

Effect of the Combined Oral Contraceptive Pills (Yasmin) on the Fetal Malformation in Pregnant Mice (Mus Musculus)

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ABSTRACT

Background: Combined oral contraceptive pills (Yasmin) is an great contraceptive which used a very big vary of female worldwide , and it consists of synthetic estrogen (ethinyl estradiol) and a synthetic progesterone (drospirenone).

Aim of study:- This study was designed to inspect the effect of Yasmin on the deformation of fetus of pregnant mice when treated with the drug before pregnancy .

Methods:- In this study, 25 female mice were used, divided to three groups, the first group is control group (A) consisting of 5 pregnant mice, while the second group (B) and the third group (C) are the treatment group, each group consists of 10 pregnant mice which treated before pregnancy occurs with drug Yasmin for 10 days for group (B) and 20 days for group (C) at a dose (0.1ml) of concentration .

Results:- The results showed macroscopic congenital malformations in both treatment groups compared with control group (A), which include (exophthalmia, congestion in the brain, enlarged head size, pros encephalon, rough skin, micromelia, foot rotart, increased number of fingers, and Tail torsion), in addition to the presence of (bleeding at the end of the tail area) in third group. And the percentage of malformations in (group C) was higher than the (group B) . And observed that there was no extensive difference ($P \geq 0.05$) in the rate of fetus weight, head, trunk and body length for the group (B), and there was extensive difference ($P \leq 0.05$) in the group (C) contrast to the control group(A). And there was extensive difference in the length of the tail and hind limbs in both groups (B , C), and there was no extensive difference in average length of fore limbs of both groups (B ,C), contrast to the control group(A).

Conclusion:- The results of the current study suggest the use of contraceptive pills (Yasmin) immediately before pregnancy occure has the potential to cause deformities in fetuses in the event of pregnancy occure .

Introduction

Researchers developed in the early 1900s, the birth control pill Hormonal for women (Rosenberg *et al* .,2007). Since beginning of manufacturing combined contraceptive pill in the 1960's, it has come to be a major and essential part of pregnancy control (Fraser, 2000) .More than 100 million women use combined oral contraceptive pill in worldwide, which it is one of the most common techniques of contraception (Fu *et al* .,1999). Combined oral contraceptive pill (Yasmin) is a synthetic estrogen and progesterone containing (0.03mg) of ethinyl estradiol and

(3.0mg) of drospirenone (Fathizadeh *et al.* , 2010). And both (EE and DRSP) are crystalline White to yellowish or crystalline powder, and insoluble in water (Bachmann & Kopacz , 2009). These pills contain unequal doses of estrogen and progesterone and are called biphasic pills and they are used for 21 days and then left for 7 days (Hassan, 2018). And the pills have to be taken at around the same time every day, and the effectiveness of the medicine will be affected if they are used for more than 24 hours .14% of women use combined oral contraceptive pills for reasons not related to contraception, which can be used to treat other conditions such as acne, hirsutism, and menstrual disorders. And a side effects of combined contraceptive pills are headache, nausea, breast pain, abdominal cramps, increased vaginal secretions, decreased libido, and it can also cause high blood pressure (Cooper *et al.* , 2022). Long-term use of artificial estrogen and progesterone can lead to an imbalance between the level of these hormones in the body, and it may lead to serious side effects such as cervical and breast cancer (Shukla *et al.* , 2017).

Studies indicated that the use of oral contraceptive pills leads to a decrease in concentration of folate in the blood after stopping for up to three months, and this can lead to congenital defects in the fetus in the event of pregnancy occur (Lewis *et al.* , 1998). Some studies also have confirmed that birth control pills lead to an increase in the level of homocysteine in pregnant mothers and cause deformities in the fetal body (Cerrizuela *et al.* , 2020). The process of embryonic development begins after the fertilization process and is regulated by chemical bonding between cells and their layers, and this bonding is regulated by gene expression, and when any error occurs in the translation of genetic orders, the error is translated into deformities that appear on the fetus during the stages of embryonic development (Bamforth *et al.* , 2001).

Materials and methods

Preparation of Animals

25 female Swiss white mice, belonging to the *mus musculus* strain, were used, with an average weight of (25±5) grams and ages between (10-12) weeks, obtained from the Biotechnology Research Center at Al-Nahrain University. The drug Yasmin (combined contraceptive pills) was used in the form of pills with a concentration of (3.03 mg). Each tablet contains a synthetic estrogen (ethinyl estradiol 0.03 mg) and a synthetic progesterone (drospirenone 3.0 mg), manufactured by the German company Bayer AG . The treatment mice were given oral doses in the morning once a day at a concentration of 0.1ml of the drug . Experimental animals were divided to three main groups (A, B, C) , group A was the control group contained 5 mice , while the treated groups were (B, C) which each group contains 10 mice.

A- First group: The untreated control group.

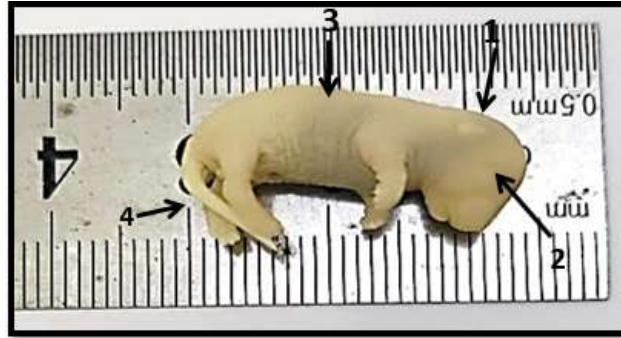
B- Second group: The group treated with the therapeutic dose of the drug for a duration of 10 days.

C- Third group: The group treated with the therapeutic dose of the drug for a duration of 20 days (dosing for 10 days, then a rest for 5 days, and dosing again for 10 days).

One day after the end of the dosing process, females and males were isolated for the purpose of mating, and to identify the extent of pregnancy occurs and the extent of effect on fetal malformation .

Extraction and changes in

The embryos uterine horns after fetal membranes anatomy tools, then



study of phenotypic embryos

were extracted from the gently removing the from them using some the embryos were

transferred to the fixative solution, then the weights of the embryos were measured using an sensitive balance, as well as measuring the dimensions of the fetus, the length of the body, the head, the torso, the tail, and the front and hind limbs) using a tape and an iron ruler, and examined all phenotypic changes with the naked eye and a Dissecting Microscope with 2X magnification, to identify the prominent changes and deformities of the fetuses compared to the control.

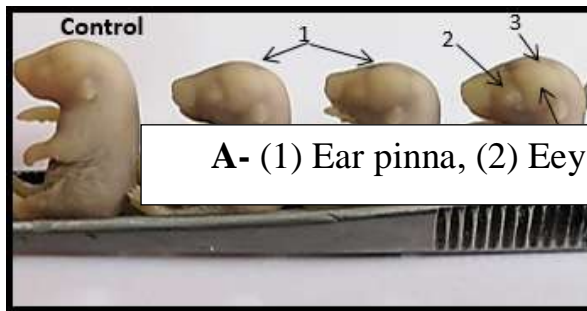
Statistical Analysis

The statistical results were analyzed by an analysis of variance (ANOVA) test , and the arithmetic average were compared based on the Duncan is Multiple Range check at a significant level ($P \leq 0.05$) .

Results

Results of the current study showed the appearance of morphological deformities in the fetus of mothers treated before pregnancy with the therapeutic dose of 0.1mL of the combined oral contraceptive pill for different periods of time in both groups of pregnant mice. Figure (1) shows normal fetus from the control group for comparison with deformed fetus . As the deformities were observed in the second group (B) , represented by exophthalmia, congestion in the brain, enlarged head size, pros encephalon, rough skin, micromelia, foot rotart, increased number of fingers, and Tail torsion, as shown in Figure (2). The same deformities were seen in the third group (C) , as in Figure (3), in addition to the presence of (bleeding at the end of the tail area). And the percentage of fetal malformations in (third group) was greater than the (second group).

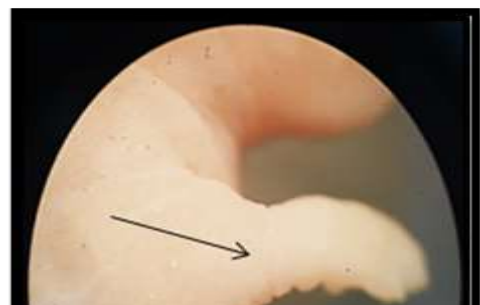
Figure (1):- side view of a normal , untreated control mouse embryo showing:



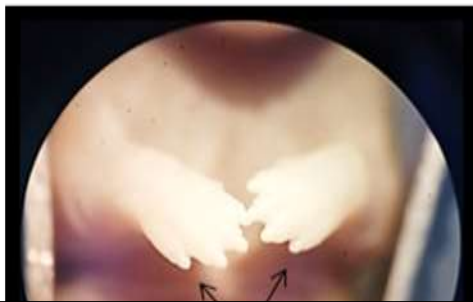
A- (1) Ear pinna, (2) Eey, (3) Trunk, (4) Tail.



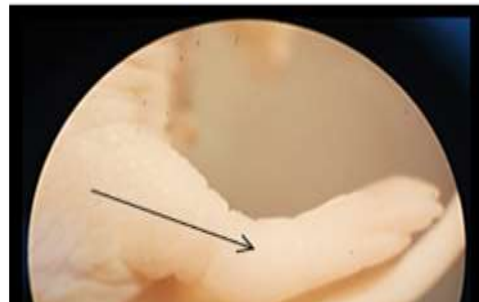
B- Normal head shape (anatomical microscope) 2X.



C- Normal upper limbs (anatomical microscope) 2X.



D- Normal number of fingers (anatomical microscope) 2X.



E- Normal lower limbs (anatomical microscope) 2X.

Figure

(2):- Mice fetus (mother treated with a concentration of 0.1ml of the drug before pregnancy for 10 days) for the second group.

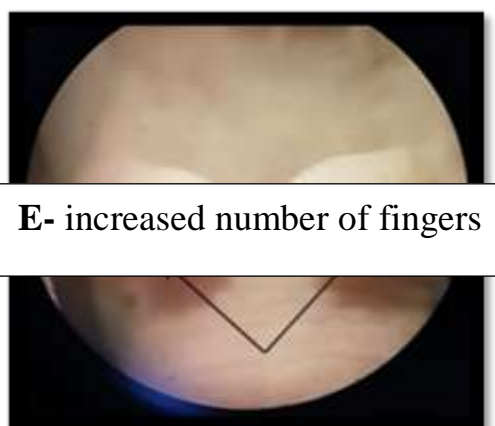
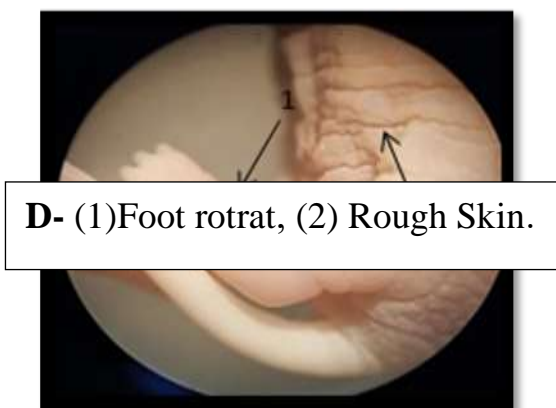
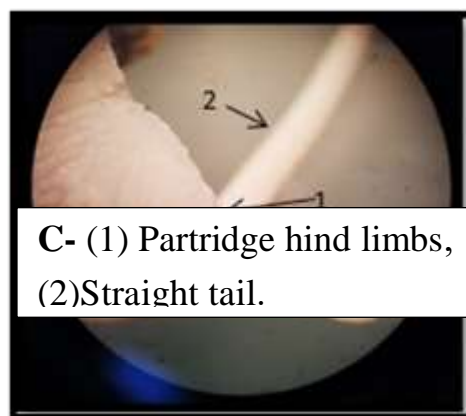
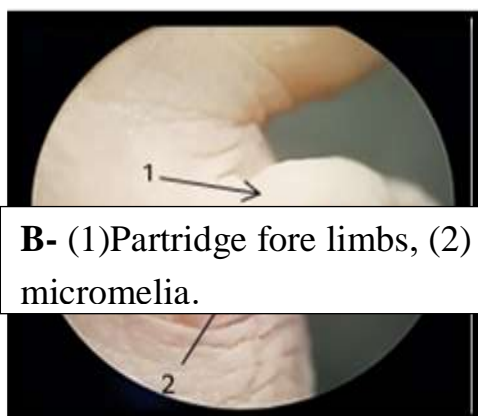
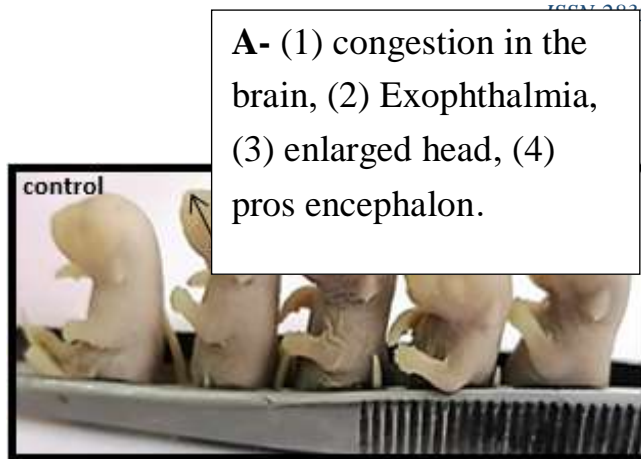
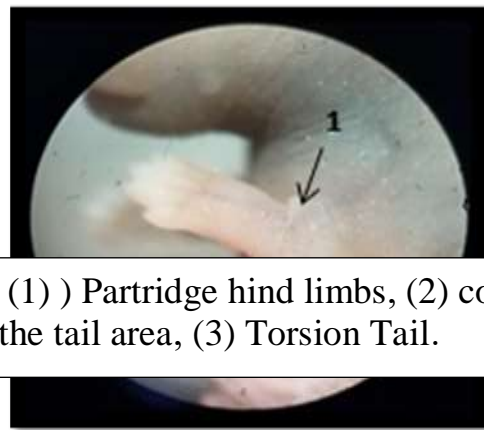
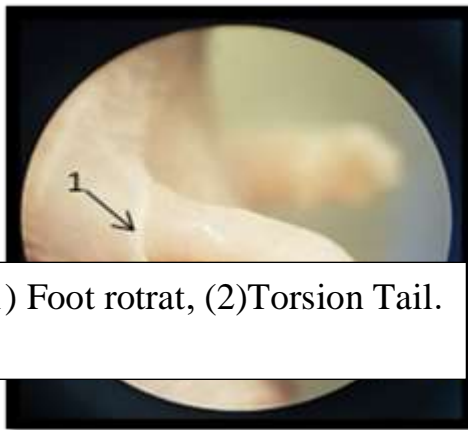


Figure (3):- Mice fetus (mother treated with a concentration of 0.1ml of the drug before pregnancy for 20 days) for the third group.

A- (1) High head, (2) congestion in the brain, (3) Exophthalmia

B-(1) Partridge fore limbs, (2) increased number of fingers.

C- (1) Partridge fore limbs, (2) micromelia.

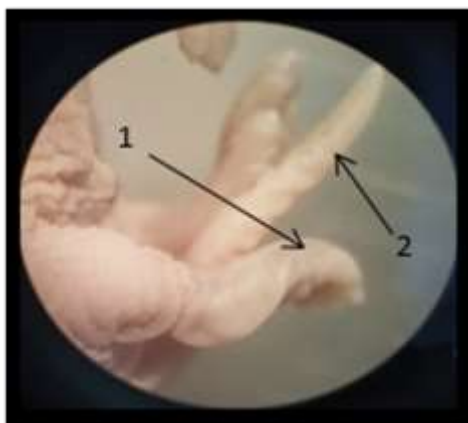


D- (1) Foot rotat, (2)Torsion Tail.

E- (1)) Partridge hind limbs, (2) congestion in the tail area, (3) Torsion Tail.

Table (1 and 2) shows the statistical results, as it was noted that there was no extensive difference of $P \geq 0.05$ in the rate of fetus weight, head, trunk and body length of the second group (B), and there was extensive difference of $P \leq 0.05$ in the third group (C) contrast to control group (A) .

As for the length of the tail and hind limbs, there



was extensive difference of $P \leq 0.05$ in both groups contrast to control group, and there was no extensive difference of $P \geq 0.05$ in the average length of the fore limbs for both groups contrast to control group.

Table(1):-Show the effect of a treatment on the average fetuse weight (gm) and the average head and trunk length (cm) \pm standard deviation .

groups		variable	Fetus weight	Head length	Trunk length
Control Pregnant			1.146±0.011 a	1.5±0.0 a	2.17±0.06 a
Experimental Pregnant	1.0 ml for 10 days		1.074±0.033 a	1.53±0.05 a	2.14±0.05 a
	1.0 ml for 20 days		0.904±0.148 b	1.4±0.1 b	2.0±0.08 b

Table(2):- Show the effect of a treatment on the average body length, tail, fore and hind limbs (cm) ± standard deviation .

groups		variable	Body length	Tail length	Fore limbs	Hind limbs
Control Pregnant			3.56±0.057 a	1.1±0.0 ab	0.8±0.0 a	0.95±0.054 a
Experimental Pregnant	0.1 ml for 10 days		3.58±0.084 a	1.1±0.07 a	0.77±0.07 a	0.87±0.05 ab
	0.1 ml for 20 days		3.3±0.1 b	0.967±0.057 b	0.74±0.05 a	0.82±0.084 b

Discussion

Results of the current study showed that treatment of pregnant mice with (Yasmin) drug before pregnancy led to the emergence of a series of fetal abnormalities in both experimental groups, including head abnormalities, limb abnormalities, and tail abnormalities. These results not consistent with (Charlton *et al* ., 2016) who note that exposure to oral contraceptives immediately before or during duration pregnancy does not appear to be related with an increased risk of major congenital defects. Also note (Waller *et al* ., 2010) that ladies who use oral contraceptives during early pregnancy do not have an increased risk for most sorts of major birth anomalies .

It has been shown that exogenous sex hormones enlarge plasma concentrations of vitamin A, which it can be teratogenic (Maden , 2001). Studies also indicate that blood folate concentrations drop after oral contraceptive use and after stopping stay low for up to three months, which can lead to a range of congenital defects (shere *et al* ., 2015) .And this is consistent with (Araki *et al* .,2021), where they indicated that folic acid regulates the gene expression of nucleic acids, and that its deficiency in the fetus causes neurodevelopmental

defects and developmental disorders, and that low folic acid may stimulate neuronal immaturity by deficient nuclear methylation in genes related with neuronal differentiation and maturation in mice. The use of low-dose oral contraceptive pills for at least three menstrual cycles increased the levels of homocysteine in the blood (Fallah *et al.* , 2012). And the abnormalities that we mentioned in the results are attributed to the high level of homocysteine in pregnant mice, which was caused by the Yasmin pill (Momeni *et al.* , 2019). And some studies have confirmed that high levels of homocysteine in pregnant mothers cause abnormalities in the body (the nervous system and the limbs of fetuses) (Cerrizuela *et al.* , 2020). Some research also indicated that there is a relationship between serious congenital defects and their prevention by folic acid, as homocysteine levels in the blood are inversely proportional to folic levels, and with folic depletion increase homocysteine levels (Brütting *et al.* , 2021).

Conclusion

The therapeutic dose of the drug is not safe for pregnant women who used it immediately before conception. The longer period of use of the drug (Yasmin), the more pathological effects increased, as its effects for a period of 20 days is greater than 10 days. We advise women not to become pregnant immediately after stopping taking the contraceptive pill (Yasmin) due to the possibility of fetal abnormalities.

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