ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 12, pp.43901-43906, December, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

DETERMINATION OF IL-17 AND IL-27 LEVELS IN SERA OF INFERTILE WOMEN WITH UNEXPLAINED INFERTILITY AND POLYCYSTIC OVARY SYNDROME SUBJECTED TO OVULATION INDUCTION/INTRAUTERINE INSEMINATION PROGRAM AND THEIR EFFECTS ON PREGNANCY OUTCOME

[1,*]Sundus Fadhil Hantoosh, [2]Dr. Muhammad-Baqir, M.R. Fakhrildin, [3]Dr. Manal Taha Meteab and [4]Dr. Rajwa Hasen Essa

[1]Training and Development Department, Research and Training Forensic DNA Centre, AL-Nahrain University
[2]Department of Medical Physiology, College of Medicine, Jabir ibn Hayyan Medical University
[3]Reproductive Physiology Department, High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, AL-Nahrain University
[4]Biology Department, College of Science, AL-Mustansiriya University

ARTICLE INFO

Article History:
Received 23rd September, 2016
Received in revised form
10th October, 2016
Accepted 15th November, 2016
Published online 30th December, 2016

Key words:

Ovulation Induction,
Intrauterine Insemination,
IL-17, IL-27,
Pregnancy Outcome.

ABSTRACT

Background: Cytokines play considerable role in reproductive system.
Objectives: Study role of interleukin-17 (IL-17) and interleukin-27 (IL-27) in ovulatory process, endometrial receptivity, and implantation processes and their pathogenic contribution to infertility and their effects on pregnancy outcomes after ovulation induction/intrauterine insemination (OI/IUI).
Materials and Methods: Twenty unexplained infertile and twenty-seven polycystic ovarian syndrome (PCOS) women subjected to (OI/IUI) and sixteen fertile women as control group were enrolled in this study. Serum IL-17 and IL-27 concentrations for study cases were measured before triggering of ovulation by human chorionic gonadotropin (hCG) administration and also were measured for control group.
Results: Only two (4.25%) PCOS women became pregnant after OI/IUI. There were significant increase in concentrations of IL-17 and IL-27 for unexplained infertility women (p=0.0004, p=0.006, respectively) and for PCOS women not became pregnant (p=0.02, 0.0041, respectively) compared to control group. The two PCOS women who became pregnant exerted no significant difference in IL-17 concentration (p=0.39) and significant increase in IL-27 concentrations (p=0.006) compared to control group.
Conclusions: Abnormal IL-17 and IL-27 concentrations adversely affected OI/IUI pregnancy outcome.

Citation: Sundus Fadhil Hantoosh, Dr. Muhammad-Baqir, M.R. Fakhrildin, Dr. Manal Taha Meteab and Dr. Rajwa Hasen Essa. 2016. "A study on environmental pollution and biodiversity in valsad and Navsari District", *International Journal of Current Research*, 8, (12), 43901-43906.

INTRODUCTION

Infertility is defined as the inability to conceive following one year of unprotected intercourse (Silverberg *et al.,* 2008). Up to 30% of couples who are unable to conceive are considered to have unexplained infertility (The Practice Committee of the American Society for Reproductive Medicine, 2006). Polycystic ovarian syndrome (PCOS) is a common endocrine disorder affecting 6-7 percent of women of childbearing ages (Cho and Atkin, 2008). Intrauterine insemination (IUI) is the first line of assisted reproductive technology for infertility treatment (Elnashar, 2004).

****Corresponding author: Sundus Fadhil Hantoosh,***
Training and Development Department, Research and Training Forensic DNA Centre, AL-Nahrain University.

Cytokines play a considerable role in reproductive system (Sarapik *et al.,* 2012). They play crucial roles in follicular growth, ovulatory process, development of endometrial receptivity, and mediate embryo implantation processes (Sarapik *et al.,* 2012; Van Mourik *et al.,* 2009).

This study aimed to

1-Study the role of the interleukin-17 (IL-17) and interleukin-27 (IL-27) in follicular growth, ovulatory process, endometrial receptivity, and implantation processes. 2-Study the pathogenic contribution of these interleukins 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 AND 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 27 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. Ages of all study and control cases ranged from 21 to 35 years old.

The diagnosis of unexplained infertility and polycystic ovary syndrome (PCOS) were done in the consultant clinic by specialist physician. The diagnostic criteria for polycystic ovary syndrome were done according to the basis of the Rotterdam criteria (2003 ESHRE/ASRM consensus) and was done by the specialist physician (Lujan *et al.,* 2008). 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 from this 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.

Blood Sampling: Informed and signed consent was obtained at the time of blood sampling from all cases involved in the study. 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^{o}C until the time of measuring IL-17and IL-27 levels using ELISA kits (CUSABIO/China). Sera obtained from the control group were stored at -20^{o}C until the time of measuring IL-17 and IL-27 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).

Ovulation Induction: Twenty infertile women with unexplained infertility and twenty-seven infertile women with polycystic ovary syndrome were subjected to one of the following three ovulation induction protocols. 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). Ovulation induction protocols were prescribed by the specialist physician. 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 human chorionic gonadortopin (hCG) injection 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.

RESULTS

Our study revealed that only two women with polycystic ovary syndrome became pregnant and none of the women with unexplained infertility became pregnant after ovulation induction/intrauterine insemination treatment. Table (1) showed significant increase in the mean levels of IL-17 and IL-27 for women with unexplained infertility subjected to ovulation induction measured immediately before triggering of ovulation by administration of hCG injection in comparison with the control fertile women group.

Table 1. Mean Levels of Cytokines IL-17and IL-27in 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 (N=20) Mean±SE (pg/ml)	Control Fertile Females Group (N=16) Mean±SE (pg/ml)	P-value
IL-17	16.02±2.36	5.36±0.56	0.0004*
IL-27	12.6±1.68	6.63±0.92	0.006*

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

Table (2) showed no significant decrease in the mean level of cytokine ratio IL-27/IL-17 for women with unexplained infertility subjected to ovulation induction programs measured immediately before administration of hCG injection compared to control fertile group.

Table 2. Mean Level of Cytokines Ratio IL-27/IL-17 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 (N=20) Mean±SE (pg/ml)	Control Fertile Females Group (N=16) Mean±SE (pg/ml)	P-value
IL-27/IL-17	1.29±0.33	1.44±0.27	0.26

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

Table (3) showed significant increase in the mean levels of IL-17and IL-27 for twenty five infertile women with polycystic ovarian syndrome did not become pregnant subjected to ovulation induction program measured immediately before triggering of ovulation by administration of hCG injection compared to sixteen control fertile group.

Table 3. Mean Levels of Cytokines IL-17and IL-27in 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) (N=25) Mean±SE (pg/ml)	Control Fertile Females Group (N=16) Mean±SE (pg/ml)	P-value
IL-17	9.47±1.63	5.36±0.56	0.02*
IL-27	13.06±1.86	6.63±0.92	0.0041*

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

Table (4) showed significant increase in the mean level of cytokine ratio IL-27/IL-17 for twenty-five women with polycystic ovarian syndrome subjected to ovulation induction program measured 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 4. Mean Level of Cytokine Ratio IL-27/IL-17in 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- ratios	Polycystic ovarian syndrome Females (not became pregnant) (N=25) Mean±SE (pg/ml)	Control Fertile Females Group (N=16) Mean±SE (pg/ml)	P-value
IL-27/IL-17	3.55±1.03	1.44±0.27	0.05*

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

Table (5) showed significant increase in the mean level of IL-27 and no significant difference in the mean levels of IL-17 for the two polycystic ovarian syndrome women who became pregnant after intrauterine insemination measured immediately before triggering of ovulation by hCG injection in comparison with the control fertile group.

Table 5. Mean Levels of Cytokines IL-17and IL-27in 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) (N=2) Mean±SE (pg/ml)	Control Fertile Females Group (N=16) Mean±SE (pg/ml)	P-value
IL-17	4.08±0.22	5.36±0.56	0.39
IL-27	18.63±4.43	6.63±0.92	0.006*

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

Table (6) revealed significant increase in the mean levels of cytokine ratio IL-27/IL-17 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 6. Mean Levels of Cytokine Ratio IL-27/IL-17 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- ratios	Polycystic ovarian syndrome Females (became pregnant) (N=2) Mean±SE (pg/ml)	Control Fertile Females Group (N=16) Mean±SE (pg/ml)	P-value
IL-27/IL-17	4.52±0.84	1.44±0.27	<0.05

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

DISCUSSION

It was dedicated that ovarian stimulation for intrauterine insemination affected circulating cytokine levels (Younis *et al.,* 2014). It was recognized that recombinant-FSH used in ovulation induction program affected cytokine release modestly (Wu *et al.,* 2007). Putowski *et al.* (2004) dedicated that immunological alterations were involved in the etiopathogenesis of women with unexplained infertility. Knebel *et al.,* (2008) demonstrated that alterations in inflammatory markers were a feature of polycystic ovarian syndrome. ELMekkawi *et al.* (2010) revealed that inflammatory mediators were significantly overproduced in polycystic ovarian syndrome women. Thus, we demonstrated that ovulation induction might affect levels of circulating cytokines and in addition immunological aberrations in the infertile women included in our study might be contributed to the interpretation of these significant cytokine levels differences seen in our study.

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). The immune system contributes to follicle maturation through the agency of T lymphocytes (Wu *et al.,* 2007). Cumulus cells express IL-17. The release and accumulation of IL-17 protein from cumulus cells and granulose cells was confirmed using Bio Plex Protein Array System (Shimada *et al.,* 2013). IL-17 induces the production of IL-1β which can lead to cytoplasmic maturation and can drive the acute inflammatory process needed for ovulation (Aggarwal *et al.,* 2002; Revelli *et al.,* 2009; Mcinnes, 2013). IL-17 mainly mediates its immune regulatory function by promoting the generation of pro-inflammatory cytokines and chemokines which leads to the attraction of neutrophils and macrophages to the inflammation site (Jin and Dong, 2013). This might well illustrate its significant role in the ovulatory process.

Upregulated 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 of these women 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 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-17 induces the production of IL-1β which increases pro-inflammatory signaling during window of implantation (Aggarwal *et al.,* 2003). IL-17 induces the production of IL-6 which its levels are the highest during the window of implantation (Aggarwal and Gurney, 2002). Stromal and epithelial cells produce IL-6 and production is maximal during decidualization which indicates its significant role in decidualization. IL-17 mainly mediates its immune regulatory function by promoting the generation of pro-inflammatory cytokines and chemokines which leads to the attraction of neutrophils and macrophages to the inflammation site (Jin and Dong, 2013). These illustrate its role in the inflammatory endometrial microenvironment during endometrial receptivity. Decidual transformation of stromal cells, which commences before implantation in women is facilitated by local dendritic cells (Plaks *et al.,* 2008). IL-27 is largely produced by antigen presenting cells such as dendritic cells and macrophages (Abdalla *et al.,* 2015). So, we showed that endometrial environment during window of implantation is inflammatory and we revealed the role of pro-inflammatory cytokines in maintaining this inflammatory environment and that they affect decidualization processes during window of implantation.

But immune regulation is crucial to maintain the required environment during window of implantation. Antiinflammatory IL-27 directly inhibits Th17 cells by inhibiting IL-6 signaling. IL-27 induce IL-10-producing type 1 regulatory T cells (Tr1) which in turn suppresses IL-17 production. The pro-inflammatory role of IL-17 is as key inflammatory mediator for Th1 differentiation and IFN-ɣ production (Abdalla *et al.,* 2015). IL-27 suppresses macrophage responses to TNF-α and IL-1β (George *et al.,* 2010). IL-27 acts directly on dendritic cells themselves. IL-27 signaling induces immunosuppressive dendritic cells to express high levels of CD39, which in turn promotes the differentiation of T regulatory cells (Wu *et al.,* 2014). All these findings showed the significant role of IL-27 in regulating the immune response during window of implantation. Altered cytokine profile measurements might affect endometrial preferable microenvironment during endometrial receptivity and thereby, hostile intrauterine environment obtained (Yavuz *et al.,* 2013). So this might be one of the interpretations for poor pregnancy outcome obtained in this study. The human embryo is a semi-allograft which means it is antigenically foreign to the mother (Wang *et al.,* 2014). 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). 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). A significant role of antigen presenting cells is in shaping the cytokine profile towards maintaining immune tolerance microenvironment at the maternal-fetal interface (Koldehoff *et al.,* 2011). IL-27 can inhibit Th17 cell development through mechanisms including the suppression of IL-6 signaling mediated IL-17 production and via induction of IL-10 production by type1 regulatory T cells (Tr1). IL-27 induces anti-inflammatory cytokine IL-35 production by T regulatory cells (Abdalla *et al.,* 2015). It was observed that IL-27 is able to induce IL-10-producing Th1 cell differentiation of naïve $CD4^+$ T cells (Niedbala *et al.,*2007). Th1 inflammatory immune response is crucial at the beginning of embryo implantation. But IL-27 can suppress Th1 immunity. IL-27 inhibits IL-2 production from T cells. As IL-2 plays a pivotal role in proliferation and survival of Th1 cells, this may explain the IL-27-mediated suppression of Th1 immunity. Suppression of Th1 cell response also can be attributed to the induction of anti-inflammatory cytokine IL-10 (Iwasaki *et al.,* 2015). IL-27 suppresses macrophages responses to the pro-inflammatory cytokines TNF-α and IL-1β (George *et al.,* 2010). These may illustrate the role of IL-27 in immune tolerance required for maintaining of pregnancy. IL-27 directly acts on dendritic cells. IL-27 signaling stimulates immunosuppressive dendritic cells to express high levels of CD39, which in turn induces the differentiation of T regulatory cells (Tregs). IL-27 signaling in dendritic cells also prevents the differentiation of Th1 and Th17 cells which indicates moving toward immune tolerance of the allograft (Wu *et al.,* 2014). Low and high cytokine concentrations are detrimental but an intermediate optimal concentrations are required for successful implantation (Van Mourik *et al.,* 2009).

The majority of pregnancy losses occurs before or during implantation (Singh *et al.,* 2011). Highly elevated IL-17 production could contribute to the initiation of maternal-fetal immunological rejection (Mcinnes, 2013). The significant high mean level of IL-17in 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 cytokine IL-17 led to failure of success of implantation process. For successful implantation of embryo to occur the balance between effector 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). It was documented that an imbalance between anti- and pro- inflammatory mediators might lead to spontaneous abortion (Schubert, 2013). A study reported that depletion of IL-27-producing-uterine dendritic cells resulted in a severe impairment of implantation and led to embryo resorption (Plaks *et al.,* 2008).

All these findings 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. It was found that excess of pro-or anti-inflammatory cytokines was detrimental to pregnancy outcome (Van Mourik *et al.,* 2009). Our study showed that the two women became pregnant their proinflammatory IL-17 mean levels showed no significant difference when compared to the control fertile group while they had aberration in anti-inflammatory IL-27mean levels. 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 found an increased recruitment of $CD3^{+}/CD4^{+}/IL\text{-}4^{+}$Th2 cells after hCG application and IFN-ɣ secreting Th1 cells were reduced (Koldehoff *et al.,* 2011). HCG is involved in T regulatory cell expansion and recruitment of T regulatory cells from peripheral blood to the deciduas (Wang *et al.,* 2014). Another 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, that 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 (Koldehoff *et al.,* 2011). All these findings dedicated 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.

Conclusion

Unexplained infertility women exhibited significant increase in the mean levels of IL-17and IL-27 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-17 and IL-27measured immediately before triggering of ovulation by hCG administration compared to healthy fertile control group. 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-27and had comparable IL-17 mean levels 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 the pro-inflammatory cytokine IL-17 mean levels were comparable to the control group and they exerted significant aberrations in the anti-inflammatory cytokine IL-27 but the administered hCG as trigger for ovulation had immunoregulatory effects and immunosuppressive effects which maintained immunotolerance needed for success of pregnancy.

REFERENCES

Abdalla A, Li Q, Xie L, Xie J. 2015. Biology of IL-27 and its role in the host immunity against *Mucobacterium tuberculosis.* Int J Biol Sci. 11(2): 168-175.

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

Cho L, and Atkin S. 2008. Management of polycystic ovarian syndrome. Trends in Urology, Gynaecology and Sexual Health. 13(6).

ELMekkawi S, ELHosseiny A, Mansour G, Abbas A, Asaad A, 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(9): 23-26.

Elnashar A. 2004. Opinion intrauterine insemination. Middle East Fertility Society Journal. 9(2): 101-106.

Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, Carmina E, Chang J, Yildiz BO, Laven JS, Boivin J, Petraglia F, Wijeyeratne CN, Norman RJ, Dunaif A, Franks S, Wild RA, Dumestic D, 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.

George K, Ivashkiv G, and lionel B. 2010. Suppression of TNF-α and IL-1 signaling identifies a mechanism of homeostatic regulation of macrophages by IL-27. Journal of Immunology. 185(11): 7047-7056.

Iwasaki Y, Fujio K, Okamura T, Yamamoto K. 2015. Interleukin-27 in T cell immunity. Int J Mol Sci. 16: 2851-2863.

Jin W, and Dong C. 2013. IL-17 cytokines in immunity and inflammation. Emerging Microbes and Infection. 2.

Knebel B, Janssen OE, Hahn S, Jacob S, Gleich J, Kotzka J, Muller-Wieland 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(8): 481-486.

Koldehoff M, Katzorke T, Wisbrun N, Propping D, Wohlers S, Bielfeld P, Steckel N, Beelen D, Elmaagacli A. 2011. Modulating impact of human chorionic gonadotropin hormone on the maturation and function of hematopoietic cells. Journal of Leukocyte Biology. 90(5): 1017-1026.

Lujan M, Chicen D, and Pierson R. 2008. Diagnostic criteria for polycystic ovary syndrome: pitfalls and controversies. J Obstet Gynaecol Can. 30(8): 671-679.

Mcinnes I. 2013. Effector mechanisms in autoimmunity and inflammation. In: Firestein G, Budd R, and Gabriel S. editors. J.KELLEY's textbook of rheumatology. 9th ed. China: ELSEVIER SAUNDERS.

Merviel P, Lourdel E, Cabry R, Boulard V, Barzakowski M, Demailly P, Brasseur F, Copin H, Devaux A. 2009. Physiopathology of human embryonic implantation: clinical incidences. FOLIA HISTOCHEMICA ET CYTOBIOLOGICA,. 47(5): 25-34.

Niedbala W, Wei X, Cai B, Hueber A, Leung B, Mcinnes I, 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 Th 17 cells. Eur J Immunol. 37: 3021-3029.

Ozkan Z, Deveci D, Kumbak B, Simek M, Sekercioglu S, 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.

Plaks V, Birnberg T, Berkutzki T. Sela S, Benyashar A, Kalchenko V, Mor G, Keshet E, Dekel N, Neeman M, Jung S. 2008. Uterine DCs are crucial for decidua formation during embryo implantation in mice. J Clin Invest. 118: 3954-3965.

Putowski L, Darmochwal-Kolarz D, Rolinski, J, Oleszczuk J, Jakowicki J. 2004. The immunological profile of infertile women after repeated IVF failure (preliminary study). Eur J Obstet Gynecol Reprod Biol. 112(2): 192-196.

Revelli A, De'lle Piane L, Casano S, Molinari E, Massobrio M, Rinaudo P. 2009. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. Reproductive Biology and Endocrinology. volume (7).

Sarapik A, Velthut A, Haller-Kikkatalo K, Faure G, Bene M, Bittencourt M, Massia F, UiboR, Salumets A. 2012. Follicular proinflammatory cytokines and chemokines as markers of IVF success. Clinical and Developmental Immunology. Volume 2012.

Schubert C. 2013. Immune imbalance leads to abortion. Biology of Reproduction.

Shimada M, Yanai Y, Okazaki T, Yamashita Y, Srirman V, Wilson M, Richards J. 2013. Synaptosomal-associated protein 25 gene expression is hormonally regulated during ovulation and is involved in cytokine/chemokine exocytosis from granulose cells. Molecular Endocrinology. 21(10).

Silverberg K, Vaughn T, and Burger N. 2008.Unexplained infertility.

Singh M, Chaudhry P, and Asselin E. 2011. Review bridging endometrial receptivity and implantation: network of hormones, cytokines, and growth factors. J Endocrinol. 210(1): 5-14.

Stahl P, Stember D, and Schlegel P. 2011. Interpretation of the semen analysis and initial male factor management. CLINICAL OBSTETRICS AND GYNECOLOGY. 54(4): 656-665.

The Practice Committee of the American Society for Reproductive Medicine. 2006. Effectiveness and treatment for unexplained infertility. Fertility and Sterility. 86(4): 111-114.

Uibo R, Salumets A, Jaakma, U, Mandar R, Laan M, Hooijkaas H. 2013. Immune activation in female infertility: significance of autoantibodies and inflammatory mediators. Dissertationes Medicinae Universitatis Tartuensis 209 Aili 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 Leukocye Biology. 85: 14-19.

Wang WJ, Liu FJ, Liu X, Hao CF, Bao HC, Qu QL, Liu M. 2014. Adoptive transfer of pregnancy-induced $CD4^{+}CD25^{+}$ regulatory T cells reverses the increase in abortion rate caused by interleukin 17 in the CBA/JX BALB/c mouse model. Hum Reprod.

Wu J, Xie A, and Chen W. 2014. Cytokine regulation of immune tolerance. Burns & Trauma. 2(1): 11-17.

Wu R, Fujii S, Ryan N, Van der Hoek K, Jasper M, Sini I, Robertson S, Robker R, Norman R. 2007. Ovarian leukocyte distribution and cytokine/ chemokine mRNA expression in follicular fluid cells in women with polycystic ovary syndrome. Human Reproduction. 22(2): 527-535.

Yavuz A, Demerci O, Sozen H, Uludogan M. 2013. Predictive factors influencing pregnancy rates after intrauterine insemination. Iran J Reprod Med. 11(3): 227-234.

Younis A, Hawkins K, Mahini H, Butler W, Garelnabi M. 2014. Serum tumor necrosis factor-α, intetrleukin-6, monocyte chemotactic protein-1 and paraoxonase-1 profile in women with endometriosis, PCOS, or unexplained infertility. J Assist Reprod Genet. 31(11): 1445-1451.

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