ORIGINAL ARTICLE

The effect of adipose tissue StAR gene Expression on ICSI among Polycystic Ovarian Syndrome cases and Matched controls: A case-control study

Short Title: StAR gene Expression In ICSI

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ABSTRACT

Objective. steroidogenesis is vital for reproduction and fertility; it is governed by the acute regulatory protein gene (StAR). Earlier work discussed a different StAR expression in the adipose tissue (AT) of pregnant polycystic ovarian syndrome (PCOS) women. This study examined the correlation of StAR expression levels in AT of PCOS women to various parameters of intracytoplasmic sperm injection (ICSI) compared to those without.

Patients and methods. A case-control study recruited 40 infertile women who had successful ICSI cycles into 2 groups: 20/40 cases and 20/40 non-PCOS cases; both groups were age and body mass index-matched for scheduled elective cesarean section. A sample of anterior abdominal wall AT was taken for a real-time PCR StAR gene expression. ICSI parameters, including hormonal, ultrasonic, stimulation drug doses and duration, and the outcome, were all retrospectively recorded and correlated with StAR gene expression.

Results. StAR gene folding was higher among PCOS-pregnant women (2.545 ±1.58 vs. 1.00 ±0.0), P-value< 0.023. estradiol hormone was correlated to StAR gene; -0.39, P<0.05. The total doses of recombinant follicle-stimulating hormone and human menopausal gonadotropin had significant correlations to StAR gene r= (-0.39, 0.60), P-value of (0.049, 0.005), respectively.

Conclusions. The significant association of StAR gene expression with stimulation drug doses suggests a clinical application, as it can reduce ovarian hyperstimulation syndrome and overall expense, which makes ICSI safer and more accessible to infertile couples. Women with PCOS present challenges in ICSI, and thus careful balance must be adjusted.

Key words

Polycystic ovarian syndrome; StAR gene; ICSI; stimulation dose.

INTRODUCTION

Assisted reproductive technique ART has revolutionized infertility treatment. However, many confounders affect its acceptance and success [1]. Polycystic ovarian syndrome (PCOS) a leading cause of female infertility and a well-known indication for in vitro fertilization (IVF). It poses many challenges in stimulation programs, from poor to hyper-responders, higher odds of oocyte incompetence, and, most importantly, poor outcomes [2,3]. To date, PCOS pathophysiology remains unclear [4]. Abnormal ovarian steroidogenesis (Estradiol and progesterone hormones) was linked to PCOS due to their indispensable role in regulating the menstrual cycle, including folliculogenesis, ovulation, and optimizing endometrium lining for implanting fertilized ova [5].

Steroidogenesis is governed initially via the expression of the steroidogenic acute regulatory protein (StAR), a protein that controls the rate-limiting step in steroidogenesis, which is the entry of cholesterol to the inner side of the mitochondria [6]. The StAR expression is itself cyclic in nature [7]. During the follicular phase (where estrogen predominates), the expression is low in preovulatory follicles. Around midcycle, where a luteinizing hormone (LH) surge occurs, StAR expression is upregulated in the dominant follicle [8]. The highest levels of StAR expression in theca-lutein and granulosa-lutein cells occur during the early and mid-luteal phases (where progesterone predominates) to promote endometrial decidualization and support a growing pregnancy. Finally, StAR expression decreases significantly at the end of the luteal phase [9].

After the giant leap in IVF in the last 40 years, our policy was revised with the introduction of mild stimulation since high doses of ovarian stimulation drugs were associated with higher burdens of IVF, particularly on the female partner [10].

An urgent need was raised to decrease IVF-related risks without compromising the positive outcome. Some sought to reduce oxidative stress, while others sought novel biomarkers that could optimize the stimulation program and predict positive outcomes [11,12].

Animal and human studies examined the StAR gene's expression in fatty tissues and its relationship to various aspects of metabolic syndrome, including obesity, diabetes, and PCOS [13,14]. However, no earlier research examined the implications of StAR gene expression on IVF programs. For that, the current study examined the effect of the StAR gene on IVF parameters among PCOS and non-PCOS women with successful IVF cycles, aiming to improve IVF program performance and acceptability.

PATIENT AND METHODS

Study type and setting:

The current study was a case-control study; it enrolled 40 eligible women who were scheduled for an elective C-section. They were infertile women who underwent successful intracytoplasmic sperm injection (ICSI) treatment in the university hospital in Baghdad, Iraq, in the period between July 2021 and March 2022, who satisfied our inclusion criteria. Patients signed consent after we briefed them about the study method and aim. Mustansiriyah University/ethics committee issued the study approval; Institutional Review Board (IRB) 165 dated October 11, 2021.

Our IVF center, like many centers, has witnessed raised demands for ICSI rather than conventional IVF. Although ICSI is originally indicated for moderate-severe male infertility [1], the procedure is conducted for non-male factors. These increased demands for ICSI are attributed to physicians' and patients' fear of fertilization failure, which is encountered in conventional IVF and not ICSI. For that, most of our referred cases underwent ICSI and not IVF.

Study Participants:

Cases were sequentially recruited into 2 groups; both groups were age and body-mass index-matched. Twenty infertile women represented the case group (20/40) who had a confirmed polycystic ovarian syndrome as the only cause of their infertility. Another twenty women represented the control group (20/40) who had infertility due to tubal factor or male factor infertility. All enrolled women (cases and control group) underwent assisted reproduction in the form of controlled ovarian stimulation and ICSI. Their demographic criteria (age, body mass index (BMI), infertility cause, and history of prior IVF trials) were taken a day before the C-section. As for the data regarding their ICSI protocol, including D2 hormonal and ultrasonic parameters, in addition to doses of stimulation drugs, duration, and the IVF outcome following stimulation, were all retrospectively collected from the IVF center files.

Definitions of inclusion criteria

Infertility was defined as failure to conceive after 1 year of unprotected regular sexual intercourse [15]. *PCOS* diagnosis was made according to Rotterdam criteria (Two out of three: oligo/anovulation, clinical/biochemical hyperandrogenemia, and ultrasonic feature of polycystic ovaries of 12 or > more follicles with a size of 2-9 mm and/or a volume >10 ml for each ovary [16]. *Tubal infertility* was confirmed with bilateral tubal blockage proved by hysterosalpingography or laparoscopy and dye. *Male Factor infertility* was based on the WHO 5th manual 2010 for normal seminal fluid parameters [17] .

Exclusion criteria

Diabetic and gestational diabetic patients, as well as cases with gestational hypertension, were excluded. History of taking medication that may affect lipids or glucose metabolism as anti-hyperlipidemic, anti-diabetic, anti-hypertensive, or glucocorticoid pharmacological products, and those who had missing data were also excluded.

Estimation of Sample size

Sample size= $(Z_{1-\alpha/2})^2 SD^2/d^2$

Z1-α/2 represents the standard normal variate which is equal to 1.96

SD represents the variable standard deviation; its value is obtained from previously published studies

d= absolute error or precision determined by the operator. Herein, we hypothesized the absolute error to be 0.1.

Sample size= $(1.96)^2 (0.3)^2 / (0.1)^2$ = (3.84* 0.09)/ 0.01= 35 participants.

So the sample size is 35 patients, and our study involved 40 participants.

Protocol for ICSI

Patients who underwent ICSI were assessed on day two(D2) of a menstrual cycle for hormonal analysis at the biochemistry laboratory of the IVF center, including:

Serum follicular stimulating hormone (FSH), LH, testosterone, anti-Mullerian hormone (AMH), Estradiol (E2), and prolactin levels. A transvaginal ultrasound was done for all participants at D2 to assess antral follicle counts(AFC) and endometrial thickness(ET).

Infertile women included in the study were exposed to flexible antagonist protocol and controlled ovarian hyperstimulation with the recombinant follicle-stimulating hormone (FSH) 150 IU/day for the PCOS group and 225-300 iu for control group. In addition to 75-150 iu human menopausal gonadotropins (HMG) starting from D2 of menstrual cycle till triggering day. Dose calculation for gonadotrophins was based on AFC and AMH levels on D2[18].

Patients were re-examined on D7 of the cycle with a trans-vaginal ultrasound, where readjustments of the dosage were made, and a 0.25 mg/day GnRH antagonist was added to the treatment (flexible antagonist protocol) when the leading follicles reached size 14 mm treatment and were continued till at least three follicles size 17 mm were reached. Then Ova triggering was done via human chorionic gonadotropin (hCG)5000 iu or with the addition of GnRH agonist once E2 levels reached 2500 pg/mL. 35 hours later, oocyte retrieval was done under general anesthesia, and all mature oocytes were injected with sperm by the ICSI procedure. Grading of cleavage stage embryos was made according to the Veeck et al. [19] grading system, while blastocyst stage embryos were graded as per the simplified grading system.

Fresh or frozen Embryo transfer with a soft transfer catheter was done under the guidance of abdominal ultrasound. Luteal support continued for 14 days with progesterone suppositories. Patients were followed after embryo transfer for 2 weeks to verify the outcome of pregnancy test. After 28 days following embryo transfer, a transvaginal ultrasound confirmed clinical pregnancy. Participants continued till the end of pregnancy according to the ANC schedule [20]

Collection of adipose tissue biopsies (samples)

During the C- section delivery, an anterior abdominal wall subcutaneous tissue sized about 2 cm3; was taken by scalpel upon incising the skin. Then they were immediately washed by normal saline 0.9%, segmented, and feezed in liquid nitrogen (-196) vial till the time of the analysis.

RNA isolation

For all collected samples, RNA was isolated from the abdominal subcutaneous tissue samples using the commercial kit (BioUltra, 83913-1EA) and then converted to cDNA by using (11117831001, Roche). According to the manufacturer's instructions

Relative gene expression

In order to estimate the gene expression level, a target sequence within the StAR gene has been amplified by using the RT-PCR along with a separate reaction of GAPDH gene. The

sequence of target gene as follow; sense primer; GCCCAAGAGCATCATCAAC and antisense; GCTGGTCTTCAACACCTG . The sequence of reference gene as follow sense primer; AGGTCATCCCTGAGCTGAA and antisense; CTGCTTCACCACCTTCTTGAT, highlighted in Table 1.

Statistical analysis

The Data normality was checked by Shapiro -Wilks test. Continuous data were presented as Means± standard deviation(M±SD). Students t-test was used to compare significant differences between means. Categorical variables were compared by Chi-square test was. The Statistical Analysis System- SAS (2018) program was used to detect the effect of different factors on study parameters [21]. The correlation coefficient tested the strength of the association between variables in this study. A P-value <0.05 was significant for all tests.

RESULTS

The study population was 40 pregnant women matched for age, and BMI divided into; the study group (20/40) PCOS cases who conceived through a successful ICSI cycle; control group (20/40) non-PCOS women who undergo successful ICSI cycle for tubal or male factor. In regard to patients' demographic criteria, neither the age nor the BMI was significant among the two groups, shown in **Table 2** Male and tubal factors represented 13(65%) and7 (35%) of the controls versus 20(100%) PCOS cases in the study group. Duration of infertility was (7.05 ±4.45) vs. (4.25 ±2.17) years in the controls and PCOS cases, respectively. This was the first trial of IVF for 11 (55%) versus 18 (90%), while history of earlier IVF was positive for 9(45%) and 2(10%) of controls and PCOS, respectively. C-sections indications vary between obstetrical causes and the patient's wish.

In **Table 3** the participant hormonal and ultrasonic parameters were shown; serum LH, testosterone, and AMH were significantly higher among PCOS cases, while FSH was higher among controls. Serum estradiol and prolactin levels were insignificant. Both AFC and Endometrial thickness (ET) showed meaningful differences among the groups; AFC was higher, and ET was lower in PCOS vs. controls, respectively. **Table 4** showed that the total dose of r fsh and HMG used in the stimulation program were significantly higher among non-PCOS cases, while days needed for achieving stimulation were insignificant. As for the IVF outcome following stimulation, the Number (No.) of transferred embryos, No. of 8- cells, No. of 4- cells, and No. of 2PN were all statistically higher among PCOS -cases, while No. of transferred embryos was insignificant.

The StAR gene expression was highlighted in **Table 5**. StAR gene folding was significantly higher among PCOS-pregnant women versus non-PCOS pregnant (2.545 ±1.58 vs. 1.00 ±0.0), P-value< 0.023.

In **Table 6,** The correlation of the StAR gene versus all study parameters was described by a correlation coefficient; BMI showed a weak positive insignificant correlation with r= 0.14, P= NS. The estradiol hormone showed a significant inverse correlation -0.39, P<0.05, while the rest of the hormones FSH, LH, testosterone, AMH, and the ultrasonic AFC and ET failed to have a significant correlation as P>0.05, r= (-0.16 , -0.12 , -0.20 , 0.11 , 0.14 ,and-0.26) respectively. Total dose of r fsh stimulation and total dose of HMG stimulation showed significant correlations

to StAR gene with r= (-0.39, 0.60), and P-value of (0.049, 0.005), respectively. Neither days needed for stimulation, No. of retrieved oocytes, No. of 2PN, No. of 4-cells, No.8-cells, and No. of transferred embryos were of statistical value in correlation with P>0.05.

DISCUSSION

Our study showed StAR gene folding was significantly higher among PCOS-pregnant women. The total dose of r fsh and HMG used in the stimulation program was significantly higher among non-PCOS cases. In contrast to BMI, which showed weak, insignificant correlations, the StAR gene showed a significant inverse correlation to estradiol hormone. The total dose of r fsh stimulation and total dose of HMG stimulation showed significant correlations to StAR gene. Although PCOS cases had statistically higher No. of transferred embryos, 8- cells, 4- cells, and 2PN it fails to show a meaningful correlation.

Earlier studies on adipose tissue expression found higher levels of StAR gene folding [14]. Furthermore, Emami et al. [22] results showed significant difference between the genetic expression of the enzymes related to steroid metabolism (including StAR gene and CYP11A1; Cytochrome P450scc enzyme) between PCOS adipose tissue and healthy controls that were taken on C-section day. Moreover, mineralocorticoids and glucocorticoid-related enzymes were also different. Emami et al. results open a horizon for more roles of adipose tissue in cortisol metabolism [22].

Tabatabaie et al. study confirmed the presence of StAR gene in the cumulus cells of infertile PCOS women who undergo IVF programs. Their study opens a new interventional horizon for PCOS; the genetic expression of StAR gene was reduced in the PCOS sub-group that received myo-inositol supplement compared to the group without [23].

Wang et al. examined the StAR gene expression in a comparative study comprising pregnant PCOS cases and healthy controls. The gene was expressed in the subcutaneous fat of both groups. As for the enzymes needed for the inactivation of steroid hormones, ; (5-reductase1 and P450 aromatase) were significantly lower in PCOS. Consequently, PCOS women had higher local testosterone up to 400 times the plasma level and lower E2. The serum levels of the aforementioned hormones were positively correlated to their levels in the adipose tissue [24].

Our data showed a significant increase in serum LH and testosterone in PCOS cases; they were positively correlated to StAR gene expression, though the P-value was not significant.

Many consider the adipose tissue as an endocrine system of its own. PCOS women have excess abdominal fat that will render local and plasma levels of androgen and E2 and further exaggerate menstrual cycle disorders, infertility disorders, and hyperandrogenemia [25]. Hyperandrogenemia promotes central obesity, the androgen type of adiposity; although our group was matched in BMI before and after pregnancy, androgen was still higher in PCOS cases, and BMI was only insignificantly related to the StAR gene. Our result signifies the role of the StAR gene in the overproduction of androgen irrespective of BMI. We speculate that if a waist-to-hip ratio was used, it would perform better than BMI. [26,27].

As for E2 serum levels, though they were insignificant on D2 hormone analysis, by regression, they were negatively correlated to StAR gene expression with a significant P<0.05. The role of

E2 in IVF outcomes was discussed earlier [28]. Immature ovarian follicles are E2 dependent. Females with low E2 production suffer from embryonic arrest. Furthermore, androgen excess can inhibit follicular maturation, which explains why small follicles in PCOS-hyperandrogenic women have lower rates of maturation, fertilization, and embryonic development when compared to immature follicles in normal females [28,29].

Another possible mechanism for how low E2 can impact IVF success rates is estrogen immunomodulatory action. Growing evidence suggests that estrogen can prevent oxidative stress (OS) production and simultaneously upregulates endogenous antioxidants like glutathione peroxidase [30].

Oxidative stress (OS) is attracting more attention as a cause of infertility disorders like PCOS. In addition, OS serves as a prognostic biomarker in IVF success. Serum E2 levels were linked to follicular OS biomarkers that inversely impact ovarian response [31].

Analysis showed that PCOS cases had statistically higher No. of transferred embryos, 8- cells, 4- cells, and 2PN than non-PCOS cases; however, it fails to show a meaningful correlation with StAR gene. That was in line with other studies where PCOS cases were expected to be a hyper- responder and to have higher No. of the retrieved oocyte with a comparable pregnancy rate and good-quality embryo [32].

Tabatabaie et al. study. has shown an equal No. of retrieved oocytes among PCOS cases and healthy controls. However, they found that the PCOS subgroup treated with inositol supplement had more M2 oocytes, higher fertilization rates, and better embryo quality, as well as lower StAR gene expression in cumulus cells [23].

Inositol is a follicular stimulating hormone 2^{nd} messenger that controls the proliferation and maturity of granulosa cells, thus promoting oocyte maturation and producing high-quality oocytes and embryos, which explains inositol's beneficial effect on ovulation. Inositol therapy should be considered based on the patient's unique symptoms, demands, and various PCOS phenotypes to properly personalize the supplements[33,34].

Comprehending the molecular biology and physiology that underlie human fertility is crucial. It may improve the successful treatment of female and male infertility, possibly by immunity modulation among infertile copuls[35,36]. Not to mention the importance of a multidisciplinary approach in management [37].

The total dose of r fsh and HMG used in the stimulation program was significantly higher among non-PCOS cases. This can easily be interpreted as; some normal women may be normal or poor responders compared to PCOS women, who are mostly hyper responders [38].

The total dose of r fsh correlated strongly and inversely with StAR gene expression, while total dose of HMG showed the most positive correlation to StAR gene expression, $r = 0.6$, $P<0.005$. Our results have practical application in daily practices; it can save considerable cost by reducing dosages of gonadotropins that are not needed in women with high expressions of StAR gene [34].

PCOS women pose many challenges in IVF programs. A delicate balance should be tuned accordingly [39-42].

Mild in-vitro fertilization was proposed and is receiving more acceptance, especially with the encouraging results of comparable pregnancy rates, fewer patients' discomfort, reduced ovarian hyperstimulation syndrome and reduced overall cost. Hopefully, it will make the IVF service more acceptable to physicians and infertile couples [10,42,43].

Study limitations: The different phenotypic expressions of PCOS women add more to the complexity of PCOS syndrome. Our study did not take these sub-divisions in consideration, which was a study limitation. We were more interested in the link of StAR gene expression to IVF parameters and outcomes irrespective of PCOS phenotype. Another drawback is a small sample size, owing to the difficulty in obtaining the sample, the strict inclusion criteria we set for enrolment, and the COVID-19 pandemic [44].

Study Strengths: StAR gene expression was discussed in PCOS women by others who looked into the molecular aspects of StAR gene expression. Our research looks at the clinical implications of that relationship in terms of hormonal parameters, IVF outcomes, and, most importantly, a practical aspect of the stimulation program. The correlation of the StAR gene with gonadotrophin dose provides a prognostic avenue for reducing the physical and financial burden of IVF on infertile couples.

CONCLUSIONS

StAR gene expression was significantly higher among PCOS women; moreover, it correlated to stimulation drug doses. Therapeutic decisions based on genetic print may optimize patient outcomes and halt some of their associated burdens. Another promising prognostic aspect is the interventional role that is introduced by adding myo-inositol, which is believed to improve the IVF outcome.

COMPLIANCE WITH ETHICAL STANDARDS

Authors contribution

ZH: study design, conception, WN manuscript preparation, drafting, and literature review. ZH Data collection, WN and ZH : manuscript preparation, statistical analysis.

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Study registration Not applicable

Disclosure of interests

We authors, declare; that we have no conflict of interest.

Ethical approval

Mustansiriyah University/ethics committee issued the study approval (IRB 165 dated October 11, 2021).All study methods were done under Helsinki tent and comparable medical standards.

Informed consent

All participants gave informed consent before participation.

Data Sharing

All data are available upon reasonable requests to the corresponding author

TABLES LEGENDS

Table 1 Real-time –PCR reaction components additions

Table 2 Demographic criteria of study participants

Table 3 Hormonal and ultrasonic parameters of the study participants in D2 of the stimulation program

Table 4 Stimulation program drugs, doses and the IVF outcome following stimulation program

Table 5 Gene expression of StAR gene in patients and control

Table 6 The correlation coefficient between StAR gene and patients parameters

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Table 1:

Component	20µL(Final volume)
Green PCR Master Mix (Applied Biosystems™, 4309155)	10µl
cDNA	3µL
Forward primer	1µL
Reverse primer	1µL
Nuclease-free water	5µL

Table 2:

Parameters	Control group (n=20)	Study group (n=20)	Confidence interval	*P*-value
Age (years)	32.60 ±4.47	32.20 ±6.39	0.28 -1.27	0.820 NS
BMI (Kg/m^2) before Pregnancy	29.84 ±4.67	29.99 ±4.59	0.17-0.84	0.916 NS
BMI (Kg/m^2) after Pregnancy	33.54±5.28	33.87±5.33	0.22-1.-07	0.83 NS

All data are presented as (means ± SD)

BMI; body mass index.

Table 3.

Parameters	Control group (n=20)	Study group (n=20)	Confidence interval	*P* -value
FSH(IU/L)	6.92 ±1.87	4.15 ±1.48	0.87-3.41	0.0001 **
LH(IU/L)	6.48 ±2.57	9.58 ±2.64	1.02-3.77	0.0006 **
Testosterone(ng/dl)	0.632 ±0.48	1.706 ±0.80	0.82-1.89	0.0001 **
AMH (ng/ml)	2.94 ±1.48	5.51 ±1.91	1.13-4.02	0.0001 **
Estradiol (Pg/ml)	34.54 ±9.44	30.98 ±11.59	0.33-1.05	0.293 NS
Prolactin (ng/ml)	19.13 ±8.81	18.84 ±8.02	0.29-0.84	0.912 NS
AFC	20.40 ±10.51	45.65 ±8.34	0.96-3.73	0.0001 **
Endometrial thickness(mm)	5.70 ±2.92	3.65 ±1.30	0.89-1.93	0.0068 **

** (P≤0.01).

All data are presented as (means ± SD) . FSH follicular stimulating hormone, LH luteinizing hormone, AMH anti-Mullerian hormone, AFC antral follicle counts.

Table 4.

Parameters	Control group (n=20)	Study group (n=20)	Confidence interval	*P*-value
Total dose of r fsh stimulation	2362.50 ±803.54	1848.75 ±8.02	0.64-1.52	0.030 *
Total dose of HMG stimulation	798.75 ±578.02	251.25 ±157.85	0.95-3.04	0.0009 **
Duration days for stimulation	10.15 ±1.30	10.00 ±1.74	0.52-1.16	0.760 NS
Number of oocytes retrieved	7.95 ±3.91	18.75 ±4.64	1.14-4.53	0.0001 **
Number of 2PN	4.85 ±3.85	12.10 ±1.94	0.86-2.33	0.0001 **
Number of 4- cells	3.85 ±2.89	8.20 ±2.26	1.09-3.87	0.0001 **
Number of 8- cells	2.50 ±2.08	7.00 ±1.86	0.92-3.18	0.0001 **
Number of transferred embryos	2.35 ±1.53	2.35 ±0.87	0.02-0.17	1.00 NS

* (P≤0.05), ** (P≤0.01).

All data are presented as (means ± SD) .

rFSH: recombinant follicle-stimulating hormone, HMG: human menopausal gonadotropin,2PN: stage 2-pronucleus (mature oocyte)

Table 5:

Group	Ct target	Ct ref	ΔCt	ΔΔ Ct	Folding
Patients	24.22	17.42	6.81	-0.595	2.545 ±1.58
Control	25.56	17.43	8.14	0.735	1.00 ±0.00
T-test	--	--	--	--	1.289
P-value	--	--	--	--	0.0225*

* (P≤0.05).

Table 6.

Parameters	Correlation with StAR gene	
BMI(Kg/m^2)	*r.*	0.14 NS
	P.	0.556
FSH(IU/L)	*r.*	-0.16 NS
	P.	0.495
LH(IU/L)	*r.*	-0.12 NS
	P.	0.616
Testosterone(pg/ml)	*r.*	-0.20 NS
	P.	0.388
AMH(ng/ml)	*r.*	0.11 NS
	P.	0.648
Estradiol(pg/ml)	*r.*	**-0.39 ***
	P.	**0.050***
AFC	*r.*	0.14 NS
	P.	0.560
Endometrial thickness(mm)	*r.*	-0.26 NS
	P.	0.263
Total dose of r fsh stimulation	*r.*	**-0.39 ***
	P.	**0.049***
Total dose of HMG stimulation	*r.*	**0.60 ****
	P.	**0.005***
Duration days for stimulation	*r.*	-0.04 NS
	P.	0.840
Number of oocytes retrieved	*r.*	0.11 NS
	P.	0.650
Number of 2PN	*r.*	0.06 NS
	P.	0.805
Number of 4-Cells	*r.*	-0.02 NS
	P.	0.924
Number of 8-Cells	*r.*	0.18 NS
	P.	0.439
Number of transferred embryos	*r.*	-0.29 NS
	P.	0.212

* (P≤0.05), ** (P≤0.01), NS: Non-Significant.

BMI; body mass index, FSH: follicular stimulating hormone, LH: luteinizing hormone, AMH: anti-Mullerian hormone, AFC: antral follicle counts. rFSH: recombinant follicle stimulating hormone, HMG: human menopausal gonadotropin ,2PN: stage 2-pronucluse (mature oocyte)