Early Serum Progesterone Measurement on 9th Day after Oocyte Retrieval can be used as a Predictor of Fresh Intracytoplasmic Sperm Injection Cycle Success

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Abstract

BACKGROUND: In intracytoplasmic sperm injection (ICSI) infertility treatment cycles, measuring serum Progesterone level at day 9 after oocyte retrieval could be used as a predictor of success.

METHODS: Sixty-nine women were prospectively included in this study, treated with fresh embryo transfer ICSI cycles. Progesterone analyses were performed on the day of oocyte pick up (day 0) at serum and follicular fluid, then re-assessment at serum on day 9 after oocyte retrieval. The data were compared to evaluate the correlation among hormones measured on day 0 and day 9 with pregnancy rate.

RESULTS: Pregnancy rate of Iraqi women was 22% (n = 15), mean serum progesterone on day 9 among pregnant ladies was (mean ± SD, 39.5 ± 13.0) which was significantly higher than that who failed to get pregnant (mean 23.2 ± 11, p = 0.001), then after adjustment of the baseline readings (day 0) estimated mean = 11.1. The differences were still significant, receiver operating characteristic curve area showed that serum Progesterone on day 9 after pick up can differentiate correctly between those who will conceive and those who will not, with a sensitivity = 0.933 and specificity = 0.519, at cut off point = 22.33 and above, (Area under the curve = 0.822, p = 0.001).

CONCLUSION: Serum progesterone on day 9 could be one of the predictors of endometrial receptivity and pregnancy, which is actually of great value for both doctors and patients during that stressful period till the date of confirmation, which might negatively affect treatment outcome, as well as the psychosocial and pharmacological impact of medication and limited activity for a failed one.

Introduction

Inspite of the advancement in medical treatment techniques, infertility remains a struggle for many clinicians, and the fear from the failure of that costly treatment intracytoplasmic sperm injection (ICSI) cycle can cause consequent stress which could negatively affect the outcome. So many researchers tried to find a method for identifying the success of treatment early which might be reflected positively on treatment outcome, in addition to studying hormones that reflect endometrial receptivity, as it is fundamental for success. In our study we studied progesterone hormone which plays a unique role in implantation and pregnancy.

The adrenal cortex and the gonads both synthesize progesterone, which is an endogenous steroid hormone [1]. During the luteal phase, the corpus luteum (CL) is formed by the luteinization of granulosa cells, which results in a significant increase in Progesterone secretion and a second rise in estradiol (E2) levels. The luteal phase of the ovary normally lasts 14 days, after which the CL regresses, resulting in a decrease in ovarian E2 and Progesterone production, this is followed by ischemia, necrosis, endometrial shedding, and bleeding [2].

Progesterone's key role during pregnancy is to maintain a low degree of vascular tone in the myometrium, it also has an effect on the production of inflammatory mediators by human T-cells in the uterus. Thus, the Loss of Progesterone causes an increase in myometrial contractility as well as a decrease in the ability to fend off immunologic challenges, increasing the chance of miscarriage and premature delivery of the fetus [3].

The controlled ovarian stimulation cycle (COS) alters the pulsatile release of luteinizing hormone, which impairs the CL's appropriate support at the anterior pituitary level [3].

Progesterone directs endometrial secretory transformation by changing gene expression and as a result, it's important for implantation and early embryologic development [4], [5]. After COS, the granulose of the CL secretes insufficient amounts

of Progesterone endogenously [6]. Due to the suction of the granulosa cells during oocyte retrieval, inhibition of the hypothalamic-pituitary axis by supra-physiological synthesis of steroids, and luteolytic effect of gonadotropin-releasing hormone (GnRH) analogs or GnRH antagonists utilized for the COS [7]. By supplementing with Progesterone, human chorionic gonadotropin (hCG), or a GnRH agonist; luteal phase support compensates for this shortage [8].

The function of steroids (Progestron and E2) is to get meiotic support and undergo regular fertilization and development to the blastocyst stage. The higher the ratios of Progesterone to E2 in follicular fluid in several species, the better embryo development [9]. The indirect effect of Progesterone can impact embryo development by binding to uterine stroma or endometrial Progesterone receptors and starting a process of events containing differences in gene expression; thus, eventually protein expression or variations in uterine permeability to ions, amino acids, or metabolites from plasma or by non-genomic effects on the uterine endometrium [10].

High Progesterone level before eggs collection is linked with a noticeable endometrial receptivity decrease. Thus, an optimal Progesterone level is essential for successful implantation and pregnancy [11].

Materials and Methods

This cross-sectional prospective study used the data from 69 ladies from the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/ Al-Nahrain University, and al-Farah Fertility private Center, in Baghdad/Iraq. It was conducted during the time between October 2020 and September 2021. The study was approved by the Arab Faculty for Medical Specialization. The participants are fully informed about the objectives and methods.

Women's ages ranged from 19 to 43 years old, infertility duration ranged from (1 to 17) years old, the cause of infertility was a malefactor in 60% of cases. This analysis included data records of 115 patients, who underwent the ICSI procedure; 29 patients who had the procedure canceled as they did not carry out all the investigations due to failure of getting oocyte or embryo, 17 patients removed as fresh cycle converted to a frozen cycle.

Inclusion criteria were any fresh transfer ICSI treatment cycle regardless of age, cause of infertility, or protocol used, in addition to the absence of endometrial or ovarian pathologies.

Exclusion criteria; treatment cycle does not result in oocytes for fertilization, no embryo suitable for transfer obtained or our cycle urgently converted to frozen one duto associated risk of fresh embryo transfer as in moderate or severe ovarian hyperstimulation syndrome and its consequences lastly if trigger day progesterone level more than 1.5 ng/mL which reflected expire implantation window.

Hormonal assessment on day 2–3 of the cycle, antral follicle count evaluated on day 5–6 of cycle patient received standard ovarian stimulation with a recombinant follicle-stimulating hormone (FSH) (rFSH: Gonal F, Follitrope) with or without human menopausal gonadotropins (HMG: Menagon, menopure).

The method used for pituitary suppression was antagonist protocol that included an antagonist drug (cetrorelix acetate-Cetrotide), which was initiated from observation of follicles size of >13 mm on ultrasound, serial transvaginal pelvic ultrasonography was used to monitor the follicle growth. Adjustment of the stimulation dose until two or more follicles larger than 18 mm, then triggering with hCG (Ovitrelle) only or agonist (Treptoreline- Decapeptyl) combined with Ovitrelle were given. After 36 h of the trigger, follicle aspiration was performed under general anesthesia, the surgery was carried out transvaginally with ultrasound guidance. The collection and processing of the sperm, as well as the ICSI procedure, were completed on the same day. 2 or 3 days following ova pick-up, the embryos were transferred with abdominal ultrasonography assistance.

Progesterone pessaries at a dose of 400 mg, along with intramuscular Progesterone injection once every 3–4 days until the day of a pregnancy test. Serum B-HCG was done 16 days after pick up to confirm pregnancy. Radioimmunoassays for Progesterone were used to measure hormone levels in the blood (nanograms per milliliter are the unit of measurement).

The samples were taken twice: first on day 0 in serum and follicular fluid respectively, then re-measurement of the hormone on the 9th day in serum at the morning time. For uniformity, the majority of our samples are forwarded to the same clinical laboratory.

Statistical analysis

Was carried out using the available statistical package of Statistical Packages for Social Sciences - version 24. Data were presented in simple measures of frequency, percentage, mean, standard deviation, and graphs were used to present descriptive statistics. The significance of difference of different means (quantitative data) was tested using the Students *t*-test analysis of covariance (ANCOVA) (which is the midpoint between analysis of variance and regression analysis, used to find out the significance of differences between different means of quantitative variables) was used on the 9th day of work.

After adjustment of day 0 reading of same variables, "receiver operating characteristic (ROC)" curve technique was used to analyze the use of any parameter as diagnostic or screening tool for disease and the

ability to determine the "cut-off value" which of optimum sensitivity and specificity for diagnosing disease. The ROCS area "area under the curve (AUC)" explanation as follows, 0.9---- "Perfect," 0.8--- "Good" 0.7--- "Fair" 0.6--- "Poor" <0.6 "Failure." Pearson correlation was used to find out the significance of correlations between related numerical variables. The p < 0.05 was considered as the discrimination point of significance.

Results

In a sample of 69 infertile couples, 15 (22%) became pregnant and 54 (78%) failed. The mean of serum P (S.P) on day 9 post oocyte pick up was 39.5 ± 13.0 among pregnant ladies which is significantly higher than that of ladies who failed to get conceptions (mean 23.2 ± 11), p = 0.001. To abolish the effect of starting serum P level (pick up day: day 0) on the 9th-day post-collection serum level, the researchers applied the ANCOVA test which shows that after adjustment of baseline readings of S. P estimated mean = 11.1 the differences between end line readings after 9 days were still significant, p = 0.001 (Table 1).

Figure 1 Using S. P on D9 after retrieval as a screening test to find out the probability of getting pregnancy after ICSI can differentiate correctly between those who got pregnant and those who failed (total AUC = 0.822, p = 0.001), at cut off point = 22.33 and above, sensitivity = 0.933 and specificity = 0.519.

Table 2 it shows that intermediate positive correlation were noticed between Trigger day E level (TDE2) and getting mature oocyte (MII) (r = 0.572, p = 0.001), Furthermore, intermediate positive correlations between TDE2 and Anti-mullerian hormone (AMH) (r = 0.359, p = 0.002). Strong positive correlation were noticed between TDE2 and total number of oocyte (TNO) (r = 0.660, p = 0.001). Intermediate positive correlation was noticed between S.P at D0 and AMH (r = 0.352, p = 0.003). Weak positive correlation was noticed between MII and Grade1 embryos. (G1) (r = 0.281, p = 0.019) Intermediate correlation was noticed MII and AMH (r = 0.324, p = 0.007). Strong positive correlation was noticed between MII and total oocytes(r = 0.835, p = 0.001). Weak positive correlation was noticed between MII and No. of embryos transferred (NET) (r = 0.239, p = 0.048). Strong positive correlation was noticed between G1 and NET (r = 0.656, p = 0.001).

Intermediate positive correlation were noticed between AMH and maternal age (r=0.302, p = 0.012). Weak negative correlation was noticed between mother age and NTO (r = –0.299, p = 0.13). Weak positive correlation was noticed between NTO and NET (r = 0.286, p = 0.017).
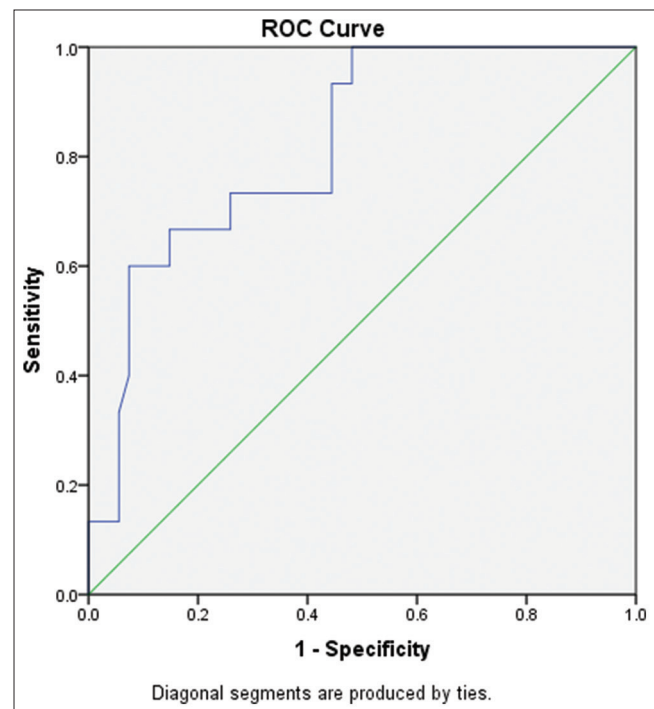

Figure 1: Area under the curve

Test result variable (s): Serum progesterone

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% confidence interval	
			Lower Bound	Upper Bound
0.822	0.056	0.0001	0.711	0.932

The test result variable (s): Serum Progesterone has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased. [a]Under the nonparametric assumption, [b]Null hypothesis: true area = 0.5.

Table 3 HCG and agonist trigger group versus HCG only group:

Significantly higher:

1. Mean of MII (7.65 ± 5.175 vs. 5.59 ± 3.256, p = 0.47)
2. Mean of G1 embryos ((2.30 ± 7.83 vs. 1.85 ± 0.894, p = 0.016).

Discussion

Endometrial receptivity has been investigated by many authors, they tried to find a non-invasive tool

Table 1: The difference between means of serum progesterone levels after 9 days of pick up (D0) after adjustment of baseline readings according to the ANCOVA test

Pregnancy	Estimated serum progesterone					p-value
	Staring mean (ng/ml)	9^{th}day mean (ng/ml)		95% confidence interval		
		Mean	Std. error	Lower Bound	Upper Bound	
Not pregnant	11.1	23.3	1.575	20.197	26.488	0.001
Pregnant		39.1	3.020	33.109	45.170	

ANCOVA: Analysis of covariance.

Table 2: Correlation between measured numerical variables

Variables	CD2E2	P FFD0	S.P D0	S.PD9	MII	TNO	G1	S.D.	AMH	M.age	NET
TDE2											
r	0.096	-0.023	-0.039	0.024	0.572**	0.660**	0.106	0.053	0.359**	-0.223	0.117
PV	0.433	0.849	0.750	0.844	0.000	0.000	0.386	0.663	0.002	0.065	0.337
CD2E2											
r		-0.111	-0.137	-0.186	0.072	0.058	-0.034	0.007	0.045	-0.004	0.133
PV		0.365	0.262	0.126	0.558	0.639	0.779	0.957	0.715	0.973	0.277
P FFD0											
r			0.151	-0.081	0.116	0.125	-0.034	0.068	0.108	0.074	-0.121
PV			0.216	0.510	0.342	0.307	0.782	0.577	0.377	0.545	0.322
S0.PD0											
r				-0.170	0.021	0.030	0.021	0.090	0.352**	-0.107	-0.010
PV				0.163	0.865	0.810	0.867	0.464	0.003	0.382	0.936
S0.PD9											
r					0.054	0.039	0.068	0.139	-0.148	-0.031	-0.027
PV					0.657	0.752	0.577	0.253	0.223	0.802	0.826
MII											
r						0.835**	0.281*	0.079	0.324**	-0.213	0.239*
PV						0.000	0.019	0.521	0.007	0.079	0.048
TNO											
r							0.235	0.007	0.447**	-0.299	0.286*
PV							0.052	0.953	0.000	0.013	0.017
G1											
r								0.048	0.087	-0.002	0.656**
PV								0.692	0.477	0.990	0.000
S.D.											
r									-0.067	0.027	0.037
PV									0.586	0.823	0.763
AMH											
r										-0.302	0.113
PV										0.012	0.353
M.age											
r											-0.007
PV											0.957

TDE2: Trigger day Estradiol (E2), CD2E2: Day 2 cycle E2 level, *P* FFD0: Progestron in follicular fluid at the fluid on day of oocyte pick up, S.PD0: S. progesterone on oocyte pick up day, S.P.D9: S. Progestron. 9 days after oocyte pick up, MII: Mature oocyte, TNO: Total no. of oocytes, G1: Grade1 embryos. S.D.: Stimulation duration, AMH: Anti-mullerian hormone, M.age: Maternal age, NET: No. of embryos transferred. Weak correlation r = <0.3, intermediate correlation r=0.3–0.6, strong correlation r = >0.6, PV<0.05 significant correlation, PV<0.01 highly significant correlation.

Table 3: Differences between means of measured numerical variables according to triggers

Lab. Investigations	Trigger	n	Mean	Std. Deviation	
TDE2	HCG	46	1351.0385	627.65825	0.326
	HCG and Agonist	23	1553.0391	865.20487	
Follicular fluid P	HCG	46	20690.22	14117.986	0.625
	HCG and Agonist	23	19017.43	11448.981	
Serum P day 0	HCG	46	10.5646	14.04643	0.670
	HCG and Agonist	23	12.1517	15.45874	
Serum P day 9	HCG	46	26.54	13.189	0.836
	HCG and Agonist	23	27.25	13.663	
Total Oocyte	HCG	46	8.78	4.427	0.088
	HCG and Agonist	23	10.96	5.788	
MII	HCG	46	5.59	3.256	0.047
	HCG and Agonist	23	7.65	5.175	
G1	HCG	46	1.85	0.894	0.016
	HCG and Agonist	23	2.39	0.783	
Freezing number Embryos	HCG	46	0.85	1.282	0.156
	HCG and Agonist	23	1.70	2.636	

HCG: Human chorionic gonadotropin, MII: Mature oocyte.

to assess the probability of pregnancy during the fresh embryo transfer cycle, As Progesterone has an essential role in controlling the luteal phase and secretary endometrial development, so it has been extensively studied in different parts of *in vitro* fertilization (IVF) cycles, with searching on a useful cut-off point to correct hormonal issues during treatments. It is known that an embryo implanted with early luteinization has a decrease in its adhesion rate. Another moment of this hormonal investigation, which motivated this study, at the luteal phase, having to wait for the day of pregnancy confirmation brings a high degree of stress to families. The determination of a Progesterone curve that enables us, 1 week before the β-HCG, to learn about treatment success or failure, would be of great value for both, doctors and patients.

The success rate of our study which included 69 ladies, their ages between 20 and 46 years old, was 22%. Because most of our patients were old which goes with the National Infertility Association success rate, IVF cycles in the United States had a success rate of 20–35% on average, with the highest success rates around 40%. For the 1st cycle of IVF, women aged 40–42 years had a 12% live birth rate (Honor *et al.*) [12].

Progesterone is advocated as one of the best predictors of pregnancy outcome in spontaneous pregnancies (Elson *et al.*) [13]. However, in IVF cycles, due to supplementation during the luteal phase, higher levels of Progesterone would be expected, even during an unviable pregnancy.

Labarta *et al.* [14], patients with an ongoing pregnancy had greater serum Progesterone levels than the rest of the patients, with an increasing tendency during the luteal phase days. Patients who had a negative outcome or had lost their pregnancy did not exhibit this behavior.

Pérez *et al.* [15] suggested that Progesterone dosage on day 9 after follicular aspiration could be one of the predictors of gestation, such information can also assist clinicians in monitoring and providing appropriate early gestational care. his study included 322 patients who underwent fresh transfer ICSI procedure, The mean Progesterone level in the pregnant group was 35.12 for pregnant women and 28.24 for non-pregnant women, and this distinction was deemed statistically significant (p = 0.02). This study revealed that using serum P test at the level of 39.1 ng/ml on (9th-day post pick up) can give the diagnostic probability of success with a sensitivity of 93.3% and specificity of 51.9%, and this finding goes with Pérez *et al.*, 2018 and Ioannidis *et al.* [15], [16].

Al-Ramahi *et al.* [17] found that a level lower than 14.2 ng/ml in early pregnancy suggests a non-viable gestation.

While Vicdan and Zeki Isik [18] suggested that there are no discernible differences between pregnant and non-pregnant women when it was measured 11 days after pick up, but, there are reports that P in spontaneous pregnancies is decreased days or weeks before an unfavorable prognosis. Thus, serum P was proposed as an early detection instrument for abnormal pregnancy (Yeko *et al.*, 1987, Hahlin *et al.*,) [19], [20].

Our study observed there is a high positive link between the level of E on the trigger day and the TNOs retrieved, which goes with Siddhartha *et al.* study [21].

The number of total oocytes had strong positive associations with the presence of top-quality embryos for transfer. Shim *et al.* [22], it goes with our study results which proved the same findings.

With good ovarian reserve, we get a high no of MIIs as the pool of total oocytes increased, and it is negatively correlated to maternal age so this is a logical finding proved by a lot of studies like that one carried out by (Broer *et al.*) [23].

AMH levels fall with chronological age, according to a slew of further investigations. The average annual drop in women aged 21 and up is 5.6% (Bentzen *et al.*). [24].

In our study, If patients are divided according to trigger type, The dual trigger group had a significantly higher mean of developed oocyte and embryo grade1 than the HCG alone group, these interesting findings correspond to a lot of new studies like Haas *et al.* [25]. Who suggested, using the dual trigger enhances the number of oocytes, MIIs, and total and top-quality oocytes.

Griffin *et al.* [26] concluded that dual triggers can raise the percentage of mature oocytes retrieved in patients with a low number of mature oocytes retrieved.

Conclusion

Serum P on the 9th-day post pick up could be one of the predictors of gestation, which is actually of great value for both doctors and patients during this period till the date of confirmation, which is associated with a lot of stress, as well as psychosocial impact of medication and limited activity.

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