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Evaluation of the Level of Stem Cell Factor in Follicular Fluid and its Effect on Oocyte Maturity, Embryo Quality and Pregnancy Rate

Ghada Firas Faisal *[1], Ula M. R. Al-Kawaz *[2], Lubna Amer Al-Anbari *[3], Huda A. R. Hussaini *[4]

* **High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq,** [1]ghadafiras99@gmail.com, [2]ulamhm@googlemail.com, [3]lubnabdgam@yahoo.com, [4]hudamhm@googlemail.com

Abstract

Stem cell factor (SCF) is one of the first growth factors derived from granulosa cells in the ovarian follicle plays a critical role in hematopoiesis and the generation of melanocytes and germ cells. SCF also serves an important role in the development of the interstitial cells of Cajal in the intestine and the learning functions in the hippocampal region of the brain. This study aimed to investigate the levels of SCF in follicular fluid (FF) and if it can be used as a potential marker for predicting oocyte, embryo quality, and pregnancy rate. A total of 44 infertile couples involved in the study. All of them underwent controlled ovarian hyperstimulation for the ICSI cycle. Antagonist protocol was used as an ovulation induction protocol. Patients were divided into two groups according to ICSI outcomes as pregnant (23 women) and non-pregnant (21 women) groups. A comparison was done in both groups based on the oocyte, embryo quality, and other ICSI cycle characteristics, and follicular fluid stem cell factor level. SCF level was measured by using an enzyme-linked immunosorbent assay. Our study showed lack of association between patients' characteristics which included (age, BMI, infertility duration, type of infertility, and causes of infertility, basal hormonal, stimulation characteristics levels), FF SCF level and the oocyte characteristics comparison (total number retrieved oocyte, germinal vesicle oocyte (GV), MI oocyte and MII oocytes, and the number of injected oocytes, fertilization rate, and embryo characteristics) between pregnant and non-pregnant groups. The present study showed that Mean follicular fluid SCF was significantly lower in pregnant women in comparison with that of non-pregnant women, 135.92±38.96 pg/ml versus 185.15±63.58 pg/ml, respectively (P=0.003). Raised SCF level in FF harms ICSI outcome.

Keywords: Stem Cell Factor, Folicular Fluid, ICSI

1. Introduction

Infertility defines as a disease condition characterized by the failure to achieve a clinical pregnancy in couples after one year of unprotected, regular sexual intercourse or on account to a person's capacity inability to reproduce, either a female or male or both according to the international glossary on infertility and fertility care (Zegers-Hochschild, et al. [1]). Stem cell factor (SCF), defines as a cytokine growth factor, that activates multiple signal pathways via binding with the c-kit receptor, this binding is important in the regulation of diverse cell proliferation, differentiation, migration, and apoptosis, including melanin cells, hematopoietic stem cells, gastrointestinal Cajal interstitial cells, mast cells, and reproductive cells (Tan, et al. [2]). The SCF/c-KIT system is expressed in several tissues of the male and female reproductive tract, namely, testis, prostate, breast, ovary, and endometrium, and its important role in regulating cell survival, proliferation, migration, and differentiation have been reported (Figueira, et al. [3]), but these studies are controversial with other several studies that have been reported about the role and the effect of SCF (Hammadeh, et al. [4], Celik, et al. [5]) who showed that there was no correlation between the concentration of SCF in serum and FF on the oocyte retrieval day with the cycle characteristics and outcome.

2. Materials and Methods

This study conducted at The High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University during the period from Oct. 2018 to Mar. 2019. 44 infertile couples were enrolled in this study; all underwent ICSI cycles, early follicular phase hormonal level of FSH, LH, cycle day 2 E_2, triggering day E_2, TSH, and prolactin (PRL). Patients with Age ≥40 years old, abnormal uterine cavity due to polyp myoma, or congenital anomalies, uncontrolled systemic disease as diabetes mellitus or uncontrolled endocrinological disorder, empty follicle syndrome were

excluded. All selected patient was subjected to the basic fertility workup of the fertility center which consists of history-taking, physical examination, ovulation detection, evaluation of tubal patency and uterine cavity, and semen analysis. All patients were enrolled in flexible antagonist protocol which started on day two of the menstrual cycle, ultrasound examination was performed to exclude those women with an ovarian cyst and assess the endometrial thickness. Patients after selection received recombinant human follicle stimulation hormone (r-hFSH) (Gonal F®; Merck Serono S.A., Geneva, Switzerland) ® containing 75 IU of FSH activity per ampoules by daily subcutaneous injection. Transvaginal ultrasound was performed on cycle day five and the subsequent scan was done every 2-3 days as required. The dose of Gonal-F® and follicle growth was monitored by serum E_2 level and transvaginal ultrasound till the day of triggering by hCG administration (Rodgers and Irving-Rodgers [6]). The GnRH antagonist used was cetrorelix acetate 0.25 mg/day (Cetrotide; Merck-Serono Ltd., Aubonne, Switzerland). Treatment with rFsh and cetrorelix acetate was continued until the day of the final oocyte maturation trigger. Ovulation induction was induced with 250 µg human chorionic gonadotropin α(r-hCG), (Ovidreal, Merck-Serono Ltd., Aubonne, Switzerland) subcutaneously when at least two lead follicles have reached 18 mm.

3. ICSI Procedures and follicular fluid collection

Oocytes were retrieved by trans vaginal ultrasound-guidance under general or regional anesthesia, oocyte aspiration was done by a gynecologist, approximately 34–36 hours after hCG injection (triggering time). A visible follicle, preferred as the start of the ova retrieval procedure, individually aspirated. All follicles within both ovaries were aspirated by ovum aspiration needle (Cook®, Australia) and follicular fluid is given directly to the embryologist to collect the retrieved oocytes-cumulus complexes in

follicular fluid. Once the OCC were collected they are rinsed with flushing media for removing any remaining blood from the follicular aspirate, transferred into drops of FertiCult Flushing media then washed by Gain medium and overlaid by paraffin/ mineral oil in an incubator at 37°C with 5-6% CO_2 and at 95% humidity. Follicular fluid samples among the several mature follicles containing oocyte were recovered during the oocyte retrieval procedure using a transvaginal follicular aspiration. The clear aspirated follicular fluid (FF) samples were collected in plain tubes and centrifuged immediately at 2000 rpm for 10 minutes to remove cell debris and granulosa cells from the samples. The prepared follicular fluid was stored immediately at -20 °Ċ until the time of the assay to prevent any sample alteration. Semen samples obtained from patients' husbands by masturbation collected after 2 to 7 days of sexual abstinence. ICSI usually performed 4 to 6 hours following transvaginal ultrasound-guided oocyte retrieval, the cumulus corona cells are removed by a combined enzymatic and mechanical treatment to denude the oocytes from the cumulus cells. Each oocyte is carefully assessed, noting the presence or absence of germinal vesicle or the first polar body. Only (metaphase II) and morphologically intact were suitable for microinjection. The injected oocyte was evaluated within 16-18 hours after intracytoplasmic sperm injection. In normal fertilization assessment, oocytes were spherical and have two polar bodies and two pronuclei (2 PN). On day 3 post oocytes retrieval or day 5 post ova pick up for blastocysts, best quality embryos were transferred into the uterine cavity under trans-abdominal ultrasonographic guidance using a flexible catheter (Cook-Ireland Ltd) which pass through the vagina and the cervix into the uterine cavity, then patient received luteal support by cyclogest 400 mg/day for 2 weeks, after that if a pregnancy test was positive, transvaginal ultrasound was done 2 weeks later to confirm gestational sac with + fetal heart activity. Follicular fluid obtained on

the day of oocyte retrieval was estimated for the SCF Level by using Simple Step ELISA® (Enzyme-Linked Immunosorbent Assay) kit that provides a quantitative measurement of SCF protein concentration.

4. Statistical Analysis

Data were collected, summarized, analyzed, and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Qualitative (categorical) variables were expressed as number and percentage, whereas, quantitative (numeric) variables were first evaluated for normality distribution using Kolmogorov-Smirnov test, and then accordingly normally distributed numeric variables were expressed as mean (an index of central tendency) and standard deviation (an index of dispersion), while those numeric variables that are not normally distributed were expressed as median (an index of central tendency) and inter-quartile range (an index of dispersion).

5. Results

Patients enrolled in the present study were divided into two groups, the first group included women who succeeded to get pregnant (n=23) and the second group includes women who failed to get pregnant (n=21). The demographic data of the pregnant and non-pregnant groups are shown in Table 1. The statistical analysis showed no significant difference among the two groups concerning the age, Body mass index (BMI), duration of infertility, types of infertility, and cause of infertility. Regarding oocytes and embryo characteristics (embryos number, GI embryos number, GII embryos number, and GIII embryos number) comparison between both groups shown in Table 2, there is also no significant difference concerning the number of oocytes, GV, MI and MII except for abnormal and ruptured oocytes was significantly lower in women who succeeded to get pregnant when compared to that of women who failed to

Table (1): Demographic characteristics of sub-fertile women enrolled in the present study

Characteristic	Pregnant *n* = 23	Non pregnant *n* = 21	*P-Value*
Age (Years)			
Mean ±SD	29.52 ±4.79	27.95 ±5.82	0.333 † NS
<35, *n* (%)	20 (87.0 %)	19 (90.5 %)	1.000 ¥ NS
≥ 35, *n* (%)	3 (13.0 %)	2 (9.5 %)	
BMI (kg/m^2)			
Mean ±SD	26.25 ±3.16	26.29 ±3.87	0.971 † NS
Normal, *n* (%)	8 (34.8 %)	9 (42.9 %)	0.802 € NS
Overweight, *n* (%)	11 (47.8 %)	8 (38.1 %)	
Obese, *n* (%)	4 (17.4 %)	4 (19.0 %)	
Duration of infertility (years)			
Mean ±SD	8.52 ±4.22	6.67 ±4.24	0.154 † NS
Type of infertility, *n* (%)			
Primary	19 (82.6 %)	17 (81.0 %)	1.000 ¥ NS
Secondary	4 (17.4 %)	4 (19.0 %)	
Cause of infertility, *n* (%)			
Male factor	10 (43.5 %)	10 (47.6 %)	0.508 € NS
Female factor	6 (26.1 %)	7 (33.3 %)	
Combined	1 (4.3 %)	2 (9.5 %)	
Unexplained	6 (26.1 %)	2 (9.5 %)	

n: number of cases; SD: standard deviation; BMI: body mass index; †: independent samples t-test; ¥: Yates correction; €: Chi-square test; NS: not significant at P ≤ 0.05

Table (2): Oocyte and Embryo characteristics

Characteristic	Pregnant (*n* = 23)	Non-pregnant (*n* = 21)	*P* †
Number of oocytes	8.48 ±3.73	9.81 ±6.27	0.392 NS
Ruptured and abnormal	0.52 ±1.04	1.43 ±1.66	0.034 S
GV	1.17 ±1.64	0.71 ±1.10	0.287 NS
M I	1.39 ±1.23	1.29 ±1.23	0.778 NS
M II	5.22 ±2.19	6.38 ±4.76	0.297 NS
Number of injected oocyte	6.43 ±2.35	7.00 ±4.37	0.592 NS
Number of embryos	4.52 ±1.95	4.67 ±2.96	0.847 NS
Number of GI embryos	3.09 ±1.53	2.43 ±1.29	0.133 NS
Number of GII embryos	0.96 ±1.11	1.86 ±1.93	0.062 NS
Number of GIII embryos	0.48 ±0.95	0.48 ±1.03	0.994 NS

n: number of cases; †: Independent samples t-test; NS: not significant at P ≤ 0.05; S: significant at P ≤ 0.05; GV: germinal vesicle; MI: metaphase I oocytes; MII: metaphase II oocytes

Table (3): Stem cell factor in follicular fluid in pregnant and non-pregnant groups

SCF Characteristic	Pregnant (*n* = 23)	Non pregnant (*n* = 21)	*P*
Follicular fluid SCF (pg/ml)	135.92 ±38.96	185.15 ±63.58	0.003 HS

SCF: stem cell factor; *n*: number of cases; †: Independent samples t-test; HS: Highly significant at *P* ≤ 0.05.

get pregnant, (P=0.034). The present study showed that mean level of follicular fluid SCF was significantly lower in pregnant women in comparison with that of non-pregnant women, 135.92±38.96 pg/ml versus 185.15±63.58 pg/ml, respectively (P=0.003), as shown in Table 3. Regarding non pregnant group, follicular fluid SCF was not significantly correlated to any of women characteristics with the exception of highly significant positive correlation to endometrial thickness. Regarding pregnant group, follicular fluid SCF was not significantly correlated to any of

Table (4): Correlations of Follicular fluid SCF to all other characteristics in non-pregnant group and pregnant group

Characteristic	SCF in F.F in non-pregnant group		SCF in F.F in pregnant group	
	r	*P*	*r*	*P*
Age	0.034	0.884	0.055	0.803
BMI	0.054	0.816	-0.118	0.592
Duration	-0.115	0.620	-0.098	0.657
Infertility type	-0.032	0.890	-0.038	0.863
infertility cause	-0.015	0.949	0.268	0.216
FSH	0.224	0.328	-0.511	0.013*
LH	0.285	0.210	0.072	0.743
PRL	0.057	0.807	-0.087	0.694
TSH	0.221	0.337	0.082	0.710
E_2/ CD_2	-0.100	0.667	0.094	0.671
E_2/Trigger day	0.153	0.508	0.518	0.011*
Dose of gonadotropin	0.043	0.853	-0.198	0.365
Duration of stimulation	0.167	0.469	-0.057	0.797
Thickness of endometrium	0.593	0.005**	-0.081	0.712
oocyte No.	0.210	0.361	0.406	0.054
rub/abnormal oocyte	-0.033	0.886	0.365	0.087
GV	0.234	0.307	0.184	0.401
M I	0.097	0.674	0.022	0.921
M II	0.209	0.363	0.459	0.028*
Number of injected oocytes	0.223	0.332	0.451	0.031 *
embryo No	0.446	0.043	0.231	0.290
No. of G1	0.323	0.153	0.235	0.281
No. of G2	0.245	0.284	-0.081	0.712
No. of G3	0.393	0.078	0.190	0.385
FR	0.262	0.251	-0.190	0.386

women characteristics with the exception of significant negative correlation to FSH and significant positive correlation to estradiol at day of trigger, number of MII oocytes and number of injected oocytes, as shown in Table 4.

6. Discussion

The level of stem cell factor infertile women gained significant attention as a factor that may affect the pregnancy rate, following assisted reproduction, in a significant manner; however, substantial contradictory and controversial results existed that justified the planning and conduction of the current study. To our knowledge, this is the first study that compares the SCF level in embryo media between patients undergoing ICSI treatment. The main finding of our study was the presence of significant differences in the SCF levels in embryo media between ladies who succeed to get pregnant and those who fail to get pregnant, being lower in pregnant women. Studies that investigated the association of SCF status with IVF outcome revealed inconsistent results. (Tan, et al. [2]), found that levels of SCF in FF and GCs were positively associated with oocyte maturation, normal fertilization, cleavage, embryo quality, and clinical pregnancy outcome. While the result of other study showed that the SCF level related to the number of the follicles (SCF/f) in FF and serum in patients who fail to get pregnant was significantly higher than in those who succeed to get pregnant (Salmassi, et al. [7]) which is agreed with the results of this study. Others suggested that there is no correlation between the concentration of SCF in serum and FF on the oocyte retrieval day with the cycle characteristics and outcome. These results are in agreement with the current result (Hammadeh, et al. [4], Celik, et al. [5]). Some studies have linked the activity of SCF to the synthesis of AMH as a negative association with the pathway of SCF (Hu, et al. [8], Fu, et al. [9]). Other study showed that SCF level was raised from oocyte retrieval day through the transfer of the

embryo to implantation and its highest concentration was reached at pregnancy confirmation day (2 weeks after ET), after the implantation, during gestation, SCF level reached a plateau (3–4 weeks after ET) (Salmassi, et al. [10]). The difference was not confirmed in this study as SCF levels measured on oocyte pick up day only. It has been shown that serum and FF SCF kinetics can be influenced by gonadotropins amount administered for ovarian stimulation, one study has indicated a positive association between the gonadotropins total dose administered, levels of serum estradiol, serum and FF SCF concentrations (Salmassi, et al. [10]), another study showed that different gonadotropins or inhibition not significantly alter SCF concentrations (Celik, et al. [5]); however, this issue remains controversial. (Gizzo. et al. [11]) suggested that SCF level in serum could be a promising biomarker, especially in patients of poor-responder, for making the clinical decision on whether to proceed or to cancel oocyte pickup, it could be helpful to avoiding empty follicular retrievals where no MII oocyte are obtained. (Kalem, et al. [12]) suggested that serum and follicular fluid levels of SCF were decreased in patients with PCOS, however, raised SCF levels in PCOS patients are correlated with increased maturation of oocyte and rates of clinical pregnancy. This study concludes that raised SCF levels in FF harm ICSI outcome.

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Author Contribution

Faisal GF performed the study, Al-Kawaz UMR, Al-Anbari LA, and Hussaini HAR supervised the work.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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Biography

Ghada Firas Faisal

She was born in Baghdad. Iraq in 1992. She received the B.Sc. Degree in Veterinary Medicine and Surgery in 2015, the M.Sc. in Applied Embryology in 2019. She is a member of Veterinarians Syndicate and the Iraqi Fertility Society.

Dr. Ula M. R. Al-Kawaz

He is a Professor of Urology at Al-Nahrain University. he served as the dean of the high institute of infertility diagnosis and ARTs, Al Nahrain University. He is a fellow of the Iraqi Board for Medical Specialization, Council of Urology since 2006, and a fellow of the European Board of Urology, since 2009. He published more than 27 articles both local and international. He supervised many M.Sc. and Ph.D. students.

Dr. Lubna Amer Al-Anbari

She received the M.B.CH.B., the Higher diploma from the college of medicine, University of Baghdad, in 2004, and 2011, respectively. She received the board in infertility from the Iraqi society of medical specialists in 2012. She published many kinds of research both local and international. She supervised many students. She held many academic positions.

Dr. Huda A. R. Hussaini

She received the M.B.Ch.B: Babylon University, college of medicine, in 2000. The diploma in Radiology from the University of Baghdad in 2007. She is a fellow of the Iraqi Board for Medical Specialization, Council of Radiology since 2008. Currently, she is an assistant professor. Al Nahrain University, College of Medicine, department of surgery (specialist radiologist). She is a Member of staff in the high Institute of infertility diagnosis and ARTS. Including the ultrasound department in the institute since 2018. She has Participations in a lot of annual meetings, medical conferences. Seminars, continuous learning, and thesis discussion. She is a member of the Iraqi medical association, the Iraqi Society of radiologist and medical imaging, Iraqi Journal of Embryos infertility researches, European Society for Hybrid, Molecular, and translation imaging. She published more than articles and has supervised many students.

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