

The effects of Agnucaston and Metformin on the chromosomes of pregnant females and their embryos

Hanaa M.Roshdy

Cell Biology Department, National Research Centre, Giza, Cairo

Abstract: Background: Agnucaston (chaste berry fruits) and Metformin are the most common medications in the treatment of polycystic ovary syndrome (PCOS) and hormonal imbalance. The safe use of Agnucaston and Metformin in the virgin females and in the pregnant females and their embryos has not been adequately studied. **Aim** of the study is to evaluate the cytogenetic effects of Agnucaston and Metformin before and during pregnancy. **Materials and Methods:** The sample of this study was female mice (Virgin and pregnant) divided into two groups control (did not administer any medication) and treated group (administered Agnucaston and Metformin orally) by doses of 0.3 and 1.3 mg/kg/day respectively for (15) consecutive days. After one day from the last treatment the females were sacrificed and cytogenetic analysis were conducted. **Results:** females treated with Agnucaston (Virgin and pregnant) showed increase in frequencies of chromosomal aberrations significantly and also in their embryos but these increases were highly significant in pregnant females than virgin. While, the females treated with Metformin there was a slight significant increase in the frequencies of the chromosomal aberrations in the pregnant females and embryos but there was no significant increase in the virgin females treated with Metformin before pregnancy. **Conclusion:** Our results indicate that Agnucaston has a mutagenic effects on the females (Virgin and pregnant) and on the embryos while Metformin has a slight mutagenic effects on the pregnant females and their embryos but does not have a mutagenic effects on the virgin females. [Nature and Science 2010;8(7):1-7]. (ISSN: 1545-0740).

Key words: (polycystic ovary syndrome), Agnucaston, Metformin, chromosomal aberrations, mice, embryos.

Introduction

Polycystic ovary syndrome (PCOS) a common endocrine-pathy and is the major cause of menstrual disorder and an ovulate infertility. It affects more than 15 to 20% of girls and young women in the world. Rotterdam (2004). The most common cause of (PCOS) is the hormonal imbalance in the brain and in the ovary at the same time. The pituitary gland in the brain produce excess Luteinizing hormone (LH) which increases the production of male hormones (testosterone) by the ovary. This high level of male hormones increases the risk of metabolic syndrome (high blood pressure, high cholesterol and high insulin in the blood). Because the level of male hormones remain high, so some of it may be converted to estrogen causing increase in its levels in the blood without change in progesterone level causing hormonal imbalance, leading to stop ovulation and irregular menstrual cycle.

The use of Agnucaston is the most common form in treatment of (PCOS). It is (a herbal medication) that contains the (chaste berry). Chasteberry has antioxidants, flavonoids and other chemical properties that act on the neurotransmitter dopamine in the brain which correct the hormonal imbalance and lower the level of Luteinizing hormone which also lower the level of testosterone in the blood so help to imbalance the level of progesterone and the level of estrogen in the blood, Milewicz. et al (1993)

and Lauritzen et al (1997). Chaste berry is sometimes called (the women's herb). It is used in regulation of the menstrual cycle, premenstrual disorder, female infertility and treating polycystic syndrome. Chasteberry seems to affect many hormones that regulate women's reproductive cycles, Jarry et al (1994). Metformin is another medication which decreases insulin level. because the high insulin level initiates ovaries to produce more testosterone hormone, Dunaif et al (1989). Only one of the two medications are required for the treatment of PCOS but most women are treated with the both in the same time.

At present no adequate data illustrates the safety use of Agnucaston and Metformin in the females before and during pregnancy is available. So, in the present study, we aimed to study the cytogenetic effects of Agnucaston and Metformin if given orally for about 15 consecutive days to the females before pregnancy and during pregnancy. In addition, their effects on their embryos. Results were compared with the controls.

2-Materials and Methods:

2.1.Materials:

2.1.1. Chemical drugs:

- a) Metformin hydrochloride: was obtained from a pharmacy in Egypt under a trade name (Amophage). Its chemical name is (N, N-dimethylimidodicarbonimidic

hydrochloride). It is not chemically or pharmacologically related to any other classes of oral anti-hyperglycemic agents. Its molecular formula is $C_{14}H_{11}N_5.HCl$ and a molecular weight is 165.63. It is freely soluble in water.

Amophage is used to management diabetes (type II diabetics as also used for treating the polycystic ovary syndrome (PCOS).

The recommended dose of Amophage is 500mg/kg/day for human,. Norman (2003).

b) **Agnucaston:**

Was obtained from (Bionorica) (Germany).

Agnucaston is a film coated tablets (125mg) contains: Dry extract of chaste tree fruit. Each tablet contains 125 mg of chastetree. It is soluble in water.

Chaste tree is sometimes called (the women's herb) it is used for menstrual disorders and polycystic syndrome (PCOS) Chaste berry seems to control production of many chemicals (dopamine) and hormones in the brain that regulate women's reproductive cycles. The recommended dose of Agnucaston is 125 mg/kg/day, for human, Hobbs and Blumenthal (1999).

2.1.2. Study sample:

Virgin female mice weighting 25-30 gm were acquired in pathogen-free, well-ventilated room in order to enable the animals to acclimatize to their environment. Drinking water and food supplied ad libitum. They were divided into two groups; first group were used for studying the effects of the treatments (in virgin females) and the second group were used for studying the effect of the treatments (in pregnant females) as following:

1- The first group was divided into three parts. The first part of virgin females administrated orally a single dose of Amophage 1.3mg/kg/day. This dose equal to the recommended dose for human after modified to suit the small weight of albino mice according to Pagat and Barnes (1964). The second part of virgin females administrated orally a single dose of Agnucaston 0.3 mg/kg/day. This dose equal to the recommended dose for human after modified to suit the small weight of albino mice according to pagat and Barnes (1964). The third part virgin females served as control administrated distilled water.

2- The second group were females housed with adult males by ratio of 3:1 after one day of mating the females which exhibiting a vaginal plug were considered as pregnant. The day of the appearance of the vaginal plug was considered as the first day of pregnancy. The pregnant females were weighted and caged individually and divided into three parts: The first part administrated orally from the day (3) to the day (18) of gestation with a dose of (1.3 mg.kgm/day)

Amophage. The second part administrated, orally from (3) to day (18) of gestation with a dose of (0.3 mg/kg/day) Agnucaston. The third part served as control were administrated distilled water orally. After (15) days of treatments with (Amophage and Agnucaston) the virgin and pregnant females were killed, the bone marrow of females were collected and (15) embryos were randomly selected from each part of pregnant females to study the chromosomal abnormalities.

2.2. Methods

2.2.1. Chromosomal preparations from bone marrow cells of females (virgin and pregnant).

Chromosomes from bone marrow cells were prepared according to the methods of HUS and patton (1969) and Yosida *et al.* (1971).

Bone marrow cells were collected in T.C.M. 199 culture media and colchicine was added (2m.L of 0.05 Colchicine) then, the cells were incubated at 37°C for 90 minutes. After centrifugation, 5ml of hypotonic solution of (0.56%) KCl was added and the pellet suspended and incubated at 37°C for 30 minutes. After centrifugation the cells were fixed in freshly prepared 3:1 methylalcohol-glacial acetic acid then two or three drops of cell suspension were dropped on a clean slide. Slides were stained with Giemsa stain 10% for 25 minutes.

2.2. 2. Chromosomes preparations from embryos cells:

Chromosomes preparations from embryonic cells were prepared according to Romagnano *et al.* (1995). Embryos livers were collected from each group and placed in 5ml T.C.M. 199 media, 2ml of 0.05 colchicine was added for each tube and incubated at 37°C for 90 minutes. An amount of 5ml of hypotonic solution of 0.56% KCl was added to the pellet and the cells were incubated at 37°C for 20 minutes .5ml of fresh fixative (3 methyl alcohol: 1 glacial acetic acid) were added to the cells. Two or three drops from the cell suspension were added to the surface of clean slides, air-dried and stained with 5% Giemsa stain, and examined for chromosomal aberrations. 50 metaphase spreads were examined for each female and embryo, scoring the different types of chromosomal aberrations (structural and numerical).

2.2.3. Statistical analysis:

The data of chromosomal aberrations in the females and Embryos were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1999). Least significant differences were used compare between means according to Waller and Duncan (1969) at probability 5%.

3-Results

3.1. Chromosomal aberration:

3.1.1. In the virgin females:

Means \pm L.S.D values and results are given in Table (1). In the group of virgin females treated with Amophage for 15 consecutive days. The frequencies of the total number of structural and numerical aberrations were in the same limit of control group, there was no significant difference between Amophage group and control group (18, 7.67) and (17, 6.33) respectively. While, in the group of virgin females treated with, (Agnucaston) there were significant increases in the frequencies of structural and numerical aberrations compared with controls (26, 13) and (17, 6.33) respectively.

3.1.2. In the pregnant females:

Means, \pm L.S.D. values and results are given in Table (2). In the group of pregnant females treated with Amophage from day (3) to day (18) of pregnancy. The frequencies of the total structural and numerical aberrations were increased significantly from that of the control group (17.67 and 8.33) and (14.33 and 7.33) respectively.

Also, in the group of pregnant females treated with Agnucaston the frequencies of the total structural and numerical aberrations were increased highly significantly from that of the control group (32.3 and 17.67) and (14.33 and 7.33) respectively.

Table (1): The effect of oral administration of Amophage and Agnucaston on (virgin females):

Treatments	Structural Aberrations								Numerical aberrations			
	Chromatid gaps	Chromatid break	Deletion	Fragment	Centromeric attenuation	Ring	Endometosis	Total structural aberrations	<40	>40	Polyploidy	Total numerical aberration
Control	3.33b \pm 0.58	0.67 \pm 0.58	1.67b \pm 0.58	2.33 \pm 0.58	3.33b \pm 0.58	0.00 \pm 0.00	3.00b \pm 1.00	14.33c \pm 1.53	3.00b \pm 1.00	4.33b \pm 0.58	0.00b \pm 0.00	7.33c \pm 1.15
Amophage	4.00b \pm 1.00	1.00b \pm 0.00	2.67b \pm 1.53	2.00 \pm 1.00	3.67b \pm 0.58	0.33 \pm 0.58	4.00b \pm 0.00	17.67b \pm 0.58	3.67b \pm 0.58	4.67b \pm 0.58	0.00b \pm 0.00	8.33b \pm 1.15
Agnucaston	6.67a \pm 0.58	3.67a \pm 0.58	5.00a \pm 0.00	3.33 \pm 0.58	6.67a \pm 0.58	1.00 \pm 1.00	6.00a \pm 1.00	32.33a \pm 0.58	7.33a \pm 0.58	8.67a \pm 0.58	1.67a \pm 0.58	17.67a \pm 0.58
L.S.D. at α 0.05	1.490	0.941	1.884	N.S.	1.53	N.S.	1.632	1.998	1.490	1.153	0.666	1.998

Means of different letters (a, b, c, d) in the same column are significantly different. The column without letters is not significant. 50 metaphase were examined from each animals.

Table (2): The effect of oral administration of Amophage and Agnucaston on pregnant females

Treatments	Numerical aberrations								Numerical aberrations			
	Chromatid gaps	Chromatid break	Deletion	Fragment	Centromeric attenuation	Ring	Endometosis	Total structural aberrations	<40	>40	Polyploidy	Total numerical aberrations
Control	4.33b \pm 0.58	1.33b \pm 0.58	2.33c \pm 0.58	2.67 \pm 0.58	2.67b \pm 0.58	0.00 \pm 0.00	3.67 \pm 0.58	17.00b \pm 1.00	2.67b \pm 0.58	3.67b \pm 0.58	0.00 \pm 0.00	6.33b \pm 1.15
Amophage	5.00b \pm 0.00	1.67b \pm 0.58	3.00b \pm 0.00	1.67 \pm 0.58	3.00b \pm 0.00	0.33 \pm 0.58	3.33 \pm 0.58	18.00b \pm 0.00	3.33b \pm 0.58	4.33b \pm 0.58	0.00 \pm 0.00	7.67b \pm 0.58
Agnucaston	6.33a \pm 0.58	3.00a \pm 0.00	4.00a \pm 0.00	2.67 \pm 0.58	4.67a \pm 0.58	0.67 \pm 0.58	4.67 \pm 0.58	26.00a \pm 1.73	6.00a \pm 1.00	6.33a \pm 0.58	0.67 \pm 0.058	13.00a \pm 1.00
L.S.D. at α 0.05	0.941	0.941	0.666	N.S.	0.941	N.S.	N.S.	2.307	1.490	1.153	N.S.	1.884

Means of different letters (a, b, c, d) in the same column are significantly different. The column without letters is not significant. 50 metaphase were examined from each animals.

3.13. In the embryos:

Means \pm L.S.D. values and results are given in Table (3).

Cytogenetic examination in embryos treated with (Amophage) showed a slight significant increase in the total number of structural and numerical aberrations as compared with embryo control group the frequencies of structural and numerical aberrations were (14.7 and 7.3) compared with that of control (12.3 and 6.0) respectively.

On the other hand, cytogenetic examination in embryos group treated with (Agnucaston) showed a highly significant increase in the total number of structural and numerical aberrations compared with the control group. The frequencies of structural and numerical aberrations were (24.3 and 12) compared with that of control (12.3 and 6.0) respectively.

Table (3): Embryo: The effect of oral administration of Amophage and Agnucaston on embryos.

Treatments	Structural Aberrations							Numerical aberrations				
	Chromoti d gaps	Chromoti d break	Deletion	Fragment	Centromeri c attenuation	Ring	Endo metosis	Total structural aberration	<40	>40	Polyploidy	Total numerical aberration
Control	3.7b \pm 0.6	0.3b \pm 0.6	0.7b \pm 0.6	2.3 \pm 0.6	3.0 \pm 1.0	0.0 \pm 0.0	2.3b \pm 0.6	12.3c \pm 1.2	2.7b \pm 0.6	3.3c \pm 0.6	0.0 \pm 0.0	6.0c \pm 1.0
Amophage	3.3b \pm 0.6	1.0ab \pm 0.0	1.7b \pm 0.6	2.7 \pm 0.6	3.0 \pm 1.0	0.0 \pm 0.0	3.0b \pm 0.0	14.7b \pm 0.6	3.3b \pm 0.6	4.0b \pm 0.0	0.0 \pm 0.0	7.3b \pm 0.6
Agnucaston	5.3a \pm 0.6	1.7a \pm 0.6	3.3a \pm 0.6	3.7 \pm 0.6	4.7 \pm 0.6	1.0 \pm 0.0	4.7a \pm 0.6	24.3a \pm 0.6	5.3a \pm 0.6	6.0 \pm 0.0	0.7 \pm 0.6	12.0a 0.0
L.S.D. at α 0.05	1.153	0.941	1.153	N.S.	N.S.	N.S.	0.941	1.632	1.153	0.666	N.S.	1.331

Means of different letters (a, b, c, d) in the same column are significantly different. The column without letters is not significant. 50 metaphase were examined from each animals.

3.2. Comparison between the effect of all groups (control and treated) before pregnancy and during pregnancy:

3.2.1. Comparison between control groups in the virgin and pregnant Females:

Means \pm L.S.D. and results are given in Table (4). There were no significant differences between all the types of structural and numerical aberrations in the two control groups of the virgin and pregnant females.

3.2.2. Comparison between the effect of Amophage treatments on the females before and during pregnancy.

Means \pm L.S.D. and results are given in Table (5) cytogenetic examination showed that there were no significant differences between all types of structural and numerical aberrations and also between the total structural and numerical aberrations in the two Amophage treatment groups of virgin and pregnant females. This means that the effect of Amophage is the same before and during pregnancy.

3.2.3. Comparison between the effect of Agnucaston treatments on the females before and during pregnancy:

Means \pm L.S.D. and results are given in Table (6) when comparing the frequencies of the total chromosomal aberrations (structural and numerical) between the virgin females and pregnant females treated with Agnucaston we found that mothers (pregnant females) had more frequent chromosomal aberrations than those of virgin females. This means that the effect of Agnucaston is more frequent during pregnancy than before pregnancy.

3.2. Comparison between the effect of all groups (control and treated) before pregnancy and during pregnancy:

3.2.1. Comparison between control groups in the virgin and pregnant Females:

Means \pm L.S.D. and results are given in Table (4). There were no significant differences between all the types of structural and numerical aberrations in the two control groups of the virgin and pregnant females.

3.2.2. Comparison between the effect of Amophage treatments on the females before and during pregnancy.

Means \pm L.S.D. and results are given in Table (5) cytogenetic examination showed that there were no significant differences between all types of structural and numerical aberrations and also between the total structural and numerical aberrations in the two Amophage treatment groups of virgin and pregnant females. This means that the effect of Amophage is the same before and during pregnancy.

3.2.3. Comparison between the effect of Agnucaston treatments on the females before and during pregnancy:

Means \pm L.S.D. and results are given in Table (6) when comparing the frequencies of the total chromosomal aberrations (structural and numerical) between the virgin females and pregnant females treated with Agnucaston we found that mothers (pregnant females) had more frequent chromosomal aberrations than those of virgin females. This means that the effect of Agnucaston is more frequent during pregnancy than before pregnancy.

Table (4): Comparison between control groups in the females before and during pregnancy.

Treatments	Structural Aberrations								Numerical aberrations			
	Chromatid gaps	Chromatid break	Deletion	Fragment	Centromeric attenuation	Ring	Endo metosis	Total structural aberration	<40	>40	Polyploidy	Total numerical aberration
Before pregnancy	4.33 \pm 0.58	1.33 \pm 0.58	2.33 \pm 0.58	2.67 \pm 0.58	2.67 \pm 0.58	0.00 \pm 0.00	3.67 \pm 0.58	17.00 \pm 1.00	2.67 \pm 0.58	3.67 \pm 0.58	0.00 \pm 0.00	6.33 \pm 1.15
During pregnancy	3.33 \pm 0.58	1.67 \pm 0.00	3.00 \pm 0.00	1.67 \pm 0.58	3.00 \pm 0.58	0.33 \pm 0.58	3.33 \pm 0.58	18.00 \pm 1.73	3.33 \pm 1.00	4.33 \pm 0.58	0.00 \pm 0.58	7.67 \pm 1.00
L.S.D. at α 0.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Means of different letters (a, b, c, d) in the same column are significantly different. The column without letters is not significant. 50 metaphase were examined from each animals.

Table (5): Comparison between the effect of oral administration of Amophage in the females before and during pregnancy.

Treatments	Structural Aberration								Numerical aberration			
	Chromatid gaps	Chromatid break	Deletion	Fragment	Centromeric attenuation	Ring	Endo metosis	Total structural aberration	<40	>40	Polyploidy	Total numerical aberration
Before pregnancy	5.00 \pm 0.00	1.67 \pm 0.58	3.00 \pm 0.00	1.67 \pm 0.58	3.00 \pm 0.00	0.33 \pm 0.58	3.33 \pm 0.58	18.00 \pm 0.00	3.33 \pm 0.58	4.33 \pm 0.58	0.00 \pm 0.00	7.67 \pm 0.58
During pregnancy	4.00 \pm 1.00	1.00 \pm 0.00	2.67 \pm 1.53	2.00 \pm 1.00	3.67 \pm 0.58	0.33 \pm 0.58	4.00 \pm 0.00	17.67 \pm 0.58	3.67 \pm 0.58	4.67 \pm 0.58	0.00 \pm 0.00	8.33 \pm 1.15
L.S.D. at α 0.05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Means of different letters (a, b, c, d) in the same column are significantly different. The column without letters is not significant. 50 metaphase were examined from each animals.

Table(6): Comparison between the effect of oral administration of Agnucaston in the females before and during pregnancy.

Treatments	Structural Aberration								Numerical aberration			
	Chromatid gaps	Chromatid break	Deletion	Fragment	Centromeric attenuation	Ring	Endo metosis	Total structural aberration	<40	>40	Polyploidy	Total numerical aberration
Before pregnancy	6.33 \pm 0.58	3.00 \pm 0.00	4.00 \pm 0.00	2.67 \pm 0.58	4.67 \pm 0.58	0.67 \pm 0.5	0.67 \pm 0.58	26.00 \pm 0.73	6.00 \pm 1.0	6.33 \pm 0.58	0.67 \pm 0.58	13.00 \pm 1.00
During pregnancy	6.67 \pm 0.58	3.67 \pm 0.58	5.00 \pm 0.00	3.33 \pm 0.58	6.67 \pm 0.58	1.00 \pm 1.0	6.00 \pm 1.00	32.33 \pm 0.58	7.33 \pm 0.58	8.67 \pm 0.58	1.67 \pm 0.58	17.67 \pm 0.58
L.S.D. at a 0.05	N.S.	N.S.	N.S.	N.S.	1.308	N.S.	N.S.	2.927	N.S.	1.308	N.S.	1.851

Means of different letters (a, b, c, d) in the same column are significantly different. The column without letters is not significant. 50 metaphase were examined from each animals.

4-Discussion:

Polycystic ovary syndrome (PCOS) is one of the most common causes of menstrual disorder in the world and a leading cause of infertility. Polycystic ovary syndrome (PCOS) is caused by an imbalance in the hormones of the brain and ovary. The most important hormones that caused (PCOS) called the (LH) Luteinizing hormone and insulin hormone. When the levels of the two hormones were increased in the blood, extra production of testosterone by the ovary result which caused a menstrual disorder and infertility in the girls and women.

The most common drugs for the treatment of (PCOS) are Agnucaston and Amophage (Metformin hydrochloride). Agnucaston is a herbal drug contains a dry extract of the fruit of the chaste tree which taken to decrease the level of (LH) in the blood leading to regulate the menstrual periods and treatment of (PCOS). Also (Metformin hydrochloride) was used to increase the sensitivity of the body to insulin hormone leading to decrease the insulin level in the blood.

The present study was carried out in order to evaluate the cytogenetic effects of Agnucaston and Metformin on the Bone marrow cells of virgin, pregnant females (mothers) and their embryos.

In the present study, the administrating of virgin females with (Amophage once daily for 15 days showed no significant increase in the chromosomal aberrations in the bone marrow cells compared with the control group.

While, the administration of virgin females with Agnucaston for 15days caused a significant increase in the chromosomal aberrations of bone marrow cells compared with the control group. These results is in agreement with George and George et al (2003) who observed that Metformin has a mutagenic or clastogenic effect when administrated orally to the human.

Wherever, negative results were obtained by Prilepkaya et al (2009) who found that no clastogenic or mutagenic effects was observed when Agnucaston administrated orally in a recommended dose to human females

In addition, cytogenetic and developmental toxicity in embryos may occur through a direct effect of some chemicals or hormones on the embryo, fetus or indirectly through toxicity of the drug to the mothers and the placenta, or most commonly as a combination of the two concepts. Maternal conditions are capable of adversely affecting the developing organism in the uterus Khera (1981) and (1984).

In our study, the administration of Metformin with a dose equal the recommended dose in human given to the female mice during pregnancy caused slightly significant increase in the chromosomal aberrations (structural and numerical) to the pregnant

females and their embryos. These finding was in agreement with Glueck et al (2002) who observed that after oral administration of Metformin to the females during pregnancy no clastogenic effect on the females and their embryos was observed.

Also, in the present study, we found that when pregnant females were administrated orally with a dose of Agnucaston equal to the recommended dose in human during pregnancy caused a highly significant increase in the total chromosomal aberrations (structural and numerical) in the pregnant females and in the embryos.

These findings were in agreement with Jerry et al (1991) who reported that Agnucaston should be prevented during pregnancy because it caused embryo toxicity though the placenta.

Also, positive results were obtained by Jerry et al (1991) who observed that Agnucaston when administration to females during pregnancy caused an abortion and bleeding.

However, negative results were observed by Christiansen et al (2005) who found that Agnucaston did not cause any toxicity when administrated orally to the females during pregnancy.

In the present study when comparing the cytogenetic effect of oral administrations of the two drugs (Metformin and Agnucaston). We found that in the females treated with Metformin before and during pregnancy there were no significant difference in the frequencies of chromosomal aberrations (structural and numerical). However, in the females treated with Agnucaston there were significant differences in the frequencies of chromosomal aberrations (total structural and numerical).

These results were in agreement with Jakubowicz, et al (2002) who reported that Metformin has no toxic effects in the females (during pregnancy). In addition, these results was in agreement with Hobbs and Blumeutual, (1999) who observed that agnucaston may has a toxic effects if taken during pregnancy.

In conclusion, our results indicated that Agnucaston had a significant mutagenic and cytotoxic effects on females (before and during pregnancy) and on the embryos. In the present study we found that the treatment of female mice with Metformin had no mutagenic or cytotoxic effects to the female mice (before pregnancy). However, the treatment with Metformin during pregnancy caused a slight significant increase in the total chromosomal aberrations of pregnant females and embryos. This may be due to cross of Metformin into female placenta causing slight cytotoxic effects to the mothers and embryos. So, we concluded that (Metformin) should be taken under medical control but Agnucaston should be avoided during pregnancy and lactation.

5-References

- Christiansen, Nybo A. et al. (2005).** Evidence-based investigations and treatments of recurrent pregnancy loss. *Fertility and sterility* 83(4):827-839.
- Dunaif S. Segal K.R.; Futterwet, W. (1989).** Profound peripheral insulin resistance, independent of obesity in polycystic ovary syndrome *Diabetes*, 38: 1165-74.
- George S.S., George K, et al. (2003).** Sequential treatment of Metformin and clomiphene citrate in women with polycystic syndrome. *Human Repro* 18, 299-304.
- Glueck C.J., Wang P. and et al. (2002).** Pregnancy outcomes among women with polycystic ovary syndrome treated with Metformin. *Human Reprod*, 17: 2858-2864.
- Hobbs, C. and M. Blumenthal (1999).** *Vitex agnus-castus: Aliteratural Review Herbal Gram* (47).
- HSU, T.I.C and Patton, J.L (1969).** Bone marrow preparations for chromosome studies, In *Bernischek comparative mammalian cytogenetics* springerverlag, pp 454-460.
- Jakubowicz D., Luorno M.I. et al. (2002).** Effects of Metformin on early pregnancy loss in the polycystic ovary syndrome. *J. Clin. Endocrinol metab*, 87: 524-529.
- Jarry, H.S. Leonliardt, C. et al. (1994).** In vitro prolactin but not LH and FSH Release is inhibited by compounds in extracts of agnus castus: *Exp Clin. Endocrinol* 102(6): 448-454.
- Balen, A. Michelmore, K. (2002). What is Polycystic ovary syndrome *Hum Repro*, 17:2219-2227.
- Jerry, H, Hoff W., et al (1991).** Chastree significantly inhibits the stress induced secretion of prolactin in male rats. *Phytother.* (12): 77-82.
- Khera, K.S. (1981).** Common fetal aberration and their teratogenic significance, a review. *Fund Appl Toxicol*, 1: 13-18.
- Khera, K.S. (1984).** Maternal toxicity: a possible factor in embryo-fetal deaths and fetal malformations of rodent-rabbit. *Teratology*, 31:129-153.
- Lauritzen, C. et al. (1997).** Treatment of premenstrual tension syndrome with vitex agnus castus. Controlled, doubled blind study versus pyridoxine. *Phytomedicine* 4: 183-189.
- Milewicz, A. et al. (1993).** Vitex agnus castus extract in the treatment of luteal phase defects due to latent hyperprolactinemia results of controlled double-blind study *Arzne imforsch* 43(7): 752-756.
- Norman R.J. (2003).** Metformin in polycystic ovary syndrome: systematic Review and Meta. Analysis *BMJ*. 327-954.
- Pagat and Barnes (1964).** Evaluation of Drug activities, Vol. 1. Academic press.
- Romagnano, A., Richer, C.L. and Perrove, MA. (1985).** A direct technique for the preparation of chromosomes from embryo equine embryos. *Can. J. Genet. Cytol*, 27: 365-369.
- Rotterdam, (2004).** Consensus of diagnostic criteria and long-term health risks Related to polycystic ovary syndrome. *Fertil-Steri* 81-19-25.
- Snedecor, G.W. and Cochran, W.G. (1990):** *Statistical methods* 9th ed Iowa state Univ. Press, Lova, U.S.A.
- Prilepskaya V., Ledina A. and et al. (2009).** Vitex agnus castus. Successful treatment of moderate to severe premenstrual syndrome. *Maturitas*, spp. 555.563.
- Waller, A. and Duncan, D.B. (1969).** Multiple range and multiple test.. *Bionetries*, 11: 1-24.
- Yosida, T. H; Truchiya, K and moiwaki, K (1971).** Frequency of chromosome polymorphism of *Rattusrattus* collected in Japan. *Chromosoma*, 30:33.

4/15/2010