create conditions which assist in fertilization. This makes fertilizing more likely, and allows the semen to survive longer than it would anywhere else. Progesterone also prepares uterus for implantation of the fertilized ovum and it helps prepare the lining of the uterus (endometrium) to receive the egg if it becomes fertilized by a sperm [10]. Progesterone causes the abrupt change in the mucus which occurs immediately after ovulation and defines the peak symptom. If the egg is not fertilized, progesterone levels drop and menstrual bleeding begins. During pregnancy, the placenta also produces high levels of progesterone, starting near the end of the first trimester and continuing until the baby is born. Levels of progesterone in a pregnant woman are about 10 times higher than they are in not pregnant woman. Progesterone also forestalls the shedding of the endometrium (where implantation occurs), helping to prevent miscarriage in the early weeks of pregnancy.

Pregnancy recognition signaling

In rodents, mating induces release of prolactin from the anterior pituitary and it is the initial luteotrophic signal for corpus leutium (CL) formation and production of progesterone to about day 12 of pregnancy, and then lactogenic hormones from conceptuses and uterine decidua act on luteal cells to maintain their function and secretion of progesterone. The gestation period for rats, mice and hamsters is 20–22days and functional CL must produce progesterone through day 17[1].

Uterine receptivity to implantation varies among species and involves changes in modification of phenotype of uterine stromal cells, silencing of receptors for progesterone and estrogen, expression of genes that are coordinate with attachment of trophectoderm to uterine luminal and superficial glandular epithelia, suppression of genes for immune recognition, alterations in membrane permeability to enhance conceptus-maternal exchange of factors, angiogenesis and vasculogenesis, increased vascularity of the endometrium, activation of genes for transport of nutrients into the uterine lumen, and enhanced signaling for pregnancy recognition. Differential expression of genes by uterine epithelial and stromal cells in response to progesterone and others may influence uterine receptivity to implantation in mammals [11].

The studies indicate that progesterone-induced advances in transport of select nutrients, particularly Arg. and glucose, into the uterine lumen on Days 9 and 12 of pregnancy are coordinate with advanced conceptus development. Progesterone secretion during the luteal phase influences oviductal and endometrial functions which are essential for embryo viability and implantation in a number of species including rats and primates [12]. In related studies [13, 14], an early increase in circulating levels of progesterone accelerates blastocyst growth and development in ewes that is associated with increases in total recoverable glucose, aspartate (acidic amino acid), Arg and lysine (basic amino acids), and citrulline, asparagine, serine, Gln, beta-alanine and alanine (neutral amino acids) in uterine flushing on day 9 of pregnancy compared with control ewes [15].

Metronidazole

Metronidazole (MTZ) is an antimicrobial agent that has been used in clinical medicine for >45 years. It was originally indicated for the management of infection caused by *Trichomonas vaginalis* and was then shown to be effective against other protozoal infections, such as amebiasis and giardiasis and it kills a wide variety of bacteria that are known collectively as anaerobic bacteria. It

works by entering bacterial and protozoal cells and interfering with their genetic material (DNA). It damages the DNA and also prevents the bacteria and protozoa from forming new DNA. This ultimately results in metronidazole killing the micro-organisms, which clears up the infection. MTZ is used to prevent infection following surgery, particularly gynaecological surgery and surgery on the gut, where many anaerobic bacteria may be found. MTZ has shown evidence of carcinogenic activity in a number of studies involving chronic, oral administration in mice and rats [16, 17].

Materials and Methods

The animals: Forty eight no pregnant female rats *Rattus norvegicus*, aged 7-9 weeks old, weighting 200-250 gms each were used in this study which completed in Alrazzaza and West Alfurat Researches Unit, Karbala University, Iraq, on 2012. They were randomly divided into four groups, the rats were maintained under light program of LD 12:12 and fed *ad libitum*. The animals were mated and the day on which spermatozoa were found in the vaginal smear and the presence of vaginal plug was designated day zero of pregnancy as outlined in table (1). All female rats were divided into two main groups: The G1 group was the rats sacrificed on day 7 of pregnancy (dpc). The G 2 group was the rats sacrificed on day 9 dpc. Each group was subdivided into three subgroups in accordance to administration of drug.

Calculation of the dose and Drug Administration:

each tablet of drug containing 500 mg of the active ingredient Metronidazole, MTZ, (From Sanofi Aventis Co. France). The dose for the rat was calculated according to Paget and Barnas equation[18]. MTZ 9 mg/day was given for 200 gm body weight rat as therapeutic dose. The calculated dose (therapeutic, double and triple therapeutic dose) of suspension solution in distilled water of MTZ drugs were administered as an oral dosage one time daily for six days (G1group 1) to the animal sacrificed on the morning of day 7 dpc, and for eight days (G2 group 2) to the animals sacrificed on the morning of day 9 dpc. Distilled water was administered via the same route to the control animals group of both groups G1 and G2.

Table 1: The arrangement of experimental design.

days post coitum (dpc)	Subgroups			
	Control (Distilled water) (T1)	Therapeutic dose of MTZ 9mg (T2)	Double therap. Dose of MTZ 18mg (T3)	Triple therap. Dose of MTZ 27mg (T4)
G1: 7 dpc	n : 6	n : 6	n : 6	n : 6
G2: 9 dpc	n : 6	n : 6	n : 6	n : 6

Hormonal assay:

Blood samples were obtained directly from the heart in Ethylene diamine tetraacetic acid (EDTA) Free Tubes, and centrifuged to obtain the serum which transferred to Vidas® apparatus for quantitative assay of Progesterone and Estradiol assay, using the special kit of Vidas® Estrodiol II (E2 II), technique of Enzyme-Linked Fluorescent Immunoassay for The Quantitative Determination (ELFA), and the kit of Progesterone Assay (PRG).

Results

Estrogen Hormone:

The results statically revealed that taking treatment dose (T2) and double dose (T3) MTZ for 7 dpc group cause decrease the level value of estrogen hormone in blood serum but this decrease didn't reach significant level $p>0.05$ comparing with control group (T1), but it was significant $p<0.05$ at the triple dose (T4) comparing with groups T1, T2, T3. This result was confirmed by the correlation factor (r) between MTZ dose and estrogen hormone level during both periods 7 and 9 days of pregnancy period, and the correlation r was not significant $p>0.05$ inversely for 7dpc and

significant p<0.05 inversely for the period of 9 dpc. And there were no significant effect p>0.05 of the pregnancy periods on the mean of hormone level in blood serum which was 29.92 and 27.35 Pg/ml during the both pregnancy periods 7 and 9 dpc respectively.

Table 2: Effect of the drug in the level of blood serum estrogen hormone of pregnant rats.

Groups / period	T1 Control Dist. Water (Pg/ml).	T2 MTZ 9 mg (Pg/ml).	T3 MTZ 18 mg (Pg/ml).	T4 MTZ 27mg (Pg/ml).	Mean of hormone for pregnancy period (Pg/ml).
7dpc	31.25 ± 3.72	32.44 ± 2.91	28.71 ± 2.74	27.29 ± 2.33	29.92 1.46±
9dpc	33.00 ± 3.14	30.60 ± 2.57	24.97 ± 2.42	20.82 ± 3.12	27.35 1.59±
Mean of hormone for treatments ± SE	32.13 2.36± A	31.52 1.89± A	26.84 1.83± AB	24.06 2.04± B	

Mean of ± SE
Large different letters indicates the presence of significant differences at P < 0.05.

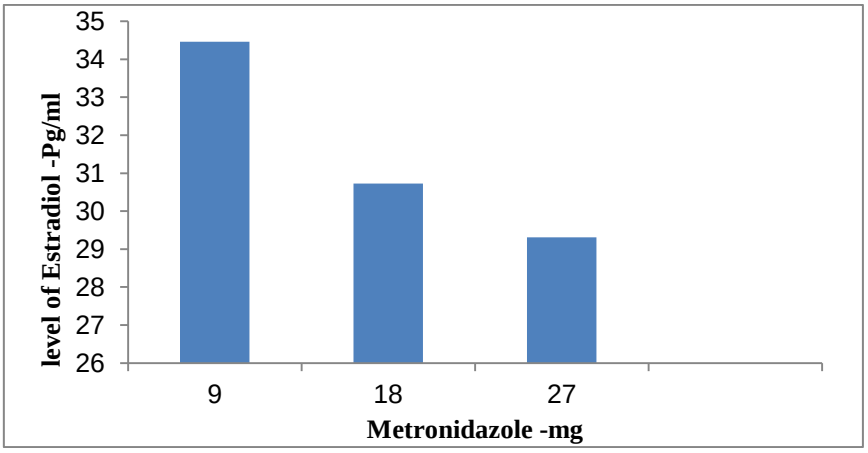

Fig. 1 : The correlation between the drug dose in age 7dpc and the level of blood serum estrogen hormone of pregnant rat.

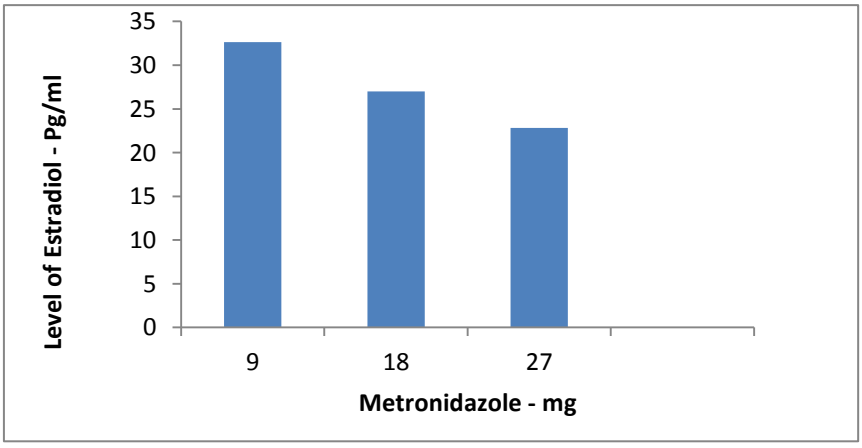

Fig. 2: The correlation between Metronidazole dose in age 9dpc and the level of blood serum estrogen hormone of pregnant rat.

Progesterone Hormone:

Table (2) showed that the treatment dose T2 double T3 and triple dose T4 of MTZ has causes significant decrease $p<0.05$ in the value of blood serum level compared with control T1 as clear from the correlation between MTZ and Progesterone blood serum level during the pregnant periods 7 and 9 dpc, and the correlation between the MTZ dose and progesterone level was inversely nonsignificant $p>0.05$. There was significant affect $p<0.05$ for the mean of progesterone level which was in blood serum 50.87 and 43.91 ng /ml during the pregnancy periods 7 and 9 dpc respectively.

Table – 3: Effect of the drug in the level of blood serum progesterone hormone of pregnant rat.

Groups / period	T1 Control (ng/ml)	T2 MTZ 9mg (ng/ml)	T3 MTZ 18mg (ng/ml)	T4 MTZ 27mg (ng/m)	Mean of hormone for pregnancy period
7dpc	51.08 ±3.10 a	52.79 ± 2.51 a	50.57 ± 3.01 a	49.02 ± 2.65 a	50.87 ±1.37 a
9dpc	59.89 ± 3.88 b	40.62 ± 251 b	38.32 ± 2.53 b	37.80 ± 3.31 b	43.91 ±2.30 b
Mean of hormone for treatments ± SE	55.49 ±2.86 A	46.21 ±2.76 B	44.45 ±2.79 B	43.41 ±1.81 B	

Mean of ± SE
Small different letters indicates the presence of significant differences at $P < 0.05$.

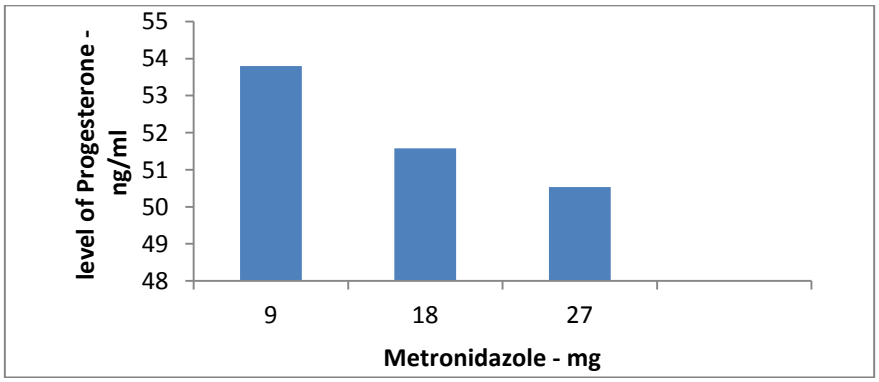

Fig. 3: The correlation between the drug dose in age 7dpc and the level of blood serum progesterone of pregnant rat.

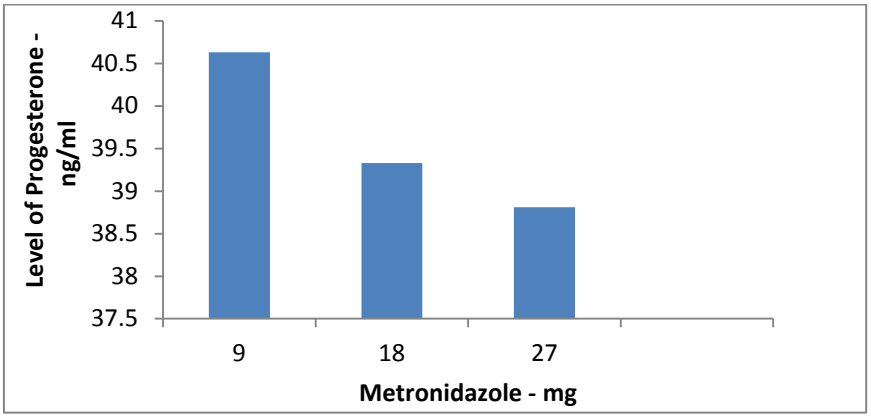

Fig. 4: The correlation between Metronidazole dose in age 9dpc and the level of blood serum progesterone of pregnant rat.

Discussion and Conclusions:

The results of hormonal levels values of the present work together with the analysis of the results statistically were in favor of considering that there was no harmful effect of the drug MTZ on the process of implantation on days 7 and 9dpc, even the estrogen level was decreased when the MTZ dose was increased, as shown in Figs. 1 and 2, and the MTZ dose increased the serum level of progesterone would be decreased as in table 3 and as in figs .3, 4,this result was adapted with [13,19 and 20].

The studies performed to date have mostly included only small groups of human being patients with a lack of fertile controls, and only a few prospective, controlled trials have been carried out. Therefore, definite conclusions about the clinical value of the MTZ on the level of progesterone and estrogen hormones in the assessment of endometrial function and prognosis for pregnancy after artificial reproductive therapy cannot be drawn at present. Further evaluation of their importance for and function during implantation is needed.

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