

**EFFECT OF HORMONAL CHANGES IN BLOOD AND OVARIAN FOLLICULAR FLUID ON SUBFERTILITY: A STUDY ON WOMEN SUBJECTED TO INTRACYTOPLASMIC SPERM INJECTION(ICSI)**

**تأثير التغيرات الهرمونية في الدم و السائل الحويصلي المبيضي على العقم :دراسة على مريضات يخضعن للحقن المجهرى للحيمين**

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البحث مستل

**Abstract:**

Sixty two infertile women with ovulatory cause of infertility (group A) who undergo ICSI were enrolled in the study . The study also includes 30 women with male cause of infertility, who also undergo ICSI and regarded as control group (group B). Both groups of women were subjected to controlled ovarian hyperstimulation methods . At the day of ovum pickup hormone analysis of blood and follicular fluid (FF) were done to measure estradiol (E2) , testosterone , leptin and antimullerian hormones (AMH) . Blood and FF leptin and testosterone levels were significantly higher (  $P < 0.05$ ) in group A women in comparison with group B while FF and blood AMH level were significantly lower (  $P < 0.05$ ) in group A in comparison with group B. Blood and FF leptin, and testosterone levels were significantly higher (  $P < 0.05$ ) group A women with BMI equal or more than  $25\text{kg/m}^2$  in comparison with group A women with BMI less than  $25\text{kg/m}^2$  . Blood and FF estradiol and AMH level were significantly higher (  $P < 0.05$ ) in group A women aged 17 – 29 years in comparison with group A women aged 30 – 42 years. Blood and FF testosterone and leptin levels were significantly higher (  $P < 0.05$ ) in group A women with polycystic ovarian syndrome (PCOS) in comparison with group A with non PCOS . There were significant correlations between blood and FF leptin, E2, and AMH levels in group A. There were also significant correlations between blood testosterone and AMH , and between blood E2 and AMH in group A . There were a significant correlation between FF testosterone and E2, between FF testosterone and AMH, between FF testosterone and leptin, and between FF estradiol and AMH of group A women .

**الخلاصة**

اثنتان وستون امراة عقيمة (اسباب انثوية) (مجموعة A) و ثلاثين امراة عقيمة (اسباب ذكرية) (مجموعة B) اللواتي يخضعن للحقن المجهرى للحيمين اشركن في هذه الدراسة . كلتا المجموعتين اخضعتا لتحفيز المبيض . في يوم الحقن المجهرى للحيمين تحليل هرمونات الدم و السائل الحويصلي المبيضي ، الهرمونات تشمل اىستراىداىول ، تيسوستيرون ، لبتين و انتي موليريان هرمون . النتائج اظهرت ان هرموني اللبتين و التيسوستيرون مرتفعين بينما هرمون انتي موليريان منخفض عند النساء في المجموعة A مقارنة بالمجموعة B. عند دراسة مستوى الهرمونات عند النساء في المجموعة A اظهرت النتائج ما يلي : ان هرموني اللبتين و التيسوستيرون اعلى معنويا عند النساء في المجموعة (AII) (المجموعة A الاقل وزنا) مقارنة بالمجموعة AI (المجموعة A الاكثر وزنا). وجدت الدراسة ان هرمون اىستراىداىول و نسبة اللاستراىداىول الى التيسوستيرون و انتي موليريان هرمون اعلى عند النساء في المجموعة AI (المجموعة A التي تتراوح اعمار النساء فيها بين 17 و 29 سنة) مقارنة بالنساء في المجموعة AII (المجموعة A التي تتراوح اعمار النساء فيها بين 30 و 42 سنة). الدراسة اظهرت ان هرموني اللبتين و التيسوستيرون مرتفعين عند النساء في المجموعة AI (المجموعة A المصابات بتكيس المبايض) مقارنة بالنساء في المجموعة AII (المجموعة A الغير مصابات بتكيس المبايض) . الدراسة بينت علاقة معنوية بين مستوى اىستراىداىول ، لبتين و انتي موليريان هرمون بين الدم و السائل الحويصلي المبيضي عند النساء في المجموعة A. كذلك وجود علاقة معنوية بين هرموني تيسوستيرون و انتي موليريان هرمون و علاقة معنوية بين هرموني اىستراىداىول و انتي موليريان هرمون ، و بين هرموني اللبتين و التيسوستيرون و بين هرموني اىستراىداىول و تيسوستيرون في السائل الحويصلي المبيضي عند النساء في المجموعة A.

**Introduction:**

Infertility affects about 10-15% of couples and is a medical concern for 2.7 million women of reproductive age in U.S (Bili *et al.*,2001),and some studies suggest a range of lifetime prevalence of infertility percentage range from 6.6 to 32.6% (Edmonds,2012). Although there has been no change in the prevalence of fertility problems more couples seek help than did previously. The causes of fertility problems in women include disorder in male or female partner, in 39% of couples a problem will be found in both partners and no identifiable cause is found in one third of couples trying for baby. Polycystic ovarian syndrome (PCOS) is one of the most common female endocrine disorders affecting approximately 5 -10% of women of reproductive age and is thought to be one of the leading causes of female infertility (Brassard *et al.*, 2008). Fertility treatment may be medical , surgical or involve assisted conception where by the egg and sperm are brought into close proximity to facilitate fertilization. Approximately 50% of couples will conceive after receiving advice and simple treatment, but the reminder require more complex assisted conception technique, and 4% of couples will remain childless. The chance of conception over the first 6 months of marriage is approximately 60% . At the end of first year 85% of couples will have conceived (Campbell and Monga ,2004) . Studying some of hormones that related to fertility state is of major benefit to identify the causative factors and search for appropriate treatment for these fertility problems. The hormones synthesized by follicular cells during the process of follicle maturation accumulate in follicular fluid (FF) and association is believed to be exist between the hormonal content of FF and the quality and degree of oocyte maturity, and therefore FF hormonal content is presumed to be related to fertilization and embryo development and implantation rate (Hill *et al.* ,2007). Early studies designed to investigate the role of leptin in obesity and appetite regulation also gave rise to an appreciation of the relationship between reproductive function and body energy reserves. It is now suggested that leptin may serve as the critical link between the body's adipose tissue and the hypothalamo-pituitary axis, indicating whether adequate energy stores are sufficient to support normal reproductive function (Mantzoros *et al.* ,2000) . Anti-Mullerian hormone (AMH) is quantitative marker for ovarian reserve. Although AMH levels are independent of the classical endocrine fluctuations of the menstrual cycle , AMH plays a role in the regulation of ovarian function during both early and late follicle development . It can be considered a factor reflects the depletion rate of the primordial follicle pool and affects the maintenance of the pool of growing follicles(La Marka *et al.*, 2010) . It is well known that a predominantly intrafollicular estrogenic environment is associated with good follicular growth and has anti-atresia effects. Elevated estradiol (E2) in FF indicate a more advanced stage of oocyte maturation and have been repeatedly found to be associated with a higher chance of achieving pregnancy(Doi *et al.*,2005). Elevated FF testosterone levels were associated with lower quality oocytes, and it cause tendency of these oocytes toward lower cleavage rates after fertilization (Pasquali ,2006).

**Aim of study:** Is to measure some hormones in blood and follicular fluid and detect their role in fertility.

**Materials and methods:** Sixty two infertile women with ovulatory cause of infertility(group A) were enrolled in this study . These women were selected randomly from those attending fertility centre in Al-Sader Teaching Hospital in Al-Najaf city during the period from January 2012 to September 2012. The cause of infertility in these selected women was ovulatory disorders. The study also included group B women represented by 30 women with normal investigations (hormones (FSH ,LH ,testosterone ,E2), ultrasound and hysterosalpingeography) but with male cause of infertility indicated for ICSI, e.g. sever oligozoospermia (Tomlinson *et al.* 2001 ) . The age of these women (both groups) ranges between 17-42 years , and their BMI range between 18-31kg/m<sup>2</sup> . According to the BMI the infertile women in group A were divided into two subgroups:First group include 25 women with female cause of infertility with BMI less than 25 kg/m<sup>2</sup> . Second group include 37 women with female cause of infertility with BMI more than or equal 25kg/m<sup>2</sup>. The infertile women in group A were also divided according to their ages into two groups First group include 29 women with female cause of infertility age from 17 to 29

years. Second group include 33 women with female cause of infertility age from 30 to 42 years. Thirty five (56%) out of 62 women ( group A) included in this study classified as women with PCOS and 27 (44%) of them classified as women with non PCOS. This classification was based on the joint ESHRE/ASRM (European Society for Human Reproduction and Embryology / American Society for Reproduction Medicine definition of POS as the presence of two out of the following three criteria 1. oligo- and / or anovulation (that is oligomenorrhea or amenorrhea) 2. Hyperandrogenism (clinical feature and/or biochemical elevation of testosterone) 3. polycystic ovaries assessed by ultrasound (Edmonds, 2012). The duration of infertility ranges between 1-20 years. Full history was taken from the infertile women regarding their age, parity, duration of infertility. Careful menstrual history was taken regarding regularity, duration and timing the day of the cycle. Also, full history was taken about any medical disease or previous surgical intervention especially pelvic surgery, in addition to drug history. The measurement of the weight and height were done to measure BMI. All these women were subjected to the same investigations and medical and surgical procedures.

**Equipments and Reagents:** 1. SIEMENS versa ultra sound pro machine 2. Apparatus for height and weight measurement. Measurement of the weight and height for each woman is done for the calculation of their body mass index (BMI). 3. Enzyme linked immunosorbent assay (ELISA) system (Bio Tek). 4. Kits for hormone measurement, kits used for ELISA technique for detection of estradiol, testosterone, leptin and AMH levels in blood and follicular fluid 5. Centrifuge (EBA 20): For centrifugation of blood and FF

**follicular fluid collection** : All women (group A or group B) participated in this study were subjected to controlled ovarian hyperstimulation method by the gynecologist using the short protocol (antagonist cycles) by daily dose GnRH antagonist alongside with gonadotrophin stimulation. Transvaginal ultrasound scan was performed to measure the size of the leading follicles and to estimate when it will reach (16-22) mm in diameter, then hCG injection to trigger ovulation, ovum collection was performed under general anesthesia with a transvaginal Wallace needle under ultrasound guide. The ova and their FF were taken and perform isolation of ova and transported into special media, 10 milliliters of FF were collected, centrifuged at 3000 round per minute for 5 minutes. Then FF was separated into disposable plain tubes and kept frozen for future analysis. Meanwhile, ovum was stripped of their cumulus oophorus cells by the embryologist and after that ICSI was performed. Measurements of blood and FF leptin, E2, testosterone, and AMH were done using Elisa technique. At the day of pickup of oocytes, patient fasting blood and follicular fluid level of leptin, estradiol, testosterone and AMH were measured.

**Statistical analysis** The data was reported as mean  $\pm$  standard deviation (SD) for each variable. Statistical study is done by using unpaired t-test and ANOVA for the comparison of means, statistical significance was assumed  $P < 0.05$ . Pearson's correlation coefficients (r) were calculated to estimate the correlations between parameters. All statistical analyses were performed using SPSS (Statistical Package for social Science) version 19.0.1 multilingual program (2010), IBM-USA.

## **Results**

**Comparison of blood and follicular fluid hormone levels between group A and group B women** The results of the study show that blood and FF leptin and testosterone levels were significantly higher ( $P < 0.05$ ) in group A women (infertile women with ovulatory cause of infertility) in comparison with group B (women with male cause of infertility), while FF and blood AMH level was significantly lower ( $P < 0.05$ ) in group A women. There was no significant difference in blood and FF estradiol level between group A and group B women, as shown in table 1.

Table1.Comparison of different blood and follicular fluid hormone levels between group A and group B women (Mean± Standard deviation).

Hormone level (Mean± Standard deviation)	Group A N=62	Group B N=30
Blood estradiol (pg/ml)	392±224	403.7±217
F F estradiol (pg/ml)	2760±235	2884±278
Blood testosterone (ng/ml)	6.3±2	2.6±1.2 *
FF testosterone (ng/ml)	19.8±5.2	9.9±5.5 *
Blood leptin ng/ml	31.1±15	20.2±10.6 *
F F leptin (ng/ml)	33.5±17	22.8±10.8 *
Blood AMH ng/ml	2.4±1.7	3.62±2.1 *
FF AMH (ng/ml)	5.5±4	8±4.9 *

\*significant at p-value ( P< 0.05)

**Comparison of blood and follicular fluid hormones levels in different BMI subgroups of group A women .** There were statistically no significant differences in the level of blood or FF E2 between group A women with BMI less than 25 kg/m<sup>2</sup> and group A women with more than or equal 25 kg/m. The results show a statistically significant difference in the level of blood and FF testosterone between these two groups . Also ,there was a significant difference in the level of blood and FF leptin between these two groups bu no significant difference in the level of blood or FF AMH between these groups (at level of significance P < 0.05), as shown in table 2.

Table 2. Comparison of blood and FF hormone levels between different BMI subgroups of group A women . (Mean± Standard deviation)

Hormone level Mean± Standard deviation	group A women with BMI less than 25 kg/m <sup>2</sup> N=25	group A women with more than or equal 25 kg/m N=37
Blood estradiol (pg/ml)	404±144	393±155
FF estradiol (pg/ml)	2858±130	2986±141
Blood testosterone (ng/ml)	2.7±1.9	5.6±1.6 *
FF testosterone (ng/ml)	7±3.6	12.6±4.8 *
Blood leptin (ng/ml)	22±5	40±12 *
FF leptin (ng/ml)	28±2	42.3±5 *
Blood AMH (ng/ml)	2.5±1	2.6±1.1
FF AMH (ng/ml)	6±3	6.3±4

\*significant at p-value ( P< 0.05)

**Comparison of blood and FF hormones level in different age subgroups of group A women .**According to the results shown in (table 3 ) , there were a significant differences in blood and FF estradiol level between group A women with age 17-29years and group A women with age 30-42years . The results show that there were significant differences in blood and FF AMH between these two age groups . The results reveal no significant difference in blood and FF testosterone and leptin between these two groups (at level of significance P < 0.05).

Table 3.Comparison of blood and follicular fluid hormone levels between different age subgroups of group A women . (Mean± Standard deviation)

Hormone level Mean± Standard deviation	group A women with age17-29 years (N=29)	group A women with age 30-42 years (N=33)
Blood estradiol (pg/ml)	469 ± 25	316 ± 21 *
FF estradiol (pg/ml)	2978± 81	2155 ± 83 *
Blood testosterone (ng/ml)	2.4 ± 0.6	2.6 ± 1
FF testosterone (ng/ml)	9.6 ± 5	9.7 ± 4.7
Blood leptin (ng/ml)	30 ± 18	32 ± 12
FF leptin (ng/ml)	30 ± 22	30 ± 13
Blood AMH (ng/ml)	3.4 ± 2	1.5 ± 0.3 *
FF AMH (ng/ml)	6 ± 2.6	2.1 ± 0.4 *

\*significant at p-value ( P< 0.05)

**Comparison of blood and FF hormones level between group A women with PCOS and group A with non PCOS** According to the results shown in (table 4 ) there were no significant difference in blood and FF E2 and AMH between group A women with PCOS and group A women with non PCOS . The results show that there were significant differences between testosterone level in blood and FF between these two groups . Also, the results reveal significant differences in leptin level in blood and follicular fluid between these two groups( at level of significance P < 0.05)

Table (4) Comparison of blood and follicular fluid hormone levels between PCOS and non PCOS group A women . (Mean± Standard deviation)

Hormone level Mean± Standard deviation	group A women with PCOS (N=35)	group A women with non PCOS(N=27)
Blood estradiol (pg/ml)	403 ± 251	404 ± 244
FF Estradiol (pg/ml)	2719 ± 259	2858 ± 230
Blood testosterone (ng/ml)	6.6 ± 1.3	2.7 ± 0.9 *
FF testosterone (ng/ml)	11 ± 5.4	7 ± 3.6 *
Blood leptin (ng/ml)	34 ± 14	22 ± 10 *
FF leptin (ng/ml)	35 ± 15	25 ± 12 *
Blood AMH(ng/ml)	2.4 ± 0.7	2.5 ± 1.1
FF AMH (ng/ml)	5.9 ± 1.6	6 ± 2.2

\*significant at p-value ( P< 0.05)

**Correlation between hormone level in blood and FF in group A women** According to the results shown in table 5 , there were no significant correlation between blood and FF testosterone in group A women. The results show that there were significant correlations between E2 between blood and FF of group A women . Also ,the results reveal significant correlation in leptin and AMH between blood and FF of group A women.

Table 5. Correlation between hormone level in blood and FF in group A women (N=63)

Hormone	FF Testosterone	FF Estradiol	FF Leptin	FF AMH
Blood Testosterone	r =0.119 P =0.357			
Blood Estradiol		r =0.319 * P=0.012		
Blood Leptin			r =0.872 * P=0.001	
Blood AMH				r =0.932 * P=0.001

**\*significant at p-value <0.05**

**Correlations between different hormones in the blood of group A women** According to the results shown in (table 6 ) ,there were no significant correlation between blood estradiol and testosterone or between testosterone and leptin or between estradiol and leptin or between leptin and AMH in group A women. The results show that there were significant correlation between testosterone and AMH in blood of group A women . Also ,the results reveal significant correlations between estradiol and AMH in blood of group A women .

Table (6 )Correlations between different hormones in the blood of group A women. Number=63.

Hormone	Estradiol	Leptin	AMH
Testosterone	r=0.016 P=0.904	r=0.151 P=0.240	r=0.331 P=0.009 *
Estradiol		r=0.004 P=0.976	r=0.282 P=0.026 *
Leptin			r=0.096 P=0.460

**\*significant at p-value ( P< 0.05)**

**Correlation between different hormones in the FF of group A women** According to the results shown in (table 7 ) ,there were a significant correlation between FF E2and testosterone and between E2 and AMH and between testosterone and AMH of group A women. The results also show that there were significant correlations between testosterone and leptin hormone but show no significant correlations between leptin and AMH or between E2 and leptin in FF of group A women.

(Table (7) Correlations between different hormones in FF of group A women number =63).

Hormone	Estradiol	Leptin	AMH
Testosterone	r=0.219 P=0.027 *	r=0.253 P=0.048 *	r=0.230 P=0.035 *
Estradiol		r=0.46 P=0.99	r=0.22 P=0.041 *
Leptin			r=0.29 P=0.091

\*significant at p-value ( P< 0.05)

## Discussion

**Blood and follicular fluid hormones in group A and B women** As shown by the results expressed by table (1) , there were no significant differences in blood or follicular fluid estradiol levels between group A and group B women . This could be due to the fact that both groups of women were subjected to controlled ovarian hyperstimulation which causes growth of high number of follicles and consequently increment in estradiol level in both groups. Also Chian *et al.* (2001) reported high levels of E2 in blood and FF in infertile women who undergo ICSI and subjected to controlled ovarian hyperstimulation . The results reveal that there were significant differences in blood and follicular fluid testosterone between group A and group B women . Several investigators have demonstrated that mature oocytes with a higher potential for fertilization are associated with lower testosterone levels in FF at the time of follicular rupture .Pregnancy was associated with follicles showing a significantly higher estradiol to testosterone ratio than follicles in which the oocyte failed to implant or did not cleave in vitro. The results of the study show that there were a significantly higher leptin levels in blood and follicular fluid in group A women (which includes large number of infertile women with PCOS ) . Higher level of leptin is usually associated with polycystic ovarian syndrome (Gurbuz *et al.*,2005 and Hill *et al.*,2007). Elevated leptin concentrations were associated with reduced ovarian response to stimulation, reduce follicular maturation and reduce pregnancy success (Anifandis *et al.* ,2005) .Some reports indicated that elevated serum and follicular fluid leptin levels may be used as predictive markers of assisted reproductive technology failure (Mantzoros *et al.*,2000).Although similar results recorded by Welt *et al.*, (2003) ; however they did not consider blood and FF leptin suitable markers of oocyte maturation or embryo quality . Unkila *et al.* (2001) show the presence of higher serum leptin concentrations 12 days after embryo transfer is one cause of pregnancy success.. The interactions of FF leptin with estradiol and growth hormone , as well as the direct inhibitory action of high leptin levels on the ovaries that lead to ineffective follicular maturation possibly the mechanisms by which leptin affects fertility . (Antczak and Van Blerkom ,2000 and Agarwal *et al.*, 2000). The results revealed significantly lower AMH in blood and follicular fluid of group A women.AMH reflects ovarian reserve , so that higher level of AMH is associated with good ovarian reserve and better chance of fertility (De Vet *et al.*,2002 and La Marca *et al.*,2010) and lower AMH in group A may be one cause of lower fertility.

**Blood and follicular fluid hormones level and body weight of group A women** The results of this study (table 2) reveal that there were significantly higher testosterone level( both in blood and FF )in group A women with higher BMI.

Kezele and Skinner ( 2003) revealed similar changes , because adipose tissue possesses aromatase, an enzyme that converts androstenedione to estrone and testosterone to estradiol and the excess of adipose tissue in obese patients create excess androgens and estrogens . Also PCOS is associated

with obesity and hyperandrogen state (Puurunen *et al.* ,2009) .The results show that there were significantly higher leptin in blood and FF in women with higher BMI , such observation appear to be logical because leptin hormone is product of adipose tissue . Messinis *et al.*, (2000) show that leptin level significantly related to BMI. The findings of this study are in agreement with the results reported by Brannian *et al.*, (2001) and Anifandis *et al.*, (2005) , who reported a significantly higher level of leptin in blood and FF of infertile obese women, and significantly lower level of leptin in blood and FF of infertile women with low BMI . Furthermore, obese women with the polycystic ovarian syndrome tend to have higher leptin concentrations than women with the polycystic ovarian syndrome who had normal BMI. Thus they tend to account the higher leptin concentrations found in these infertile women with the polycystic ovarian syndrome at least in part to be related to high body content of adipose tissues (Christos *et al.*,2000 and Unkila *et al.*,2001).

**Blood and follicular fluid hormones level and age of group A women** The results shown in table (3 )revealed that E2 in blood and FF was significantly higher in younger women of group A. This could be due to more follicular activity in younger women. The estrogens synthesized by follicular cells during the process of follicle maturation accumulate in follicular fluid , so that an association is believed to exist between the hormonal content of FF and the quality and degree of oocyte maturity. Elevated E2 in FF indicate a more advanced stage of oocyte maturation and have been repeatedly found to be associated with a higher chance of achieving pregnancy. The results of the study (table (4.3 ))show no significant change in testosterone level in blood or follicular fluid of women of different age groups . Other researchers show that blood levels of ovarian androgens decrease only slightly and remain relatively stable until menopause, while the decrease in adrenal androgens can already be observed after the age of 30 years (Piltonen *et al.*, 2004 and Puurunen *et al.* ,2009).The results of table (4.3) show significantly higher levels of AMH in younger women of group A, as AMH is age related and is higher with better ovarian reserve in women of younger age . The result of this study indicates a reduction in AMH level with increasing age. The reason of this phenomenon is the decrease in the number of follicles as women aging . The result of this study is in agreement with the result of La Marca *et al.* (2010) who reported that circulating levels of AMH decline with age which may reflect the age-associated depletion of ovarian follicles. The age-related decrease in AMH levels was also supported by the results of previous studies in which a negative correlation between age and serum AMH levels has been reported (De Vet *et al.*, 2002 ; Fanchin *et al.* ,2005) .

**Blood and FF hormones level and polycystic ovarian syndrome in group A women** The results of the study (table 4)show a significantly higher blood and FF testosterone levels in infertile women with PCOS . Polycystic ovarian syndrome causes state of hyperandrogenism so that elevation of testosterone hormone (Brannian *et al.*, 2001; Silfen *et al.* ,2003 and Puurunen *et al.* ,2009) . Other studies show a significant increase in the serum and FF testosterone level in women with PCOS (Alvarez *et al.* 2006 and Abbott *et al.* 2009) .The association between oocyte maturity and low testosterone concentration in FF seems to be expected, since atresia of the follicles and of their oocytes is usually associated with granulosa cell degeneration and with an increased testosterone/estradiol ratio .There was no significant change in blood or follicular fluid estradiol in women with PCOS , while other researches show that an increase in estradiol in women with polycystic ovarian syndrome due to enhanced peripheral aromatization of androgens to estrogens (mainly testosterone to E<sub>2</sub>) in extraglandular tissues in the presence of androgen excess (Carmina *et al.* ,2003 and Kezele *et al.* ,2003) .Also leptin hormone is significantly higher in group A women with polycystic ovaries . This is probably due to the fact that most of these women have higher BMI . Cook *et al.* (2002) show that women with polycystic ovaries have higher leptin concentrations than women without such a diagnosis .This association appears to be due to the positive correlation between leptin and BMI. It was found that women with polycystic ovaries who



became pregnant had lower FF leptin concentrations than those with polycystic ovaries who did not succeed to become pregnant. ( Mantzoros *et al.*, 2000).

There are no significant differences in blood or FF AMH were found between group A women with PCOS and women without such diagnosis . This could be the effect of ovarian hyperstimulation , as AMH decreases when follicular maturation progress ; and as sample collection was done when most follicles are mature so that AMH decreased but not significantly differ . Other studies show that serum AMH levels increased by 2 to 4 folds in women with polycystic ovaries. High levels of AMH were reported also in the FF of women with polycystic ovaries in addition to the high serum level (McGee and Husch ,2000 and Pigny *et al.* 2006 ) . While, Eldar-Geva *et al.* (2005) reported that women with polycystic ovaries have higher serum AMH levels during ovarian hyperstimulation . However, recent data indicated that women in whom AMH levels fall after ovarian stimulation have the best response to induction. (Silberstein *et al.* , 2006).

**Correlation between blood and FF hormones of group A women** As shown in table (5), there was no significant correlation between blood and FF testosterone in group A women . Testosterone secretion in female is mainly occurs by adrenal cortex and to lower extent by the ovaries so that no correlation can be exist between blood and FF testosterone levels (Lasley *et al.*, 2002 ; Li and Lin ,2005) Blood and FF estradiol levels was significantly correlated as E2 secretion is greatly dependant on follicular secretion especially in case of ovarian hyperstimulation . It is suggested that in human ovaries up to 90% of the estradiol in FF may originate from the granulosa cells (Bili *et al.*,2001 and Abbott *et al.* ,2009). While Micah *et al.* (2007) show that FF estradiol correlated with serum estradiol only in pregnant women and was unrelated in non-pregnant women. The results show a significant correlation in blood and FF leptin. The result of this study in agreement with the result of Bützow *et al.* (2000) who reported the similar correlation between blood and FF leptin in

infertile women who undergo intracytoplasmic sperm injection. Agarwal *et al.* (2000) in their study show that FF leptin concentrations was significantly correlated with the serum leptin concentrations and highly related to adiposity as the adipocytes have been shown to be a major source of leptin in the body, but leptin synthesis has also been demonstrated in ovarian granulosa cells .Blood and FF AMH were significantly correlated . AMH is mainly secreted by ovarian follicles and accordingly the change in FF level of AMH can create a similar changes in blood level of AMH .Other researchers found the same results (Josso *et al.* 2001 and Pellat *et al.* 2007).

**Correlation between different hormones in blood and FF of group A women:** The results expressed by table (6 )and (7) reveal that there was a negative correlation between estradiol and AMH in blood and in FF. The probable explanation may be as follow : when follicular maturation progresses estradiol secretion increases while AMH decreases . Similar observations have been recorded by other researchers, (Grujters *et al.* ,2003 and Silberstein *et al.*, 2006),who found that serum and FF AMH values are also correlated significantly with serum and FF estradiol levels . The proposed functions of AMH are inhibition of the initial recruitment of primordial follicles and inhibition of aromatase activity in granulosa cells, thus reducing the production of estradiol (Pigny *et al.* 2006) . The results show a significantly negative correlation between blood and FF testosterone and AMH. The result of this study in agreement with the result of Pigny *et al.*(2006 )and La Marca *et al.* (2010) , who reported the similar correlation between blood and FF testosterone and AMH . This could be due to fact that AMH inhibit testosterone production by granulosa (Josso *et al.*, 2001).The results of the present study show that there were a significant correlation between FF testosterone and estradiol . This could be cause by the fact that androgens ( testosterone in particular) are immediate precursors of estradiol, thus, if a reduction in testosterone production by thecal cells is observed this fact definitely contributes to the reduction of estrogen synthesis observed in parallel. FF hormones reflect ovarian production not peripheral production of hormones and so these changes are more prominent in FF . Also the positive association between estradiol and testosterone in FF was shown by Dor *et al.* (2000) and Cha *et al.* (2000).

Also the results of this study reveal a correlation between FF testosterone and

leptin . This correlation could be caused by the effect of PCOS which produce excess testosterone and related to high leptin .These changes are found in FF as FF hormones reflect ovarian production not peripheral production of hormones. Other researches show the same changes (Lory *et al.* 2004 and McCartney *et al.*2007). The results show no significant correlations between estradiol and leptin in blood or FF . Also Agarwal *et al.* (2000) and Anifandis *et al.* (2005) found no correlation between serum leptin and estradiol. The lack of correlation suggested that estradiol and leptin might have opposing actions during ovarian stimulation. (Bützow *et al.* , 2000) . Messinis *et al.*(2000) suggested that high intra-ovarian leptin levels suppress the ovarian response to gonadotropins, ovarian steroidogenesis , follicle maturation so that reducing the overall pregnancy rate due to the fact that leptin interferes primarily with aromatase expression or activity . The results of some studies have suggested a positive relationship between estrogen and leptin Others speculate that the inhibitory action of leptin in the ovary might partially explain why obese individuals require higher doses of gonadotropin for ovarian hyperstimulation (Unkila *et al.*,2001 and Anifandis *et al.*2005) .

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