Relationship of Beta -hCG in Embryo Culture Media with Embryo Quality and Pregnancy Rate

Jinan F. Abdullah¹, Noor M. Ali², Lubna A. Al-Anbari³

¹B.Sc, Science of Biology, Al-Karkh birth hospital, Ministry of Health, Baghdad, Iraq
²Ph.D, Chemistry and biochemistry, College of Medicine, Al- Nahrain University, Baghdad- Iraq
³Assistant professor Dr. High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al- Nahrain University, Baghdad- Iraq

Corresponding author: Jinan F. Abdullah, Email: genanfaisal@gmail.com

Abstract

Background: Despite the high cost of ICSI treatment, its success rate is still poor. Furthermore, it has been shown that the standard criterion for embryo selection based only on morphology is insufficient to represent 'Embryo Quality'.

Objective: The objective of this study was to assess the concentration of human chorionic gonadotropin in embryo culture media and its effect on the development of embryos and whether it can be used as a non-invasive biomarker to predict pregnancy rate and assist in embryo selection during ICSI cycles.

Patients, Materials and methods: In the report, forty infertile women receiving ICSI treatment were included. For ovulation induction, an antagonist procedure was used. Patients were classified into two groups: (pregnant n=18), and (non-pregnant n=22) women. The embryo culture media samples were collected at the day of embryo transfer. Chemiluminescent Immunoassay (CLIA) equipment has been used to determine human chorionic gonadotropin levels in embryo culture media.

Results: The mean level of beta-human chorionic gonadotropin in the embryo media of pregnant women was significantly higher compared to the mean level of beta-human chorionic gonadotropin in non-pregnant women (3.896 ±1.868 versus 2.94 ±0.625). In addition, beta-hCG levels in embryo culture media were not correlated with the total number of embryos, GI, GII, and GIII in the two groups.

Conclusion: The concentration of beta-human chorionic gonadotropin in embryo culture media plays a significant and influential role in pregnancy rates, although the level of embryo culture media hCG showed no association with the total number and quality of embryos in the pregnant and non-pregnant groups.

Keyword: Beta-hCG, Infertility, Embryo Quality, ICSI Cycle

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Introduction

Infertility is “a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse” (WHO, 2019). Fertility decline in females already begins about the age of 25–30 and the median age at last birth is 40–41 years (Vander and Wyns, 2018). In healthy young couples, the possibility of pregnancy in one year is typically 85-90 percent, the majority in six months, and about 20-25 % in one
reproductive cycle, therefore infertility affects about 10-15% of couple (Jungwirth et al., 2015). Although ART plays a decisive role in reducing infertility, In vitro fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI) are two famous techniques that widely used among ART’s, which have nearly the same stages (Hafiz et al., 2017) in infertile couples. Intra-cytoplasmic sperm injection (ICSI) has contributed to a major increase in the treatment of these patients (Abdolmaleki and Joorsaraei, 2019). Intracytoplasmic sperm injection (ICSI): is typically performed in patients with oligospermia or semen consistency abnormality. It requires the direct injection of single spermatozoa into the egg to facilitate fertilization (Al-Ghazali and Al-Jarrah, 2013; Sheriff, 2019). Embryos that lead to successful pregnancy typically vary in their metabolic profiles compared to embryos that do not, and as a result, studies are underway to establish a molecular signature that can be identified by non-invasive assessment of the medium of embryo culture (Butler et al., 2013). During in vitro fertilization procedures, embryo culture represents the culture of human gametes and embryos. The process is performed when eggs and sperm are mixed in a culture dish and the resulting embryos are transferred to the uterus (Youssef et al., 2015). The first trophoblast signal found in the maternal blood, human chorionic gonadotropin (hCG), is used to diagnose pregnancy and induces pregnancy-sustaining steroid progesterone secretion (Fournier, 2020). Between the 10th and 11th week of pregnancy, which results in the greatest hCG values, hCG levels decline but remain high by the end of the first trimester relative to non-pregnant women (Than et al., 2018).

It is part of the glycoprotein hormone family that is disulfide-rich heterodimers, with a communal \( \alpha \)-chain and distinctive \( \beta \)-chains unique to their specific G-protein linked receptor (Larson, 2019). With the exception being the Carboxy Terminus Peptide (beta-CTP) containing four glycosylated serine residues that are responsible for hCG’s longer half-life, beta-hCG is mostly similar to beta-LH (Esteves et al., 2015).

Five different isoforms of hCG have been described: - regular hCG (r-hCG), free-\( \beta \) hCG (hCG\( \beta \)), hyperglycosylated hCG (H-hCG), hyperglycosylated free-\( \beta \) hCG (H-hCG\( \beta \)), and pituitary hCG (p-hCG), all of them with special biological functions (Fournier, 2020).

**Methodology**

**Subjects and data collection**

This study was performed in the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University In the duration between August (2019) and January (2020), and it was involved recruitment of forty infertile women undergoing ICSI cycles. The study was approved by the Local Medical Ethical Committee of high institution for infertility diagnosis and Assisted Reproductive Technologies Al-Nahrain University, and agreement was got from the patients to contribute in the study. Women with Prior documentation of intolerance or allergy to any gonadotropin, Endometriosis, uterine fibroids or a uterine anomaly and hydrosalpinges were excluded.

**Intracytoplasmic sperm injection (ICSI) Technique**

The flexible GnRH antagonist procedure, including the administration of gonadotropin (r-hFSH) (Gonal F \( \circledR \); Merck Serono, Switzerland) containing 75 IU of FSH action per ampoule on the 2nd day of the menstrual cycle, was used in all patients undergoing ICSI. When follicle size reached 14 mm in diameter, follicle development was tested by serum E2 level and transvaginal ultrasound, then down-regulation of the pituitary gland with a GnRH antagonist (cetrorelix; merck-Serono, Switzerland). Treatment with (cetrorelix acetate 0.25mg) and (rFSH) per day was continued until the
day of the final oocyte maturation trigger then Ovulation was induced with human chorionic gonadotropin (Ovidreal, Merck- Serono, Switzerland) (250 μg = 6500 IU), once a minimum of two follicles have reached (18 mm). After 36 hours of injection with hCG, ovarian follicle aspiration was done. The ICSI procedure was done, and on day 3 of embryo culture in both groups, the best quality embryos were transferred. Serum βhCG was tested two weeks after embryo transfer, and Positive test patients received progesterone (Cyclogest) 400 mg daily for 3 months and clinical pregnancy was reported by the presence of a gestational sac with cardiac activity in ultrasonography between weeks (4-6).

**Embryo Grading System**

Embryo grading was assessed (24-72) hours after fertilization and depends on morphological assessment systems, in addition to the classical parameters of blastomere cell number, the ratio of fragmentation and blastomere symmetry, the developed embryos were graded in accordance with the embryo grading system.

- **Grade I:** Embryo with less than 10% fragmentation, stage-specific cell size, no multinucleation
- **Grade II:** Embryo with (10–25%) fragmentation, stage-specific cell size for majority of cells, no evidence of multinucleation
- **Grade III:** Embryo with severe fragmentation (<25%), cell size not stage specific, evidence of multinucleation.

**Embryo culture media collection**

Culture media samples were collected from the four-well dish after embryo transfer (ET) cycles for each patient and centrifuged directly at (2000) rpm for (10) min to separate mineral oil layer from culture media and stored at –20°C to prevent any alteration of sample. Chemiluminescent Immunoassay (CLIA) instruments used in culture media for quantitative determination of total B-hCG

**Statistical analysis**

Using the SPSS (Statistical Packages for Social Sciences) program, version 20, the data entered and analyzed. Descriptive data including chi-square test for qualitative and independent sample t-test for quantitative variables. A Pearson correlation test was used to correlate Beta-hCG in cultural media with the total number and quality of embryos. Probability value (P value) ≤ 0.05 was set as statistically significant.

**Result**

In this study, there was no significant difference in demographic characteristics between pregnant and non-pregnant women as shown in Table 1.1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pregnant n = 18</th>
<th>Non-pregnant n = 22</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>28.39 ± 5.532</td>
<td>30.59 ± 5.795</td>
<td>0.230† NS</td>
</tr>
<tr>
<td>&lt;35, n (%)</td>
<td>15 (83.33 %)</td>
<td>16 (72.73 %)</td>
<td>0.341 YNS</td>
</tr>
<tr>
<td>≥ 35, n (%)</td>
<td>3 (16.67 %)</td>
<td>6 (27.27 %)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.96 ± 5.325</td>
<td>29.13 ± 4.417</td>
<td>0.416 NS</td>
</tr>
<tr>
<td>Underweight</td>
<td>1 (5.56 %)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Normal, n (%)</td>
<td>6 (33.33 %)</td>
<td>5 (22.73 %)</td>
<td>0.471 NS</td>
</tr>
<tr>
<td>Overweight, n (%)</td>
<td>5 (27.80 %)</td>
<td>9 (40.91 %)</td>
<td></td>
</tr>
<tr>
<td>Obese, n (%)</td>
<td>6 (33.33 %)</td>
<td>8 (36.36 %)</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.2: embryo media HCG in pregnant and non-pregnant groups

<table>
<thead>
<tr>
<th>HCG Characteristic</th>
<th>Pregnant n = 18</th>
<th>Non pregnant n = 22</th>
<th>† P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo culture media HCG</td>
<td>3.896 ±1.868</td>
<td>2.94 ±0.625</td>
<td>0.030S</td>
</tr>
</tbody>
</table>

hCG: human chorionic gonadotropin; n: number of cases; †: Independent samples t-test;

Predictive role of hCG embryo culture media in the outcome of pregnancy after assisted reproduction:
The above results showed that there was a statistically significant differences in the mean level of hCG in embryo culture media between pregnant and non-pregnant women, so a curve analysis of the receiver operator characteristic (ROC) was performed. The cutoff value of embryo culture media HCG was ≥3.115 mIU/ml and had a highly significant level (P < 0.003), with a sensitivity and specificity levels of 61.9% and 73.7%, respectively.

Table 1.3: Characteristics of ROC curves

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Embryo culture media HCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutoff value</td>
<td>≥3.115</td>
</tr>
<tr>
<td>AUC (95 % CI)</td>
<td>0.771 (0.626- 0.917)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>61.9</td>
</tr>
<tr>
<td>Specificity</td>
<td>73.7</td>
</tr>
<tr>
<td>P-value</td>
<td>0.003S</td>
</tr>
</tbody>
</table>

HCG: human chorionic Gonadotropin; CI: confidence interval; AUC: area under curve

Correlations between hCG embryo culture media and the total number and quality of embryo in pregnant and non-pregnant women
Embryo culture media HCG were not significantly correlated with the total number and quality of embryo (GI, GII, GIII) in pregnant and non-pregnant women.
Table 1.4: Correlations between hCG embryo culture media and the total number and quality of embryo in pregnant and non-pregnant women

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Embryo Media hCG in non-pregnant women</th>
<th>Embryo Media hCG in pregnant women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>embryo No</td>
<td>0.303</td>
<td>0.171</td>
</tr>
<tr>
<td>No. of GI</td>
<td>0.143</td>
<td>0.527</td>
</tr>
<tr>
<td>No. of GII</td>
<td>0.254</td>
<td>0.254</td>
</tr>
<tr>
<td>No. of GIII</td>
<td>-0.229</td>
<td>0.306</td>
</tr>
</tbody>
</table>

Discussion

In the current research, pregnancy results were found to have no association between BMI and mean age. This result is in agreement with the result of another study which showed that maternal age does not impact pregnancy or delivery outcomes for couples using testicular sperm from ozoospermic patients (Choi et al., 2016), also, another study has shown that embryo consistency is not influenced by the age of women (Stensen et al., 2010). As well, another study showed that there was no impact of BMI on endometrial receptivity or oocyte quality, and then on the outcome of pregnancy (Banker et al., 2017). Moreover, Üçkardesler et al. (2009) reported that no correlation was noted between BMI and rates of oocyte maturation and fertilization. In comparison, some studies have shown that higher BMI ranges have a detrimental effect on pregnancy results after IVF / ICSI (Poonam Bhojwani et al., 2016). With respect to the causes of infertility, the major cause of infertility was the male factor. Several other researchers came up with findings identical to our study results, explaining that unexplained infertility obtained identical reproductive results as cases of male infertility throughout the use of ICSI (Misra and Mishra, 2017; Alasmari et al., 2018). However, other studies have shown that the risk of adverse results for ART pregnancies varies widely depending on the cause of infertility (Kuivasaari-Pirinen et al., 2012; Al–Ghazali and Al-Jarrah, 2013).

The main finding of the current study showed that hCG levels in embryo culture media were higher in women who were pregnant relative to those who won't be able to become pregnant. In contrast, no significant correlation was observed between hCG levels in embryo culture media and total number and quality (GI, GII, GIII) of embryo in pregnant and non-pregnant women. Another articles reported that as the number of blastomeres increased, the concentration of hCG in spent culture media increased gradually and decreased as the morphological grade of the embryo was depressed and It correlates positively with the status of early embryo development and implantation rate and thus serves as a useful biomarker in the IVF-ET procedure for embryo selection (Butler et al., 2013; Wang et al., 2014). In addition, Xiao-Yan et al. (2013) found that beta-hCG concentration in the day 5 groups was higher than in the day 3 group. In addition, blastocysts leading to successful implantation and pregnancy showed better morphological
grading and were positively associated with higher levels of hCG-β in culture media relative to embryos not implanted (Chen et al., 2019; Balakier et al., 2020). Also, during the early implantation process, hCG secreted by embryonic blastocyst cells can directly modulate endometrial receptivity and differentiation (Evans, 2016). Yu et al. (2015) stated that HCG secreted from the embryo stimulates human peripheral blood mononuclear cells (PBMC) at the implantation site, whereby PBMC from pregnant women controls embryo invasion. Sarhan et al reported that LH showed no impact on the outcome of fertilization and follicular LH levels were not correlated with oocyte development potential and embryo grading because embryos of Grade A and Grade C were similar in LH levels lower than LH in embryos of Grade B (Sarhan et al., 2017).

Conflict of interest: Authors declared there is no conflict of interest.

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