IRAQI JOURNAL OF EMBRYOS AND INFERTILITY RESEARCHES (IJEIR)
Jinan et al., Vol. 14, Issue 2, Pp. 70-84 (2024) Original Research
DOI: http://doi.org/10.28969/IJEIR.v14.i2.r5.24 E-ISSN: 2616-6984 P-ISSN: 2218-0265

Relationship of Beta-hCG in Follicular Fluid with The Oocyte Quality and Pregnancy Rate in ICSI Cycle

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ABSTRACT

Human chorionic gonadotropin is a glycoprotein hormone produced by the placenta. It exists in several isoforms with long half-lives (hours) and angiogenic potential; it maintains the corpus luteum to produce progesterone. This study aimed to evaluate Human chorionic gonadotropin concentration in follicular fluid and its effect on oocyte maturity and pregnancy rate, and whether it can be used as a noninvasive biomarker for predicting oocyte competence and pregnancy rate during intracytoplasmic sperm injection cycles. Infertile women undergoing ICSI treatment were included in the study. An antagonist protocol was used for ovulation induction. Patients were divided into two groups: pregnant women (n=18) and non-pregnant women (n=22). Samples of follicular fluid were collected on the day of oocyte pick-up. Chemiluminescent Immunoassay (CLIA) equipment was used for the quantitative determination of total β-human chorionic gonadotropin in follicular fluid. The mean beta-human chorionic gonadotropin level showed a highly significant increase relative to the mean level of non-pregnant women (110.24 ±20.50) versus (56.195 ±29.45). Moreover, there was no significant relationship between the levels of beta-human chorionic gonadotropin follicular fluid and the total number of oocytes and oocyte quality in the two groups. The Beta Human chorionic gonadotropin concentration in the follicular fluid plays an important and influential role in the prediction of pregnancy rates. Despite this, follicular fluid hCG level showed no relationship with the total number and quality of oocytes in the pregnant and non-pregnant groups.

Received:
07-Oct- 2024
Accepted:
30-Oct-2024
Published:
08-Dec-2024

How to cite:

Jinan F. Abdullah, Lubna A. Al-Anbari, Faten N. Mohammed1; Relationship of Beta-hCG in Follicular Fluid with The Oocyte Quality and Pregnancy Rate in ICSI Cycle; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2024); 14(2): 80-42.
Doi: http://doi.org/10.28969/IJEIR.v14.i2.r5.24

KEYWORD

Beta-hCG, Follicular Fluid, Oocyte Quality, ICSI Cycle

1. Introduction

According to the World Health Organization (WHO), infertility is a disease that generates disability as an impairment of function (Zegers-Hochschid et al.,2017[1]). Generally, it is described as not conceiving without the use of birth control after 12 months of regular sexual intercourse (Feskov et al, 2019[2]).

The invention of intracytoplasmic sperm injection (ICSI) has been responsible for more than three million babies worldwide (AL-Ghazali and AL-Jarrah, 2013[3]). It is usually done in patients with oligospermia or with abnormal semen quality. Including oocyte extraction from ovaries, then fertilizing them in the laboratory by inserting a single spermatozoon directly into the cytoplasm of the oocyte using a glass micropipette, then transferring a certain number of embryos that develop into the uterus in an effort to induce pregnancy (Palermo et al., 2014[4]).

The oocyte plays a very important role in the quality of the embryos and hence the effects of IVF / ICSI. The oocyte is a highly specialized cell responsible for developing, activating, and regulating the embryonic genome and promoting fundamental processes such as cellular homeostasis, metabolism, and cell cycle progression in the early embryo, cytoplasm, and polar body (Hoshino, 2018[5]).

LH and hCG are involved in controlling various physiological functions by their action through the same receptor, and clinical experience derived from the use of immediately available hCG in Hypogonadotropic hypogonadism treatment, and controlled ovarian stimulation (COS) for ART (Carsarini et al., 2018[6]). In this latter case, gametogenesis could be clinically supported by the administration of exogenous FSH and hCG, thereby replacing LH action at least partially (Santos et al., 2018[7]).

Human chorionic gonadotropin (hCG) is the first trophoblast signal identified 10 days after fertilization in the maternal circulation and activates pregnancy-sustaining steroid progesterone secretion (Larson and Mcpherson, 2019 [8]). It represents one of the first molecular messages transmitted by the pre-implantation embryo to modulate the implantation site and to ensure a timely initiation of the nidation process. Although CGB gene expression has already been demonstrated in the embryo at the 8-cell level, active hormone secretion begins at the blastocyst stage (Schumacher and Zenclussen, 2019[9]). It is a part of a family of disulfide-rich heterodimer glycoprotein hormones, with a typical α-chain and distinctive β- The α-chain is unique to their G-protein-related receptor (Fournier, 2020) , and the β-chain is responsible for hCG's longer Half-life (Cole, 2012; Esteves and Alviggi, 2015[11],[12]).

2. Patients and Methods

The present study recruited 40 Iraqi infertile women from infertile patients undergoing ICSI cycles and was performed in the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies /Al-Nahrain University from August 2019 to January 2020. The study was approved by the Local Medical Ethical Committee of the High Institution for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, and consent was obtained from the patients to participate in the study.

Women with empty follicle syndrome, Prior documentation of intolerance or allergy to any gonadotropin, Endometriosis, a uterine anomaly or uterine fibroids and hydrosalpinges, uncontrolled systemic disease, such as diabetes mellitus, and women with no fertilized oocyte were excluded.

Intracytoplasmic sperm injection (ICSI) Technique.

All patients undergoing ICSI have been treated with a flexible GnRH antagonist protocol, including gonadotropin administration (r-hFSH) (Gonal F®; Merck Serono, Switzerland) containing 75IU of FSH activity per ampoule on 2ed day of the menstrual cycle. The follicles' growth was checked by serum E2 level and transvaginal ultrasound, then down-regulation of the pituitary gland with GnRH antagonist (cetrotide; Merck-Serono, Switzerland) when follicle size reached 14 mm in diameter.

Treatment with rFSH and cetrorelix acetate 0.25mg per day was continued until the day of the final oocyte maturation trigger, then Ovulation was induced with (250 µg = 6500 IU) of human chorionic gonadotropin (Ovidrel, Merck-Serono, Switzerland). When a minimum of two follicles have reached 18 mm.

Oocyte pick up

Ovarian follicle aspiration was performed after 36 hours of injection with hCG. Within 3 hours after follicular aspiration, the cumulus oophorus and corona radiata were extracted from the oocytes. After oocyte denudation, a rapid morphological evaluation is performed by an inverted microscopic, including evaluation of the zone pellucid, cytoplasm and perivitelline space (PVS) for any abnormality, and evaluation and Classification by nuclear maturity as metaphase II (MII), metaphase I, presence or absence of the first polar body or germinal vesicle. When the first polar body (PBI) is visible in the PVS, mature oocyte is presumed to be at the MII stage, Whereas the presence of an intracytoplasmic nucleus known as the 'germinal vesicle' (GV) is characteristic of the first meiotic prophase I, The immature oocytes (MI)with neither visible GV nor PBI.

The ICSI procedure was performed, and the best-quality embryos were transferred on day 3 of embryo culture in both groups. Two weeks after embryo transfer, serum β-hCG was assessed, and Patients with positive tests received progesterone (Cyclogest) 400mg daily for three months. Clinical pregnancy was confirmed by the presence of a gestational sac with cardiac activity in ultrasonography between weeks 4-6.

Sample collection

For each patient, Follicular fluid samples were collected after oocyte aspiration and put in plain tubes. Next, the clear aspirated follicular fluid (FF) samples were centrifuged immediately for 10 minutes at 3000 rpm. Subsequently, the supernatants were taken to Eppendorf tubes and stored at 20°C.

Chemiluminescence Immunoassay (CLIA) equipment is used for the quantitative determination of total b-hCG in follicular fluid.

3. Statistical analysis

Data were entered and analyzed using the SPSS (Statistical Package for Social Sciences) program, version 20. Descriptive data, including an independent sample t-test for quantitative and a chi-square test for qualitative variables. Correlation of follicular fluid to oocyte characteristics was made using the Pearson correlation test. A probability value (P value) less than or equal to 0.05 was set as statistically significant

4. Results

Demographic characteristics of the study groups

Forty ladies were included in this study, 18 (45%) were pregnant, and 22 (55%) were non-pregnant. The mean age of the pregnant women (28.39 ± 5.532 years), in comparison with that of women who failed to get pregnant (30.59 ± 5.795 years), was not significantly different (P = 0.230).

Also, the difference in proportions of women < 35 and ≥ 35 years of age between pregnant and non-pregnant ladies, 15 (83.33%) versus 16 (72.73%) and 3 (16.67 %) versus 6 (27.27%), respectively, was not significantly different (P = 0.341). The mean BMI of pregnant ladies was 27.867±5.325 kg/m2, and that of non-pregnant ladies was 29.134±4.417 kg/m2; there was no significant difference in mean BMI between the two groups (P = 0.416).

The ladies enrolled in this study were categorized, according to the BMI, into underweight (<18.5 kg/m2), normal weight (18.5- 24.9 kg/m2), overweight (25-29.9 kg/m2), and obese (≥ 30 kg/m2). Both the pregnant and non-pregnant ladies were within the overweight range (27.867±5.325 kg/m2 and 29.134±4.417, respectively) with no significant difference between them (P=0.416). There was also no significant difference in the distribution of ladies according to BMI between the two enrolled groups (P = 0.471).

There was no significant difference in the proportions of women with primary or secondary infertility between the two groups, 12 (66.67%) versus 15 (68.18%) and 6 (33.33%) versus 7 (31.82%), respectively (P = 0.592).

There was no significant variation in the distribution of women in both groups according to the causes of infertility (P=0.348). Male factor showed in 13 (72.22%) versus 11 (50%), female factor was found in 3 (16.67%) versus 6 (27.27%), whereas, unexplained causes were seen in 2 (11.11%) versus 5 (22.73%), in pregnant and non- pregnant ladies, respectively. (p=0.460), and serum AMH levels (p= 0.288).

Follicular fluid hCG in pregnant and non-pregnant ladies:

The difference in the mean level of hCG in the follicular fluid of pregnant and non-pregnant women was statistically significant, as shown in Table 1.3. Mean follicular fluid hCG was higher in pregnant women than in non-pregnant women: 110.24 ±20.50 mIU/ml versus 56.195 ±29.45 mIU/ml, respectively. The difference was highly significant (P = 0.000).

Predictive role of follicular fluid hCG in pregnancy outcome following assisted reproduction

A receiver operator characteristic (ROC) curve analysis was carried out. The cutoff value of follicular fluid hCG was ≥88.87 mIU/ml and had a highly significant level (P < 0.000), with sensitivity and specificity levels of 90% and 100%, respectively.

Correlations of Follicular fluid hCG with the number of oocytes and oocyte quality

Follicular fluid HCG was not significantly correlated with the total number of oocytes, abnormal oocytes, ruptured oocytes, GV, MI, M II, Number of injected oocytes, fertilized oocytes, and fertilization rate in pregnant and non-pregnant women.

***Table (1)*: Demographic characteristics of women enrolled in the present study**

Characteristic	Pregnant n = 18	Non pregnant n = 22	*P value*
Age (years)			
Mean ±SD	28.39 ± 5.532	30.59±5.795	0.230 NS
<35, *n* (%)	15 (83.33 %)	16 (72.73 %)	0.341 ¥ NS
≥ 35, *n* (%)	3 (16.67 %)	6 (27.27%)	
BMI (kg/m2*)*			
Mean ±SD	27.867±5.325	29.134±4.417	0.416 †
Underweight	1(5.56%)	0	0.471 € NS
Normal, *n* (%)	6 (33.33%)	5 (22.73%)	
Overweight, *n* (%)	5 (27.78%)	9 (40.91%)	
Obese, *n* (%)	6 (33.33%)	8 (36.36%)	
Type of infertility, *n* (%)			
Primary	12 (66.67%)	15 (68.18%)	0.592 ¥ NS
Secondary	6 (33.33%)	7 (31.82%)	
Cause of infertility, *n* (%)			
Male factor	13 (72.22%)	11 (50%)	0.348 € NS
Female factor	3 (16.67%)	6 (27.27%)	
Unexplained	2 (11.11%)	5 (22.73%)	

N: number of cases; **SD:** standard deviation; **BMI:** body mass index; **†:** independent samples t-test; **¥:** Yates correction; **€**: Chi-square test; **NS:** not significant at P >0.05

Table 2: Follicular fluid hCG in pregnant and non-pregnant groups

hCG Characteristic	Pregnant n = 18	Non pregnant n = 22	P-Value
Follicular fluid HCG	110.24 ±20.50	56.195 ±29.45	0.00 HS

hCG: human chorionic gonadotropin; **n:** number of cases; **†:** Independent samples t-test; **HS:** Highly significant at P ≤ 0.01

Table 3: Characteristics of ROC curves

Characteristics	Follicular fluid HCG
Cutoff value	≥88.87
AUC (95 % CI)	0.957 (0.879 - 1.035)
Sensitivity	90
Specificity	100
P-value	0.000 HS

hCG: human chorionic gonadotropin; **AUC:** area under curve; **CI**: confidence interval; **HS**: Highly significant at $P \leq 0.001$; S: significant at $P \leq 0.05$

Table 4: Correlations of Follicular fluid hCG with the number of oocytes and oocyte quality in non-pregnant and pregnant groups

Characteristic	HCG in F.F. in non-pregnant		CG in F.F in pregnancy	
	r	p	r	p
Oocyte No.	-0.024	0.915	0.054	0.832
Abnormal oocyte	-0.262	0.239	-0.020	0.938
Ruptured oocyte	0.981	0.006	0.702	-0.086
GV	0.542	0.161	0.795	-0.059
M I	0.596	-0.134	0.361	-0.205
M II	0.925	0.024	0.173	0.302
Number of injected oocytes	0.885	0.037	0.148	0.319
Fertilized oocyte	0.593	0.135	0.344	0.212
FR	0.547	0.152	0.247	0.258

5. Discussion

The findings of this study showed significant differences in hCG levels in follicular fluid between pregnant women and those who failed to get pregnant, with higher levels in pregnant women. In contrast, there was no significant association between B-hCG follicular fluid with the total number of oocytes and the quality of the oocytes.

Other researchers had a similar result; Ghasemi et al. found clinical pregnancy to be significantly correlated with follicular fluid beta-hCG, although no relationship was observed in the mean beta-hCG levels between mature and immature oocytes (Ghasemi et al., 2019[13]). Also, Nagata et al. reported that the ratio of hCG levels in the follicular fluid to serum hCG levels strongly predicts ovarian responsiveness and subsequent pregnancy (Nagata et al., 1999[14]).

In other studies, Balakier et al stated that in synchronizing endometrial and fetal growth, hCG plays a critical role (Chen et al., 2019; Balakier et al., 2020[15]). Sarhan et al also stated that LH had no effect on the outcome of fertilization and follicular LH levels were not connected to the potential for oocyte growth and embryo grading (Sarhan et al., 2017[16]). Additionally, Schumacher and Zenclussen described that T-lymphocytes from pregnant women expressed hCG/luteinizing hormone receptor genes and that hCG has immunoregulatory properties that alter human lymphocytes functions and it regulates biological aspects of these early pregnancy events as helpers in the establishment of an adequate embryo-endometrial relationship (Schumacher andZenclussen, 2019[17]).

In contrast, some other research findings showed that elevated follicular LH concentration was correlated with high-quality embryos and normally fertilized oocytes, indicating that LH may influence the oocyte far beyond fertilization by secreting substances, mainly steroids (Ilhan et al.,2014[18]).

The explanation for the contradiction of the present results with other results is that this result may be because few patients underwent the ICSI cycle.

Acknowledgement

We would like to acknowledge the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/ Al Nahrain University, Baghdad, Iraq

Funding

This work received no funding .

Author Contribution

Jinan F. Abdullah, performed the study, Lubna A. Al-Anbari, Faten N. Mohammed, supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure .

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