DOI Number: 10.37506/v14/i1/2020/ijfmt/193094

Evaluation of Humoral Immunological Profile in Infertile Women after IVF Failure in Baghdad City

Heba Mohammed Abed Al-Tamimi[1], Farhan Abood Risan[1]

[1]College of Health and Medical Technology, Middle Technical University

Abstract

Background: An (in Vitro) Fertilization (IVF), means fertilizing an ovum with a spermatozoon outside the body in a culture dish under controlled culture conditions. And It was taken a principle option to treat infertility, which is define as a disease of the reproductive system can be diagnosis by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. The cause of infertility may be immunological or genetic. A progress was made an in vitro fertilization (IVF) techniques, However the majority of transferred embryos fail to implant. Morphology embryo scoring is the standard procedure for most IVF centers for choosing the best embryo, but remains limited since even the embryos classified as (top quality) may not implant. The initial investigation on the cause recurrent (in vitro) fertilization failure ignites the attention on the reproductive immunology. The production of antibodies is an immunomodulatory factor that causes failure of embryo implantation, and the most commonly studied antibodies in women with implantation failure after IVF procedure , include antiphospholipid (aPL), antisperm antibodies (ASA), and antinuclear antibodies (ANA).

Objectives: The aim of this study is to determine the immunological aspects of patients after IVF failure, such as ; antiphospholipid antibodies (aPLs), antinuclear antibodies (ANAs), and anti-sperm antibodies (ASA).

Patients, Materials and Method: This prospective study was undertaken on ninety infertile women undergoing in vitro fertilization (IVF) programe. Their age was from 20years to 49 years. Blood specimens were collected from all women, on the day of egg pick up, and screened for all studied parameters by ELISA, and were statistically analyzed.

Results: In the present study clinical pregnancy rate was 27.8%. And the present study showed a highly significant difference ($P<0.01$) in aPL (IgM, IgG) between patients and control group.The data in the present study, demonstrated a highly significant difference ($P<0.01$) in Antinuclear antibodies (ANAs) between the studied groups. Also a highly significant difference ($P<0.01$) in Antisperm antibodies (ASAs) between the studied groups.

Conclusions: In vitro fertilization (IVF) failure, precisely failed embryo implantation, associated with produced auto-antibodies (anti-phospholipid antibodies, anti-sperm antibodies, and anti-nuclear antibodies), in the sera of the female.

Key words: *In vitro fertilization, immunological profile and infertility.*

Introduction

Infertility is usually defined as the disappointment of a couple to conceive after one year unprotected regular intercourse. It has been assessed that 10 –15% of couples look for medical help for fertility assessment, and the problem is obviously equally shared between male and female associates, most of the time, childlessness is some level of subfertility where 1 in 7 couples need specialist help to conceive [18]. The infertility is divided in to; primary infertility, when never the couple have had a live birth, and secondary infertility, which means a failure to achieve live birth after having a live birth or an abortion [1].

The etiology of infertility is believed to be multifactorial, The realized hazard components incorporate genetic abnormalities, ovulatory disorders, tubal damage, uterine or peritoneal issues, and male factors [4]. Unexplained infertility is characterized as absence of conception notwithstanding a year of unprotected intercourse, not clarified by anovulation, poor sperm quality, tubal pathology, or any known reason for infertility, The most applied treatments for unexplained infertility is (in vitro) fertilization, includes using standard protocols for controlled ovarian stimulation, oocyte retrieval with ultrasound management, insemination, embryo culture and trans-cervical replacement of embryos at cleavage or blastocyst-stage [13]. Repeated (in vitro) fertilization (IVF) failure has been characterized as the lack of implantation after transfer of an embryo, which can cause physical, emotional, and financial distress for couples looking to begin family [9]. For a significant number of 'unexplained' failures of IVF treatment, an immunologic basis has been suspected for a long time, and thinking about a few distinctive in vitro immune parameters, the abnormalities are related with reproductive failure in clinical cases [16]. One of the most unexplained infertility issue in female is recurrent implantation failure (RIF), RIF is determined when transferred embryos fail to implant following several (in vitro) fertilization (IVF) treatment cycles [19].

There are several autoimmune factors have been associated with implantation failure result, And certain investigations concentrated on relationship between the autoimmune system and the IVF/ICSI result highlighting the role of autoantibodies during treatmen [4]. The predominance of antiphospholipid antibodies (aPLs), and antinuclear antibodies (ANAs) was increased in unexplained infertility women [15] . That the aPL have been described with increased frequency in women with recurrent implantation failure after (in vitro) fertilization (IVF) [17]. If aPL contained in the serum, it will have a significant impact on ovulation, fertilization and/or early embryonic development. The aPL have been presented to interact in the maternal-fetal interface causing a defective endovascular trophoblastic invasion, That aPL may induce infertility through their negative effects on implantation [8].

Immune or immunological infertility is identified when produced antibodies bind to the antigens spontaneously, which is occurring on either the male or female gametocytes. Antibodies bind to seminal proteins or structures present on the sperm or oocyte. So, anti-sperm antibodies (ASA) have been recognized more frequently than anti-oocyte antibodies [3]. And the presence of ANAs is associated with immunologically estimated infertility, that the female, with high levels of ANA in their sera have also higher ANA levels in their follicular fluids, and these levels will be negatively correlated with the number of good quality embryos obtained in IVF/ICSI cycles [21]. That ANAs could directly impact oocyte maturation and embryo development resulting in infertility [20]. found that approximately 50% of spontaneous abortion women express ANAs, so that ANAs could be related to infertility, premature ovarian failure, and embryo transfer failure [21]. The presence of ANAs consider a risk factor for infertile women and can be included in the mechanism, resulting in embryo implantation failure, and could impair the fertilization rate and the number of good quality embryos and thus could lead to IVF/ICSI failure [19]

The pathogenic mechanisms for antiphospholipid antibodies (aPL)-mediated pregnancy loss

1- aPL and intraplacental thrombotic phenomena

placental thrombosis and infarction were described. It was suggested that such an effect might be cause by the in vitro capacity of aPL, mostly anti-ß2 glycoprotein 1 antibodies (anti-ß2GP1), to induce a pro-coagulant state by disruption of annexin A5 shield on trophoblast and endothelial cell monolayers [14] .

2- aPL and direct placental damage

Numerous evidence showed that alternative aPL, mediated pathogenic mechanisms which is directly affect placental tissue. The observation of β2GPI reactivity with trophoblast cell membranes, human stromal decidual cells, and human endometrial endothelial cells (HEECs), suggested the placental tropism of anti-β2GPI antibodies. aPL Inhibit trophoblast differentiation, which exposed via the reduced secretion of women chorionic gonadotrophin (hCG), aPL estimate trophoblast damage and apoptosis [5]

aPL and inflammation-mediated damage

Acute inflammatory events can be result in a negative pregnancy outcome by the activity of pro-inflammatory mediators, for example, complement, tumor necrosis factor-α [TNF-α], and chemokines that have been shown to have a role in animal models of aPL

result in fetal loss [12]

The pathogenic mechanisms for antisperm antibodies mediated pregnancy loss:

It was established that antispermatozoal antibodies impairment fertility by different mechanisms; The antispermatozoal antibodies may perhaps mask some antigens which are essential for the penetration of the spermatozoa into the ovule, Spermatozoa and antibody, can procedure complexes on uterine tissue, which triggers the excretion of histamine and makes the exclusion of the implanted embryo. It is assessed that around 5% of infertility cases are of immunological origin and related to the presence of ASAs in both women or/and men [6]

The pathogenic mechanisms for antinuclear antibodies - mediated pregnancy loss

In the early stages after fertilization, the determining whether the fertilized egg would successfully develop into an embryo done by stability of nucleus, which consider the key factor in this process. In the process of mitosis, new components of the cells will be produced. Some of these components, as well as proteins, polysaccharides, and glycoproteins, may get exposed at the surface of the cells. In normal conditions, these components would not be known by the immune system. However, in an imbalanced immune system, these components may trigger the activation of autoantibodies. These autoantibodies generally associated with the components of nucleus, and ANAs have been supposed as an important immune cause of the implantation failure[10] .

Subjects, materials and methods

Ninety infertile couples undergoing (in vitro) fertilization (IVF) program from the Kamal Al-Samaraee IVF Center, Ministry-of Health in Baghdad-Iraq and The Institute for Infertility Diagnosis and Assisted Reproductive Technologies Al-Nahrain University in Baghdad-Iraq, enrolled in this study through the period from November / 2018 - June /2019. All couples were subjected to the basic fertility work-up of the fertility center which consists of history- taking, physical examination, ovulation detection, evaluation of tubal patency and uterine cavity, and semen analysis. The patients were divided into two groups according to success or failure IVF, into sixty five infertile women have implantation failure and didn't get pregnant , and twenty five infertile women managed to get pregnant after where the embryos is placed in the uterine cavity in order to implant(control group). Venous blood samples were collected from all patients and healthy controls. Chemical and biological materials used in this study (to estimate to measure serum markers as ASA, aPL, and ANA) , including ; Phospholipid screen IgG/IgM ELISA, ANA Screen ELISA, and Anti-Spermatozoa Antibody (ASA) ELISA.

Results

The current study showed a highly significant difference (P<0.01) between patients (IgM +v) and control group (aPL IgM -ve), That the mean level of of non-pregnant women and control group was (A 15.76±0.15 and B 3.5±0.31 u/ml respectively) . Also result of estimation aPL(IgG) in sera of another patients and control group show that the mean of non-pregnant women with (IgG +ve) was (A19.7±3.22)u/ml, and for pregnant women (control IgG) was (B 0.85±0.25)u/ml. The statistical analysis shows a highly significant difference (P<0.01) between pregnant (control group) and non-pregnant with positive result for aPL IgG (Table 1). These results were in agreement with study done by [17], that they compared with fertile control women, significant differences in the prevalences of aPLs in the IgG and IgM. All all of the IgG aPLs studied were significantly elevated over fertile control values. These results were in agreement with study done [2], who found that the results consistently showed significantly higher levels of aPL among women experiencing IVF failure.

Table (1) Estimation of aPL in the sera of patients and control group

Test groups	Statistic	Range			
	Mean ± SD	Minimum	Maximum	NO(%)	Std. Error
*Patients group (IgM +ve)	A 15.76± 0.15	15.63	15.93	*1(1.1)	0.08

Cont... **Table (1) Estimation of aPL in the sera of patients and control group**

Patients group (IgM -ve)	B 3.8±0.47	3.21	4.81	17(18.9)	0.11
Control (Igm)	B 3.5±0.31	3.19	3.98	7(7.8)	0.12
F ratio (P value)	113(0.000)				
* Patients group (IgG +ve)	A 19.7±3.22	16.79	23.16	3(3.3)	1.85
Patients group (IgG -ve)	B 1.2±0.49	0.43	2.20	44(48.9)	0.07
Control (IgG)	B 0.85±0.25	0.53	1.32	18(20)	0.06
F ratio(P value)	91(0.000)				

* Two valid default values were computed to complete the statistical analysis for the comparison purpose , noting that the number of positive samples is only one and its value 15.73.

The data demonstrated in table (2) showed that The mean in non-pregnant with ANA+ve, and control group was (A 1.67±0.3) s/co, (B 0.55±0.08)s/co respectively. This data demonstrated a highly significant difference (P<0.01) between the studied groups. The current study was in agreement with study done by [10] , which found, the ANA expression in the infertile patients was higher than that in the fertile group. Another Study showed reveals the presence of ANA may exert a detrimental effect on the outcome of IVF impaired oocyte and embryo development and decreased pregnancy and implantation rate [21] .

Table (2): Estimation of ANA in the sera of patients and control group.

Study groups (s/co)	**Statistic**	**Range**			
Patients group	Mean ± SD	Minimum	Maximum	NO(%)	Std Error
Positive samples	A 1.67 ± 0.3	1.28	2.21	14(15.5)	0.08
Negative samples	B 0.57 ± 0.06	0.41	0.69	51(56.7)	0.009
Control	B 0.55 ± 0.08	0.43	0.69	25(27.8)	0.016
F ratio(P value)	77(0.000)**				

And the data demonstrated in Table (3) showed that The Mean in non-pregnant with ASA+ve was (A 121.26±13.98)U/mL , and the Mean for the pregnant (control group), was (B 31.00±4.34) U/mL. This data demonstrated a highly significant difference (P<0.01) between the studied groups.The current study was in agreement with study done by [7] in Baghdad, suggested that detections of antisperm antibodies in the serum of infertile women were which is significantly (p<0.05) higher from control group, the conclusions of this study was These higher levels of complement components may be due to activation of classical pathway by ASA that directed against sperm antigens ending in defect in function and motility of the sperms .

Table (3): Estimation of level of ASA in the sera of patients and control group.

Study groups (U/ML)	Statistic	Range			
Patients group	Mean ± SD	Minimum	Maximum	NO(%)	Std. Error
Positive samples	A 121.26±13.98	108	155	12(13.3)	4.035
Negative samples	B 31.87±4.67	23	40	53(58.9)	.642
Control	B 31.00±4.34	23	38	25(27.8)	.869
F ratio(P value)	49 (0.000)**				

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under College of Health and Medical Technology, Middle Technical University and all experiments were carried out in accordance with approved guidelines.

References

1. Benksim A , Elkhoudri N , Addi RA , Baali A , Cherkaoui M. Difference between primary and secondary infertility in Morocco: frequencies and associated factors. International Journal of Fertility & Sterility, 2018;12(2):142.
2. Boutzios G , Papadimitriou E , Mathioudakis A , Vlachoyiannopoulos P , Mastorakos G. Presence of antiphospholipid antibodies is associated with increased failure rates of assisted reproductive techniques: a meta-analysis. 20th European Congress of Endocrinology, 56. BioScientifica. 2018.
3. Brazdova A , Senechal H , Peltre G , Poncet P. Immune aspects of female infertility. International Journal of Fertility & Sterility, 2016;10(1): 1.
4. Chen X , Mo M-L, Huang C-Y , Diao L -H , Li G -G , Li Y-Y , Zeng Y. The etiology of infertility is thought to be multifactorial. The known risk factors include genetic abnormalities, ovulatory disorders, tubal damage, uterine or peritoneal problems, and male factors. Journal of Reproductive Immunology, 2017;122:14–20.
5. Di Simone N , Castellani R , Raschi E , Orietta Borghi M , Meroni P L , Caruso A.. Anti-beta-2 glycoprotein I antibodies affect Bcl-2 and Bax trophoblast expression without evidence of apoptosis. Annals of the New York Academy of Sciences, 2006;1069(1): 364–376.
6. Heng BC , Huang W , Zhong X , Yin P , Tong GQ. Roles of antiphospholipid antibodies, antithyroid antibodies and antisperm antibodies in female reproductive health. Integrative Medicine International, 2015;2(1–2): 21–31.
7. Ibrahim DS , Caitano AE , Maki B , Salih WH , Mahdi BM. Determination some of complement components in infertility women with antisperm antibodies. Iraqi Journal of Medical Sciences, 2010;8(2):18–23.
8. Kutteh WH , Stanic AK , Schust DJ. Immunology and reproduction. In Yen and Jaffe's Reproductive Endocrinology 2019;301–321. Elsevier.
9. Li J , Chen Y , Liu C , Hu Y , Li L. Intravenous immunoglobulin treatment for repeated IVF/ICSI failure and unexplained infertility: a systematic review and a meta-analysis. American Journal of Reproductive Immunology, 2013;70(6):434–447.
10. Li Y , Wang Y , Ma Y , Lan Y , Jia C , Liang Y , Wang S. Investigation of the impact of antinuclear antibody on the outcome of inávitro fertilization/ intracytoplasmic sperm injection treatment. Taiwanese Journal of Obstetrics and Gynecology, 2015;54(6):742–748.
11. Mahdi BM , Salih WH , Caitano AE , Kadhum BM , Ibrahim DS. Frequency of antisperm antibodies in infertile women. Journal of Reproduction & Infertility, 2011;12(4):261.
12. Meroni PL , Borghi MO , Raschi E , Tedesco F. Pathogenesis of antiphospholipid syndrome:

understanding the antibodies. Nature Reviews Rheumatology, 2011;7(6): 330.

13. Pandian Z , Gibreel A , Bhattacharya S. In vitro fertilisation for unexplained subfertility. Cochrane Database of Systematic Reviews, 2015; (11).
14. Rand JH , Wu XX, Quinn AS , Taatjes DJ. The annexin A5-mediated pathogenic mechanism in the antiphospholipid syndrome: role in pregnancy losses and thrombosis. Lupus, 2010;19(4): 460–469.
15. Reimand K , Talja I , Metsküla K , Kadastik Ü , Matt K , Uibo R. https://www.iasj.net/iasj?func=article&aId=79822. Journal of Reproductive Immunology, 2001;51(2):167–176.
16. Sanmarco M , Bardin N , Camoin L , Beziane A , Dignat-George F , Gamerre M , Porcu G. Antigenic profile, prevalence, and clinical significance of antiphospholipid antibodies in women referred for in vitro fertilization. Annals of the New York Academy of Sciences, 2007;1108(1):457–465.
17. Sauer R , Roussev R , Jeyendran RS , Coulam CB. Prevalence of antiphospholipid antibodies among women experiencing unexplained infertility and recurrent implantation failure. Fertility and Sterility, 2010;93(7): 2441–2443.
18. Schattman G L , Esteves SC , Agarwal A. Unexplained infertility: Pathophysiology, evaluation and treatment. Unexplained Infertility: Pathophysiology, Evaluation and Treatment, 2015;1–347. https://doi.org/10.1007/978-1-4939-2140-9
19. Simopoulou M , Sfakianoudis K , Maziotis E , Grigoriadis S , Giannelou P , Rapani A, Vlahos N. The Impact of Autoantibodies on IVF Treatment and Outcome: A Systematic Review. International Journal of Molecular Sciences, 2019;20(4):892.
20. Yanase K , Madaio MP. Nuclear localizing anti-DNA antibodies enter cells via caveoli and modulate expression of caveolin and p53. Journal of Autoimmunity, 2005;24(2):145–151.
21. Ying Y , Zhong Y , Zhou C , Xu Y , Wang Q , Li J , Wu H. Antinuclear antibodies predicts a poor IVF-ET outcome: impaired egg and embryo development and reduced pregnancy rate. Immunological Investigations, 2012;41(5): 458–468.