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Using progesterone to follicular index ratio is better correlated with intracytoplasmic sperm injection outcome than using serum progesterone level alone

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ABSTRACT

Objectives: To investigate the possibility of using the ratio of serum progesterone level to the number of follicles, according to ovarian response in the day of human chorionic gonadotrophin trigger, as a predictor of cycle outcome.

Material and methods: A prospective intervensional study was conducted at Kamal Al-Samarai Hospital for Infertility Treatment and IVF during the period from December 2020 to September 2021. Ninety infertile women underwent intracytoplasmic sperm injection (ICSI) cycles using antagonist protocol. Moreover, once the patient reached triggering criteria, meticulous recording of follicular index together with serum estrogen level and serum progesterone level are measured. Fresh embryo transfer of cleavage stage embryo is done once serum progesterone level was less than 1.5 ng/mL. A follow-up to confirm pregnancy rate and cycle outcome was done.

Results: The study showed a positive pregnancy rate of 28.9%. The relationship between progesterone follicular index (Prog/FI) ratio and (ICSI) outcome was highly significant with a p value of 0.001. Additionally, an inverse relationship, as the ratio was lower the pregnancy rate was improved, was documented. The receiver operating characteristic (ROC) curve for progesterone follicular index ratio was 0.711 with a cut off value of 0.0354 ng/mL in addition to a sensitivity of 65.6 and a specificity of 65.4.

Conclusions: The serum progesterone level is an independent factor for the prediction of intracytoplasmic sperm injection (ICSI) outcome, whereas the progesterone follicular index ratio can be used as a potential marker for predicting ICSI outcome in fresh embryo transfer.

Keywords: follicular index; ICSI; outcome; progesterone level; ratio

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INTRODUCTION

During in vitro fertilization and intracytoplasmic sperm injection, progesterone elevation has been observed in about 38% of the cycles regardless of their stimulation protocol [1]. This progesterone elevation is probably not due to premature luteinization as Gonadotropin-releasing hormone agonists or an antagonist is used to prevent luteinizing hormone surge, might be due multiple causes of unclear origin. However, many reports suggest that it is due to increased granulosa cell estrogen production [2–4]. This progesterone elevation, whether it has a deleterious effect on cycle outcome or not, remains a grey area. Meta-analysis has suggested that there is a negative effect of progesterone elevation on pregnancy rate with fresh embryo transfer following controlled ovarian stimulation [2], in contrast other studies showed there is no negative effect of progesterone elevation on pregnancy rate [5, 6].

Over the last several years so many studies give different cut-off values for elevated progesterone level in controlled ovarian stimulation ranging from 0.8 to 2.0 ng/mL [7–9] and this difference is due to variable ovarian response to stimulation from patient to patient [9–11]. It was hypothesized to use progesterone level secreted by each follicle termed as progesterone follicular index ratio rather than using total serum progesterone alone to be more accurate and logic to predict the cycle outcome according to the response of

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the ovary to stimulation and not to uniform the cut off value for all patients. Follicles of 14 mm and above are the major contributors for the total amount of progesterone secreted by the ovary. Hence, an increase in progesterone could be observed in the venous effluent of the ovary containing the preovulatory follicle as early as day 10 of the cycle when the preovulatory follicle size was reached [12]. Follicular stimulating hormone, induces further luteinizing hormone receptor on the granulosa cells of these large follicles, contributes in preovulatory rise of progesterone level during normal menstrual cycle [1].

Objectives

The aim of the study was to evaluate which parameter can better predict the ICSI outcome. Each patient has its own ovarian response to stimulation regarding the number of follicles and progesterone production per follicle. Collectively this can help us whether to proceed with fresh embryo transfer or defer to a frozen cycle.

MATERIAL AND METHODS

A prospective interventional study was conducted at Kamal Al-Samarai Hospital for Infertility Treatment and IVF. The study received an approval from the local Medical Ethical Committee of the Arab Board Council/Obstetrics and Gynecology department. Each participant signed a consent form after being informed in detail about the study and the potential usefulness of the results.

Sample was collected according to the inclusion and exclusion criteria from ninety infertile women planned for intracytoplasmic sperm injection (ICSI) cycles.

Inclusion criteria are age 23–39 years, ICSI cycle for mild male factor, tubal factor infertility and unexplained infertility**,** normal or poor responder patients**,** antagonist protocol**,** fresh embryo transfer of cleavage stage embryo**,** and informed consent.

Exclusion Criteria are polycystic ovary syndrome (PCOS) patients, uterine cavity abnormality, endometriosis, uncontrolled systemic disease, trigger day progesterone level of 1.5 ng/mL or more, blastocyst fresh embryo transfer, frozen cycle, and agonist protocol.

Ovarian stimulation on day two of the cycle was achieved by a recombinant form of follicular stimulating hormone (rFSH), (Gonal F., Merck Serono; Folitrip., LG). The starting dose was individualized according to the women's age and body mass index (BMI), sixty patients were normal responder with starting dose of 150 to 225 IU/day, and the other thirty patients of poor responder had a starting dose of 300 to 400 IU/day according to their reserve or a previous poor response to stimulation.

The follow up, to measure the follicular growth, was conducted sonographically every other two days. Once the patient reached the triggering criteria, which is at least three follicles 16–22 mm in diameter, a meticulous recording of the follicular index (FI) was conducted. FI is defined as the total number of follicles reaching 14 mm and above on the trigger day. An intravenous blood sample was taken from each patient to measure serum estrogen and progesterone level by enzyme linked immunosorbent assay (ELISA) method in the same day of trigger, and the results were recorded. Progesterone to follicular index (prog/FI) ratio was calculated for each patient by dividing serum progesterone level to the follicular index.

Triggering was performed with human chorionic gonadotrophin (HCG) (Pregnyl 10,000 IU, Organon; or Ovitrelle 250 mg, Merck-Serono) administered 35 hours prior to transvaginal ovum pickup.

Ovum pickup was done under general anesthesia, and fertilization performed using ICSI cycle. Fresh embryo transfer of cleavage stage embryo was done 72 hours post ovum pickup. The number of transferred embryos was from one to two embryos depending on maternal age, medical history and past ICSI trial history if present.

The follow-up, continued until confirmation of pregnancy or not to calculate the ICSI outcome, was documented. The pregnancy was diagnosed by rising serum B-HCG level and confirmed ultrasound of positive fetal heart.

The available statistical tool SPSS-27 was used to analyze the data (Statistical Packages for Social Sciences — version 27). Simple frequency, percentage, mean, standard deviation, and range measurements were used to present the data from minimum to the maximum values.

The significant difference between two independent means in the form of quantitative data was determined using the Students' *t*-test or the ANOVA test for the difference between more than two independent means. If p value was equal to or less than 0.05, statistical significance was considered [13].

The receiver operating characteristic (ROC) curve technique was used to establish the utility of any parameter as a diagnostic or screening tool for disease, as well as the possibility to find the optimal "cut-off value" for diagnosing disease. The following is an explanation of the ROCS area "area under the curve" (AUC):

- 0.9 — "Perfect";
- 0.8 — "Good";
- 0.7 — "Fair";
- 0.6 — "Poor";
- < 0.6 — "Fail" [14].

RESULTS

Of the 90 participants' patients, the ICSI outcome of the current study was 26 (28%) patients with positive pregnancy rate. The mean ± SD of each parameter was shown in the

Table 1. The relationship between follicular index (FI), number of oocytes, progesterone level, estrogen level and progesterone follicular index (Prog/FI) ratio with the cycle outcome

	Pregnancy test		p value
	Positive 26 (28.9%)	Negative 64 (71.1%)	
	Mean ± SD (range)	Mean ± SD (range)	
FI	14.88 ± 4.70 (7–25)	12.86 ± 3.82 (7–22)	0.036#
Number of oocytes	12.2 ± 3.7 (5–20)	10.5 ± 3.1 (4–18)	0.029#
Progesterone [ng/mL]	0.48 ± 0.21 (0.10–0.77)	0.58 ± 0.27 (0.29–1.32)	0.078
Estrogen [pmol/L]	1608.8 ± 604.6 (600–3046)	1596.8 ± 569.0 (594–2691)	0.929
Prog/FI ratio	0.032 ± 0.012 (0.0124–0.0556)	0.049 ± 0.024 (0.0150–0.1125)	0.001#

#Significant difference between two independent means using Students *t*-test at 0.05 level; SD — standard deviation

table with its range for the sample collected. The relationship between follicular index, number of oocyte and progesterone follicular index ratio (Prog/FI) with ICSI outcome were statistically significant with p value of 0.036, 0.029 and 0.001 respectively as shown in Table 1.

The relationship between age, progesterone level and ICSI outcome were not statistically significant, all p values were more than 0.05, although increasing age especially 35-years-old and above had higher progesterone production but still not statistically significant. The relationship between stimulation dose, progesterone level and ICSI outcome were not statistically significant; all P values were more than 0.05.

The relationship between age, prog/FI ratio and ICSI outcome was statistically significant with p value of 0.001 as those with negative pregnancy rate had significant higher Prog/FI ratio and those with older age group of 35-years-old and more had significant higher Prog/FI ratio compared to positive pregnancy with p value of 0.005 in the same age group. The relationship between stimulation dose, Prog/FI ratio and ICSI outcome is statistically significant with p value of 0.001, those with negative pregnancy had significant higher Prog/FI ratio and this ratio increased with stimulation dose increment as shown in Table 2.

The receiver operating characteristic curve and AUC value for estrogen was 0.505 and for progesterone was 0.584. They failed to predict ICSI outcome in fresh embryo transfer. AUC for FI was 0.627 poor to predict ICSI outcome, while AUC for Prog/FI ratio was 0.711 which is fair predictor for ICSI outcome in fresh transfer. The proposed cut off value of Prog/FI ratio was 0.0354 ng/mL for prediction of ICSI outcome with a sensitivity of 65.6 and a specificity of 65.4 as shown in Figure 1.

DISCUSSION

The present study showed that Prog/FI ratio had been a good predictor of ICSI outcome in cleavage stage fresh embryo transfer cycle while the trigger day serum progesterone level alone was not a good predictor for the cycle outcome. The present study showed the ICSI outcome was 26 (28.9%) of positive pregnancy rate that nearly simulates the pregnancy rate in most of the world *In Vitro* Fertilization (IVF) centers.

The current study showed there were positive significant correlations between follicular index and number of oocytes with ICSI outcome, the p value was 0.036 and 0.029 respectively, these findings go with other study that reported pregnancy improved with an increasing number of oocytes [15]. This could be explained by increasing the number of oocytes will increase the number of the embryos, as well as improve the chance of a good quality embryo, that we had gotten.

The current study showed the relationship between progesterone level and ICSI outcome had no statistical significant difference p value 0.078, and this goes with study who observed that elevated progesterone level on the trigger day doesn't negatively affect the cycle outcome [16], in contrast study showed elevated progesterone level associated with negative effect on outcome [4, 9] and this could be explained by different exclusion criteria for progesterone level at the trigger day like including agonist protocol or involvement of blastocyst embryo transfer in the study.

The current study showed the relationship between estrogen level and ICSI outcome had no statistically significant difference, p value 0.929, this goes with other study [17], in contrast to other study that showed the increase in estrogen level associated with higher pregnancy rates [18], this could be explained due to the involvement of the hyper responders or different protocols used.

The current study showed the correlation between Prog/FI ratio and ICSI outcome, P value of 0.001, was highly significant with inverse relationship. This finding goes with other studies that showed Prog/FI ratio is a better predictor of IVF-ICSI outcome than trigger day serum progesterone level [19, 20].

As each mature follicle will secrete its own progesterone, therefore we cannot uniform a cut off value of progesterone for

Table 2. The relationship between age, stimulation dose with progesterone and with progesterone follicular index (Prog/FI) ratio and its effect on the outcome

		Progesterone [ng/mL]				p value
		Positive pregnancy		Negative pregnancy		
		No	Mean ± SD	No	Mean ± SD	
Age [years]	20–24	2	0.63 ± 0.19	1	0.39	0.499
	25–29	9	0.49 ± 0.23	18	0.57 ± 0.30	0.501
	30–34	8	0.36 ± 0.18	22	0.56 ± 0.29	0.073
	35–39	7	0.56 ± 0.21	23	0.63 ± 0.24	0.488
	p value		0.207		0.732	
Stim. dose	150	6	0.43 ± 0.23	10	0.50 ± 0.30	0.629
	225	13	0.49 ± 0.21	31	0.56 ± 0.26	0.408
	300	3	0.52 ± 0.29	11	0.72 ± 0.32	0.353
	375	2	0.62 ± 0.16	9	0.54 ± 0.19	0.610
	400	2	0.35 ± 0.21	3	0.78 ± 0.14	0.067
	p value		0.759		0.207	
		Prog/FI ratio				
Age [years]	20—24	2	0.031 ± 0.010	1	0.022 ±	0.498
	25—29	9	0.031 ± 0.012	18	0.038 ± 0.016	0.262
	30—34	8	0.029 ± 0.013	22	0.044 ± 0.024	0.120
	35—39	7	0.038 ± 0.009	23	0.064 ± 0.022	0.005#
	p value		0.552		0.001^	
Stim. dose	150	6	0.026 ± 0.013	10	0.036 ± 0.018	0.273
	225	13	0.032 ± 0.009	31	0.042 ± 0.021	0.091
	300	3	0.038 ± 0.017	11	0.060 ± 0.021	0.121
	375	2	0.035 ± 0.002	9	0.059 ± 0.023	0.194
	400	2	0.040 ± 0.022	3	0.093 ± 0.017	0.054
	p value		0.500		0.0001^	

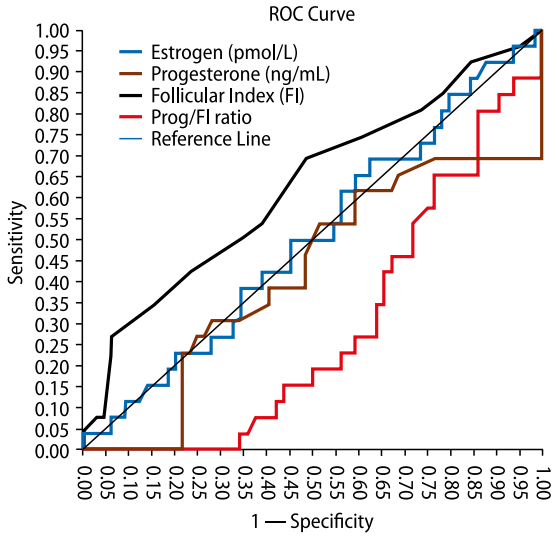
#Significant difference between two independent means using Students-t-test at 0.05 level; ^ Significant difference among more than two independent means using ANOVA-test at 0.05 level; SD — standard deviation; Stim. — stimulation

an ovary which produces for example 17 oocytes and another ovary which produce three or four oocytes. The lower pregnancy rate, which was associated with a higher Prog/FI ratio, could be explained by a higher ratio could cause an endometrial-embryo asynchrony as showed in study in which progesterone elevation affect the implantation window [21], rather than associated with bad quality oocytes or embryos, as many studies showed when serum progesterone is elevated was not associated with negative effect on pregnancy outcome as frozen transfer is used [9, 22]. Another study showed no relation between elevated progesterone level and IVF outcome in fresh transfer of donor cycle [23], this will further confirm this finding because it transferred embryos to a uterus not exposed to a high progesterone level during the period of oocytes development.

The current study found the age increment, especially 35-years-old and above, had higher progesterone production and lower pregnancy rate and this might be explained by change in ovarian response to stimulation or using a higher doses of gonadotrophin stimulation although this was not statistically significant, this goes with other study [24].

The current study found as the stimulation doses increased, progesterone level getting higher and pregnancy rate getting lower although this was not statistically significant, this finding goes with other studies that showed a positive correlation between high late follicular phase progesterone level and the administered FSH dose [1, 25], which could be explained by a higher dose causes further pressure on the granulosa cells to produce more progesterone.

The current study showed the relationship between age, Prog/FI ratio and ICSI outcome is statistically significant, p value of 0.001. Increased age associated with higher progesterone production per follicle, increased prog/FI ratio,



Test result variables	AUC	SE	p value	95% CI	
				Lower bound	Upper bound
Estrogen [pmol/L]	0.505	0.067	0.943	0.374	0.636
Progesterone [ng/mL]	0.584	0.070	0.216	0.447	0.720
Follicular Index	0.627	0.067	0.060	0.495	0.759
Prog/FI ratio	0.711	0.054	0.002*	0.604	0.818

Figure 1. The receiver operating characteristic (ROC) curve of estrogen progesterone, follicular index and progesterone follicular index (Prog/FI) ratio of the studied sample for the prediction of the outcome with the AUC values; *significant at P 0.05 and less; AUC — area under the curve; CI — confidence interval; Prog/FI ratio — progesterone follicular index ratio; SE — standard error

that adversely affect the pregnancy rate especially those with age group of 35-years-old and more, these findings go with other studies [19, 20]. This could be explained as much as the age increased there is a change in ovarian response to stimulation, and usually doctors use higher doses of gonadotrophins to maximize the number of growing oocytes.

In the present study showed the relationship between stimulation dose, Prog/FI ratio and ICSI outcome is statistically significant (p value is 0.001) means as the dose increased there was increase in prog/FI ratio and thus a reduced pregnancy rate,

The present study showed the ROC curve for estrogen and progesterone were 0.505, 0.584 respectively, they failed to predict ICSI outcome. This is the same finding of a study [9], *i.e.*, progesterone is a weak marker of IVF outcome. ROC curve for FI was 0.627 which was poor to predict ICSI outcome, while ROC curve for PFI ratio was 0.711 which is fair predictor for ICSI outcome in fresh transfer, these findings go with other studies [19, 20].

The present study showed the proposed a cut off value of PFI ratio of 0.0354ng/ml for prediction of ICSI outcome with a sensitivity of 65.6 and a specificity of 65.4, our PFI ratio cut off value was lower than that found in other studies [19, 20]. This could be due to involvement of agonist protocol in their studied sample which is associated with higher progesterone production [26] compared to antagonist protocol that we used only in our study. In addition, a different race or a different laboratory technique was implemented. Further meta-analyses, to prove the effectiveness of using Prog/FI ratio as a predictor of ICSI outcome and the proposed Prog/FI ratio that can be used according to the types of patients and their protocol, are required.

CONCLUSIONS

So many studies in the last several years are trying to find markers to predict the outcome of IVF cycles. Serum progesterone level alone is not a predictor of cycle outcome. Therefore, we suggest using Prog/FI ratio as a potential marker for predicting ICSI outcome. Once the ratio is high, it is better to segment the cycle and proceed with frozen embryo transfer.

Article information and declarations

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Conflict of interest

All authors declare no conflict of interest.

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