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Evaluation of Anti-zona Pellucida Antibodies in Serum and Follicular fluid for Polycystic ovarian women undergoing Intracytoplasmic Sperm Injection Programme

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ABSTRACT

This study aims to evaluate the reproductive performance of women with PCO undergoing ICSI treatment by checking the presence of anti-zona pellucida antibody (AZA) in blood serum and follicular fluid and correlating it to ICSI outcome. This prospective study was conducted at the Fertility centre in AL-Najaf Al-Ashraf city over a period of one year. The results of this study showed that the mean value and range of the age in the studied groups were (29.71±6.62) (18-45year) while the mean value for BMI was (27.70±4.05) Kg/m^2, ranging between (19 -35.7). The mean duration of infertility was (8.40±4.16), ranged between 2-18years. Quite a large percent, i.e. 86.7% of the subjects had a history of primary subfertility. Normal range values were documented for Basal Hormonal Levels. While peak E_2 was within normal values range, but E2 on CD2 showed higher significance as found in the (non PCO) group. It was found that both the follicular & blood serum AZA was reportedly higher among women with (Non-PCO) of infertility than their counterparts but without any significant difference. Also, the peak means value for follicular AZA in subjects reporting with non PCO group came around 10.29 IU/L, while that for ovulatory cause followed along. The results of present study conclude that the AZA levels (both follicular & blood serum) may be used as one important marker of fecundity in cases of IVF/ICSI procedural candidates to increase successful pregnancy rates and reduce cancellation cycles. Infertile women must be treated for the clinical condition before starting ICSI procedure for successful ICSI outcomes. Thus, even remotest immune activation is detected by blood serum & follicular AZA and treated thoroughly afterwards, may affect ICSI outcome positively.

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INTRODUCTION

Infertility is a worldwide medical & social issue is affecting approximately 10% of all reproductive-aged couples as reported. Amongst the causes of infertility, Polycystic ovarian syndrome (PCO) is found to be 30% as given by ASRAM, 2012. The Non PCO causes may include tubal defects, amenorrhea, endometriosis etc. While fecundity, i.e. being able to reproduce has been considered an important parameter of reproductive health (Boivin *et al.,* 2007), Infecundity is the failure of a reproductive-aged couple in achieving conception or to have a live birth even after more than or equal to one year of regular sexual intercourse (unprotected) for women ageing less than 35 years and

the duration is 6 months for women ageing more than 35 years. (Wasiu *et al.,* 2012).

As the physiological process of conceiving states, any successful conception is always the result of many complex interactions, specifically between the female uterus & mature blastocyst under immune-hormonal control, along with other factors (interactions between different body systems) that control conception (Saito, 2000).

The female ovary in Humans can be a site of the autoimmune attack, for example, in the circumstances like organ-specific or any systemic autoimmune conditions. The ovarian dysfunction that follows many times clinically shows symptoms and conditions like an ovarian failure (premature). Along with this cause, other clinical conditions or pathologies of female ovaries, like endometriosis, PCOS, repeatedly unsuccessful IVF attempts are likely to result in preeclampsia (PE), also called as spontaneous miscarriages (Reimand *et al.,* 2001). It also has been found to be associated with anti-ovarian autoimmunity (Luborsky, 2002).

Presently human reproduction and its context with immunology are receiving many fold medical attention within the community. Also one can find numerous papers being published in journals of immunology, on even bits & wholes of every related thing on reproductive immunology (Uibo *et al.,* 2012). The previous researches available concludes that cellular, particularly, humoral nature autoimmunologic disquests are the causes that result in female infertility.

Coming to what autoimmunity has to do in conception, the autoimmunity must be understood as Human immune system's loss of ability to differentiate self from non-self-tissues. The result of this immune loss is characterized by symptoms of inflammation, which is a cellular/ humoral antibody & immune-system-mediated processes requiring both active& passive or adaptive immunity (Cho and Gregersen, 2011). The hormonal or endocrine autoimmunity often clinically is found to be organ specific, but in many cases, it is found to be related to polyclonal activation (Jasani, 1999), as well as non-endocrinal autoimmunity (Tagoe *et al.,* 2012).

It is reported that subclinical autoimmunity anticipates clinical autoimmune ailments forehead months to years. It is also reportedly related to a decline in fecundity & increase in abortion risk in reproductive females even at subclinical stage (Gleicher, 1999).

The present literature shows that anti-ZP antibodies are assured to be a reason for infertility in females as they have blocking effects (sperm–ZP binding) (Takamizawa *et al.,* 2007). But the anti-zona pellucida antibodies seems promisingly essentials autoantibodies (Nishimoto *et al.,*1980), more so in cases of unexplained infecundity (Shivers and Dunbar, 1977; Ulcova- Gallova *et al.,* 2004). The cellular ZP surrounds oocyte during the process of ovulation and stays there till implantation which comprises cellular receptors (ZP1, ZP2, ZP3) (Kamada *et al.,* 1992) to species-specific sperm cells (Luborsky *et al.,* 1999). The sperm & egg combination is thus a receptor-mediated physiological process (Hovav *et al.,* 1994).

Some particular antibodies have the undesired effect to completely or partially obstruct primary sperm-egg connection & further sperm infiltration in egg, which may very well be the reason for natural or artificial fertilization (Dunbar *et al.,* 1998).

ICSI- Intra-cytoplasmic sperm injection (ICSI) is a" form of micromanipulation including the injection of only one motile (live) sperm cell directly into the ooplasm of MII stage oocyte (De Vos, 2000)". ICSI has been proven relief for many couples struggling with infertility. Though it is relatively within reach for patients nowadays, the inclusion criteria include severe male infertility issues like low sperm count or quality, female issues like blocked fallopian tubes, ovarian failure (primary or secondary), decreased ovarian reserve, endometriosis (Huang and Rosenwaks., 2012).

METHODOLOGY

Study design

Prospective analytical cohort.

Sample

A total of 36 subjects were selected from females being treated at the fertility centre from November 2017 to October 2018, which were further divided into women with (polycystic ovary syndrome) in one group and women with (non PCO) reasons of infertility in other groups. Both groups had 18 subjects each.

Procedure: Completely sub-fertile reproductive-aged couples were evaluated through semen examination for men while complete medical, surgical and gynaecological history was documented for women to conceive the cause of their infertility, i.e. if it is a polycystic ovarian syndrome or not. Other than this, thorough physical examination along with anthropometric measures like weight (in kgs), height (in cms) and body mass index (BMI) (in kg/cm) was done. Complete gynaecological examination had been done at day 2 of the cycle, USG (ultrasonography), blood tests for hormones like LH, FSH, AZA, prolactin, E2, mid-luteal progesterone levels were also checked.

Selection Criteria

- Male subject: who are suffering from infertility along with ejaculated semen and who otherwise had normal zoo spermia.
- Female subject: subjects are having infertility problems (PCO or others) were included in the study.
- Female subjects are undergoing ICSI cycles.
- Subjects must show negative for screening tests for HIV & hepatitis B & C.
- Males having azoospermia or those having epididymal or percutaneous epididymal or percutaneous aspired or testicular sperm collection were excluded from the study
- Females with ICSI cycles without follicle aspiration and embryo transfer were also excluded from the study.

Measurements

Subjects Preparation

Trans-vaginal (U/S): Trans-vaginal USG was done by a gynaecologist on second cycle day infertility centre to evaluate count of antral follicles (AFC) using" real-time ultrasound device, using" vaginal probe (5-7 MHZ)". Follicles measuring 2-10 mm were counted to assess the antral follicles, also to measure the endometrial thickness and to exclude the presence of the ovarian cyst.

Evaluation of Serum and Follicular (AZA): On second cycle day**,** Blood & serum were separated by using a serum separator tube, also permitting tests to the cluster to two hours toward room temperature or overnight toward 2- 8°C. Then it was Centrifuged to 3000 rpm for 10 minutes, after that eliminating serum and test instantly or store tests at 20- 80°C to further assess the amount of serum AZA by using special human Elisa kit (My BioSource/ USA).

The follicular fluids which were obtained on the day of egg retrieval were centrifuged at 3000 rpm for about ten mins. The procedure was done to eliminate the cell parts and analyze the concentration of the follicular fluid AZA by using the same kit.

Ovarian stimulation protocols: Single infusion Decapeptyl; 0.1 m.g., a Gn RH have been administered subcutaneously on 2nd cycle day while waiting for the outcomes of FSH, LH and E2.

Antagonist Protocol: Stimulation to start with r. FSH infusion (Gonal- f) on 2 or 3rd day of menstrual cycle which lasted till HCG day. Subsequently, HMG was also started. Serial USG (ultrasonography) have been done for assessment of follicular development.

HCG administration: For normal responders, HCG as a rule, was injected at the E2 level which must be more than 400 pg/ml for 3 days and the diameter of 2 or more follicles arrived at 17 mm or bigger. Ovulation was triggered by Pregnyl (5000-10000 IU) i. m, so ovulation happened in about thirty-six to forty hours following infusion.

Statistical Analysis

Statistical analysis was done by using SPSS (statistical package for social sciences) version 20 (2011). The mean, standard deviation, numbers and percentages as descriptive statistics. Further chi-square test was used for categorical data and independent sample t-test, analysis of variance (ANOVA) with Bonferroni correction and Pearson correlation coefficient for continuous variables.

RESULTS AND DISCUSSION

Despite the reasons of infertility (PCO or Non-PCO), the AZA concentrations, follicular count, pregnancy rates and other ICSI outcomes vary when observed in both groups, i.e. group with PCO subjects & Non PCO subjects respectively. Also, there are less number of studies analyzing ICSI outcomes in female subjects with PCO that too when USG evidence of PCO has been found to affect 20-30% of females (Balen *et al.,* 1995; Farquhar *et al.,* 1994; Polson *et al.,* 1988).

As the result of the present shows, the AZA concentrations, from both follicular fluid (FF) and serum in subjects with Polycystic syndrome & Non Polycystic syndrome, did not show any significant differences statistically. But the values were found to be slightly higher in non PCO group when compared to their counterpart between groups analysis. Along with these, the concentrations of AZA were found to be significantly raised in FF and serum of subjects of non PCO group again. Shivers & Dunbar discovered that the presence of serum anti-zona activity in about thirty-two percent of infertile females while normal reproductive females have no like this reactivity. (Shivers and Dunbar, 1977).

Correlation analysis of serum and follicular fluid AZA in the PCO and Non PCO groups of sub-fertile women viewed a positive without statistically significant difference.

In Non-PCO group of females, the pregnancy rate was (40%), and in a group of patients with PCO originating infertility was (30%). This may be due to the reason that amongst the subjects needing ICSI, the woman at times is relatively young and fertile (good egg quantity and quality) as compared to some of the women having IVF for other reasons. The outcomes of ICSI were discussed by

Table1 : Intracytoplasmic sperm injection outcomes in polycystic and non-polycystic ovarian women

Intracytoplasmic sperm injection outcomes	Polycystic ovarian group (N=18)	Non-polycystic ovarian group (N=18)	P value
Follicle count	13.40±4.52	15.87±4.52	0.199
Retrieved oocyte.	9.21±3.36	11.61±7.12	0.198
Egg at M II	6.55±2.8	8.51±5.01	0.151
Number of injected oocyte	6.79±2.6	13.40±4.52	0.317
2PN	4±2.41	8.1±5.2	0.804
Fertilization rate	57.02±29.6	61.03±27.53	0.693
Number of Embryo	3.74±1.78	5.1±3.15	0.102
Grade I (embryo)	2±0.3	2±0.6	0.739
Grade II (embryo)	2.13±1.2	3.35±2.60	0.112
Cleavage rate	98.02±22.43	90.65±22.98	0.392
Pregnancy rate %	30%	40%	0.591

Table2 : Anti-zona pellucida antibodies (AZA) concentration in follicular fluid (F.F) and serum for polycystic and non-polycystic ovarian women undergoing ICSI program

AZA concentration	Polycystic ovarian group (N=18)	Non-polycystic ovarian group (N=18)
Follicular fluid	133.35±49.07	112.46±32.46
Serum	90.83±25.90*	88.92±32.09

Table3 : AZA concentration in f.f and serum for pregnant and non-pregnant women undergoing ICSI program

AZA concentration	Pregnant women (N=11)	Non-pregnant women (N=25)
Follicular fluid	103.328± 35.657	126.201±42.399
Serum	80.521± 24.672	91.771±32.434

some other researchers as well in context with PCOs.

A study done by Swanton et al. reported a significant reduction in total FSH and a significant increase in upper limit E2 levels as well as eggs collected in the PCO group. It also showed reduced fertilization rates for PCO group. (Swanton *et al.,* 2010)

However, Esaizadeh et al. showed no difference what so ever in clinical pregnancy rates between PCO and Non-PCO group (with 95% confidence interval) And thus was unable to relate it to ICSI outcomes between two groups statistically. (Esmaizadeh *et al.,* 2005).

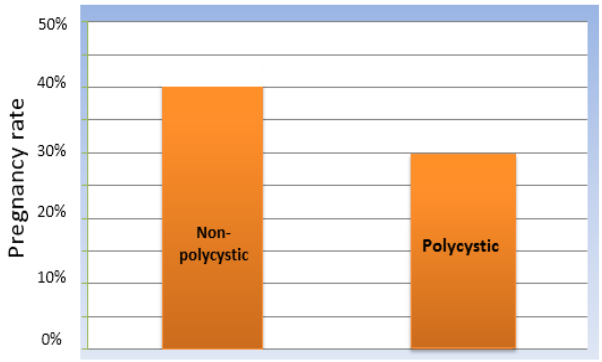

Figure 1: Pregnancy rate in polycystic and non-polycystic ovarian women undergoing ICSI program

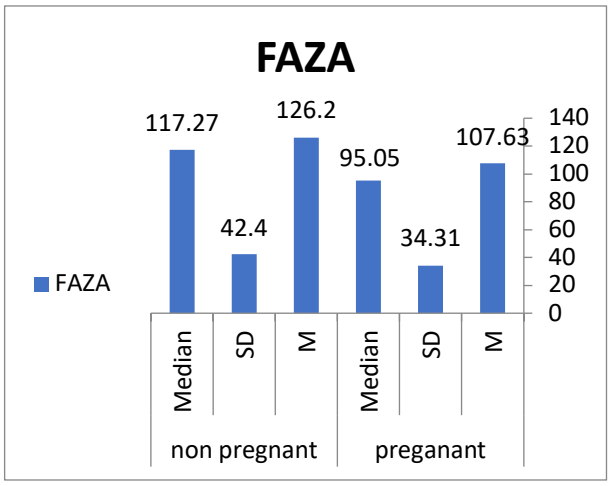

Figure 2: Correlation relations of follicular AZA concentrations pregnant and non-pregnant

However, relating AZA antibodies to PCO has not been shown statistically relevant in this study and almost negligible direct references were available but in this study we assessed the higher concentrations of AZA in FF and sera of infertile women with Non PCO reasons of infertility than in patients with PCO in relation to ICSI outcomes and pregnancy rates. Additionally, there was no significant connection between the appearance of AZA and no. of oocytes, embryos, FR and clinical PR. Further research may be needed on larger sample size to generalize the findings.

CONCLUSION

The causative connection of high concentration of AZA antibodies and failure to respond to medications in women with infertility caused by PCO is concluded from the results of this study.

REFERENCES

A. Swanton, L. Storey, E. Mcveigh, T. Child (2010) IVF outcome in women with PCOS, PCO and normal ovarian morphology. *Eur J Gynaecol Reprod Med,* 149 (1) 68-71

A.H. Balen, G.S. Conway, G. Kaltsas, K. Techatrasak, P.J. Manning, C. West, *et a., 1995.*Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients; *Hum Reprod, 10 (8). 2107-2111*

Boivin J., Bunting L., Collins JA. and Nygren KG., 2007. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod; 22 (6): 1506-12.

C.M. Farquhar, M. Birdsall, P. Manning, J.M. Mitchell, J.T. France, 1994*.; Aust N Z J Obstet Gynecol, 34 (1) 67-72*

Cho J. H. & Gregersen P. K., 2011. Genomics and the multifactorial nature of the human autoimmune disease. *N. Engl. J. Med.* 365, 1612–1623.

D.W. Polson, J. Adams, J. Wadsworth, S. Franks, 1988. Polycystic ovaries—a common finding in normal women, Lancet, *1 (8590) 870-872*

De Vos A., 2000. Intra-cytoplasmic sperm injection (ICSI). *Hum Reprod*, 15 (4): 59-64.

Dunbar BS., Avery S., Lee V., Prasad S., Schwahn D., Schwoebel E., Skinner S., Wilkins B., 1994. The mammalian zona pellucida: its biochemistry, immunochemistry, molecular biology, and developmental expression. *Reprod. Fertil Dev*.; 6 (3): 331-47.

Gleicher N., 1999. Reproductive failure prior to the onset of clinical autoimmune disease. *Rheumatology (Oxford)* 38, 485–487.

Hovav Y., Almagor M., Benbenishti D., Margalioth EJ., Kafka I., Yaffe H., 1994. Immunity to zona pellucida in women with low response to ovarian stimulation, in unexplained infertility and after multiple IVF attempts. *Hum Reprod*, Apr; 9 (4): 643-5.

Huang JYJ. and Rosenwaks Z., 2012. In vitro fertilization treatment and factors affecting success. *Best Pract Res Clinical Obstet and Gynecol*, 26: 777-788.

Jasani B. *et al.,* 1999.Natural antibody status in patients with Hashimoto's thyroiditis. *J. Clin. Lab.Immunol.* 51, 9–20.

Kamada M., Daitoh T., Mori K., Maeda N., Hirano K., Irahara M., Aono T. and Mori T., 1992. The etiological implication of autoantibodies to zona pellucida in human female infertility. *Am J Reprod Immunol,* Sep; 28 (2): 104-9.

Luborsky J., 2002." Ovarian autoimmune disease and ovarian autoantibodies," *Journal of Women's Health and Gender-BasedMedicine*, vol. 11, no. 7, pp. 585–599.

Luborsky J., Llanes B., Davies S., Binor Z., Radwanska E., and Pong R., 1999." Ovarian autoimmunity: greater frequency of autoantibodies in premature menopause and unexplained infertility than in the general population," *Clinical Immunology*, vol. 90, no. 3, pp.368–374.

Nishimoto T., Mori T., Yamada I. and Nishimura T., 1980. Autoantibodies to zona pellucida in infertile and aged women. *Fertil Steril*., Dec; 34 (6): 5526.

Raivo Uibo, 1, 2 Andres Salumets, 2, 3 and Gilbert Faure, 2012. Immunological Aspects of Human Reproduction.

Reimand K., Talja I., Metsküla K., Kadastik Ü., Matt K. and Uibo R., 2001. Autoantibody studies of female patients with reproductive failure. *Journal of Reproductive Immunology*. 51 (2): 167–176.

S. Esmaizadeh, M. Faramarzi, G. Jorsarai 2005. Comparison of *in vitro* fertilization outcome in women with and without sonographic evidence of polycystic ovarian morphology; *Eur J Gynaecol Reprod Med, 121 (1)*. 67-70.

Saito S., 2000." Cytokine network at the feto-maternal interface," *Journal of Reproductive Immunology*, vol. 47, no. 2, pp. 87–103.

Shivers CA. and Dunbar BS., 1977. Autoantibodies to the Zona pellucida: A possible cause for infertility in women. Science; 197: 1082-1086.

Tagoe C. E., Zezon A. and Khattri S., 2012. Rheumatic manifestations of autoimmune thyroid disease: the other autoimmune disease. *J. Rheumatol.*39, 1125–1129.

Takamizawa S., Shibahara H., Shibayama T., Suzuki M., 2007. Detection of Arizona pellucida antibodies in the sera from premature ovarian failure patients by a highly specific test. *Fertil. Steril.* 88, 925–932.

Ulcova- Gallova Z., Babcova K., Novakova P., Micanova Z., Rokyta Z., 2004. Anti-zonal antibodies in ovulatory cervical mucus and serum of patients with fertility disorders, *Ceska Gynekol*., May; 69 (3): 215-8.

Wasiu Eniola O., Adebayo Adetola A. and Taiwo Abayomi B., 2012. A review of female infertility: important etiological factors and management. *J Microbiol Biotech Res*, 2 (3): 379-385.