Al-Kufa University Journal for Biology / VOL.10 / NO.1 / Year: 2018

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Effect of iron status on fertility of woman in relation with certain hormones and other parameters

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Abstract:

Seventy six subfertile women and thirty fertile women (control group) enrolled in this study. The subfertile women subdivided into two subgroups according to the cause of subfertility: first group ovarian dysfunction (OD) either due to polycystic ovary syndrom (PCOS) or due to other cause (OC) likes anovulation, hyperprolactenemia, premature ovarian failure. Second group: unexplained infertility, their age range (16-40) yr. At day 3 of menstrual cycle (MC) estimation of serum level of multiple hormones like luteinized (LH), follicle stimulating (FSH), estradiole (E2), prolactin (PRL), testosterone, iron status (serum iron, serum ferrtin and total iron binding capacity TIBC) , while at day 13 of MC, estimation of serum levels of LH, serum leptin, glutathione, iron status .Comparison of iron status between fertile and subfertile women at day 13 of MC revealed significant increase in serum ferritin level in women with PCOS. Assessment the serum levels of certain hormones in fertile and subfertile women in those having serum ferritin level less than normal value at day 3 of MC show the following: significantly higher serum testosterone level in PCOS group, also significantly higher serum prolactine level in group of OD due to O.C. as compared with fertile group. Assessment the serum levels of certain hormones in fertile and subfertile women in those having serum ferritin level higher than normal value at day 3 of MC show significantly higher serum prolactine level in group of OD due to O.C. as compared with fertile group. Assessment the serum levels of certain hormones in those having serum ferritin level less than normal value at day 13 of MC, shows significant decrease in LH in women with OD due to O.C. as compared with fertile group.

Conclusions: It was concluded that the decrease in serum ferritin levels in the blood may be associated directly or indirectly with subfertility caused by ovarian dysfunction

Key words: Iron status, Serum ferritin, polycystic ovarian syndrome

{Citation: Sami Rehem Al-Katib, Maisem Mohammed Hussein Al-Kaabi and Aseel J. Al-Bderi. Effect of iron status on fertility in relation with certain hormones and other parameters**.** American Journal of Research Commuication, 2014.

Introduction:

Subfertility defined as the inability to conceive after a certain period of time (the length of which vary) [1]. Twelve months is the lower reference limit for Time to Pregnancy by the World Health Organization [2]. In both men

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

and women the fertility process is complex. Subfertility affects about 10% of all couples. Even under ideal circumstances, the probability that a woman will get pregnant during a single menstrual cycle is only about 30% and when conception does occur, only 50-60% of pregnancies advance beyond the 20th week. The inability of a woman to produce a live birth (because of abnormalities that cause miscarriages) is called infecundity [3] [4]. About a third of subfertility problems are due to female causes and another third are due to male causes. In the remaining cases, subfertility affects both partners or the cause is unclear (unexplained). It is equally important for both partners to be tested at the same time [5].

Iron is an element of crucial importance to living cell and exists in a range of oxidation states, the most common being ferrous (Fe^{+2}) and ferric ($Fe^{+3)}$) forms. Iron can be associated with proteins; bind to oxygen (O^2), transfer electrons and mediate catalytic reactions. Enzymes of the citric acid cycle (succinate dehydrogenase and aconitase) are iron-dependent. Iron is a critical component of heme in hemoglobin (Hb), myoglobin, cytochromes as well as iron–sulfur complexes of the electron transport chain [6]. Iron is also required for activity of ribonucleoside reductase, the rate-limiting enzyme of the first metabolic reaction committed to DNA synthesis. Therefore, iron plays an important role in metabolic processes including O^2 transport, electron transport, oxidative phosphorylation and energy production, xenobiotic metabolism, DNA synthesis, cell growth, apoptosis, gene regulation and inflammation [7][8]. It is also a necessary cofactor for the synthesis of neurotransmitters, dopamine, norepinephrine and serotonin [9]. Although iron comprises only 0.008% of the body's mass (approximately 6 g for 75-kg for adult male), we cannot live without this important element in our bodies. In child bearing age women, the average daily iron absorption is about twice that in men, largely because of gestation, lactation periods and the blood loss during menstruation (around 20 mg iron/period) [10]. 12% of all women of child-bearing age have an iron deficiency. Estimations of iron status before pregnancy can help to avoid deficiency which is a common condition during pregnancy. Serum ferritin is the most reliable test for body's iron stores [11]. A small clinical trial testing the efficacy of an iron-containing supplement among women who had unsuccessfully tried to become pregnant documented a higher pregnancy rate in the treatment group [12]. Dietary iron presents in food in two forms as heme and non-heme iron. Mathur and Sharma (1995), found that treating iron deficiency anemic patients with 500 mg of vitamin C/ twice daily resulted in an increase in average haemoglobin level by 8%, increase serum iron concentration by 17%, transferrin saturation to 23% and a decrease in total iron binding capacity by 7% [13]. Consumption of iron supplements and non-heme iron from non animal sources may decrease the risk of ovulatory infertility. Women who consumed iron supplements had a significantly lower risk of ovulatory infertility than women who did not use iron supplements [11]. The majority of infertility cases due to

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

ovulation disorders may be preventable through modifications of diet and lifestyle. Infertility can be reduced by consuming non-heme iron, which is found in iron supplements, multivitamins and spinach. Researchers studying a large group of women found that women with a high daily intake of non-heme iron (41-51 mg) had lower rates of infertility due to ovulatory disorders [7][14].

Materials and Methods

The subfertile women enrolled in this study were collected from the outpatient department of fertility center in the gynecological and obstetrical teaching hospital in Karbala city and from multiple gynecologic/obstetric privet clinics while the control fertile women obtained from relatives and some friends. The obtained total number for this study about 106 which divided into 2 groups: subfertile women(that account 76) and fertile or control women(that account 30). The subfertile women subdivided into three subgroups according to the cause of subfertility : ovarian dysfunction group which subdivided into OD due to polycystic ovary syndrome (PCOS) and OD due to other cause (OC) like anovulation, hyperprolactenemia, premature ovarian failure. All of them at reproductive age (16-40) years and their body mass index (BMI) ranged from 20-45 kg/m^2. The study was achieved throughout a period which extends from July 2012- October 2013.

Equipments and Kits:

Height and weight measurement apparatus: Measurement of height and weight for each patient was done to calculate their body mass index

Ultrasound device: Ultrasound used called Ultra mark ATL9.

Blood Samples collection: Ten milliliters of venous blood was drawn using a disposable needle and plastic syringes from each woman. The blood sample was left at room temperature about 10 minutes for complete clotting, centrifuged at 3000 round per minute (RPM) for 5 minutes, and then the serum was separated and transported into new disposable plain tubes and kept frozen for future analysis.

Chemical kits: By using different enzyme techniques for determination the concentrations of the following: serum iron, serum TIBC, serum ferritin, serum glutathione ,leptin ,LH , FSH , E2, testosterone ,PRL,

Methods: History taken from each women including her parity ,type of subfertility (primary or secondary) and its duration, menstrual history(date, duration, frequency, regularity and amount of bleeding) , past obstetrical and gynecological history like ectopic pregnancy, ovarian cyst, miscarriages .Evaluation of her investigations, like hormonal assay, ultrasound, hystrosalpingiograph, and semen analysis for her husband. If suspected PCOS, in addition to menstrual irregularity, ask about gaining weight and hirsutism. Nutritional history, good or poor nutrition through asking her about quality, quantity and frequency of fruit, vegetables, meats and dairy products, and their availability, to be ingested. At day 3±1 of menstrual cycle and estimation of

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

serum LH, FSH, E2, prolactin, testosterone, iron status , while at day 13 of MC estimation LH , serum Leptin, glutathione, iron status .

Statistical Analysis: Type of study is case-control study .Statistical analysis was done by using SPSS (statistical package for social sciences) version 20 in which we use chi square test for categorical data and independent sample T-test and ANOVA (analysis of variance) with LSD for measurement data. We set P value <0.05 as significant.

Results:

Assessment the relation between the levels of iron status for fertile and subfertile women at day 3 of MC: Comparison show there is no significant difference between all groups,as in table 1.

Table (1) Serum levels of iron status for fertile and subfertile women at day 3 of MC (mean±SD).

Variable	Fertile women (n=30)	Subfertile women (n=76)		
		Ovarian dysfunction(n=49)		Unexplained (n=27)
		O.C.(n=40)	PCOS (n=9)	
S. iron (µg/dl)	61.66±30.8	58.47±24.15	61.46±24.59	67.54±22.19
S. ferritin (ng/dl)	14.89±11.77	12.70±12.58	18.12±10.87	17.47±11.54
T.I.B.C (µmol/L)	323.4±59.38	315.35±51.53	311.77±31.11	322.14±62.05

No significant difference (P >0.05) in the variables between all groups.

Assessment the relation between the levels of iron status for fertile and subfertile women at day 13 of MC: Table (2) revealed no significant difference between all groups except serum ferritin which was significantly higher (P<0.05) in PCOS as compared with fertile women**.**

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Table (2) Serum levels of iron status for fertile and subfertile women at day 13of MC (mean±SD).

Variable	Fertile women (n=30)	Subfertile women (76)		
		Ovarian Dysfunction (n=49)		Unexplained (n=27)
		O.C. (n=40)	PCOS (n=9)	
S. iron (µg/dl)	66.09±41.07	60.80±24.27	78.57±19.95	75.03±22.41
S.ferritin (ng/dl)	16.45±15.32	14.91±14.14	27.48±17.09 *	14.91±8.63
T.I.B.C (µmol/L)	289.03±61.47	299.8±48.41	287±30.54	301.407±53.54

* indicate significant difference (P <0.05) as comparison between fertile and subgroups of subfertile women .

Assessment the serum levels of certain sex hormones in fertile and subfertile women in those having serum ferritin level less than normal value at day 3 of MC: Table (3) explain the significant increase (P<0.05) in serum testosterone level in PCOS group . Also there is significant increase (P<0.05) in serum prolactine level in group of ovarian dysfunction due to O.C.

Table (3) Assessment the serum levels of certain sex hormones in fertile and subfertile women in those having serum ferritin level less than normal value at day 3 of MC (mean ±SD):

Variables	Fertile women (n=21)	Subfertile women (n=56)		
		Ovarian dysfunction(n=32)		Unexplained (n=24)
		O.C. (n=27)	PCOS (n=5)	
LH (mIU/ml)	3.5±1.29	3.82±2.14	5.03±4.33	3.95±1.83
FSH(mIU/ml)	6.7±3.48	6.12±3.68	5.86±1.742	5.7±2.06
Estrogen (pg/ml)	59.41±19.3	50.13±23.47	43.5±24.9	64.83±30.95
Testosterone (ng/ml)	0.53±0.15	0.48±0.25	0.73±0.262 *	0.32±0.15
Prolactine (ng/ml)	14.94±6.3	24.22±13.88 *	16.32±12.3	20.55±10.5

* indicate significant difference (P <0.05) as comparison between fertile and subgroups of subfertile women.

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Assessment the serum levels of certain sex hormones in fertile and subfertile women in those having normal serum ferritin level at day 3 of MC: Table (4) shows no significant difference between fertile and subfertil womn in all variables except in serum prolactine level is significantly higher (P<0.05) in of ovarian dysfunction due to O.C.

Table (4) Assessment the serum levels of certain sex hormones in fertile and subfertile women in those having normal serum ferritin level at day 3 of MC (mean ±SD):

Variables	Fertile women (n=9)	Subfertile women (n=20)		
		Ovarian dysfunction(n=11)		Unexplained (n=9)
		O.C. (n=7)	PCOS (n=4)	
LH (mIU/ml)	4.01±1.49	3.94±2.5	6.30±4.52	6.99±9.92
FSH (mIU/ml)	5.72±1.95	4.56±2.16	3.87±2.16	6.11±3.35
Estrogen (pg/ml)	61.47±11.3	57.25±25	43.21±31.2	50.23±17.7
Testosterone (ng/ml)	0.52±0.19	0.56±0.25	0.47±0.29	0.40±0.29
Prolactine (ng/ml)	19.13±8.18*	29.84±15.6 *	13.9±10.84	19.66±9.7

* indicate significant difference (P <0.05) as comparison between fertile and subgroups of subfertile women.

Assessment the serum levels of certain parameters in fertile and subfertile women in those having serum ferritin level less than normal at day 13 of MC: The result table (5) in shows significant decrease (P<0.05) in serum level of LH for group of ovarian dysfunction due to O.C. as compared with fertile group.

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Table (6) Assessment the serum levels of certain parameter in fertile and subfertile women in those having serum ferritin level less than normal
At day 13 of MC (Mean ±SD)

Parameter	Fertile Women (n=21)	Subfertile women (n=51)		
		Ovarian dysfunction(n=30)		Unexplained (n=21)
		O.C.(n=28)	PCOS (n=2)	
LH(mIU/ml)	9.95±2.85	6.09±2.17*	9.19±7.36	9.95±7.59
Leptin (pg/ml)	158.64±75.03	188.85±133.01	132.52	165.36±93.01
Glutathione (microg/ml)	33.77±13.76	36.02±14.91	27.219	34.86±18.15

* indicate significant difference (P <0.05) as comparison between fertile and subgroups of subfertile women.

Assessment the serum levels of certain parameters in fertile and subfertile women in those having normal value of serum ferritin level at day 13 of MC:
Table (6) shown no significant differences (P>0.05) between all groups.

Table (6) Assessment the serum levels of certain parameters in fertile and subfertile women in those having normal value of serum ferritin level
At day 13 of MC (Mean ±SD):

Parameter	Fertile Women (n=9)	Subfertile women (n=25)		
		Ovarian dysfunction (n=19)		Unexplained (n=6)
		O.C.(n=12)	PCOS (n=7)	
LH(mIU/ml)	9.78±2.13	8.98±3.98	7.31±4.34	9.06±3.02
Leptin (pg/ml)	178.43±99.14	184.69±116.8	177.31±70.52	141.47±23.9
Glutathione (microg/ml)	34.16±19.52	40.53±17.18	30.09±17.28	43.88±24.16

Discussion: The results of statistical analysis shown that serum ferritin level less than normal value which range between (20-159 ng/ml) [15] in all groups at day 3 of M.C even there is no significant difference when compared between fertile and subfertile group, but at day 13 of M.C. the serum ferrtin level slightly increased and the difference in value between the 2 periods due to blood loss that lead to decrease in iron storage (ferritin) during M.C. and this significant

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

increases in serum ferritin day 13 of MC. more clear in PCOS as compared with fertile groups and this may be related to lower activity of ovary in PCOS and oligomenorrhea. The presence of transferrin and its receptor in granulosa cells and oocytes has been documented. More recently, it has been reported that granulosa cells can synthesize transferrin, which may be translocated to the oocytes. Although it is possible that transferrin and transferrin receptor are redundant in the ovary or do not play an important role in local iron metabolism, it has been suggested that these proteins are essential for ovum development and are required to support the increased iron demand of the developing follicle [16][17]. This result agrees with a study done by Sharifi *et al.* (2011) shows that overweight and obese cases with PCOS had higher ferritin levels than BMI matched controls and found that oligomenorrhea and less blood loss in PCOS subjects might be the best explanation for their higher ferritin levels [18]. A similar result has been reported by another two studies suggested that increased body iron stores, expressed as increased serum ferritin concentrations, are present in women with PCOS [19][20]. Sathiyanarayanan *et al.* (2014)studies show that women who do not get sufficient amounts of iron may suffer anovulation (lack of ovulation) and possibly poor egg health, which can inhibit pregnancy at a rate 60% higher than those with sufficient iron stores in their blood. When the blood does not get enough iron, anemia, or an insufficient number of red blood cells, may develop. Since the insufficiency of red blood cells that deliver oxygen to all of the body ´s tissue and organs including the ovaries and uterus may cause the eggs stored in the ovaries to weaken over time and become unviable [21]. Also, the subfertile women may have iron deficiency that associated with low serum ferritin before they get try for pregnancy as explained by researchers [22] who found that up to 12 percent of all women of child-bearing age have an iron deficiency and the principle cause of iron deficiency anemia (IDA) in premenopausal women is blood lost during menses. A small clinical trial testing the efficacy of an iron-containing supplement among women who had unsuccessfully tried to become pregnant documented a higher pregnancy rate in the treatment group [11][12].

Serum testosterone level was significantly increases in PCOS which is the most common cause of ovulation disorders [23], this result agree with Nisenblat and Norman(2009)[24] who show that hyperandrogenemia is a common disorder in PCOS . Elevated free circulating levels of bioactive androgen results from either direct increases of ovarian production or an inhibition of hepatic synthesis of sex hormone-binding globin in PCOS patients [25].

Serum prolactine was significantly increase ($P<0.05$) in group of ovarian dysfunction other than PCOS as compared with fertile whom having low serum ferrtin ,while Shibli *et al*,(2011)[26] found no significant relations between ovarian dysfunction and serum prolactin as compared with fertile when the level of serum ferritin within normal value at day 3 of MC. Klibanski (2010)[27] show that increases the secretion of the prolactin hormone inhibits ovulation in women

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

through negative modulations of pituitary hormones secretion responsible for gonadal function, including LH and FSH, this prevents the onset of a new pregnancy too soon, and so breast feeding was used in the past as a method of contraception. If a non-pregnant woman has abnormally high levels of PRL, it may cause her difficulty in becoming pregnant. It is considered as the most frequent cause of anovulatory cycles, although spontaneous pregnancy may occur occasionally [28]. A recent study done by [29] in mice, explained that hyperprolactinaemia directly inhibits the secretion of kiss-peptin, a neurohormone, that are essential to GnRH functioning and by preventing the secretion of GnRH which effectively blocks ovarian cyclicity. By administering kiss-peptin, we can restore the release of GnRH and restart ovarian cyclic functioning and ovulation despite hyperprolactinaemia [27].

Serum level of LH significantly decreased in group of ovarian dysfunction , also other researchers show that serum ferritin level and iron deficiency have direct or indirect effect in decreasing level of LH hormone in preovulatoury period (day 13 of M.C.) that causing ovulation problem [16][30]. Environment can have large impact on the HPG axis; women with eating disorders, stress, physical exercise and weight loss are suffering from oligomenohrrea and secondary amenorrhea. Starvation from anorexia nervosa causes the HPG axis to deactivate causing women's ovarian and uterine cycles to stop [31].

The study show no significant changes in leptin levels in relation to serum ferritin changes .High leptin levels is closely associated with decreased oocyte maturity, poor fertilization and embryo quality, and lower pregnancy rates in PCOS patients [32][33][34]. Others suggest that elevated leptin levels in the ovary may block estrogen production, disturbing follicular development and oocyte maturation [35]. The study show no significant changes in Glutathion levels in relation to serum ferritin changes .Glutathion is the most abundant non protein thiol in mammalian cells and participates in multiple functions vital to the physiology of the cells, acting as a reducing agent, antioxidants, and free radical scavenger and is involved in the metabolism and detoxification of xenobiotics, so alterations in Glutathion and metabolism have been associated with different human disease [36].

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