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RESEARCH ARTICLE

Correlation of LIF and Glycodeline an in Prediction of Embryo Implantation of Infertile Women with and without PCOS

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Abstract

Implantation of the blastocysts into the maternal uterus is a crucial step in mammalian reproduction, which is controlled by a number of complex molecules like hormones, cytokines, and growth factors and their cross talk. A network of these molecules plays a crucial role in preparing receptive endometrial and blastocysts. This study aimed to found out the role of Glycodeline A,LIF gene expression, concentration, in the endometrial that may interfere with implantation process of polycystic ovary syndrome (PCOS) and non-PCOS women, A convenient blood sample of 80 infertile women undergoing *in vitro* fertilization (IVF) program were intentionally divided according to the cause of infertility into 40 healthy women their husbands complaining from male infertility factors, and 40 infertile women with polycystic ovary syndrome. Glycodeline A, LIF were measured on ovulatory and luteal phase of cycle (CD14-CD16,17) at the day of ovarian pickup and embryo transfer by using quantitive polymerase chain reaction (qPCR) and Elisa technique , Results of the present study showed that The gene expression of *PAEP* ,LIF in addition to levels of serum(Glycodeline A, LIF) were more valuable in predicting the pregnancy out come in infertile PCOS women than in non-PCOS women This result can be utilized to be used as predictors of implantation window for successful implantation and pregnancy.

Keywords: *Implantation, Glycodeline A, PCOS, IVF program, LIF.*

Introduction

Infertility is defined as the failure to achieve a clinical pregnancy after 12 months or more of regularly unprotected sexual intercourse [1], The infertility is either primary when never the couple having had a live birth or secondary infertility which is failure to realize a live birth after having had alive birth or abortion [2], The cause may be related to a problem with the man, woman or both [3],

In females, one of the most infertility problem is PCOS which described as endocrine disorder that may associated with hyper-androgenism and chronic an ovulation [4], failure of PCOS treatment may solute by *in* vitro fertilization (IVF) programs Implantation can occur during only a very short time period, known as the "window of implantation, During this window, the embryo fuses itself to the endometrium, giving it access to the maternal blood supply. This process is enhanced by many markers and factors [5] .Implantation failure is related to either maternal factors or embryonic causes. Maternal factors include uterine anatomic abnormalities, thrombophilia, non-receptive endometrium and immunological factors [6].

Many factors may interfere with implantation process. Progesterone associated endometrial protein(*PAEP*) is one of these factors which is a glycoprotein (Glycodeline A) that plays an important role in implantation that belongs to the lipocalin superfamily, Glycodelin-A, a progesterone-regulated glycoprotein, is highly expressed during the secretory phase in the human endometrium.

The concentration of Gd A gradually increases in the endometrial glands 4 to 5 days after ovulation and reaches its peak on day 10, coinciding with the implantation window [7], Leukaemia inhibitory factor (LIF) is another factor which is a glycoprotein that plays an important role in implantation, but also has a variety of functions in different organ systems [8]. LIF was first identified from its ability to induce differentiation of myeloid leukemia cells into macrophage-like cells, but LIF is in fact produced and secreted by a variety of cell types, including epithelial and stromal cells in the endometrium [9].

Repeated implantation failure (RIF) is determined when embryos of good quality fail to implant following several in vitro fertilization (IVF) treatment cycles. Implantation failure is related to either maternal factors or embryonic causes. Maternal factors include uterine anatomic abnormalities, thrombophilia, non-receptive endometrium and immunological factors [10].

Subjects, Materials and Methods

Patients

Eighty infertile women undergoing IVF program were selected from Kamal Al-Samaria IVF Hospital, Ministry of Health in Baghdad-Iraq and involved in this prospective study through the period from February 2017 to February 2018. Convenient blood sample of 80 infertile women undergoing IVF program were intentionally divided according to the cause of infertility into 40 healthy women their husbands complaining from male infertility factors, and 40 infertile women complaining from polycystic ovary syndrome. Blood samples were taken from the patients in oocyte pick up and embryo transfer.

The *PAEP* and LIF were measured on ovulatory and luteal phase of mensrual cycle (CD14-CD16, 17) at the day of ovarian pickup and embryo transfer, respectively. Every participant woman was interviewed and asked to answer information including hormones, age, and type of infertility and duration of infertility. Venous blood samples (6ml) were collected from each woman for both groups. Each blood sample was divided into two tubes:

- EDTA tubes for molecular studies.
- Gel plain tube for serological test: the serum obtained by putting the blood samples in gel plain tube, the tubes centrifuged at 5000rpm for five minutes, serum was collected and kept in freezer until used.

RNA Extraction

RNA was extracted from collected blood samples by using AccuZol™ kit Reagent Applied Bio system\ USA [11]. A total RNA 10pg (18 µl) was reversely transcribed to a complementary DNA (c DNA) by using Accu Power ᴿ Rocket Scrript ™ RT Premix kit (Bioneer Company). The procedure was carried out in a reaction volume of 20µl according to the manufacturer with modifications, PCR Program for CDNA synthesis program, as follow Primer annealing 30 ºC for10 minutes, CDNA synthesis 42 ºC for 30minutes, heat inactivation 95 ºC for 5minute.

Quantitative Real Time PCR (qRT–PCR)

The expression level of *LIF* gene was performed by Two Step RT- QPCR to confirm the expression of target gene, quantitative real time qRT-PCR SYBR Green assay was used. This assay was performed using a syber green master mix (Go Tag q PCR Master Mix, Promega, USA), In 10 µl reaction volume in (Table 1).

Table 1: Thermal Cycling Protocol al Cycling Protocol

Steps	°C	m:s	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:15	45
Annealing	55	00:30	
Extension	72	00:30	

Primers used for Quantitative Real Time PCR

Primers used for quantitative real time PCR are listed in table (2). Primers for used for *CD62L* (ID: 6402) *LIF* (ID: 3976), primer was designed according to National Center for Biotechnology Information (NCBI) http://www.ncbi.nlm.nih.gov/genbank, and stored lyophilized at (-20°C). (Table2).

Table 2: Primers of CD62L, LIF used for Quantitative PCR

Primer Name (*CD62L*)	**Seq.**
Forward primer	5'-CTTCTTCAGCCACCTCTCTTT-3`
Reverse primer	5'-CGCAGGCTATTTCTCTCTTCTC-3`
Primer Name (*LIF*)	**Seq.**
Forward primer	5'-CCAACAACCTGGACAAGCTA-3'
Reverse primer	5'-GGGGTTGAGGATCTTCTGGT-3`

Gene Expression Calculation

Standard Curve: stander curve was performed as following as described by [12]:

Eleven of 0.2 ml tube prepared, 90 µl of Nuclease Free Water was added to each tube then made a serial dilution by added 10 µl from sample of 41*1010 1/ µl copy No. to the first tube and made a serial dilution by transferred 10 µl from first tube to second tube and so on. The standard curve reaction started from the third tube (41*108 1/ µl copy No.) to the tube number eleven (41 1/ µl copy No.).

ELISA Assay

Measurement the concentration of Glycodeline A (ng/ml*),* LIF (ng/ml), serum level by using Enzyme-linked immunosorbent assay Elisa kit (cusabio, catalog number CSB-E-12071h)

Statistical Analysis

The Data were analyzed using SPSS statistical package for Social Sciences (version 20.0 for windows, SPSS, Chicago, IL, USA).All values were expressed as mean ± standard deviation. Independent samples t-test was used to compare between means of the studied groups. Qualitative relations were evaluated using Chi-square test a *p* value < 0.05 was considered as statistically significant for all analyzed data [13].

Results and Discussion

Glycodeline a Concentration in PCOS vs. non PCOS Women in oocytes Picks up Stage and Embryo Transfer

The mean of Glycodelin A in the serum of pregnant and non- pregnant of two groups at the time of oocyte pick up and embryo transfer (shown in Table 3 and Table 4). There was no significant (P=0.35) increase in the mean of Glycodelin A at the time of oocyte pick up in the pregnant of PCOS group compared to non- pregnant (0.095±0.01, 0.08±0.007 respectively) and non –PCOS pregnant women (0.114± 0.04) and the p value was 0.23.

No Significant (p= 0.54) increase in the mean of Glycodelin A of pregnant PCOS women was noticed compare to non- pregnant PCOS women .The level of Glycodelin A in the serum of pregnant PCOS at the time of oocytes pick up was shown significant (p=0.03) differences compared to non- PCOS women of the corresponding group as shown (Table 3). On the other hand, the mean of Glycodelin A in the serum of pregnant women of PCOS at the time of embryo transfer was high compared with non-pregnant women (0.088±0.005, 085±0.006, respectively).

However, statistically no significant (p=0.54) differences was observed between them. There was no significant (p=0.42) differences in the level of Glycodelin A in pregnant PCOS (0.088±0.005) and pregnant non-PCOS groups (0.091±0.007).The level of Glycodelin A in the serum of pregnant non-PCOS at the time of ET was shown no significant (p=0.36) differences compared to non-pregnant women of the corresponding group as shown in (Table 4) [28].

who reported the Glycodelin A is a factor Immunomodulatory that leads to successful implantation and leads to pregnant, the immune system plays an important role, immunomodulaty factors such as a Glycodelin A needed for formation of receptive endometrium and placenta identification this factor has led to their use as marker of implantation that may identify defect causing sub fertility, many studies reported that Abnormal levels of glycodelin-A in the endometrium, uterine flushings, and/or maternal serum correlate with unexplained infertility, early pregnancy loss, and recurrent miscarriage.[14,:15; 16; 17].

And the role of glycodelin-A in placental development and fetomaternal tolerance in early pregnancy It has been proposed that glycodelin egg/preembryo because of its ability to suppress the activity of natural killer cells in [14, 15].

Table 3: Glycodeline A concentration in PCOS vs. non PCOS women in oocytes picks up stage

Parameter	Pregnancy state	Groups				*P* value
		PCOS		Non PCOS		
		Mean	SD	Mean	SD	
Glycodeline A concentration (ng/ml) (at time of oocyte pick up)	Pregnant	0.095	0.01	0.114	0.04	0.35
	Non pregnant	0.08	0.007	0.085	0.013	0.522
	P value	0.03*		0.23		

*Analyses were performed by Independent samples t-test

Table 4: Glycodeline A concentration in PCOS vs. non PCOS women in embryo transfer stage

Parameter	Pregnancy state	Groups				P value
		PCOS		Non PCOS		
		Mean	SD	Mean	SD	
Glycodeline A concentration (ng/ml) (at time of embryo transfer)	Pregnant	0.088	0.005	0.091	0.007	**0.42**
	Non pregnant	0.085	0.006	0.086	0.007	0.79
	P value	0.54		0.36		

* Analyses were performed by: - Independent samples t-test

LIF Concentration in PCOS vs. non PCOS Women in oocytes Picks up Stage and Embryo Transfer

The mean of LIF in the serum of pregnant and non –pregnant of two groups at the time of oocyte pick up and embryo transfer (shown in Tables 5 and 6).There was a significant (p=0.044) increase in the mean of LIF at the time of oocyte pick up in the pregnant of PCOS group compared to non-pregnant (0.158±0.028, 0.14±0.02 respectively) and non PCOS pregnant women (0.219±0.037) compare to non-pregnant (0.166±0.04) and the P value was a significant (p=0.044) increase in the mean of LIF concentration of pregnant non-PCOS women was noticed compare to PCOS women. On other hand, the mean of LIF in the serum of pregnant women of PCOS at the time of embryo transfer was highly compared with non-pregnant women (0.195±0.02, 0.164±0.029 respectively), however, statistically no significant (P=0.092) differences was observed between them, there was no significant (P=0.43) difference in the concentration of LIF in pregnant PCOS (0.195±0.02) and pregnant non-PCOS groups (0.226±0.08). The concentration of LIF in the pregnant non-PCOS at the time of ET was shown no significant (P=0.95) differences compared to non-pregnant women of the corresponding group as shown in Table 6. LIF production measure endometrial cultures from idiopathic female factor infertile women are reduced compared with fertile women [18]. Similar to the results of current study, it has been found that LIF can also be detected in uterine flushing, and its level is significantly lower in women with unexplained infertility [19]. Endometrium of infertile women produces significantly less LIF during the period of receptivity [20].

This results explain LIF plays a central role in the control of implantation and when the gene lacking function their blastocysts fail to implant and do not give rise to the development of clinical gestation [20]. *LIF* plays a critical role in the process of blastocyst implantation. Therefore, the aberrant LIF production is linked to implantation failure [19]. The same observation was noticed by [21] when reported that LIF concentrations were lowered in both serum and follicular fluid of infertile compared with the healthy one.

Table 5: LIF concentration in PCOS vs. non PCOS women in oocytes picks up stage

Parameter	Pregnancy status	Group				P value
		PCOS		Non PCOS		
		Mean	SD	Mean	SD	
LIF concentration at time of oocyte pick up	Pregnant	0.158	0.028	0.219	0.037	0.044*
	Non pregnant	0.14	0.02	0.166	0.04	0.35
	Pvalue	0.46		0.09		

* P<0.05 *= Significant Analyses were performed by: - Independent samples t-test

Table 6: LIF concentration in PCOS vs. non PCOS women at time of embryo transfer

Parameter	Pregnancy Status	Group				P value
		PCOS		Non PCOS		
		Mean	SD	Mean	SD	
LIF concentration at time of embryo transfer	**Pregnant**	0.195	0.02	0.226	0.08	0.43
	Non pregnant	0.164	0.029	0.223	0.04	0.057
	P value	0.092		0.95		

* Analyses were performed by Independent samples t-test

Comparison of *CD62L* in Pregnant PCOS and non PCOS Women at the Time of Oocyte Pick up and Embryo Transfer

The Mean of CD62L of pregnant and non-pregnant of the two groups in oocytes picks up shown in table 7. There was a no significant (p=0.026) decrease in the mean of CD62L at the time of oocyte pick up in the pregnant of PCOS group compared to non-pregnant women (100.4±70, 1066.6±483, respectively) and non PCOS pregnant women (627.9±316) compared to non-pregnant (2355±746) and the P value was 0.15 (Figure 4.7).

However there was no significant (p=0.53) decrease in the mean of CD 62L expression of pregnant PCOS women was noticed compared to non –PCOS women as shown in (Table 7). On the other hand, the mean of CD62L of pregnant women of PCOS at the time of embryo transfer was low compared with non- pregnant women (476.5±250, 2718±1150, respectively). However, statistically no significant (p=0.115) differences was noticed between them. There was no significant (p=0.53) differences in the level of CD62L in pregnant PCOS (476.5±250) and pregnant non-PCOS groups (313.7±199).The level of CD 62 L of pregnant non-PCOS at the time of ET was shown no significant (p=0.26) differences compared to non-pregnant women of the corresponding group as shown in (Table 8).

Because PAEP gene was expressed just in endometrial, thus it will not find real RNA level in blood, although the find product protein i.e. Glycodelin A, can identify in blood. Therefore, it can proposed that the main function of this gene may be inhibition of immune cells such as natural killer cells that attack the embryo in endometrial and made failure of implantation these cells called PNKC (CD56 bright) [22; 23].

It's have subset CD26L so that the reason of measurement the expression the CD26L was to identify the expression of PAEP gene. It's the reverse relationship between PAEP and CD 62L.It has been known that Glycodelin A is a progesterone-induced endometrial glycoprotein which has been amply documented to play a role in down-modulation of the maternal immune response to fetal allo-antigens and to be indispensable for the maintenance and progression of pregnancy. The effect of glycodelin on T cells, Glycodelin-induced apoptosis in activated T cells occurs his effect of glycodelin on the cells of the innate immune system, namely monocytes and NK cells.

The present study found that glycodelin A induced apoptosis in monocytic cells before their differentiation to macrophages, Glycodelin induced apoptosis in NK cells. Natural killer cells constitute 50–90% of lymphocytes in human uterine decidua in early pregnancy. This results agree with other studies [22, 23, 24] .

Consequently ,the present data means that the PAEP gene is high level in pregnant group than non- pregnant in both studied groups when the CD62L was high expression in non-pregnant compare to pregnant that's mean my gene expression in this group low and cannot inhibition the NKC leading to failure of implantation and results in non- pregnant.

The corresponding group which the expression of CD62L in pregnant with low level compare with non-pregnant so that PAEP gene expression is high and can inhibit the NKC leading to successful implantation then a pregnant. Successful pregnancy depends largely on adequate placentation and maternal tolerance of the fetus. Glycodelin-A is a glycoprotein abundant in the decidua during early pregnancy. It plays an important role in placental development and feto-maternal defense [9].

Table 7: *CD62L* expression in pregnant and non-pregnant women complaining from PCOS and non-PCOS in oocyte pick up

Parameter	Pregnancy state	Groups				*P* value
		PCOS		Non PCOS		
		Mean	SD	Mean	SD	
CD62L gene expression (at time of oocyte pick up)	Pregnant	100.4	70	627.9	316	0.095
	Non pregnant	1066.6	483	2355	746	0.203
	P value	0.026		0.15		

Table 8: *CD62L* expression in pregnant and non-pregnant women complaining from PCOS and non-PCOS in embryo transfer

Parameter	Pregnancy state	Groups				*P* value
		PCOS		Non PCOS		
		Mean	SD	Mean	SD	
CD62L gene expression (at time of embryo transfer)	Pregnant	476.5	250	313.7	199	0.53
	Non pregnant	2718	1150	1056	500	0.257
	P value	0.115		0.26		

LIF Expression in PCOS and non-PCOS Women at the Time of oocytes Pick up and Embryo Transfer

The mean of LIF expression of pregnant and non –pregnant of two groups at the time of oocyte pick up (shown in Tables 9 and 10). There was no a significant (p=0.061) increase in the mean of LIF at the time of oocyte pick up in the pregnant of PCOS group compared to non-pregnant (24.79±4.32, 23.95±1.80 respectively) and non PCOS pregnant women (25.90±1.67) compare to non-pregnant (23.71±2.68) and the P Value was no significant (p=0.061) increase in the mean of

LIF expression of pregnant non-PCOS women was noticed compare to PCOS women (The expression of LIF in the pregnant non-PCOS at the oocyte pick up was shown significant (P=0.045) differences compared to non-pregnant women of the corresponding group as shown in Table 9 On other hand, the mean of LIF expression of pregnant women of PCOS at the time of embryo transfer was highly compared with non-pregnant women (24.79±4.32, 23.95±1.80 respectively). However, statically no significant (P=0.71) differences was observed between them .There was no significant (P=0.061) difference in the expression of LIF in pregnant PCOS (24.79±4.32) and pregnant non-PCOS groups (25.90±1.67). The expression of LIF in the pregnant non-PCOS at the time of ET was shown no significant (P=0.17) differences compared to non-pregnant women of the corresponding group as shown in Table 10. LIF regulates multiple processes prior to and during implantation such as uterine transformation into a receptive state, decasualization, blastocyst growth and development, embryo-endometrial interaction, trophoblast invasion, and immune modulation the same results obtained by other researchers [25, 26].

It has been noticed that the LIF may also be involved in immune tolerance through regulation of HLA-G, a class1 MHC molecule especially expressed by invasion cytotrophoblast cells [26]. The LIF secreted from the uterus is regarded an important factor in embryo implantation, and the maximal expression of LIF in endometrial is during implantation window [27, 28, 29]; therefore the LIF expression was highly level in pregnant women compared to non-pregnant.

Table 9: LIF expression in PCOS vs. non-PCOS at the time of oocyte pick up

Parameter	Pregnancy state	Groups				P value
		PCOS		Non PCOS		
		Mean	SD	Mean	SD	
LIF gene expression (at time of oocyte pick up)	Pregnant	27.72	3.99	28.14	3.13	0.86
	Non pregnant	25.14	3.27	24.15	0.54	0.521
	P value	0.297		0.045		

* P<0.05 = Significant Analyses were performed by: - Independent samples t- test

Table 10: LIF expression of PCOS vs. non-PCOS at the time of embryo transfer

Parameter	Pregnancy state	Groups				
		PCOS		Non PCOS		
		Mean	SD	Mean	SD	P value
LIF gene expression (at time of embryo transfer)	Pregnant	24.79	4.32	25.90	1.67	0.061
	Non pregnant	23.95	1.80	23.71	2.68	0.88
	P value	0.71		0.17		

* Analyses were performed by: Independent samples t-test.

Table 11: The spearman Rank correlation analysis of (non PCOS, pregnant) group

	LIF (pg/ml) (O.P.U)	LIF (pg/ml) (E.T.)	LIF gene expression (O.P.U.)	LIF gene expression (E.T.)	Glycodeline A (ng/ml) (O.P.U.)	Glycodeline A (ng/ml) (E.T.)	CD62L gene expression (O.P.U)	CD62L gene expression (E.T.)
Type of Infertility	0.236	-0.866	-0.289	-0.289	-0.444	-0.296	0.296	0.740
S. FSH (mIU/ml)	-0.949	0.60	0.20	0.00	0.718	0.051	-0.872	0.359
S.LH (mIU/ml)	-0.632	0.10	-0.50	0.30	0.667	0.205	-0.616	0.359
S.E2 (pg/ml)	0.738	0.300	0.10	0.00	0.103	-0.103	0.205	**-0.975****
Prolactin (ng/ml)	0.316	0.00	0.80	-0.80	-0.308	-0.667	0.051	0.154
S.TSH (mmol/L)	-0.316	0.60	-0.20	0.30	0.872	0.205	-0.718	-0.205
LIF (pg/ml) (O.P.U)	1	-0.632	0.211	-0.316	-0.50	-0.50	0.833	-0.50
LIF (pg/ml) (E.T.)	-0.632	1	0.60	-0.10	0.667	-0.051	-0.667	-0.462
LIF Expression (O.P.U.)	0.211	0.60	1	0.70	0.154	-0.564	-0.359	-0.154
LIF expression (E.T.)	-0.316	-0.10	0.70	1	-0.205	**0.975****	0.359	-0.103
Glycodeline A (ng/ml) (O.P.U.)	-0.50	0.667	0.154	-0.205	1	-0.289	**-0.921***	-0.158
Glycodeline A (ng/ml) (E.T.)	-0.50	-0.051	-0.564	**0.975****	-0.289	1	0.368	-0.026
CD62L expression (O.P.U)	0.833	-0.667	-0.359	0.359	**-0.921***	0.368	1	-0.132
CD62L expression (E.T.)	-0.50	-0.462	-0.154	-0.103	-0.158	-0.026	-0.132	1

Data were shown as correlation coefficient (R2). Correlation analyses were performed by Spearman Rank correlation test. * $p<0.05$; ** $p<0.01$; no asterisk: $P>0.05$.

The Spearman Rank Correlation Analysis of (non PCOS, pregnant) group

The non-parametric Spearman rank correlation coefficient is calculated for each combination of all parameters with (concentration and expression of LIF, Glycodelin A, CD62L at the oocytes pick up and embryo transfer) Correlation coefficients are between -1 and 1, with positive numbers indicating a positive correlation and the negative numbers indicating a negative correlation.

The LIF expression (E.T.) showed positive highly significant correlation with Glycodeline A (ng/ml) (E.T.) (R^2=0.975, $p<0.01$) pregnant non-PCOS group Glycodelin A is a factor Immunomodulatory that leads to successful implantation and leads to pregnant the immune system plays an important role, immunomodulaty factors such as a Glycodelin A needed for formation of receptive endometrium and placenta identification this factor has led to their use as marker of implantation that may identify defect causing sub fertility [14;15,14] who reported that Abnormal levels of glycodelin-

A in the endometrium, uterine flushings , and/or maternal serum correlate with unexplained infertility, early pregnancy loss, and recurrent miscarriage, therefore the Glycodelin A was a good correlation for pregnancy outcome, The results of the current study similar to other studied (9). On other hand The LIF expression (E.T.) showed positive correlation in pregnant non-PCOS group associated increase with Glycodelin A concentration, so that the LIF expression (E.T.) increase to pregnant groups The same observation was noticed by (30) when reported the LIF may also be involved in immune tolerance through regulation of HLA-G , a class1 MHC molecule especially expressed by invasion cytotrophoblast cells, It has been reported that the LIF secreted from the uterus is regarded an important factor in embryo implantation, and the maximal expression of LIF in endometrial is during implantation window[28].

The Glycodeline A (ng/ml) (O.P.U.) showed negative significant correlation with CD62L expression (O.P.U) (R^2=-- 0.921, $p<0.05$) in pregnant non-PCOS group. Glycodeline secretion is a known determinant of endometrium maturity, which is also an important factor in successful embryo implantation Glycodeline is a key component of endometrial secretions and its expression is regulated by progesterone [29].

The role of glycodelin-A in placental development and fetomaternal tolerance in early pregnancy It has been proposed that glycodelin egg/preembryo because of its ability to suppress the activity of natural killer cells in [9]. So that the increase of Glycodeline A leads to decrease the CD62L was the subset of PNKC (CD56 bright) that attack the embryo in endometrial and made failure of implantation so that the decrease of CD62L was good correlation for pregnancy outcome these results are similar to [23] and elevated the Glycodelin A a good correlation by inhibition NKC to success the implantation and pregnant outcome.

Table 12: The spearman Rank correlation analysis of (PCOS, pregnant) group

	LIF (pg/ml) (O.P.U)	LIF (pg/ml) (E.T.)	LIF gene expression (O.P.U.)	LIF gene expression (E.T.)	Glycodeline A (ng/ml) (O.P.U.)	Glycodeline A (ng/ml) (E.T.)	CD62L gene expression (O.P.U)	CD62L gene expression (E.T.)
Type of Infertility	0.236	-0.866	-0.289	-0.289	-0.444	-0.296	0.296	0.740
S. FSH (mIU/ml)	-0.949	0.60	0.20	0.00	0.718	0.051	-0.872	0.359
S.LH (mIU/ml)	-0.632	0.10	-0.50	0.30	0.667	0.205	-0.616	0.359
S.E2 (pg/ml)	0.738	0.300	0.10	0.00	0.103	-0.103	0.205	-0.975**
Prolactin (ng/ml)	0.316	0.00	0.80	-0.80	-0.308	-0.667	0.051	0.154
S.TSH (mmol/L)	-0.316	0.60	-0.20	0.30	0.872	0.205	-0.718	-0.205
LIF (pg/ml) (O.P.U)	1	-0.632	0.211	-0.316	-0.50	-0.50	0.833	-0.50
LIF (pg/ml) (E.T.)	-0.632	1	0.60	-0.10	0.667	-0.051	-0.667	-0.462
LIF Expression (O.P.U.)	0.211	0.60	1	0.70	0.154	-0.564	-0.359	-0.154
LIF expression (E.T.)	-0.316	-0.10	0.70	1	-0.205	0.975**	0.359	-0.103
Glycodeline A (ng/ml) (O.P.U.)	-0.50	0.667	0.154	-0.205	1	-0.289	-0.921*	-0.158
Glycodeline A (ng/ml) (E.T.)	-0.50	-0.051	-0.564	0.975**	-0.289	1	0.368	-0.026
CD62L expression (O.P.U)	0.833	-0.667	-0.359	0.359	-0.921*	0.368	1	-0.132
CD62L expression (E.T.)	-0.50	-0.462	-0.154	-0.103	-0.158	-0.026	-0.132	1

Data were shown as correlation coefficient (R2). Correlation analysis were performed by Spearman Rank correlation test * $p<0.05$; **$p<0.01$; no asterisk: $P>0.05$.

The Spearman Rank Correlation Analysis of (PCOS, Pregnant) Group

The CD62Lwas the subset of PNKC (CD56 bright) that attack the embryo in endometrial and made failure of implantation so that the decrease of CD62L was good correlation for pregnancy outcome these results are similar to [23; 30].LIF arise (pg/ml) in (E.T.) concentration that LIF can also be detected in uterine flushing, and its level is significantly lower in women with unexplained infertility [17].

Endometrium of infertile women produces significantly less LIF during the period of receptivity. [18], this result explain LIF plays a central role in the control of implantation LIF expression (E.T.) showed negative significant correlation with CD62L expression (O.P.U) in (PCOS, pregnant group). (R^2=0.975, $p<0.05$) that a good correlation for increase the LIF expression in The same observation was noticed by [31] when reported the LIF may also be involved in immune tolerance through regulation of HLA-G , a class1 MHC molecule especially expressed by invasion cytotrophoblast cells,

It has been reported that the LIF secreted from the uterus is regarded an important factor in embryo implantation, and the maximal expression of LIF in endometrial is during implantation window[29],on other hand the correlated showed decrease the CD62L expression (O.P.U) in pregnant women ,the CD62Lwas the subset of PNKC(CD56 bright) that attack the embryo in endometrial and made failure of implantation so that the decrease of CD62L was good correlation for pregnancy outcome these results are similar to [30;31].

It is concluded that PAEP gene was expressed just in endometrium, but not detected in real RNA level of blood. Although, the Glycodelin A, was identify in blood. The study found that PNKC (CD56 bright) have subset CD26L.which is resemble Natural killer cells that attack the embryo in endometrium and can cause failure of implantation therefore Glycodelin A and LIF found to be markers plays a role in the prediction of successful pregnancy ,depends largely on its adequate amount in maternal side. At the same time, the gene expression of PAEAP and LIF more valuable in predicting the pregnancy out come in infertile PCOS women than in non-PCOS women.

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