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DETERMINATION OF IL-1B AND IL-35 LEVELS IN INFERTILE WOMEN WITH UNEXPLAINED INFERTILITY AND POLYCYSTIC OVARY SYNDROME SUBJECTED TO OVULATION INDUCTION/ INTRAUTERINE INSEMINATION

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ABSTRACT
The study aim to find out the Pathogenic effects of hormonal levels and interleukin-1β (IL-1β) and interleukin-35 (IL-35) levels in infertility and their contribution in poor pregnancy outcome after ovulation induction/intrauterine insemination (OI/IUI). Twenty unexplained infertility and thirty polycystic ovary syndrome (PCOS) women subjected to OI/IUI and sixteen healthy fertile women as control group were enrolled in this study. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol 2 (E2), testosterone, and prolactin (PRL) levels for study cases and FSH and E2 levels for control group on cycle day 2 (CD2) were measured. Interleukin-1β and IL-35 levels were measured before triggering of ovulation for study cases and for control group. Two (4%) PCOS women became pregnant. On CD2, Compared to control group, unexplained infertility women showed no significant increase in FSH and significant decrease in E2 while PCOS women not became pregnant exhibited no significant decrease in FSH and significant increase in E2 levels. Women became pregnant showed comparable FSH and significant increase in E2 levels compared to control group. Unexplained infertility and PCOS women not became pregnant showed significant increase in IL-1β while IL-35 levels were comparable in unexplained infertility women and PCOS women not became pregnant exhibited significant increase levels compared to control group. Women became pregnant had significant increase in IL-1β and IL-35 levels compared to control group. Altered hormonal levels affected oocyte qualities and endometrial receptivity. Abnormal cytokine levels adversely affected oocyte qualities, endometrial receptivity, and implantation process. Administered hCG might modulate the immune response to right way in the two women became pregnant.

KEY WORDS: ovulation induction/intrauterine insemination, hormones, interleukin-1β, interleukin-35, pregnancy outcome.

INTRODUCTION

Intrauterine insemination (IUI) is the first line of assisted reproductive technology. It is a technique where the semen is processed into highly concentrated motile sperm and then inseminated into the uterus through the cervix using a fine catheter. Intrauterine insemination is widely used for the treatment of infertile patients (Azantee *et al.,* 2011). The indications for intrauterine insemination include unexplained infertility factors, low sperm quality, unilateral tubal blockage, cervical factors, ovulatory dysfunction, and immunological causes of infertility (Azantee *et al.,* 2011; Marcus, 2010). Intrauterine insemination may be performed in either a natural or stimulated cycles (Marcus, 2010). Ovulation induction with intrauterine insemination is an effective treatment for infertile patients.

Cytokines play a considerable role in reproductive system. They play crucial roles in follicular growth, ovulatory process, development of endometrial receptivity, and mediate embryo implantation processes (Van Mourik *et al.,* 2009).

Aims of Study

1- Measure the levels of the hormones follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol 2(E2), prolactin (PRL), and testosterone on menstrual cycle day 2 (CD2) and their contribution in infertility in infertile women with unexplained infertility or polycystic ovary syndrome subjected to ovulation induction/ intrauterine insemination program.
2- Study the role of the cytokines IL-1β and IL-35 in follicular growth, induction of ovulation, ovulatory process, endometrial receptivity, and implantation processes.
3- Study the pathogenic contribution of these cytokines in improper preovulatory oocyte qualities, improper endometrial receptivity, and implantation failure for infertile women with unexplained infertility or polycystic ovary syndrome subjected to ovulation induction/intrauterine insemination program.

MATERIALS & METHODS

Study Subjects

This study was conducted with study subjects and controls at the consultant clinic of Higher Institute for Infertility Diagnosis and Assisted Reproductive Technologies at AL-Nahrain University in Baghdad/ Iraq during April 2015 to February 2016. The study cases involved 20 infertile women with unexplained infertility and 30 infertile women with polycystic ovary syndrome (PCOS) subjected to ovulation induction (OI) and intrauterine insemination (IUI). The control group comprised 16 fertile women. All study and control cases were chosen randomly. The diagnosis of unexplained infertility and polycystic ovary syndrome (PCOS) were done in the consultant clinic by specialist physician.

Inclusion Criteria

The diagnosis of unexplained infertility was done by specialist physician. Inclusion criteria for the study cases with unexplained infertility were as follows: 21-35 years old body mass index (BMI) <30 Kg/m², FSH<10mIU/ml, and E2<50pg/ml on day 2 of the menstrual cycle (CD2).

The diagnostic criteria for polycystic ovary syndrome were done according to the basis of the Rotterdam criteria (2003 ESHRE/ASRM consensus) (Lujan *et al.,* 2008). Polycystic ovary syndrome diagnosis was done by the specialist physician. Exclusion of other etiologies of androgen excess and anovulatory infertility was necessary.

Inclusion criteria for the control cases were as follows: 21-35 years old, one live birth less than 2 years before, regular menstrual cycle (24-35 days), body mass index (BMI)<30Kg/m², FSH<10mIU/ml, and E2<50pg/ml on day 2 of the menstrual cycle (CD2).The chosen control fertile group was done under supervision of the specialist physician.

Women with endometriosis, tubal factor infertility, anatomical uterine pathological conditions, male factor infertility, and women with previous implantation failure or recurrent spontaneous abortion history were excluded.

Information involved ages was obtained from the files of infertile women included in the study.

All husbands were with adequate seminal fluid analysis parameters according to the reference values published by the World Health Organization in 2010 (Stahl *et al.,* 2011). Seminal fluid analysis was done by biologist in the seminal fluid analysis room at the consultant clinic.

Body Mass Index (BMI)

Body mass index (BMI) was calculated by the weight in kilograms divided by the height in meters squared (Kg/m²). Body mass index was classified as normal weight (18.5-24.9), excessive weight (25.0-29.9) and obesity as having a body mass index equal or greater than 30.0 Kg/m² (Nassaji *et al.,* 2015).

Blood Sampling

Informed and signed consent was obtained at the time of blood sampling from all cases involved in the study. Peripheral venous blood samples were drawn on day 2 of the menstrual cycle (CD2). The samples were centrifuged at 2500rpm for 15 minutes. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol 2 (E2), testosterone, and prolactin (PRL) were measured for all study cases on cycle day 2 (CD2). The hormones were measured by using commercially available mini-VIDAS kits (BIOMERIEUX/France). For the control group the obtained sera on cycle day2 were used for measuring FSH and E2 using commercially available mini-VIDAS kits (BIOMERIEUX/France).

On the day of triggering of ovulation immediately before administration of hCG injection (OVITRELLE), peripheral venous blood samples were obtained from all study cases and centrifuged at 2500rpm for 15 minutes. The obtained sera were stored at -20°C until the time of measuring IL-1β and IL-35 levels using ELISA kits (CUSABIO/China).

Sera obtained from the control group were stored at -20°C until the time of measuring IL-1β and IL-35 levels using ELISA kits (CUSABIO/China). Fourteen days following intrauterine insemination (IUI), peripheral venous blood was obtained from all study cases included in the study and was centrifuged at 2500rpm for 15 minutes for performing pregnancy test in serum using mini-VIDAS HCG kit (BIOMERIEUX/France).

TABLE 1: showed normal levels for the hormones included in this study according to the leaflets found in the hormone kits

Normal levels for the hormones involved in the study.

Hormones	Women Cycle		
	Follicular Phase	Ovulatory Phase	Luteal Phase
FSH (miu/ml)	2.9-12.0	6.3-24.0	1.5-7.0
LH (miu/ml)	2.0-8.0	9.6-80.0	0.2-6.5
PRL (ng/ml)	5.0-35.0		
E2 (pg/ml)	18.0-147.0	93.0-575.0	43.0-214.0
Testosterone (ng/ml)	Age>19-50 years: 0.23-0.73		
β-hCG (miu/ml)	Pregnant>10.0		

Ovulation Induction

Thirty infertile women with polycystic ovary syndrome and twenty infertile women with unexplained infertility were subjected to one of the following three ovulation induction protocols. Ovulation induction protocols were prescribed by the specialist physician. First ovulation induction protocol involved the administration of clomiphene citrate (clomid) (Patheon France S.A./France) only, second involved injectable FSH (Gonal-f) (Merck Serono S.A. / Schweiz), and third involved clomid and injectable FSH product (Gonal-f).

Any of the stimulation protocols was cancelled when more than three follicles larger than 12 mm in diameter were present.

Ultrasound Examination

Transvaginal ultrasound scan was performed to measure endometrial thickness and follicular parameters. Transvaginal ultrasound examination was initiated on day

10-12 of the menstrual cycle and then repeated every 1-2 days until one to two or three follicles were with a diameter of 16 to 18 millimeters before hCG administration (OVITRELLE).

Triggering of Ovulation

Trigger of ovulation was done with 10000 units of hCG (OVITRELLE) (Merck Serono S.P.A./Italy) when one to two or three follicles with a diameter of 16 to 18 millimeters were present.

Male Partner Preparation

On day of IUI the semen samples were collected after three days of abstinence in a wide mouth polypropylene container, the method of collection was done by masturbation. After semen liquification by incubation in the incubator, seminal fluid analysis was done and semen parameters were measured according to 2010WHO reference values (Stahl *et al.,* 2011). Then either direct swim-up or simple wash sperm preparation techniques were performed for semen samples using culture medium (FertiCult ^{TM} Flushing Medium) (FertiPro/Belgium).

Intra-uterine Insemination (IUI) Procedure

Intrauterine insemination was carried out 36-40 hours post hCG administration. Intrauterine insemination was carried out by specialist physician using intrauterine catheter (Gynetics/Belgium) with one milliliter syringe. A two weeks course of daily treatment with progesterone vaginal gel was prescribed for luteal support after intrauterine insemination.

Pregnancy Test

To confirm pregnancy, after 14 days of intrauterine insemination, serum hCG levels were measured by using mini-VIDAS HCG kit (BIOMERIEUX/France).

Statistical Analysis

Statistical analysis was performed using SAS (Statistical Analysis System-version 9.0). Unpaired t-test was used to compare difference between means. $P<0.05$ was considered statistically significant (SAS, 2010).

RESULTS

Twenty women with unexplained infertility and thirty women with polycystic ovarian syndrome (PCOS) subjected to ovulation induction/intrauterine insemination (OI/IUI) protocol was included in this study. Our study demonstrated only two out of fifty women (4%) subjected to ovulation induction/intrauterine insemination protocol became pregnant. None of women with unexplained infertility subjected to OI/IUI became pregnant and only two women with PCOS subjected to OI/IUI became pregnant. All male partners enrolled in our study showed adequate sperm parameters considered fit for insemination. Ages of unexplained infertility women, polycystic ovarian syndrome women, and healthy fertile control group women ranged from twenty one to thirty five years old.

Of twenty women with unexplained infertility involved in this study, seven (35%) were with normal weight and thirteen (65%) were excessive weight women. Of thirty women with polycystic ovary syndrome involved in this study, two (6.67%) were normal weight, ten (33.33%) were excessive weight and eighteen (60%) were obese.

Table (2) showed all twenty women with unexplained infertility subjected to OI/IUI program had normal mean levels of FSH, LH, PRL, E2, and testosterone on cycle day 2.

TABLE 2: Hormonal Profile on Cycle Day 2 for Twenty Females with Unexplained Infertility Subjected to OI/IUI Program Included in This Study

Hormone	Hormone Level (Mean ±SE)
FSH (mIU/ml)	6.38±0.46
LH (mIU/ml)	4.02±0.39
PRL (ng/ml)	17.03±1.8
Testosterone (ng/ml)	0.3±0.02
E2 (pg/ml)	29.62±1.17

Value: Mean ±Standard Error.

TABLE 3: showed none significant increase in FSH levels on cycle day 2 for the twenty women with unexplained infertility to be subjected to OI/IUI when compared with FSH levels on cycle day 2 for sixteen females as control group.

TABLE 3: FSH Levels on Cycle Day 2 for Twenty Females with Unexplained Infertility Subjected to OI/IUI compared to FSH Levels on Cycle Day 2 for Sixteen Females as Control Group

FSH Levels	FSH Levels (mIU/ml) Mean ±SE	Number of Females
Unexplained Infertility	6.38 ±0.46	20
Control	5.5 ±0.24	16
Unpaired t-test	NS	
P-value	0.1	

P=probability, ($p<0.05$) was designated as significant. NS: non- significant. Value: Mean ±Standard Error.

Table (4) demonstrated significant decrease in E2 concentrations for women with unexplained infertility subjected to OI/IUI program on cycle day 2 when compared with the E2 concentrations for the control group on cycle day 2.

TABLE 4: E2 Levels on Cycle Day 2 for Twenty Females with Unexplained Infertility Subjected to OI/IUI Program Compared to E2 Levels on Cycle Day 2 for Sixteen Females as Control Group

E2 Levels	E2 Levels Mean ±SE (pg/ml)	Number of Females
Unexplained Infertility	29.62 ±1.17	20
Control Group	33.27 ±1.35	16
Unpaired t-test	S	
P-value	0.04	

P=probability, ($p<0.05$) was designated as significant. S: Significant. Value: Mean ±Standard Error.

Table (5) showed increase in LH: FSH ratio mean levels for twenty-eight women with polycystic ovary syndrome who did not become pregnant while the two women with polycystic ovary syndrome who became pregnant sowed LH: FSH ratio mean levels within normal ranges. Out of thirty women with polycystic ovarian syndrome subjected to OI/IUI program four women (13.33%) showed considerably elevated mean LH: FSH (>2:1) ratio (2.45±0.14). All polycystic ovarian syndrome women included in our study were with normal mean levels of prolactin. There was marked increase in testosterone mean levels for polycystic ovarian syndrome women who did not become pregnant while the mean testosterone levels for the two women became pregnant were within normal levels. Of thirty females with polycystic ovary syndrome subjected to OI/IUI program fifteen females (50%) their testosterone levels exceeded 0.73ng/ml with mean levels (0.82 ±0.02)ng/ml.

TABLE 5: Hormonal Profile on Cycle Day 2 for Twenty Eight Females with Polycystic Ovarian Syndrome Subjected to OI/IUI Program and did not Become Pregnant and for Two Females Subjected to OI/IUI Program and Got Pregnancy

Hormone	Hormone Level For Females Got Pregnancy (number=2) (Mean ±SE)	Hormone Level For Females Did not Become Pregnant (number=28) (Mean ±SE)
FSH (mIU/ml)	5.81±1.39	4.85 ±0.25
LH (mIU/ml)	3.09 ±0.91	5.59 ±0.50
LH:FSH	0.6 ±0.3	1.21 ±0.12
PRL (ng/ml)	24.13 ±7.02	19.27 ±1.61
Testosterone (ng/ml)	0.48 ±0.13	0.67 ±0.04
E2 (pg/ml)	43.07 ±4.7	58.98 ±4.39

Value: Mean ±Standard Error.

Table (6) showed non-significant decrease in FSH levels on cycle day 2 for females with PCOS subjected to OI/IUI program and who did not become pregnant compared with FSH levels on cycle day 2 for sixteen healthy women chosen randomly as control group.

TABLE 6: FSH Levels on Cycle Day 2 for Twenty Eight Females with Polycystic Ovarian Syndrome Subjected to OI/IUI Program and Did not Become Pregnant Compared to Sixteen Healthy Females as Control Group

FSH Levels	FSH Level (mIU/ml) Mean ±SE	Number of Females
PCOS Females (not Pregnant)	4.85 ±0.25	28
Control Group	5.5 ±0.24	16
Unpaired t-test	NS	
P-value	0.05	

P=probability, ($p<0.05$) was designated as significant. NS : Non-significant. Value: Mean±Standard Error.

Table (7) revealed that the two cases that became pregnant had comparable FSH levels on cycle day 2 when compared to the FSH levels on cycle day 2 for the healthy control group.

TABLE 7: FSH Levels on Cycle Day 2 for Two Females with PCOS Subjected to OI/IUI and Became Pregnant Compared to Sixteen Healthy Females as Control Group

FSH Levels	FSH Level (mIU/ml) Mean ±SE	Number of Females
PCOS Females (Pregnant)	5.81±1.39	2
Control Group	5.5 ±0.24	16
Unpaired t-test	NS	
P-value	0.43	

P=probability, ($p<0.05$) was designated as significant. NS: Non-significant. Value: Mean ±Standard Error.

Tables (8) and (9) exhibited significant increase in mean serum levels of E2 on cycle day 2 for both pregnant and non-pregnant women with PCOS subjected to OI/IUI program when compared to control fertile group.

TABLE 8: E2 Levels on Cycle Day 2 for Twenty Eight Females with Polycystic Ovary Syndrome Subjected to OI/IUI and did not Become Pregnant Compared to Sixteen Healthy Females as Control Group

E2Levels	E2 Level Mean ±SE (pg/ml)	Number of Females
PCOS Females (Non-pregnant)	58.98 ±4.39	28
Control Group	33.27 ±1.35	16
Unpaired t-test	S	
P-value	0.02	

P=probability, ($p<0.05$) was designated as significant. S: Significant. Value: Mean ±Standard Error.

TABLE 9: E2 Levels on Cycle Day 2 for Two Females with Polycystic Ovary Syndrome Subjected to OI/IUI and Became Pregnant Compared to Sixteen Healthy Females as Control Group

E2 Levels	E2 Level (pg/ml) Mean ±SE	Number of Females
PCOS Females (Pregnant)	43.07±4.7	2
Control Group	33.27±1.35	16
Unpaired t-test	S	
P-value	0.03	

P=probability, ($p<0.05$) was designated as significant. S: Significant. Value: Mean ±Standard Error.

We are the first to study the effects of IL-35 for women with unexplained infertility and polycystic ovarian syndrome subjected to ovulation induction/intrauterine insemination program. Table (10) showed significant increase in the mean levels of IL-1β for women with unexplained infertility subjected to ovulation induction immediately before triggering of ovulation by administration of hCG injection in comparison with the control fertile women group. The mean levels of IL-35 were comparable for both the women patients and the control group.

TABLE 10: Mean Levels of Cytokines IL-1β and IL-35 in Serum of Twenty Infertile Females with Unexplained Infertility measured immediately before Triggering of Ovulation with hCG Injection in Comparison with Sixteen Control Fertile Females.

Interleukins	Unexplained Infertility Females Mean ±SE (pg/ml)	Control Fertile Females Group Mean ±SE (pg/ml)	P-value
IL-1β	56.88 ±6.18	36.20 ±5.14	0.018
IL-35	43.15 ±4.64	42.82 ±5.58	0.75

IL-: interleukin, P=probability, ($p<0.05$) was designated as significant. Value: Mean ±Standard

Table (11) showed no significant decrease in the mean levels of cytokine ratio IL-35/IL-1β for women with unexplained infertility subjected to ovulation induction program immediately before triggering of ovulation by administration of hCG injection compared to control fertile group.

TABLE 11: Mean Level of Cytokine Ratio IL-35/IL-1β in Serum of Twenty Infertile Females with Unexplained Infertility Measured Immediately Before Triggering of Ovulation with hCG Injection in Comparison with Sixteen Control Fertile Females.

IL- ratio	Unexplained Infertility Females Mean ±SE (pg/ml)	Control Fertile Females Group Mean ±SE (pg/ml)	P-value
IL-35/IL-1β	0.98 ±0.16	1.33 ±0.16	0.14

IL-: Interleukin. P=probability, ($p<0.05$) was designated as significant. Value: Mean±Standard

Table (12) showed significant increase in the mean levels of IL-1β and IL-35 for twenty five infertile women with polycystic ovarian syndrome did not become pregnant subjected to ovulation induction program immediately before triggering of ovulation by administration of hCG injection compared to sixteen control fertile group.

TABLE 12: Mean Levels of Cytokines IL-1β and IL-35 in Serum of Twenty-Five Infertile Females with Polycystic Ovarian Syndrome did not Become Pregnant Measured Immediately before Triggering of Ovulation with hCG Injection in Comparison with Sixteen Control Fertile Females.

Interleukins	Polycystic Ovarian Syndrome Females (not became pregnant) Mean ±SE (pg/ml)	Control Fertile Females Group Mean ±SE (pg/ml)	P-value
IL-1β	58.77 ±5.84	36.2 ±5.14	0.01
IL-35	73.32±7.9	42.82±5.58	0.0031

IL-: interleukin, P=probability, ($p<0.05$) was designated as significant. Value: Mean ±Standard

Table (13) showed non-significant increase in the mean levels of cytokine ratios IL-35/IL-1β for twenty-five women with polycystic ovarian syndrome subjected to ovulation induction program immediately before

triggering of ovulation by administration of hCG injection and who did not become pregnant after intrauterine insemination compared to control fertile group.

TABLE 13: Mean Level of Cytokine Ratio IL-35/IL-1β in Serum of Twenty-Five Infertile Females with Polycystic Ovarian Syndrome did not Become Pregnant Measured Immediately before Triggering of Ovulation with hCG Injection in Comparison with Sixteen Control Fertile Females.

IL- ratio	Polycystic ovarian syndrome Females (not became pregnant) Mean ±SE (pg/ml)	Control Fertile Females Group Mean ±SE (pg/ml)	P-value
IL-35/IL-1β	1.98 ±0.41	1.33 ±0.16	0.15

IL-: Interleukin. P=probability, ($p<0.05$) was designated as significant. Value: Mean±Standard

Table (14) showed significant increase in the mean levels of IL-1β and IL-35 for the two women with polycystic ovariay syndrome who became pregnant after intrauterine insemination measured immediately before triggering of ovulation by hCG injection in comparison with the control fertile group.

TABLE 14: Mean Levels of Cytokines IL-1β and IL-35 in Serum of Two Infertile Females with Polycystic Ovarian Syndrome Became Pregnant Measured Immediately Before Triggering of Ovulation with hCG Injection in Comparison with Sixteen Control Fertile Females.

Interleukins	Polycystic Ovarian Syndrome Females (became pregnant) Mean ±SE (pg/ml)	Control Fertile Females Group Mean ±SE (pg/ml)	P-value
IL-1β	60.38 ±8.33	36.20 ±5.14	0.004
IL-35	80.67 ±5.60	42.82 ±5.58	<0.0001

IL-: interleukin, P=probability, ($p<0.05$) was designated as significant. Value: Mean±Standard

Table (15) revealed no significant increase in the mean level of cytokine ratio IL-35/IL-1β for the two polycystic ovarian syndrome women became pregnant after intrauterine insemination measured immediately before triggering of ovulation by hCG injection compared to the control fertile group.

TABLE 15: Mean Level of Cytokine Ratio IL-35/IL-1β in Serum of Two Infertile Females with Polycystic Ovarian Syndrome Became Pregnant Measured Immediately Before Triggering of Ovulation with hCG Injection in Comparison with Sixteen Control Fertile Females.

IL- ratio	Polycystic ovarian syndrome Females (became pregnant) Mean ±SE (pg/ml)	Control Fertile Females Group Mean±SE (pg/ml)	P-value
IL-35/IL-1β	1.34 ±0.09	1.33 ±0.16	>0.05

IL-: Interleukin. P=probability, ($p<0.05$) was designated as significant. Value: Mean±Standard

DISCUSSION

A study demonstrated that the overall pregnancy rate following ovulation induction/intrauterine insemination (OI/IUI) was 4.7 % (Yavuz *et al.,* 2013). This result was comparable with ours. In our study, none of women with unexplained infertility subjected to OI/IUI became pregnant and only two women with PCOS subjected to OI/IUI became pregnant. It was recognized better pregnancy rates following OI/IUI were among PCOS patients (Soria *et al.,* 2012). This agreed with our results since the women became pregnant were polycystic ovarian syndrome women. In our study, the two cases became pregnant were subjected to combination of clomiphene citrate and Gonal-f as ovulation induction program before intrauterine insemination. It was found that fertility was improved in intrauterine insemination cycles when clomiphene citrate was combined with gonadotropins as ovulation induction program (Arcaini *et al.,* 1996). This was the same as our results since the two women became pregnant were subjected to combination of clomiphene citrate and Gonal-f as ovulation induction program. The FSH change depends on the quality of the antral follicles, which depends on ovarian health over the previous months as well as ovarian reserve (McCulloch, 2014). Higher FSH concentrations affect oocyte quality and indicate a less favorable prognosis for treatment even though the cycle may appear to be regular (Soria *et al.,* 2012). The higher FSH concentrations seen in the unexplained infertility patients in our study hypothesized a decrease in ovarian reserve consistent with a decline in fertility. The significant decrease in E2 concentrations for women with unexplained infertility subjected to OI/IUI program on cycle day 2 when compared with the E2 concentrations for the control group on cycle day 2 in our study indicated they had abnormal endometrial receptivity which might be due to decreased levels of progesterone receptors promoted by the low levels of E2 (Elnashar and Aboul-Enein, 2004). So these women with unexplained infertility included in our study might suffer from decreased endometrial receptivity and this could explain why none of these women became pregnant after OI/IUI program.

Decreased levels of FSH in PCOS women indicated persistence of the negative feedback to such extent that not a single follicle was permitted to mature enough for ovulation to occur. The fact the cycles in PCOS women are anovulatory and irregular. Elevated serum LH suggested the presence of polycystic ovarian disease. Having a high LH level would lead to increased ovarian testosterone production, altered estrogen production, and abnormalities with ovulation in polycystic ovarian syndrome women (McCulloch, 2014). In our study, out of thirty women with polycystic ovarian syndrome subjected to OI/IUI program four women (13.33%) showed elevated mean LH: FSH (>2:1 ratio) (2.45 ±0.14). A study showed polycystic ovarian syndrome patients were with mean PRL levels within normal range (Ramanand *et al.,* 2012). This agreed with our findings. Both obese and non-obese polycystic ovarian syndrome women are insulin resistant and hyperinsulinemic with positive correlation between degree of hyperandrogenism and that of hyperinsulinism. Hyperinsulinemia causes hyperandrogenism by increasing ovarian androgen production and decreasing sex steroid-binding globulin serum concentrations, resulting in an increased bioavailability of androgens (Cho and Atkin, 2008). Higher follicular fluid androgen levels (testosterone) were associated with lower quality oocytes and in particular with oocytes showing a trend toward lower cleavage rates after fertilization. It was documented that predominantly androgenic intrafollicular environment might lead to follicular atresia but a certain amount of intrafollicular androgen was needed to obtain optimal follicular growth (Revelli *et al.,* 2009). Thus, lower quality oocytes contributed to high testosterone levels in polycystic ovarian syndrome women enrolled in our study might explain the poor pregnancy outcome following IUI.

The high levels of E2 seen for polycystic ovarian syndrome women in our study compared to healthy fertile control group could be explained by increased levels of testosterone and their raised conversion rate to E2. Granulosa cells derived from follicles of women with anovulatory polycystic ovary syndrome produce more estradiol in response to FSH than normal granulose cells and respond prematurely to LH. Coupled with the higher E2 levels, this can cause follicle growth arrest (Wu *et al.,* 2007). It was mentioned that measurement of cytokine profiles were influenced by exposure to r-FSH therapy (Wu *et al.,* 2007). This indicated that ovarian stimulation for intrauterine insemination affected circulating cytokine levels. It was dedicated that immunological alterations were involved in the etiopathogenesis of women with unexplained infertility (Putowski *et al.,* 2004). It was demonstrated that alterations in inflammatory markers were a feature of polycystic ovarian syndrome women (Knebel *et al.,* 2008). A study revealed that inflammatory mediators were significantly overproduced in polycystic ovarian syndrome women (ElMekkawi *et al.,* 2010). We demonstrated that ovulation induction might affect levels of circulating cytokines and those immunological aberrations in infertile women with unexplained infertility and polycystic ovarian syndrome women enrolled in our study should be considered considerably in the interpretation for these significant differences found in our study. In addition, Sixty percent of polycystic ovarian syndrome women enrolled in our study were obese. It was found that IL-1β was elevated in obese polycystic ovarian syndrome women compared to obese control group (Knebel *et al.,* 2008). This was consistent with our finding. Cytokines as the modulators of the immune system, participate in the regulation of the ovarian cycle by supporting follicular growth as well as guiding the infiltration and activation of leukocytes necessary for ovulation and tissue remodeling during follicular rupture, luteinization, and luteolysis (Revelli *et al.,* 2009). Ovulation is considered as an inflammation-like process in a sense that it involves increased vascular permeability, immune cell infiltration, expression of pro-inflammatory cytokines and swelling of the follicular tissue (Uibo *et al.,* 2013). Based on the presence of prostaglandins and leukotriens, it has been suggested that a modified inflammatory process was involved in ovulation. Nitric oxide has been proposed to induce ovulation by exerting an IL-1 induced cytotoxic effect on the granulose cell complex with succeeding hyperacmia and rupture of the follicle. There is evidence of a periovulatory surge in neutrophils in the theca of the leading follicle contributing to follicular rupture and dissociation of the ovarian wall (Buscher *et al.,* 1999). IL-1 was thought to be responsible for neutrophil chemotaxis during inflammation, as in ovulatory process, although now it appears that IL-1 stimulates macrophage release of IL-8 which may explain the ability of IL-1 to induce neutrophilic infiltration. The chemotactic activity is specific for neutrophils (Elmslie *et al.,* 1991). It was found that IL-1β could lead to cytoplasmic maturation and could drive the acute inflammatory process needed for ovulation (Revelli *et al.,* 2009).Following follicular rupture; an immunosuppressive effect has to be assumed antagonizing the deleterious effects of IL-1. The receptor antagonist suppresses the IL-1α and IL-1β mediated reactions of the immune system against ovulatory tissue damage. Thus, the ovulation appears to be a cytokine-regulated process of an inflammation followed by anti-inflammatory reaction (Buscher *et al.,* 1999). In our study, there was significant increase in the mean levels of IL-1β for both twenty women with unexplained infertility and twenty-five polycystic ovarian syndrome women who did not become pregnant subjected to ovulation induction/ intrauterine insemination treatment. Unregulated expression of pro-inflammatory cytokines by granulose cells had been detected in cases of infertility (Sarapik *et al.,* 2012). It was mentioned that changes in follicular fluid levels of main cytokines regulating folliculogenesis implied to ongoing impaired inflammatory reactions that negatively affected folliculogenesis and subsequent in vitro fertilization (IVF) treatment outcome in patients. It was reported that unexplained infertility patients had higher ovarian follicular apoptosis rate (Uibo *et al.,* 2013). Systemic inflammation is common to women with polycystic ovarian syndrome (Fauser *et al.,* 2012). According to this, a rise in the levels of pro-inflammatory mediators in follicular fluid can be expected (Uibo *et al.,* 2013). Respective imbalance could contribute to folliculogenesis defects commonly seen with polycystic ovarian syndrome women (Fauser *et al.,* 2012). So one interpretation for our poor pregnancy outcome after ovulation

induction/intrauterine insemination treatment was that although all menstrual cycles were ovulatory in the patients subjected to ovulation induction/intrauterine insemination protocol included in our study, abnormal mean pro-inflammatory cytokine levels adversely affected oocytes' qualities.

During window of implantation the endometrium is in a pro-inflammatory state (Schubert, 2013). IL-1has several functions in the window of implantation. IL-1 increases the expression of the integrin B3 subunit, an adhesion molecule that plays an important role in apposition and adhesion. IL-1 has important function in decidualization (van Mourik *et al.,* 2009). Local macrophages in the endometrial receptivity secrete specific cytokines including LIF and IL-1β to induce elevated epithelial cell expression of fucosyl transferases which in turn increase cell surface fucosylated structures that allow trophectoderm attachment (Jasper *et al.,* 2011). IL-1β is involved in the activation and migration of lymphocytes and endothelial cells (Uibo *et al.,* 2013). IL-18 which its production greatly increases during decidualization, induces the production of IL-1β to increase pro-inflammatory signaling during window of implantation. Stromal and epithelial cells produce IL-6 and production is maximal during decidualization which indicates its significant role in decidualization. IL-6 expression is under the control of several factors including IL-1 (van Mourik *et al.,* 2009). Immunologic aberrations initially present in unexplained infertility and polycystic ovarian syndrome women altered cytokine profile measurements which might affect endometrial preferable micro environment during endometrial receptivity and thereby, hostile intrauterine environment obtained and this was one of the interpretations for poor pregnancy outcome obtained in our study.

From 1980 to the 1990, maternal immune tolerance to the semi-allograft (the embryo) was interpreted by the predominance of Th2 immunity over Th1 immunity which protected the fetus from maternal immune attack. Th1 predominance was recognized in recurrent and spontaneous miscarriages and preeclampsia. Th2 predominance was also seen in cases of recurrent abortions. This revealed the Th1/Th2 classic paradigm was not enough to interpret the mechanism that would prevent fetal allograft rejection. According to these findings, authors expanded the classic paradigm by involving Th17 and T regulatory cells responses (Moreli *et al.,* 2012). On the endometrium, expression of the adhesion molecules (integrins), which are essential for embryo attachment during implantation, is controlled by IL-1 (van Mourik *et al.,* 2009). Studies revealed that matrix metalloproteinases (MMPs) and their inhibitors, as the tissue inhibitor of metalloproteinase, are essential during implantation and are regulated by several cytokines including IL-1 system which is secreted by decidual stromal cells and trophoblast cells. The mechanism for cellular invasion includes tissue remodeling of the extracellular matrix which is regulated partly by MMPs. IL-1 has indispensable role for embryo implantation at the embryo-maternal interface by regulating stromal cell expression of MMP-9 activity which is a significant enzyme involved in the degradation of the basement membrane, an indispensable mechanism required for the invasion of trophoblast cells (Huang, 2006). Two phases are required for implantation of embryo to occur: an initial inflammatory maternal immunological reaction against the allograft (the embryo), followed by development of immunological tolerance towards the allograft (Merviel *et al.,* 2009). T helper1 cell activity is crucial during early implantation period (van Mourik *et al.,* 2009). Interleukin-1has two effects on T lymphocytes, first, IL-1 stimulates synthesis and secretion of IL-2 by IL-1 induces synthesis of IL-2 receptors which in turn allow the T cell to respond to IL-2 by undergoing clonal expansion (Elmslie *et al.,* 1991). Interleukin-1 stimulates the production of leukemia inhibitory factor (LIF) which is a pro-inflammatory cytokine. Leukemia inhibitory factor is secreted in the luminal epithelium during days 18-28 of the menstrual cycle (mid-late secretory phase) and it participates in both adhesive and invasive phases of implantation due to its anchoring effects on the trophoblast. Interleukin-1β induces the production of insulin-like growth factor binding protein-1 (IGFBP-1) which is secreted by endometrial stromal cells and which plays an important role in the communication between the embryo and the endometrium. Elevated levels of IGFBP-1 cause implantation failure due to the lack of aggression, a process in which the embryo forces the endometrium to accept it (van Mourik *et al.,* 2009). Thus IL-1 is a crucial mediator for human embryo implantation. Anti-inflammatory cytokines (as IL-35) are able to inhibit the release of pro-inflammatory cytokine IL-1β by stimulating the production of IL-1 receptor antagonist (IL-1ra) (Cavaillon, 2001). Interlewukin-1β induces IL-17-producing-Th17 cell differentiation (Niedbala *et al.,* 2007). Interleukin-1affects T lymphocytes since it induces IL-2 secretion by T cells and induces the synthesis of IL-2 receptors which in turn allow the T cells to respond to IL-2 undergoing clonal expansion. This indicates that IL-1 plays an important role in IL-17-Th17 cells' expansion (Elmslie *et al.,* 1991). IL-17 stimulates the production of many cytokines such as IL-6, IL-1β, and TNF-α required for the inflammatory response crucial for initiating embryo implantation, and the production of prostaglandins necessary at the beginning of embryo implantation (Aggarwal and Gurney, 2002).

For immune homeostasis, the balance between effector and regulatory cells is necessary (Ozkan *et al.,* 2014). IL-35 is an anti-inflammatory cytokine secreted by regulatory T cells suppresses immune response through expansion of T regulatory (Tregs) cells and suppression of Th17 cell development (Niedbala *et al.,* 2007; Ozkan *et al.,* 2014). IL-35 can regulate Th1 immune response (Ozkan *et al.,* 2014). Interleukin-35 can prevent rejection of the embryo by suppressing harmful effector cells (Niedbala *et al.,* 2007). IL-35 could have three ways to mediate maternal-fetal tolerance. First, the expansion of $CD4^+$ $CD25^+$ T regulatory cells was induced or effector cells were converted into iTr (induced T regulatory)35 cells to upregulate immunosuppressive cytokine IL-10 to mediate maternal-fetal tolerance. Second, trophoblast cells and Th2 cells were induced to secrete inhibitory cytokines (IL-10 and TGF-β) to mediate maternal-fetal tolerance. Third, uterine natural killer (uNK) cell function was affected through currently ways to mediate maternal-fetal tolerance

(Jin *et al.,* 2014). Low and high concentrations are detrimental but intermediate optimal concentrations of cytokines are required for successful implantation (van Mourik *et al.,* 2009). Our study showed significant increase in the mean levels of pro-inflammatory cytokines IL-1β in women with unexplained infertility and polycystic ovary syndrome who did not become pregnant subjected to ovulation induction /intrauterine insemination program measured on the day of triggering of ovulation. The significant high mean levels of IL-1β and in the women in our study could cause excessive inflammatory process at the beginning of implantation of embryo which could lead to failure of completing the implantation process. This meant that even if the ova were fertilized the abnormal increase in the inflammatory cytokines (IL-1β) led to failure of success of implantation process. For immune homeostasis and for successful implantation of embryo to occur the balance between effectors cells and their secreted cytokines and regulatory cells and their released cytokines is necessary (Ozkan *et al.,* 2014). It was reported that imbalance in cytokine profile in infertile patients might contribute to implantation failure (Uibo *et al.,* 2013). The anti-inflammatory cytokines regulate the immune responses through controlling the pro-inflammatory cytokine immune responses. Our study revealed comparable results of mean levels of IL-35 for women with unexplained infertility when compared with the control fertile group enrolled in the study. Also, our study showed significant increase in the mean levels of IL-35 for polycystic ovarian syndrome women who did not become pregnant compared to the control fertile group included in this study. It was documented that an imbalance between anti- and pro- inflammatory mediators might lead to spontaneous abortion(Schubert, 2013). This illustrated that immune imbalance between pro- and anti-inflammatory cytokines could lead to implantation failure and this might explain the failure to be pregnant in the women did not become pregnant included in our study. Additionally, these polycystic ovarian women who did not become pregnant after ovulation induction / intrauterine insemination complained from hormonal imbalance which considerably and adversely affected intrauterine insemination results. Moreover, unexplained infertility women were with unfavorable follicle-stimulating hormone and estradiol2 levels (although were within normal ranges) which played considerable role in addition to immunologic aberration for pregnancy failure in these women.

It was found that excess of pro-or anti- inflammatory cytokines was detrimental to pregnancy outcome(van Mourik *et al.,* 2009). Our study showed the two women who became pregnant had significant increase in the mean levels of the pro-inflammatory cytokine IL-1β before triggering of ovulation with hCG injection. However, it was reported that high levels of IL-1β was detected in pregnant patients subjected to controlled ovarian stimulation/intracytoplasmic sperm injection protocol(Rehman *et al.,* 2014). Our study showed that these two women had aberration in anti-inflammatory cytokine IL-35 mean level before triggering of ovulation by administration of hCG injection. So, to explain how they got pregnancy was as the administered hCG had immunoregulatory properties it supported implantation process of the fetus in the maternal endometrium. A study conducted by Koldehoff *et al.* (2011) found an increased recruitment of $CD3^{+}/CD4^{+}/IL\text{-}4^{+}$Th2 cells after hCG application and IFN-ɣ secreting Th1 cells were reduced. Also this study documented increase of anti-inflammatory cytokine IL-10 serum levels in women after hCG application, which directly inhibited the differentiation of Th cells and maintained the suppressive activity of T regulatory cells and at the same time there was no significant changes in pro-inflammatory cytokine serum levels of IL-1β, IL-2, and TNF-α which were crucial for the initiation of inflammatory response required at the beginning of implantation process. Further, this study recorded an increase in IL-27 mRNA expression and decrease in IL-17 mRNA expression in mononuclear cells of women received hCG prior to scheduled in vitro fertilization (Koldehof *et al.,* 2011). It was found that through hCG, the blastocyst might increase inflammation by LIF and at the same time could keep inflammation in check by inhibiting pro-inflammatory cytokine IL-6 (Wilczynski, 2005). All these findings showed immunoregulatory functions of hCG and that it might modulated the immune response and let the immune responses in the right way in these two women who became pregnant. Besides that, their hormonal profile was within normal ranges which played a significant role in getting pregnancy.

CONCLUSION

Although estradiol 2 mean levels on cycle day 2 for unexplained infertility women were within normal ranges, they exerted significant decrease in estradiol2 mean levels in comparison with healthy fertile control group which indicated they suffered from abnormal endometrial receptivity and consequently adversely affected OI/IUI results. Hormonal imbalance in PCOS women negatively affected OI/IUI results. Following OI/IUI treatment, none of females with unexplained infertility became pregnant and the two females became pregnant were with PCOS which indicated that etiology affected IUI results and women with anovulatory cycles were more responsive to treatment. Combination of clomiphene citrate and Gonal-f as ovulation induction program yielded better results. Unexplained infertility women exhibited significant increase in the mean levels of IL-1β and showed comparable mean levels of IL-35 measured immediately before triggering of ovulation by hCG administration compared to healthy fertile control group. Polycystic ovarian syndrome women who did not become pregnant after IUI revealed significant increase in the mean levels of IL-1β and IL-35 measured immediately before triggering of ovulation by hCG administration compared to healthy fertile control group. Ovulation stimulation affected cytokines mean levels. Immunologic aberrations detected in these cases had considerable negative effects on OI/IUI results. The two cases became pregnant after IUI demonstrated significant increase in the mean levels of IL-1β and IL-35 measured immediately before triggering of ovulation by hCG administration compared to healthy fertile control group. The explanation for the two PCOS cases how became pregnant was that they were with

normal hormonal levels and although they exerted significant aberrations in the anti-inflammatory IL-35 the administered hCG as trigger for ovulation had immunoregulatory effects and immunosuppressive effects which maintained immunotolerance needed for success of pregnancy.

RECOMMENDATIONS

Study the effect of IL-1β and IL-35 on pregnancy outcome following other assisted reproduction technology (ART) treatments such as in vitro fertilization (IVF), gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT), and cryopreservation.

REFERENCES

Aggarwal, S. and Gurney, A.L. (2002) IL-17: prototype member of an emerging cytokine family. Journal of Leukocyte Biology. 71, 1-8.

Arcaini, L., Bianchi, S., Baglioni, A., Marchini, M., Tozzi, L. and Fedele, L. (1996) Superovulation and intrauterine insemination vs. superovulation alone in the treatment of unexplained infertility. A randomized study. J Reprod Med. 41, 614-618.

Azantee, Y.W., Embry, M., Murad, Z., Roszaman, R., My Hayati and Norsina, M. (2011) Associated factors affecting the successful pregnancy rate of intrauterine insemination at international Islamic university Malaysia (IIUM) fertility centre. Med J Malaysia. 66, 195-198.

Buscher, U., Chen, F., Kentenich, H. and Schmiady, H. (1999) Cytokines in the follicular fluid of stimulated and non-stimulated human ovaries; is ovulation a suppressed inflammatory reaction. Human Reproduction. 14, 162-166.

Cavaillon, J.M. (2001) Pro-versus anti-inflammatory cytokines: myth or reality. Cell Mol. Biol. 47.

Cho, L. and Atkin, S. (2008) Management of polycystic ovarian syndrome. Trends in and Sexual Health. 13.

ELMekkawi, S., ELHosseiny, A., Mansour, G., Abbas, A., Asaad, A. and Ali, K. (2010) Effect of metformin therapy on serum interleukin-6 and interleukin-18 levels in patients with polycystic ovary syndrome. Nature and Science. 8, 23-26.

Elmslie, R., Dow, S. and Ogilvie G. (1991) Interleukins: biological properties and therapeutic potential. Journal of Veterinary Internal Medicine. , 5283-5293.

Elnashar, A. and Aboul-Enein, G. (2004) Endometrial receptivity. Middle East Fertility Society Journal. 9, 10-24.

Fauser, B.C., Tarlatzis, B.C., Rebar, R.W., Legro, R.S., Balen, A.H., Lobo, R., Carmina, E., Chang, J., Yildiz, B.O., Laven, J.S., Boivin, J., Petraglia, F., Wijeyeratne, C.N., Norman, R.J., Dunaif, A., Franks, S., Wild, R.A. , Dumesic, D. and Barnhart, K. (2012) Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-sponsored 3rd PCOS Consensus Workshop Group. Fertil Steril. 97,28-38.

Huang H. (2006) The cytokine network during embryo implantation. Med J. 29, 25-36.

Jasper, M.J., Care, A.S., Sullivan, B., Ingman, W.V., Aplin, J.D and Robertson, S.A. (2011) Macrophage-derived LIF and IL-1β regulate alpha (1,2) fucosyltransferase2 (fut2) expression in mouse uterine epithelial cells during early pregnancy. Biol Reprod. 84, 179-188.

Jin, E., Wang, C., Hu, Q., Jin, G. and Li, S. (2014) The regular distribution and expression pattern of immunosuppressive cytokine IL-35 in mouse uterus during early pregnancy. Rom J Morphol Embryol. 55, 1353-1361.

Knebel, B., Janssen, O. E., Hahn, S., Jacob, S., Gleich, J., Kotzka, J. and Muller-Wield, D. (2008) Increased low grade inflammatory serum markers in patients with polycystic ovary syndrome (PCOS) and their relationship to PPAR gamma gene variants. Exp Clin Endocrinol Diabetes. 116, 481-486.

Koldehoff, M., Katzorke, T., Wisbrun, N., Propping, D., Wohlers, S., Bielfeld, P., Steckel, N., Beelen, D. and Elmaagacli, A. (2011) Modulating impact of human chorionic gonadotropin hormone on the maturation and function of hematopoietic cells. Journal of Leukocyte Biology. 90, 1017-1026.

Lujan, M., Chicen, D. and Pierson, R. (2008) Diagnostic criteria for polycystic ovary syndrome: pitfalls and controversies. J Obstet Gynaecol Can. 30, 671-679.

Marcus, S. (2010) Intrauterine insemination. In textbook of in vitro fertilization and assisted reproduction. The Bourn Hall guide to clinical and laboratory practice. Brinsden P. 3rd edition. Informa Healthcare. UK.

McCulloch, F. (2014) Natural treatments for autoimmune infertility concerns. NDNR Journal.

Merviel, P., Lourdel, E., Cabry, R., Boulard, V., Brzakowski, M., Demailly, P., Brasseur, F., Copin, H. and Devaux, A. (2009) Physiopathology of human embryonic implantation: clinical incidences. Folia Histochemica Et Cytobiologica. 47, 25-34.

Moreli, J., Ruocco, A., Vernini, J., Rudge, M. and Calderon, I. (2012) Interleukin 10 and tumor necrosis factor-alpha in pregnancy: aspects of interest in clinical obstetrics. Obstetrics and Gynecology. Volume 2012.

Nassaji, M., Gorbani, R., Tamadon, M. and Masomeh. (2015) Association between body mass index and urinary tract infection in adult patients. Nephrourol Mon. 7.

Niedbala, W., Wei, X., Cai, B., Hueber, A., Leung, B., Mcinnes, I. and Liew, F. (2007) IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis

through the expansion of regulatory T cells and suppression of Th17 cells. Eur. J Immunol. 37, 3021-3029.

Ozkan, Z., Deveci, D., Kumbak, B., Simek, M., Ilhan, F., Sekercioglu, S. and Sapmaz, E. (2014) What is the impact of Th1/Th2 ratio, SOCS3, IL-17, and IL-35 levels in unexplained infertility?. Journal of Reproductive Immunology. 103, 53-58.

Putowski, L., Darmochwal-Kolarz, D., Rolinski, J., Oleszczuk, J. and Jakowicki J. (2004) The (preliminary study). Eur J Obstet Gynecol Reprod Biol. 112, 192-196.

Ramanand, S., Ghongane, B., Ramanand, J., Patwardhan, M., Patwardhan, V. and Ghanghas, R, (2012) Hormonal profile of polycystic ovary syndrome (PCOS) in Indian women. RJPBCS. 3, 1159-1172.

Rehman, R., Fatima, S., Khan, T., Hussain, M. and Khan, R. (2014) Effect of endometrial thickness on pregnancy outcome after ICSI. Society for Endocrinology BES, 24.

Revelli, A., Delle Piane, L., Casano, S., Molinari, E., Massobrio, M. and Rinaudo P. (2009) Follicular fluid content and oocyte quality: from single biochemical metabolomics. Reproductive Biology and Endocrinology. Volume (7):40, DOI: 10.1186/1477-7827-7-40.

Sarapik, A., Velthut, A., Haller-Kikkatalo, K., Faure, G., Bene, M.C., Bittencourt, M., Massin, F., Uibo, R. and Salumets, A. (2012) Follicular proinflammatory cytokines and chemokines as markers of IVF success. Clinical and Developmental Immunology, Vol. (2012). 1-10.

Schubert, C. Immune imbalance leads to abortion. Biology of Reproduction. 2013. DOI:10.1095/ biolreprod. 113.10 8035.

Soria, M., Paradillo, G., Garcia, J., Roman, P., Castillo, A. and Jordana, C. (2012) Pregnancy predictors after intrauterine insemination: analysis of 3012 cycles in 1201 couples. J Reprod Infertil. 13, 158-166.

Stahl, P., Stembe,r D. and Schlegel, P. (2011) Interpretation of the semen analysis and initial male factor management. Clinical Obstetrics and Gynecology. 54, 656-665.

Uibo, R., Salumets, A., Jaakma, U., Mandar, R., Laan, M. and Hooijkaas, H. (2013) Immune activation in female infertility:significance of autoantibodies and inflammatory mediators. Dissertationes Medicinaa Universitatis Tagoma.

Van Mourik, M., Macklon, N. and Heijnen, C. (2009) Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment. Journal of Leukocyte Biology. 85, 14-19.

Wilczynski, J. (2005) Th1/Th2 cytokines balance –yin and yang of reproductive immunology. Eur J Obstet Gynecol Reprod Biol. 122, 136-143.

Wu, R., Fujii, S., Ryan, N., Van der Hoek, K., Jasper, M., Sini, I., Robertson, S., Robker, R. and Norman, R. (2007) Ovarian leukocyte distribution and cytokine/ chemokine mRNA expression in follicular fluid cells in women with polycystic ovary syndrome. Human Reproduction. 22, 527-535.

Yavuz, A., Demerci, O., Sozen, H. and Uludogan, M. (2013) Predictive factors influencing pregnancy rates after intrauterine insemination. Iran J Reprod Med. 11, 227-234.