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The Correlation between Follicular Fluid Levels of Progesterone and Anti-Müllerian Hormone and Pregnancy Rate in ICSI-Cycle

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ABSTRACT

To measure follicular fluid level of progesterone and Anti-Müllerian Hormone (AMH) and correlate it with pregnancy rate in ICSI-Cycle. Fifty infertile women managed with Intra-cytoplasmic sperm injection (ICSI) in Kamal Al-Samurai infertility center between January 1st and June 30th 2021 were investigated for follicular fluid levels of AMH and progesterone, and their correlation with pregnancy rate performed two weeks after ova pick up was analyzed, correlation of other parameters like antral follicular count (AFC) with pregnancy rate was also assessed. Both AMH and progesterone levels in follicular fluid have highly significant correlation ICSI outcome with p value less than 0.001. Follicular AMH and progesterone levels had highly significant correlation with pregnancy rate, while weight, duration and type of infertility and AFC showed no significant correlation.

1. INTRODUCTION

Infertility is a common medical problem and may cause a major social and psychological impact on infertile couples. It is defined as failing to conceive after one year in spite of regular unprotected sexual intercourse [1]. In vitro fertilization (IVF) is a common assisted reproductive technique used to achieve pregnancy [2]. Since 1992 intracytoplasmic sperm injection (ICSI) is increasingly used to improve fertilization [3]. Controlled ovarian hyperstimulation is used to induce ovulation by multiple follicles using fertility medications [2]. Follicular fluid (FF) contains a variety of molecules whose primary role is to modulate oocyte and follicular maturation. Proteomic analysis of follicular fluid is a noninvasive method to obtain information concerning follicular development and oocyte quality [4]. Selecting oocyte based on morphological criteria is subjective and inaccurate [5] and identification of oocyte competence biomarkers is one of the main targets of current studies to help in choosing the best oocyte. Metabolomics assessment of FF gives an ideal source of noninvasive predictive factors of the quality of oocyte that resulted in pregnancy [6], [7].

Anti-Müllerian Hormone (AMH) expression is restricted to the granulosa cells of the ovary. In FF, AMH is started with the recruitment of primordial follicles by inhibiting it and its expression is the highest in preantral and small antral follicles and then decline with the selection of follicles until no longer expressed during the FSH dependent stages of follicular growth [8]. Serum AMH level is highly correlated with the number of growing follicles and ultrasonographic measure of the antral follicular count (AFC) [9], [10]. Progesterone P4 is also a follicular steroid that plays critical roles in ovulation and trigger meiosis resumption in oocytes, implantation and maintenance of pregnancy. Clinically, it can be used in the female reproductive system as luteal support during IVF [11]. It was found that P4 levels in follicular fluid and its ratio to the levels of estrogen are highly associated with oocyte quality and maturity [12]. However, the effect of P4 on in vitro oocyte maturation (IVM) is still controversial [13]. The aim is to assess the correlation between FF - AMH, and P4 levels and ICSI outcome in a sample of Iraqi women enrolled in ICSI programs.

2. Patients and method

This is a prospective cohort study, included fifty patients complaining from infertility that were managed in Kamal Al-Samurai infertility center starting on January the 1st to June the 30th 2021. The study was approved by ethical committee of Arab board for specialized medical studies, and written consent was obtained from all patients recruited in the study. All patients with regular menstrual cycle, aged 20-40 years, with primary or secondary infertility were included, any patient with one or more of the following criteria were excluded, patients with PCOS, endometriosis, History of diabetes, hypertension, renal impairment or thyroid function abnormalities, cycles in which no oocytes were retrieved on the day of aspiration, Abnormal hormonal essay (AMH, progesterone, FSH, E2) and non- compliance with the protocol. AFC was assessed by transvaginal ultrasound on the second day of cycle. Then every patient underwent controlled ovarian stimulation with antagonist protocol, trigger with 10,000 IU subcutaneous HCG was given when at least one follicle is 18mm in addition to two or more follicles of 17mm, determined by transvaginal ultrasound, ova pickup (OPU) was done 35 hours after trigger administration under general anesthesia. At the day of OPU, FF aspirated from the first follicle, centrifuged and then frozen in temperature of -20 ˚C until being measured by specialized lab for AMH and progesterone with ELISA kits. ICSI was done afterwards. Three days after oocyte retrieval up to 3 embryos were transferred; luteal phase support was done by 50 mg progesterone daily for 2 weeks starting from the day of OPU. Pregnancy test (PT) is performed for all recruited ladies two weeks after OPU, and accordingly they were grouped into two groups (PT +ve, PT -ve). Specially designed forms were plotted for every patient, and data were analyzed using computerized statistical software; Statistical Package for Social Sciences (SPSS) version 25. Descriptive statistics written as (mean ± standard deviation) and frequencies presented as percentages. Multiple contingency tables conducted and appropriate statistical tests are performed, Chi-square used for categorical variables and t-test was used to compare between two means, significance.

3. Results

Fifty infertile ladies were recruited in this study, they were divided in to two groups according to pregnancy test (PT) performed two weeks after embryo transfer 33 (66%) ladies were PT +ve, the remaining 17 (34%) were PT –ve. Demographic characteristics of both groups showed no significant differences regarding age, body mass index (BMI), duration, type and cause of infertility, and AFC (tab. 1). The mean concentrations of AMH in (FF) of ladies in PT +ve group was significantly higher than in PT –ve group (3.24± 0.72 Vs 1.16 ±0.35 pg/ml) respectively (P =<0.001) (table 2). So were the mean concentrations of P4 (6.81±1.6 Vs 3. 38±1.74 pg/ml), respectively (P<0.001) (table 2). The sensitivity of the AMH test to predict positive PT at level ≥ 3.02 (pg/ml) was (76%), specificity (78%), negative predictive value (NPV) (73%) so the false negative was (27%), positive predictive value (PPV) (90%) so the false positive was (10%), and the

accuracy of the test was (75%). (tab. 3). The sensitivity of the P4 test to predict positive PT at level ≥5.71 (pg/ml) was (32%), specificity (81%), NPV (91%) so the false negative was (9%), PPV (88%) so the false positive was (12%), and the accuracy of the test was (77%) (Table 3). Combining both AMH at ≥ 3.02 pg/ml and P4 at≥ 5.71 pg/ml to predict positive PT, the sensitivity was (86%), specificity (89%), NPV (99%) so the false negative was (1%), PPV (95%) so the false positive was (5%), and the accuracy of the test was (90%) (tab. 3).

4. Discussion

Follicular fluid (FF) is produced in growing antral follicles, has a major influences on oocyte to acquire its competence. AMH, and P4 are two among many FF components with potential determents of this influences [3] many studies consider these factors and their influences on outcome of IVF, and ICSI. We considered PT as an early marker of successfully progressing ICSI, and studied the influences of some of demographic factors on it including age, weight, type, duration, and cause of infertility, and antral follicular count, and we found no significant effect for any one of them. In her study conducted in Iraq for influence of co-enzyme Q 10 in FF, and its relation to outcome also studied these demographic factors, and found no significant relation [14]. In his study of effect of AMH, and progesterone levels in FF on emberyonic development also found no significant effect of age, BMI, or AFC on outcome on embryonic development [15]. on the other hand, concluded that age is an independent predictor of live birth [16]. Takahashi C, also found no effect of duration, or type of infertility on outcome [17]. Our main objective was to assess the influence of AMH, and P4 levels in FF on the outcome of ICSI, and we found a highly significant effect of both parameters, these were consistent with results obtained by [15]. Takahashi C also found positive effect of AMH on outcome [17], and also found positive effect of progesterone/estradiol ratio in follicular fluid. [18], while found no influence of AMH level in FF on fate of oocytes in IVF [19] while showed that they were inversely correlated with oocyte maturation and development potential such as, fertilization, embryo quality and clinical pregnancy rate [20]. We could not explain these differences in results of different studies, but probably related to the sample size and different patients' criteria may be potential causes.

5. Limitations

The limitations of the current study include small number of samples, and short period of outcome follow up, in addition to absence of follow up of morphological development of embryos

6. Conclusion

AMH and P4 level in FF have significant positive correlation with pregnancy rate in ICSI cycle.

7. References

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Table 1: Demographic characteristics of ladies enrolled in the study

Characteristic	**PT +ve (*n* = 33)**	**PT -ve (*n* = 17)**	***P value***
Age (Years)			
Mean ±SD	28.59 ±5.86	28.88 ±6.64	0.8
<35, *n* (%)	24 (72.7 %)	10 (58.8%)	0.4
≥ 35, *n* (%)	9 (27.3 %)	7 (41.2%)	
BMI (kg/m2)			
Mean ±SD	28.34 ±3.06	28.31 ±4.62	0.9
≤18.4	0	0	0.7
18.5-24.9 *n* (%)	9 (27.2%)	6 (35.3%)	
25-29.9, *n* (%)	16 (50.0%)	8 (47.1%)	
≥30, *n* (%)	8 (22.8%)	3 (17.6%)	
Duration of infertility (years)			
Mean ±SD	6.52 ±3.15	5.82 ±3.17	0.4
Type of infertility, *n* (%)			
Primary	25 (75.8%)	9 (52.9%)	0.1
Secondary	8 (24.2%)	8 (47.1%)	
Cause of infertility, *n* (%)			
Male factor	19 (57.6 %)	10 (58.8 %)	0.8
Female factor	14 (42.4 %)	7(41.2%)	

Antral Follicle Count			
Mean ±SD	16.3±10.4	13.7±8.1	0.37

n: number of cases; SD: standard deviation; BMI: body mass index. level of significance at P≤0.05.

Table 2: follicular fluid levels of AMH, and P4 in PT +ve compared to PT -ve groups

FF Hormone level (Pg/ml)	**PT +ve (n=33)**	**PT -ve (n=17)**	**P value**
AMH	3.24±0.72	1.16±0.35	<0.001*
P4	6.81±1.6	3. 38±1.74	<0.001*

n: number of cases; *: Highly significant

Table 3: Validity test of FF - AMH, and P4 levels as predictor for fertilization rate

Hormone in FF	**Cutoff (pg/ml)**	Sensitivity	Specificity	NPV	PPV	Accuracy
AMH	≥ 3.02	76%	78%	73%	90%	75%
P4	≥5.71	**32%**	**81%**	**91%**	**88%**	**77%**
AMH and P4		**86%**	**89%**	**99%**	**95%**	**90%**