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The Effect of Follicular Fluid Stem Cell Factor on Clinical Pregnancy in PCOS Patients

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Abstract:
Background: Polycystic ovarian syndrome is characterized by insufficient follicular maturation, either due to gonadotrophic imbalance or the effects of local substances; as well as a decrease in aromatase activity in granulosa cells, which may be a consequence of the low concentration of follicular stimulating hormone. **Objective:** To assess the effect of follicular fluid Stem Cell Factor (ff SCF) on clinical pregnancy in PCOS. **Patients and method:** A prospective cross-sectional study conducted at infertility treatment center at Al-Sader Teaching Hospital / Al-Najaf / Iraq, during the period from October 2019 to the end of May 2020. **Results:** in PCOS group 14/44 (31.8 %) of the patients were presented with clinical pregnancy, while clinical pregnancy in non PCOS group presented in 16/40 (40 %) of the patients. the mean level of follicular fluid was decreased significantly in pregnant PCOS group (235.8±247.4 pg/ml) than that in pregnant non-PCOS group (618.0±170.7 pg/ml) regarding clinical pregnancy, while the follicular fluid was increased significantly in PCOS group (569.2±243.3pg/ml) than that in non-PCOS group (382.9±164.17pg/ml) in patients with no clinical pregnancy.**Conclusion:** Reduced concentration of ff SCF in PCOS patients compared to non PCOS patients, as the level of ff SCF reduced lead to increase rate of clinical pregnancy.
Keyword: Polycystic ovarian syndrome, follicular fluid stem cell factor, infertility, ICIS

Introduction:

Polycystic ovary syndrome (PCOS) is the most frequent endocrine alteration in women during their reproductive age (4% - 8%); it is found in more than 30% of women with secondary amenorrhea, 75% with oligomenorrhea and additionally 75% of women with PCOS suffer from infertility. (1) This entity, classified by the WHO as normogonadotropic anovulation, presents a wide phenotypic variety with different expressions and metabolic and reproductive repercussions; being anovulation the main cause of infertility. (2) PCOS is characterized by insufficient follicular maturation, either due to gonadotrophic imbalance or the effects of local substances; as well as a decrease in aromatase activity in granulosa cells, which may be a consequence of the low concentration of FSH. This decrease in aromatase activity leads to circulating hyperandrogenism and, above all, an ovarian microenvironment with an androgenic domain that constitutes another factor for the maintenance of the inhibition of aromatase activity and the arrest of follicular maturation. (3)

Likewise, an elevation in the response threshold of granulosa cells to FSH due to counter-regulatory paracrine factors (steroidal and non-steroidal) has been suggested as an explanation for the lack of follicular maturation. Non-steroidal factors, including growth factors, insulin, IGF-1, and TGF-β (growth factor beta) would increase estradiol production from granulosa cells,

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while EGF growth factor epidermal (EGF), TGF-α (growth factor alpha), anti-Müllerian hormone (AMH) and inhibin B, would inhibit the action of FSH. [4,5]
Granulosa cells possess receptors for these peptides, and intraovarian production of some has been shown to be controlled by gonadotropins and gonadal steroids.
Stem cell factor (SCF) is a cytokine growth factor formed in the follicular phase directly previous to the ovulatory phase. It is newly revealed to play a significant duty in folliculogenesis and ovulation. Stem cell factor triggers the enrollment of the primordial follicle pool and oocyte maturation., Stem cell factor in the human ovary is produced by granulosa cumulus cells can straightly stimulate growingand variation of oocytes and theca cells, and rise steroid hormone production. [6]
Follicle-stimulating hormone is elaborated in the release of stem cell factor with the regulation of many paracrine factors from the oocytes, which interrelate with GCs throughout development of follicle. The function of follicular stimulating hormone in primordial folliculogenesis looks to happen via stem cell factor as the amount of FSH-stimulated primordial follicles is depress in the existence of a stem cell factor antibody. [7] It has been really revealed that follicles cultured with stem cell factor significantly rise the mean diameter from primordial. [8] It has been suggested that levels of follicular fluid stem cell factor may disturb folliculogenesis and then process of ovulation, throughout the time serum stem cell factor might reproduce positive stimulation with a huge maturation of follicle. Unusually, a new study has revealed that IVF cycles, the levels of stem cell factor in follicular fluid were increased suggestively in patient's follicular fluid in which at least one oocyte was retrieved in comparison to no oocyte was retrieved in others. So, they conclude that stem cell factor may be utilized as a prognostic factor for ICSI outcome, particularly in the treatment of poor-responder patients [9,10]
While study done by Tan J et al found that in PCOS patients there was decrease SCF level in serum and follicular fluid than in non PCOS patients, but increase SCF has positive link with oocyte maturation, fertilization and embryo quality [11], Hammadeh et al, found in their study that Stem cell factor had an important consideration as a factor to assess the pregnancy rate in infertile women. [12]
Aim of the study: To assess stem cell factor (SCF) levels in follicular fluid (ff) that can be used as a marker to anticipate clinical pregnancy rate.
Patients and method: A prospective cross-sectional study conducted at infertility treatment center at Al-Sader Teaching Hospital / Al- Najaf / Iraq, during the period from October 2019 to the end of May 2020. The study was approved by the local Medical Ethical Committee of the Infertility treatment center at Al-Sader teaching hospital. Forty-four infertile couples with PCOS and forty couples with non-PCOS were enrolled in this study; all underwent ICSI cycles.
All selected patient was subjected to: - ·The basic fertility workup of our fertility center which included of (history taking, physical examination, ovulation detection, Uterine cavity and tubal patency were evaluated, and analysis of semen).

Inclusion criteria:
· Age 18-40 years old.
·Early follicular phase FSH, LH, cycle day 2 E2, TSH and prolactin (PRL) hormonal level which was done as part of the work up must be within normal.
· Ultrasound, hysterosalpinography and/or hysteroscopy used for evaluation of the uterine cavity, which must be normal.

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Exclusion Criteria:
· Age > 40 years old.
·Abnormal uterine cavity due to polyp myoma, or congenital anomalies.
·Uncontrolled systemic disease as diabetes mellitus or uncontrolled endocrinological disorder.
· Women with empty follicle syndrome.
· Women with no fertilized oocyte.
· Couples with semen collection failure at oocyte retrieval day.
All patients were subjected to GnRH antagonist protocol, on the 2nd or 3rd day of the menstrual cycle gonadotropin stimulation begun with either recombinant FSH (Gonal-F, MerckSerono, Darmstadt, Germany) or with Human Menopausal Gonadotropin (HMG), (Menigon, Ferring, Saint-Prex, Switzerland), serial transvaginal sonography was done to monitor ovarian stimulation, when the leading follicle reached a mean diameter of 14 mm, 0.25mg of GnRH antagonist (Cetrotide, MerckSerono Ltd., Aubonne, Switzerland) was administered with the adjusted dose of gonadotropin until day of trigger by human chorionic gonadotrophine (HCG), Pregnyl, based on follicular size and retrieval of oocytes done 35 hrs later under transvaginal ultrasound-guidance. The fluid aspirated from the leading follicle was collected in plain tube, centrifuged immediately at 2000 rpm for 10 minutes, the supernatant follicular fluid was stored at - 20 c until the time of the ELISA assessment of quantitative SCF, the kits were purchased from BTL, Shanghai Korain Biotech, and labeled as human stem cell factor ELISA kit, the standard curve range was 5 Pg/ml-1800 Pg/ml, and 2.52 Pg/ml of sensitivity.

Statistical analysis: Data were gathering and arrange then entered into Excel file then this file was transfer to SPSS program version 25. Descriptive statistics presented as (mean ± standard deviation) and frequencies as percentages. To compare between two means we used One-way ANOVA analysis. In all statistical analysis, level of significance (p value) set at ≤ 0.05.

Results:

Table 1, show that the mean age of patients in (PCOS group) was 29.27±4.25 years while 29.9±2.48 years in non-PCOS group, BMI in (PCOS group) was 28.2±4.5 Kg/m^2 and 27.01±4.06 Kg/m^2 in non-PCOS group, level of FSH in PCOS group was (5.09±2.34 mIU/ml) and for non-PCOS was (5.95±2.3mIU/ml), LHwas (4.49±2.4 mIU/ml) in PCOS and (3.62±2.28 mIU/ml) for non-PCOS, the E2meanlevel was (38.49±13.25 pg/ml) and in non-PCOS was (36.35±16.73 pg/ml), TSH level was (2.09±1.01 µIU/L) for group PCOS and (2.16±0.77 ng/ml) for non-PCOS, The level of prolactin in PCOS was (24.68±11.69 ng/ml) and for non-PCOS was (22.86±10.74 ng/ml), and total testosterone was (1.46 ±1.22 nmol/l) in PCOS and (0.44±0.20 nmol/l) in non PCOS. The mean duration of infertility in PCOS group was (9.13±5.02 years) and (8.05±3.3 years) in non-PCOS group.

Table 1 comparison of age, BMI, pre cycle hormonal values between PCOS and non PCOS

Variables	PCOS (n=44)	Non-PCOS (n=40)
Age (years)	29.27±4.25	29.9±2.48
BMI(Kg/m^2)	28.2±4.5	27.01±4.06
FSH (mlU/ml)	5.09±2.34	5.95±2.3
LH (mlU/ml)	4.49±2.4	3.62±2.28
E2(pg/ml)	38.49±13.25	36.35±16.73
TSH (mlU/ml)	2.09±1.01	2.16±0.77
PRL (µg/L)	24.68±11.69	22.86±10.74

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Duration of infertility (year)	9.13±5.02	8.05±3.3
Total testosterone(nmol/l)	1.46±1.22	0.44±0.20

Table 2 show that mean ff SCF in PCOS group was (213.9±124.7 pg/ml) and (476.9±166.55 pg/ml) in non-PCOS group(figure 1) with highly significant difference (P<0.001), mean level of total gonadotropin dose used was (1710.2±305.5 IU) in PCOS and (2062±555.7 IU) in non-PCOS group with highly significant difference (P<0.001), mean level of total days of stimulation was (10.68±1.39) in PCOS and (10.8±1.0) in non-PCOS group with no difference (P=0.6), mean level of retrieved oocyte in PCOS (15.59±5.38) and (10.9±2.7) in non-PCOS group with highly significant difference (P<0.001), mature (MII) oocyte mean level in PCOS (10.72±5.18) and (7.55±0) in non-PCOS group with highly significant difference (P<0.001), No significant difference found regarding high quality embryo (P=0.57) between both groups, fertilization rate in PCOS (33.4±9.7%) and (43.61±10.5%)in non-PCOS group with highly significant difference (P<0.001).

Table 2 comparison of cycle – related parameters between PCOS and non PCOS

Variables	PCOS (n=44)	Non-PCOS (n=40)	P value
ff SCF(pg/ml)	213.9±124.7	**476.9±166.55**	<0.001
Total gonadotropin dose (IU)	1710.2±305.5	**2062±555.7**	<0.001
Total days of stimulation	10.68±1.39	**10.8±1.0**	0.6
Retrieved oocyte (n)	15.59±5.38	**10.9±2.7**	<0.001
Mature (MII) oocyte (n)	10.72±5.18	**7.55±0**	<0.001
High quality embryos(n)	3.36±1.67	**3.2±0.76**	0.57
Fertilization rate (%)	33.4±9.7	**43.61±10.5**	<0.001

Figure 1: Distribution of follicular fluid SCF in the studied groups

As shown in table 3, the mean level of follicular fluid was decreased significantly in pregnant PCOS group (235.8±247.4 pg/ml) than that in pregnant non-PCOS group (618.0±170.7 pg/ml) regarding clinical pregnancy, while the follicular fluid was increased significantly in PCOS group (569.2±243.3pg/ml) than that in non-PCOS group (382.9±164.17pg/ml) in patients with no clinical pregnancy.

Table 3: Relationship between clinical pregnancy (PCOS & NONPCOS) and SCF

GROUPS	VARIABLES			SCF
PCOS (n=44)	Clinical pregnancy	Yes (n=14)	Mean	235.8±247.4
			Medium (min-max)	154.87—386.01
		No (n=30)	Mean	569.2±243.3
			Medium (min-max)	352.19—1291.32
Non-PCOS(n=40)	Clinical pregnancy	Yes (n=16)	Mean	618.0±170.7
			Medium (min-max)	427.54—837.44
		No (n=24)	Mean	382.9±164.17
			Medium (min-max)	81.33—501.06

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Discussion:

It has been shown that expression of SCF and its c-kit receptor in human ovaries play a significant role in oocyte maturation in ovarian follicle Abir et al, [13] they demonstrate that SCF secreted from granulosa cells directly before the ovulation play an essential function in folliculogenesis and ovulatory mechanism that could be used as a predictor of ICSI outcome. Gizzo et al, [14] intimated that serum SCF could be used as a biomarker in poor responder patients whether to proceed or cancel oocyte pickup. In our research we compare the level of ff SCF in PCOS and non PCOS patients and its effect on clinical pregnancy.

In the present study mature (MII) oocyte mean level in PCOS were increased significantly than that in non-PCOS group, while opposite to that found in fertilization rate in PCOS when it decreased significantly than that in non-PCOS group. This is same that found by Tan J, et al. [11]

The main finding in the current study was the mean level of follicular fluid SCF is decreased significantly in PCOS group than that in non-PCOS group, this is in agreement with Tan J et al., [11] Also it is same that found in a Turkish study carried by Kalem Z et al. [15] Nonetheless it in not in agreement with Sun L et al, study that's revealed follicular fluid of SCF increase in PCOS group more significantly than that in non-PCOS, so they concluded that a positive relationship between SCF and PCOS. Moreover, Sun et al, found an association between IVF outcome and ffSCF levels in PCOS patients IVF cycles. [16]. Some authors Parrott and Skinner, [17] Packer et al, [18] found that uptake of SCF into oocytes increase as the rate of growth of oocyte increases and uptake of oocyte to SCF was greatly found in hyper responder patients compared to normal or hypo responder patients this is why ff SCF is decreased in hyper responder patients, and this finding may explain our study finding as PCOS classified as hyper responder patients.

In the current study we found that there is an association between ff SCF concentration and clinical pregnancy, ff SCF concentration was higher in pregnant than non-pregnant women of non PCOS group, which is same that mentioned in a study carried by Smikle et al, [19] in which there is a strong relationship between ff SCF level and clinical pregnancy outcomes in IVF patients and they concluded that increased ff SCF concentration might improve clinical pregnancy rates after oocyte retrieval, fertilization, and embryo transfer, but the reverse was found in PCOS group when ff SCF level was lowered in pregnant than non-pregnant women of PCOS group, this finding is consistent with Faisal et al, [20] and agree with Salmassi et al, [21] findings of a significantly reduced ff SCF concentration of pregnant women compared to non-pregnant women, while Sun et al found increase ff SCF in pregnant patients than those who not get pregnancy in both PCOS and non PCOS patients. The limitation of our study was assessment of SCF in follicular fluid only, serum SCF levels should be evaluated in future research, also follicular fluid SCF can be measured in mature and immature oocyte to understand the real effect of SCF on follicle environment and oocyte maturity in PCOS patients.

Conclusion: Reduced concentration of follicular fluid SCF in PCOS patients than in non PCOS patients, as the level of ff SCF decreased leads to increased rate of clinical pregnancy.

No conflicts of interest

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Ethical clearance: was approved by Iraqi Ministry of health- scientific committee

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