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RESEARCH ARTICLE

Evaluation of Soluble L-Selectin (CD 62L)Level in Embryo Culture Media to Embryo Quality and Implantation Rate

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Abstract

Background: Although progress was made in an *in vitro* fertilization (IVF) techniques, the majority of transferred embryos fail to implant. Morphology embryo scoring is the standard procedure for most of IVF centers for choosing the best embryo, but remains limited since even the embryos classified as (top quality) may not implant. Determining molecular mechanisms of human embryo implantation is an extremely challenging task due to the limitation of materials and significant differences underlying this process among mammalian species. L-Selectin and its ligand carbohydrate have been proposed as a system that mediates initial adhesion of human blastocysts to the uterine epithelia.

Objectives: Quantitative evaluation of soluble L-Selectin molecule in an *in-vitro* culture media used for *in vitro* fertilization. Determine cut-off value of soluble L-Selectin quantity to be as qualitative predictor of successful implantation

Patients and Methods: This prospective study was undertaken in the High Institute of Infertility diagnosis and assisted Reproductive Technologies/ Al-Nahrain University/ Baghdad/ Iraq, during the period from September2017 to April 2018.A total of 74 infertile women were underwent controlled ovarian hyper stimulation for intracytoplasmic sperm injection cycle. Flexible antagonist protocol was used as ovulation induction protocol in all the cases .culture media L-Selectin level were measured on the day of embryo transfer by using Enzyme linked immune sorbent assay (ELISA) for all cases . Comparison in culture media L-Selectin was done to all cases.

Results: There was no significant difference between pregnant and non-pregnant groups in age , BMI, and level of basal hormone FSH, LH , AMH and E2 in the day of hCG injection, and significant difference in the level of L-Selectin in pregnant compared to non-pregnant groups.

Conclusion: Level of L-Selectin concentration in embryo culture media can be used as predictor of ICSI success

Keywords: L-Selectin,Culture Media,Embryo,Quality,Implantation Rate.

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Introduction:

Blastocyst implantation involves a complex, chemically and temporally synchronized dialogue between an implantation competent embryo and a receptive uterus. The steps between fertilization and the initiation of implantation generally follow a well-conserved autonomous process resulting in the blastocyst, endometrial receptivity is controlled by the ovarian steroids, estrogen (E2), and progesterone (P4) (1). In human, there is a distinct window of implantation during mid-luteal L-Selectin phase, requiring an appropriately developed embryo and an adequately hormonal primed endometrium(2). in a 28 day cycle the window has been described to occur as narrowly as cycle day 21-23 (3,4) ,and as broadly as cycle day 20-24 (5,6) . recently, genbacev *et al*. (7) identify candidate molecules likely involved in the initial step of implantation , showing that L-Selectin expressed by human embryo at the hatching blastocyst stage, initiates interaction with the uttering lining. L-Selectin is a carbohydrate binding protein or lectin. Selectins, group of cell adhesion molecular, mediate early transient cell-cell interaction.genbacev *et al*.(8) described increased binding of trophoblasts to L-Selectin ligand-expressing uterine luminal epithelium and up regulation of oligosaccharide-based L-Selectin ligands in the endometrium, which was coincident with the expression of L-Selectin by trophoblast cells during the implantation window. These finding suggest that the interaction between L-Selectin expressed by trophoblast cell and its oligosaccharide-based ligands expressed by endometrium may comprise the initial attachment step in the implantation process.

Patients, Materials and Methods:

This study included 74 infertile female enrolled in assisted reproductive technology (ART) programs to enter ICSI cycle in high Institute for Infertility Diagnosis and Assisted Reproductive Technologies,Al-Nahrain University; during the period from September 2017 to April 2018.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, basal hormone level(FSH, LH,AMH) in cycle day 2 and estradiol at the day of hCG injection .Patient with age ≥41age , abnormal uterine cavity due to polyp, myoma, or congenital anomalies uncontrolled diabetes mellitus or un corrected endocrinological disorder excluded.All patients were enrolled in flexible antagonist protocol which started on day two of the menstrual cycle, ultrasound examination was performed in order to exclude those women with ovarian cyst and assess the endometrial thickness.After selection, women received recombinant human follicle simulation hormone(r-hFSH) (Gonal F®; Merck Serono S.A., Geneva, Switzerland)® containing 75IU of FSH activity per ampoules by daily subcutaneous injection. Starting dose of r-FSH was (75-300) IU daily and individualized according to the patients age,body mass index (BMI),antral follicle count, baseline E2,prolactin, FSH and LH concentration. Trans vaginal ultrasound was performed on cycle day 5 and subsequent scan were done every 2-3 day as The dose of Gonal-F® and follicle growth were monitored by serum E2 level and trans-vaginal ultrasound till the day of hCG administration, The GnRH antagonist used

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was cetrorelix acetate 0.25mg per day (Cetrotide; Merck- Serono Ltd., Aubonne, Switzerland)started when the lead follicle was ≥12 mm. Treatment with rFsh and cetrorelix acetate was continued until the day of the final oocyte maturation trigger. Ovulation was induced with 250 µg recombinant human chorionic gonadotropin α(r-hCG),(Ovidreal, Merck-Serono Ltd.,Aubonne, Switzerland) subcutaneously when at least two lead follicles have reached 18mm.

IVF Procedures and culture media collection :

Under general or spinal anesthesia Oocytes were retrieved by trans vaginal ultrasound-guided oocyte aspiration which was done by a gynecologist, approximately 34–36 hours after hCG administration.

Patient was placed in dorsal lithotomy position, vagina cleaned by copious saline irrigation. All follicles within both ovaries areaspirated by ovum aspiration needle (Cook®, Australia) and follicular fluid given directly to the embryologist to identify the retrieved cumulus-oocytes complexesInfollicular fluid immediately to isolate the oocyte from follicular. The oocyte incubation in bicarbonate buffer solution media in 37 c and 5% co2 for 2-3 hours.The ICSI procedure was performed (4-6) hours after oocyte retrieval, the cumulus corona cells are removed by enzymatic and mechanical treatment to denude the oocyte from the cumulus cells. Each oocyte is carefully assessed, noting the presence or absence of germinal vesicle or the first polar body. Only these ova that have been extrude the first polar body (metaphase II) and morphological intact were suitable for microinjection . Around 12-17 hours afterICSI procedure, fertilization was assessed for evidence of normal fertilization which was defined as the existence of two pronuclei(2PN).

In day of embryo transfer , good quality embryo transfer to the uterus after 2 (four cell embryo), 3(eight cell embryo) after oocyte retrieval, under trans abdominal ultrasound guidance using a flexible catheter (Cook-Ireland Ltd) which pass through the vagina and the cervix into the uterine cavity where the embryos is placed in order to implant.

About 1ml of culture media was obtain from the four well after embryo transfer and collected in plane tube. Culture media sample were centrifuged for 10 minute at 1500 rpm to isolate the media from oil and stored at -20 in deep freezer before analysis and use to evaluate level of L-Selectin in embryo culture media by using ELISA.

Measurement of Soluble L-Selectin:

Culture media obtained on the day of embryo transfer were estimated for L-Selectin (CD62L) Level by enzyme-linked immunosorbent assay (ELISA), technique using diagnostic kit (Human Selectin , Leukocyte (SELL) UK) That provides quantitative determination of L-Selectin concentration in embryo culture media.

Statistical Analysis:

The Statistical Analysis System- SAS (2012) program was used to evaluate different factors in study parameters. Least significant difference –LSD test (ANOVA) or T-test was used to significant compare between means. Chi-square test was used to significant comparison between percentages. Estimate of correlation coefficient between parameters was done, and evaluation of sensitivity and specificity . A P value < 0.05 was considered to be statistically significant(9).

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Results:

Seventy-four patients enrolled in ICSI cycle in this prospective study, women enrolled in the present study were categorized into two groups, the first group included women who succeeded to get pregnant (n=31) and the second group include women who failed to get pregnant (n=43). The demographic data of the pregnantand non-pregnant groups are shown in (table 1). The statistic analysis showed no significant different among two groups concerning the age , body mass index.

Table (1): Comparison between pregnant and non-pregnant in Age and BMI

Parameter	Total	Outcome		P value
		Pregnant	Non pregnant	
Age(years)	30.76±7.02	30.77±7.36	30.74±6.86	0.986^{NS}
BMI	28.79±4.78	28.96±5.47	28.66±4.29	0.796^{NS}

BMI: Body mass index , Ns= Non-significant different

Hormonal profile for all patient are shown in table 2, Comparison of basal hormonal levels FSH, LH,AMH and E2 at the day of hCG between pregnant and non pregnant groups revealed-non significant difference despite the presence of some minor differences in range of hormonal levels between the two groups (P>0.05).

Table (2): Comparison of basal hormonal levels between pregnant and non pregnant groups

Hormone	Total	Outcome		P Value
		Pregnant	Non pregnant	
FSH	5.98(4.4-7.8)	6.50(3.9-7.72)	5.90(4.4-8.6)	0.979^{NS}
LH	4.70(2.5-7.3)	3.60(2.76-6.7)	4.80(2.4-7.3)	0.925^{NS}
AMH	2.43(1.5-3.7)	1.89(1.21-4.17)	2.43(1.9-3.4)	0.515^{NS}
E2	1257.95(950-1910.6)	1191(887.9-2000)	1279.90(1000-1883.2)	0.617^{NS}

NS: Non statistically significant difference(P>0.05)

FSH: Follicular stimulation hormone, LH: Luteinizing hormone, AMH: Antimullerin-hormone, E2: estradiol hormone

The present study showed that mean level of L-Selectin molecule in culture medias was significantly higher in women who succeeded to be pregnant in comparison with the group of women who unfortunately failed to be pregnant,1.33(0.86-1.62) versus 0.91(0.74-1.16)ng/ml, (p=0.016) as shown in table 3

Table(3): Compare between pregnant and non-pregnant in level L-Selectin in embryo culture media

Parameter	Total	outcome		P value
		pregnant	Non pregnant	
L-Selectin	0.99(0.8-1.40)	1.33(0.86-1.62)	0.91(0.74-1.16)	0.016

The result reported 37 positive sample with equal or higher than 1pg and 37 were considered as negative below 1pg L-Selectin:

According to the received operating carve analysis for determination of L-Selectin cut off value according to IVF outcome .

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The suitable cut off value was 1pg. Accordingly culture media with 1pg and above have 56.80% positive predictive value for pregnancy, and culture media with less than 1pg have 73.00% negative predictive value for pregnancy, furthermore 1pg and above have 67.74% sensitivity and 62.79% specifty for positive pregnancy outcome. As show in table 4

Table(4): predictive value sensitivity and specificity of L-Selectin

Parameter		Outcome	
		Pregnant	None pregnant
L-Selectin	Positive (>1pg) 37	21	16
		56.80%	43.20%
	Negative (<1pg) 37	10	27
		27.00%	73.00%
Total		31	43
		41.90%	58.10%
P Value		0.009*	
Effect size	Value	95%CL	
	Sensitivity	67.74	50.14-81.43
	Specificity	62.79	47.86-75.62
	PPV	56.76	40.91-71.33
	NPV	72.97	57.02-84.6

Discussion:-

In the present study , there was no significant different in age between pregnant and non pregnant women ,this result is in agreement with the result of the other studies which proposed that age is an importantfactor in sub fertility(11), It is not very exact in predicting the reproductive potential. There is wide range in relationship between ovarian function and age and ovarian reserve(12),

The effect of BMI on reproduction related to higher induced of menstrual dysfunction and an ovulation , possibly because of altered secretion of gonadotropin releasing hormone ,sex hormone binding globulin , ovarian and adrenal androgen, lutelizing hormone and aslo because of altered insulin resistance(13), clinical observation on the effect of body Wight during IVF are more controversial , some studies found that BMI is associated with adverse pregnancy out come in women undergo ICSI treatment . include lower live birth and low implantation rate and increase miscarriage(14), Whereas, others have not-found clear relationship between BMI and IVF outcome(15) , In agreement with these results, the results of this study which shows nosignificant differences between pregnant and non pregnant patients regarding BMI table1 There's number of factor contributing to these discrepancies between studies including small sample size , difference in IVF stimulation protocol , varying BMI classification system(16).Regarding The hormonal profile, The result of these study shows non-significant difference in the FSH, and LH level between pregnant and non-pregnant ladies. This goes in line with several studies that showed no clear relationship between basal level of FSH, and LH hormone and pregnancy outcome(17,18,19)

some studies showed that AMH better predictor of pregnancy outcome after IVF treatment then other hormone (20), while others authors concluded AMH level are not associated with pregnancy rates(21), whereas its agreement with the result of these study , which showed that non significant different between pregnant and non pregnant group in the level of AMH.Some studies found decreased pregnancy rates in IVF cycle associated with higher estradiol(E2) level in the day of hCG triggering, which can induce lower endometrial receptivity (22), whereas other studies showed no significant effect (23), this study revealed non-significant difference between pregnant and non-pregnant groups in level of E2 in day of triggering.

We were able, in the current study, in demonstrating significant difference in mean level of L-Selectin concentration in culture media between group of women with successful pregnancy and the group of women who failed to achieve pregnancy, being higher in pregnant women. Human implantation is a complicated and multifactorial process involving synchrony between a healthy embryo and a receptive endometrium. Viable blastocyst and a receptive endometrium are the three essential elements for a successful implantation (24),

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molecular mechanisms involved in such a complex process may lead to improvement in the implantation rate for in vitro fertilization. the expression of L-Selectin and their ligands on the surface of the embryo has been characterized.

Results gathered from IVF showed L-Selectin expression on the surface of oocytes, 2-cell embryos and on 6-cell embryo, but not on 8-cell embryos (25).

Endometrium collected from IVF that had high level of L-Selectin ligands was associated with an increase pregnancy rate (26), in vitro implantation model demonstrated strong L-Selectin on human uterine epithelial cell line (27).

Conclusion: Level of L-Selectin concentration in embryo culture media can be used as predictor of ICSI success.

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