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Follicular Fluid IL-6 Levels in Prediction of Successful Pregnancy Outcome in Women Undergoing IVF-ET Cycles - A Prospective Study

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Abstract: ***Objective**: To investigate expediency of quantitative levels of interleukin-6 (IL-6) detected in follicular fluid (FF) of women underwent in-vitro fertilization-embryo transfer (IVF-ET) cycles. **Design**: Prospective observational study. **Method**: Study comprises evaluation of IVF/ICSI-ET cycles in relation to contribution of quantitative levels of IL-6. FF samples were collected on day of oocyte retrieval by pooling ovarian follicles of size ≥15mm for each woman (n=168). Enzyme-linked immunosorbent assay (ELISA) technique was applied for measuring quantitative level of IL-6. Demography, cycle characteristics, endometrial thickness, number of oocytes, fertilization rate, grading of embryos and IL-6 levels were compared between pregnant and non-pregnant groups of women. Student T-test, Mann Whitney U-test, Chi-squares test and logistic regression were applied as appropriate. Statistical significant level was calculated at P<0.05. **Results**: The women (n=168) were divided into pregnant (Group-A; n=75) and non-pregnant (group-B; n=93) groups. The median level of IL-6 was found lower in group-A as compared to women in group-B [33.9 pg/mL (0.1-199.8) versus 92.9 pg/mL (0.3-199.8); P<0.001 respectively]. **Conclusions**: Lower concentration of IL-6 in follicular fluid was found associated with positive pregnancy outcome and served as reliable predictive marker of successful pregnancy outcome in women underwent assisted reproduction.*

Keywords: Infertile-women, Follicular-fluid, Interleukins, IVF-ET and Pregnancy-outcome

1. Introduction

Infertility affects millions of couples all over the world. Childlessness is a life crisis and impaired fertility has been reported to affect 10-15% of couples owing to various explained and unexplained reasons [1]. World Health Organization (WHO) reported 3% of women with primary infertility and 8% women with secondary infertility including nearly 12 million infertile couples from India [2], [3].

Assisted reproductive technology (ART) has great relevance to conquer infertility and provides opportunity to cater the need of infertile couples. However, implantation failure is a concomitant aspect of ART, which is surprisingly not very well understood. During in-vitro fertilization/intra-cytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) treatment cycles women succeed to produce healthy embryos but unfortunately at the level of implantation somewhat goes wrong because of unresolved and perplexing reasons [4, 5].

Association of implantation failure has also proven with ovarian de-regulation in relation to anatomic anomalies, impaired endocrinal milieu and immunological complications generated during systemic events of IVF-ET cycles [6], [7]. In past two decades, increasing recognition has been given to the contribution of immune system in regulation of ovarian functions, including success/failure of implantation. Immuno-molecular mechanism involves during implantation that makes uterus amenable (receptive) to embryo for subsequent apposition, adhesion and invasion altogether are known as successful implantation [8]. Cytokines are one of the most important immuno-competent molecules, which are found allied with implantation. These are low molecular weight water-soluble protein and glycoprotein molecules secreted by immune cells. Based on their functions, cellular origin and target sites cytokines are classified as lymphokines, chemokines and interleukins [9].

Interleukins were originally perceived as lymphocyte's derived products mediate interactions between leukocytes. However interleukins have wide array of functions, appropriately related to endothelial fibroblasts, luteal cells and granulosa cells [10]. Ovarian interleukins, secreted by granulosa cell and other immune cells within the ovary and follicles regulate diverse functions including folliculogenesis, oogenesis, ovulation, fertilization, embryo development, implantation, corpus luteum formation and regression [11], [12, [13], [14], [15]. In view of existing knowledge, present study was conducted to address the association of follicular fluid levels of IL-6 with prediction of successful implantation in women undergoing assisted reproduction.

2. Literature Survey

Cytokines are very essential immune-molecular components found allied also with the several reproductive events such

as ovulation, fertilization, implantation, implantation failure and various pre-ovulatory and ovulatory mechanisms of the women [16]. Since, cytokines have mostly the autocrine and paracrine modes of action, various investigators studied role of intra-follicular cytokines on IVF outcome. Following the same it is considered that functions of intra-follicular (pleiotropic levels) concentrations in the ovary are better than their concentration in blood (peripheral levels) [17], [13], [18]. Numerous immuno-components molecules such as lymphokines, chemokines and interleukins implicate in mediating intra-ovarian events. These secretory molecules comprise colony-stimulating factor (CSF)-1 granulocyte-macrophage (GM)-CSF, various interleukins (IL) including IL-1, IL-6, IL-8 monocyte chemotectic protein-1 (MCP-1) and tumour necrosis factor alpha (TNF∝) [11], [19], [23], [24], [25], [26].

Granulosa cells secrete several ovarian interleukins that make up primary component of intra-follicular cells of ovarian follicles and dysfunction of these cells may contribute to abnormal ovarian pathology. Some of the cases of repeated implantation failure (RIF) were found associated with abnormal expression of various cytokines, including elevated levels of endometrial natural killer (NK) cells and de-regulation of interleukin (IL) IL-12, IL-15 & IL-18 [24].

IL-12 and Interleukin-8 appears to be an essential part of folliculogenesis, although its concentration was not found associated with fertilization or implantation rate [25]. Interleukin-11 had been detected in the follicular fluid and conditioned media from granulosa cells but absent in the serum whereas atretic follicles had higher concentration of IL-11 [26]. Cytokine profile in endometrial secretion was also investigated and appears to be conductive to clinical pregnancy. Significant associations between monocyte chemo-attractant protein-1 (MCP-1) and IFN-γ -inducible 10-coda protein (IP-10), IL-1β and tumor necrosis factor-alpha (TNF-∝) levels respectively with implantation and pregnancy [27] were observed. Association between high BMI and high TNF∝ and low IL-6 mRNA expression levels in follicular cells studied in women underwent ART cycles and it was observed that IL-6 expression levels were higher in women who subsequently achieved pregnancy [28].

Possible role and significant associations of cytokine profile with the various reproductive events including successful pregnancy as well as implantation failure were studied by different group of investigators, exclusively in cases of woman, who underwent IVF-ET cycles. Present study is an attempt to analyze the relationship between successful pregnancy outcomes based on quantitative levels ovarian interleukins detected in follicular fluid. This approach enabled us to observe the functionality of follicular fluid IL-6 levels and to analysis its role as a non-invasive predictive marker of successful pregnancy outcome in women undergoing ART cycles.

3. Problem Definition

Several reports with extremely controversial finding encouraged us to attempt similar study in Indian population to find out possible participation and the effect of quantitative levels of ovarian interleukins with implantation and implantation failure in women underwent IVF-ET cycles.

4. Methodology/ Approach

This prospectively designed observational study was conducted at IVF & Reproductive Biology Centre, Department of Obstetrics & Gynecology, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi, India. Ethical approval was obtained from Institutional Ethics Committee (IEC) of Medical College and associated Hospitals (Letter number; F.2. / IEC/ MAMC/ 09/ No.196 dated 04th Dec. 2009). Infertile women, who attended "Fertility & ART Clinic" for their infertility management, and subsequently underwent IVF-ET cycles at IVF & Reproductive Biology Centre, were enrolled on the day of oocyte retrieval. Informed consent forms were obtained from each woman.

Inclusion & exclusion criteria for the selection of patients
Women having age between 20-38 years and consecutively underwent IVF-ET cycles were enrolled on the basis of certain inclusion and exclusion criteria: Women with tubal factor, male factor, ovulatory dysfunction, polycystic ovary syndrome (PCOS), endocrinal disorders, moderate to severe endometriosis, pelvic inflammatory disease and unexplained infertility were included. Whereas infertile women with ovarian hyper-stimulation syndrome, uterine anomalies, uncompensated heart diseases, and inadequate response of endometrial lining (endometrial thickness is < 7 mm) were excluded from the study.

Sample Size
A sample size of 165 women was calculated based on 30% IVF success rate, 95% confidence level with 5% error and 15% prevalence of infertility among the general population [29]. A total of 168 hFF samples were collected from 168 different women to meet the numbers of calculated sample size as per inclusion and exclusion criteria.

IVF/ICSI-ET outcome and follow-up of successful pregnancies
Women underwent standard IVF treatment of the hospital; either with agonist or antagonist protocol of ovarian stimulation and trigger was administered when leading/dominant follicles reached to 17-18 mm of diameter in size for final maturation of oocyte(s) to M-II stage. The ultrasound guided oocyte retrieval was performed after 34-36 hour of trigger consecutively in all women by 17GA/35cm, ovum pick-up needle (ova stiff ovum aspiration needle; K-OSN-1735-B-90; William A. Cook Australia, Pty. Ltd, Brisbane, Australia). Oocyte(s) were collected and washed in pre-equillibrated fertilization medium (Vitrolife, AB, Goteborg, Sweden) with optimum pH and osmolarity. After collection, oocyte (s) were incubated at humid environment of 06% CO_2, 05% O_2 at 37°C temperature to acclimatize in culture conditions. Accordingly, depending upon sperm count/concentration, IVF or ICSI procedures were performed 2-4 hours later to oocyte collection.

Insemination of sperms was done 2-4 hour after egg collection to achieve maximum fertiltization rate. In case of

patients with severe male infertility ICSI procedure was performed . Fertilization of oocytes was assessed for the presence of two pronuclei (formation of zygotes) after 16-20 hour of IVF/ICSI. Embryos were cultured for 2 to 5 days depending upon standard management of tratment cycle of individual women. Consecutive embryo transfer procedures were carried out accordingly on Day-2 /Day-3/Day-5. On day 2, cleavage stage embryos having 4 blastomeres (4-cell stage) whereas on day 3 cleavage stage embryos with 6-8 blastomeres (6-8 cell stage) were graded [37] and 1-2 good quality embryos were choosen for their transfer (Sydney IVF embryo transfer set, K-JETS-7019-SIVF, Cook Incorporated, Bloomington, USA) to uterine cavity. In cases of day 5 embryo transfer only 2-3 embryos at 6-8 cell stage were selected for extended culture and rest of the good quality embryos (if available) were cryopreserved for future cycles. On day 5 blastocyst stage embryos were graded [37] and 1-2 good quality blastocysts having distinct inner cell mass (ICM) and numrous trophoectoderm cells, were transferred. Women underwent embryo transfers were subsequently kept on luteal support with injectable progesterone (Susten, 100mg/50mg, intramuscular injection; Sun Pharmaceuticals industries Pvt. Ltd. Mumbai, Maharashtra, India) along with oral tablets and or vaginal micronized progesterone (Tablet Duphaston Dydrogesterone IP 10 BD; Abott India Limited, Mangalam, Villianur Commune, Puducherry, India or Tablet Uterone SR 300 BD; Jagsonpal Pharmaceuticles Ltd, Pant Nagar, Rudrapur, Uttarakhand India) for next two weeks.

All women were called after two weeks for urine pregnancy test (Alere; hCG One Step Pregnancy Test Device- Abon Biopharm; Co., Ltd., Hangzhou, P.R. China) and estimation of serum ßhCG levels. Serum ßhCG level >50mIU/mL were diagnosed as positive pregnancy outcome. The blood samples were repeated after 48 hours for doubling of serum ßhCG levels for successful implantation.

Trans-vaginal sonography (TVS), were performed at 4-6 weeks of embryo transfer for the presence of gestational sac with foetal heart and outcome was diagnosed as clinical pregnancy.

Collection of the follicular fluid samples-

The human follicular fluid (hFF) samples were consecutively collected from these women on the day oocyte retrieval. Only the transparent hFF samples from individual woman were pooled immediately after egg collection. Samples of hFF, if highly contaminated with red blood corpuscles (RBCs) were not pooled and avoided. Samples were centrifugation at 3000*g* and supernatant was collected in four different pre-labeled cryo-vials of 1.8 mL volume capacity (Tarson Products Pvt. Ltd, 31 Shakespeare Sarani, Kolkata, India) for the estimation of IL-6 levels. These hFF samples were nested and stored at -80°C until assaying.

Measurement of the levels of interleukins-

The cryopreserved samples were thawed and processed for quantitative estimation of levels of IL-6. The concentrations were measured with commercially available enzyme linked immunosorbent assay (ELISA) diagnostic kits (Gen-Probe Diaclone SAS, Besancon Cedex, France, sensitivity <2.0 pg/mL, intra-assay CV 6.2% and inter-assay CV 7.9%) and ELISA reader (BoiTek Instruments, Germany). Quantitative measurements were performed (at 450 nanometer wavelength), for optical density (OD) of the concentrations (pg/mL) of IL-6 provided by assaying the samples as per standard curve of serial dilution along with controls.

The recommended standards in specific ranges on which immunoassays were conduct for measuring the IL-6 levels are as follows:

IL-6: - 200, 100, 50, 25, 12.5, 6.25, Zero and Control Quantitative levels of IL-6 were allocated to the samples of individual women and subsequently compared between pregnant and non-pregnant groups.

Other than IL-6 Levels the demographic profile and various characteristics of treatment cycles were also compared.

Statistical Analysis-

Statistical analysis for quantitative levels of ovarian IL-6 and its association with pregnancy outcome was carried out. Data obtained after quantitative measurement were collected and entered in a computerized Database. Quantitative levels of IL-6 were compared for differences in mean± SD and median± SEM with the help of SPSS 21.0 (IBM Corporation, Chicago, IL, USA) statistical software package. Women were divided into two groups and compared in relation to pregnancy outcome as successful implantation group (pregnant women; Group-A) versus implantation-failure (non-pregnant women; Group-B) group.

Both the group were analyzed and evaluated for demographic profile, cycle characteristics, hormonal levels, endometrial thickness, number of oocytes, fertilization rate, grading of embryos and prediction of pregnancy outcome based on IL-6 levels. Student T-test, Mann Whitney U-test, Chi-Squares test and logistic regression test were applied as appropriate and statistical significant level was calculated at $p<0.05$.

5. Results & Discussion

All the women (n=168) were divided into pregnant (Group-A; n=75) and non-pregnant (group-B; n=93) groups. Among them, a total of 75 (44.64 %) women were found pregnant whereas 93 (55.36%) women were found unable to achieve their pregnancies. Comparison of demographic distribution and cycle charactedistics between pregnant and non-pregnant group of women are shown in table-1.

Table 1: Comparison of the demographic parameters between pregnant and non-pregnant group of women

S. No.	Demographic Parameters	Total number of women(n=168) Mean±SD (Min. -Max.)	Pregnant women (n=75) Mean±SD (Min. -Max.)	Non-pregnant women (n=93) Mean±SD (Min. -Max.)	*P* value
1	Age (years)	30.98±4.0 (21-38)	30.19 ± 4.1 (21-38)	31.54 ± 3.9 (23 - 38)	0.037*
2	Body Mass Index (BMI)	24.83±3.40 (16.82-35.56)	24.75±3.3 (16.82-33. 78)	25.08±4.1 (17.58-35.56)	0.571
3	Duration of Infertility (years)	7.43±4.4 (1-20)	6.84±4.1 (1-20)	7.91±3.9 (1-16)	0.088

***P*<0.05** is significant

The pregnant women were found younger than non-pregnant group of women and mean age difference was found statistically significant (30.19 ± 4.1 ranging 21-38 years versus 31.54 ± 3.9 ranging 23-38; ***P* = 0.037***). Mean BMI was 24.75±3.3 and 25.08±4.1; *P* = 0.571 between pregnant and non-pregnant group of women respectively and was not statistically significant.

The mean duration of infertility in pregnant group was 6.84±4.1 (range 01-20 years) and was 7.91±3.9 (range 01-16 years) in non-pregnant group of women. The duration of infertility was marginally lower in pregnant group as compared to non-pregnant group but was found statistically non-significant *P*=0.088.

The pregnant and non-pregnant group of women were analyzed for distribution of type of infertility**.** Primary infertility was present in 70.7% (53) and secondary infertility was present in 29.3% (22) in pregnant group of women whereas in non-pregnant group 59.1% (55) women had primary infertility and 40.9% (38) women had secondary infertility (*P*=0.121). The distribution of different etiology among the women in pregnant and non-pregnant group were also compared and shown in **Table 2.**

Table 2: Distribution of etiology among pregnant and non-pregnant group of women.

Sl. No.	Etiology	Pregnant (n=75; Group-A)	Non-Pregnant (n=93; Group-B)	Total (n=168)	P Value
1.	Tubal Factor	32 (42.7%)	44 (47.3%)	76 (45.2%)	0.635
2.	Male Factor	15 (20%)	09 (9.7%)	24 (14.3%)	0.261
3.	Ovulatory Dysfunction (PCOS)	04 (5.3%)	04 (4.3%)	08 (4.8%)	0.831
4.	Unexplained	13 (17.3%)	21 (22.6%)	34 (20.2%)	0.481
5.	Endocrinal Factor	03 (4%)	03 (3.2%)	06 (3.6%)	0.832
6.	Endometriosis	05 (6.7%)	05 (5.4%)	10 (5.9%)	0.832
7.	Diminished Ovarian Reserve	03 (4%)	07 (7.5%)	10 (5.9%)	0.531
	Total	75 (100%)	93 (100%)	168 (100%)	

In pregnant group, 15 (20%) women were found to have male factor infertility, 32 (42.7%) women had tubal factor, 04 (5.3%) women were diagnosed with ovulatory dysfunction (poly-cystic ovary syndrome), 13 (17.3%) women had unexplained infertility, 03 (4%) women were with endocrinal disorders, 05 (7.31%) women had endometriosis, and 03 (6.09%) women were found to have diminished ovarian reserve (DOR).

Similarly, the distributions of various etiology factors among 93 non-pregnant women (Group-B) were also analyzed. In Group-B, 09 (9.7%) women were found to have male factor infertility, 44 (47.3%) women had tubal factor infertility, 04 (4.3%) women were Ovulatory dysfunction, 21 (22.6%) women were with unexplained infertility, 03 (3.2%) women had endocrinal disorders, 05 (5.4%) women were with endometriosis and 07 (7.5%) women had DOR. It was observed that during the study period, women with tubal factor contributed to major proportion of patients followed by unexplained infertility as shown in Table-2.

No significant difference was observed when comparisons of etiology were carried out between pregnant and non-pregnant group. Various characteristics of cycles, based on ovarian stimulation protocol, were compared between pregnant and non-pregnant groups.

The characteristics of the agonist and antagonist cycles were compared in terms of endometrial thickness on day of trigger, number of retrieved oocytes, number of fertilized oocytes, number of embryo transferred to uterine cavity and number embryos cryo-preserved (vitrified). The results are summarized in **Table 3**.

Table 3: Comparison of parameters of cycle characteristics between pregnant and non-pregnant group of women underwent agonist and antagonist cycles

Type of Ovarian stimulation Protocol	Group	ET on day of trigger (mm) Mean±SD	Number of oocytes retrieved Mean±SD	Number of Fertilized Oocytes Mean±SD	Number of embryos Transferred Mean±SD
Agonist (n=127)	Pregnant (n=58)	9.37±2.3	11.92±3.1	8.44±5.2	1.89±2.3
	Non-pregnant (n=69)	9.8±1.4	11.27±6.7	8.34±4.9	1.79±0.31
	P value	0.454	0.320	0.928	0.231
Antagonist (n=41)	Pregnant (n=17)	8.27±2.9	9.27±5.6	6.63±4.1	1.92±0.3
	Non-pregnant (n=24)	8.24±3.1	8.92±4.2	7.12±3.9	1.86±0.6
	P value	0.848	0.530	0.830	0.314

***P*<0.05** is significant

On comparison, no significant differences were observed in endometrial thickness, number of oocytes retrieved and number of transferred embryos, between the pregnant and non pregnant women underwent agonist and antagonist of ovarian stimulation as shown in table-3

The distribution of pregnant and non pregnant women was made on the basis of detected levels of the IL-6 is shown in **Table 4.**

Table 4: Pregnant and non-pregnant group of women with detected and undetected levels of ovarian interleukin-6-

Type of Interleukin	No. of FF samples for IL-6 Levels (n)	Group of women with detected IL levels		Group of women with undetected IL levels	
		Pregnant group	Non-pregnant group	Pregnant group	Non-pregnant group
IL-6	168 (100%)	50 (29.76%)	64 (38.09%)	25 (14.88%)	29 (17.26%)
		114 (67.85%)		54 (32.15%)	

On comparison, the median values of detected levels of IL-6 were found significantly lower in pregnant group as compared to non-pregnant group of women as dipicted in **Table-5**.

Table 5: Comparison of the levels of ovarian interleukin-6 between pregnant and non-pregnant group of women:

Sl. No.	Type of Interleukin (women with detected IL levels)	Pregnant women Median pg/mL (Min-Max) n	Non-Pregnant women Median pg/mL (Min-Max) n	*P* value
1	IL-6 n(114)	33.9 (0.1-199.8) n=50	92.9 (0.3-199.8) n=64	<0.001

P<**0.05** is significant

The median level of IL-6 was found lower in group-A as compared to women in group-B [33.9 pg/mL (0.1-199.8) versus 92.9 pg/mL (0.3-199.8); ***P*<0.001** respectively]. (**Table-5**).

Role of interleukin-6 in prediction of successful pregnancy outcome:

The significant levels of IL-6 were analyzed through receiver operating characteristics (ROC) curve. ROC curve was plotted to depict area under the curve (AUC) and cut points, which are shown in **Figure-1**.

Figure 1: ROC curve of the levels of IL-6 in pregnant group of women

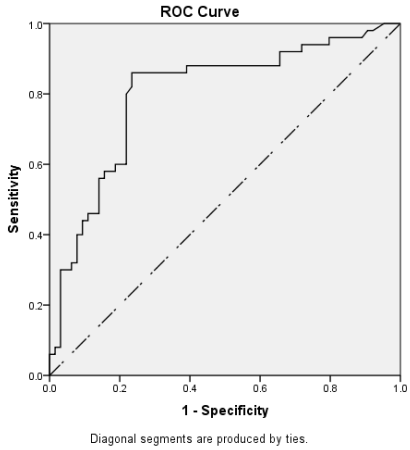

Figure 1: IL-6- Cut Point; <56.4, Area Under Curve; .800 (80.0%)

The sensitivity, specificity and AUC with cut-off values were calculated for IL-6 are summarized in **Table-6**.

Table 6: Cut-point values with sensitivity, specificity and AUC for the levels of ovarian interleukins-

S. No.	Type of Interleukins	Cut-off Values (pg/mL)	Sensitivity	Specificity	AUC
1	**IL-6 (n=114)**	<56.4	78.9%	80.0%	.800 (80.0%)

IL-6 cut-point level was <56.4pg/mL (78.9% sensitivity, 80% specificity and AUC 80%), can predict the positive pregnancy outcome. As per ROC analysis, almost 79% of the women with 80% specificity may achieve their pregnancies, if IL-6 levels remain nearly ≤56.4pg/mL as shown in **Table-6**.

Logistic regression test was applied to predict the likelihood of the pregnancy in relation to the variables those were found significant on compared between pregnant and non-pregnant group of women.

Table 7: Univariate and multivariate logistic regression for age and IL-6 to predict likelihood of the pregnancy-

Variable	Groups of women		*P* Value	Logistic regression		*P* Value
	Pregnant	Non-pregnant		Univariate or (at 95% CI)	Multivariate (Adjusted) Or (at 95% CI)	
Age (years) Mean±SD (Min-Max)	30.19±4.1 (21-38)	31.54±3.9 (23-38)	0.033	0.92 (0.85-0.99)	0.93 (0.87-0.99)	0.33
IL-6 pg/mL Median (Min-Max)	33.9 (0.1-199.8)	92.9 (0.3-199.8)	<0.001	0.98 (0.97-0.99)	0.98 (0.97-0.99)	0.03

P<0.05 is significant

The likelihood of the pregnancy with odd-ratio (OR) at 95% confidence interval (CI) was analyzed by using enter method. Univariate logistic regression of age (OR-0.92;CI 0.85-0.99 ***P*=0.033**) and IL-6 (OR-0.98; CI 0.97-0.99; ***P*<0.001**) was found significantly associated with successful implantation.

Multivariate logistic regression was used and adjusted for age and IL-6. The lower levels of IL-6 (OR-0.98; CI 0.97-0.99 ***P*=0.030**) were found associated with successful pregnancy outcome with OR at 95% CI. It was observed that per unit increase of IL-16 levels to 56.4 pg/mL will lead to reduction in successful implantation by 1.5% which was found as independently associated significant factor. It was found that the concentration of IL-6 with cut-off values (<56.4 pg/mL) with 78.9% sensitivity and 80% specificity can alone predict the likelihood of pregnancy to 80.0% as dipicted in **Table-7**.

IL-6 quantitative levels were independently found associated with the successful pregnancy outcome in ART cycles. In the present report, contributions of the pleiotropic cytokine (IL-6) were evaluated in respect to successful implantation and implantation failure in women underwent ART cycles. The biochemical measurement of concentrations of ovarian interleukins and to examine them, as biomarkers, exists in follicular fluid samples for determinations of possible association with likelihood of successful pregnancy were studied. Particularly, the quantitative levels using multiplex assay of IL-6 was tested as follicular fluid markers of successful implantation in a cohort of 168 women underwent IVF/ICSI-ET cycles.

Several investigators conducted studies for the detection of various cytokine, chemokines and interleukins in blood samples (serum and plasma) and hFF of the women underwent IVF-ET cycles including one study documented in Indian scenario in which detection of the quantitative levels of the multiplex cytokine and chemokine profile. It was observed that patients who underwent agonist (n=23) protocol of ovarian stimulation had higher levels of IL-3 and IL-12B (p70 sub-unit) and vascular endothelial growth factor (VEGF) along with lower number of oocyte in M-II stage as compared to antagonist cycle (n=22) for group of women who were shown normal response to ovarian stimulation [30].

The contributions of the immune system to ovarian function are now well understood along with the role of gonadotrophins and other intragonadal mediators [31], [32], [33], [11], [21]. Abnormal levels of cytokine may effect and play roles in pathological conditions as diverse as endometriosis, premature ovarian failure and ovarian epithelial malignancies. The interleukins were not only assessed in stimulated cycles but in non-stimulated cycles also. Those studies were suggested that folliculogenesis is regulated by the cytokines chemokines and interleukins [13].

The objective of present study was to investigate the role of cytokines (IL-6) with successful implantation in IVF cycles. In our study concentrations of IL-6 in hFF samples were significantly lower in pregnant group than non-pregnant group of women whereas findings of Bedaiwy M. et al., contradicted our results for the concentrations of IL-6 that were significantly higher in pregnant as compared to non-pregnant group of women (P=0.0005) [35]. However, Observations of Altun T. et al., regarding IL-6 levels were found similar to our study, as women achieved their pregnancies when lower concentration of IL-6 in follicular fluid was detected whereas higher IL-6 levels were found detrimental to successful IVF-ET outcome and was also evocative that FF-IL6 levels did not exhibit any correlation with oocyte yield, nor with embryo parameters. They speculate that endometrial receptivity rather than ovarian response may be the site of detrimental influences of high FF IL-6 levels [9].

Metabolomics of the FF is dynamic quantitative assessment in for all low molecular weight substances that are present in it at a given time. Being the end products of cellular metabolism, low-molecular weight metabolites can reveal the response of follicle to all influences affecting its development [36].

Follicular fluid is a superfluous, abundant and easily available biological material in assisted reproduction cycles and consider as an optimal source to predict association of non-invasive pleiotropic markers of oocyte quality, hormonal profile and successful pregnancy outcome of treatment cycles. Metabolites represent the final products of cell regulatory processes; it is now possible to profile the metabolites secreted by the oocyte into the surrounding medium by investigating the FF or, alternatively, the compounds secreted by the oocyte into the culture medium in which it is suspended during in vitro culture after retrieval ("exometabolomics" or "secretomics"). The overall metabolic profiling of FF or of oocyte culture supernatants could be more useful than the targeted metabolic approach or the study of a selective class of substances for the assessment of oocyte quality.

We analysed only one molecule (IL-6) related to immuno-ovarian functions and observed very interesting finding to continue this type of study in future also to assess metabolomics and transcriptomics loom of the successful implantation as well as implantation failure in women undergoing assisted reproduction.

The contradictory results in relation to levels of ovarian interleukins are suggestive of conducting study with large number of sample size for better prediction and association of the levels of interleukins with successful implantation and/or implantation failure. Most studies were designed to find a good predictor marker in hFF for oocyte quality were mainly correlative and not performed on large-scale, prospective and well controlled basis. Furthermore, most of the studies analyze the correlation between specific molecules and the oocyte performance using univariate, and not multivariate, analysis algorhythms. Similar statistical tests were also applied in present study to find out the molecule or factor highly predictive and associated with the successful implantation. As a consequence, until now no single substance has been identified as a reliable marker of oocyte competence to fertilization, embryo development and pregnancy [35], [36], [9], [13].

In ART program, the predictive potential of each and every marker as assessed for successful implantation and optimum results in terms of live births are need to be evaluate precisely with the help of more and more comparative studies to be conducted for validation of existing findings, including observations of present work.

6. Conclusion

The age was found most important as significant factor in positive pregnancy outcome in present study too. The women in successful implantation group were younger than failure group. The mean age was found statistically significant on comparison.

The median levels of IL-6 were significantly lower in pregnant women as compared to non-pregnant group of women. The levels of IL6 were found associated with

successful implantation and also have their role as reliable predictive marker. Quantitative levels of IL-6 were found to have very important and independent associations with successful pregnancy outcome in ART cycles.

7. Future Scope

In present study emphasis was given to detection and measurements of the limited to only one ovarian interleukin i.e, IL-6 in follicular fluid samples to find out its possible association. Although levels of various cytokines, chemokines and growth factors in serum and plasma samples of such type of patients/infertile women, were also carried out to assess their role in relation to oogenesis, maturity of oocytes, embryo quality and pregnancy outcome by different investigator groups [13], [36], [25], [26].

Role of interleukins in the local processes of ovary is still poorly understood, off course there are evidences for their involvement in steroidogenesis, in ovulation and maturation of oocyte. The physiology of normal ovarian function is paramount in relation to reproductive age and various immune-mechanisms [28].

The present study confronted with supportive as well as contradictory findings when compared with various studies in existing literature. The possible reason of that may be endocrinal milieu, different etiologies, number of subjects (sample size) analyzed, response of women to gonadotropins therapy, and inter and intra-assay approach for estimation of the levels of IL-6 as discussed over [9], [35].

Observations of our study lead this research for evaluating the molecular aspect of ovarian interleukins in successful implantation and or implantation failure. Authors suggest and encourage other investigators for conducting similar prospective multi-centric studies on large population size to validate the results and observations of present study.

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2.	JRF (INSA-Project)	IMS,BHU,Varanasi (U.P.)	01.08.1983	09.01.1985
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