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COMPARATIVE ANALYSIS OF EMBRYO DEVELOPMENT: GROUP CULTURE VS. SINGLE EMBRYO CULTURE IN ICSI CYCLE

Omer Nazar Ramzi [1*], Dr. Amal Abdulwahid Mohammed [2], Dr. Wassan Adnan [3]
[1, 2, 3] Department of Applied Embryology, High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Al-Nahrain, IRQ

Abstract
Background: Many medical methods treat infertility under the name "Assisted Reproductive Technology" (ART). For infertile couples, ART improves their chances of conceiving. Intracytoplasmic sperm injection, along with its associated procedures like embryo culture and embryo transfer, are the most common ART techniques.
Objective: To compare the development rates and morphological quality of embryos in group culture versus single embryo culture during the ICSI cycle, plus to evaluation of β -HCG marker in both groups.
Methods: This study include 40 infertile couples undergoing ICSI at the Iraqi British IVF Centre in Baghdad. November 2023-March 2024. After microinjection, MII oocytes were separated into two groups and placed in culture media. Single and group cultural systems were designed with distinct drop patterns. β -hCG level have been also measured as marker of embryo quality after culturing in both group and individual culture systems.
Results: Group cultures showed significantly ($p \leq 0.05$) higher total fertilization rates, grade I embryos and total numbers of freezing embryos than individual culture system. The β -hCG levels in group cultures embryos were also significantly ($p < 0.001$) higher than single culture system in this study.
Conclusion: It is concluded that group cultured embryos significantly healthier than single cultured embryos. Group culture system provide a supportive environment through collective secretion of autocrine and paracrine factors that promote embryo development. The β-hCG levels in group culture embryos were found to be significantly higher than those in the single culture system.
Keyword: Assisted Reproductive Technology, Embryo culture, Single embryo culture, Group embryo culture, Intracytoplasmic sperm injection, Human chorionic gonadotropin, β-hCG.

INTRODUCTION

World Health Organization (WHO) defines infertility as "the inability of a sexually active, non-contraceptive couple to achieve pregnancy in one year.". It is a common, complex health problem (Benksim et al., 2018).

According to the definition provided by the American Center for Disease Control (CDC), assisted reproductive technologies refer to any medical procedures that involve the manipulation of eggs or embryos for the purpose of fertility treatment (Jain & Singh 2022). Tubal blockage, polycystic ovaries, endometriosis in women, azoospermia, and severe oligospermia in men are the most frequent reasons for ART. Although the most frequent side effects are ovarian hyperstimulation and multiple pregnancies (Roy, Dupras and Ravitsky, 2017).

Intracytoplasmic sperm injection is the injection of a single immobilized mature spermatozoa inside a mature oocyte in metaphase II. It was a revolution in the treatment of infertile couples with a male factor who had no hope other than donation or adoption (Rubino et al., 2016).

One of the most important steps in ICSI procedure is providing appropriate embryo culture system with ideal environment and quality control (Castillo et al. 2020). Culture media are one of the most important factors for successful pregnancy in ART. Culture media is a complex solution that's used to support cell growth that developed to mimic the natural environment of the embryo (Mestres et al., 2021). The composition of embryo culture media is varying widely, with differences in lactate, pyruvate and amino acids concentration. Blastocyst development is considered as culture media dependent and affected by the presence of protein and oxygen concentration (Morbeck et al. 2014).

The decision between single or individual culture in embryo development is controversial in ART laboratories. Individually culturing embryos has specific benefits. By segregating each individual embryo, the likelihood of any interactions that may impede the progress of development is reduced (Miguel et al. 2022). This approach provides a stable environment for each embryo, minimizing the possibility of resource depletion caused by neighboring embryos (Ebner et al. 2010) In the other hand, the group embryo culturing system has become trending as a new method for growing embryos in IVF laboratories (Gardner and Lane 2017). This approach entails cultivating embryos in tiny groups within separate droplets of culture medium, with the goal of mimicking the natural conditions of the fallopian tubes where embryos typically grow (Gardner 2021). Therefore, this study aimed to evaluate embryo development morphologically and levels of secreted βhCG in grouping and single embryo culture system.

Beta-hCG in embryo culture media predicts pregnancy rates and embryo viability during in vitro fertilization. Levels of β-hCG in embryo culture media are considerably greater in good quality embryos, suggesting their use as a noninvasive embryo selection indicator. The expression of β-hCG shown as early as the 2

pronuclear stage and connected to embryo quality (Jinan et al. 2021).

METHODS

1. Patients

The Iraqi British IVF Centre in Baghdad conducted this prospective study on forty infertile couples undergoing ICSI (240 oocyte). November 2023-March 2024, regardless of prior ICSI experience. Ages ranged from 22 to 42 for women. Both primary and secondary infertility were included. A medical history and physical examination were followed by hormone measures, endocrine problem elimination, ultrasound for females, and semen analysis for males according to world health organization 2021 recommendation. Inclusion criteria represented by females aged 20 to 42**,** male with normal and mild seminal fluid parameter, females who were subjected to an antagonist protocol**,** female carrying a minimum of six MII oocytes**,** females who suffering from infertility caused by tubal obstruction and anovulatory cycles. On the other hand, exclusion criteria include female with age more than 42 year, females underwent agonist protocol, females with uncontrolled endocrine or systemic diseases, male with severe oligoasthenospermia, azospermia or underwent testicular biopsy, and male and female with carrying genetic diseases.

2. Intracytoplasmic sperm injection cycle

The oocytes and sperms of the couple were collected and subjected to an in ICSI cycle. Each of the 40 women who were unable to conceive naturally underwent the antagonist protocol. Following the completion of the oocyte retrieval, Oocyte Denudation, processing of semen, and microinjection processes as described by Maggiulli et al. (2020) and Zhang et al. (2020), the injected MII oocytes were divided into two groups and placed in culture media.

3. Preparing Culture Dish

The culture dish (Falcon/USA) was prepared the day prior to oocyte retrieval to culture the injected oocytes, which were transferred immediately after injection. The dish contained droplets of culture medium (Global total one step, Cooper Surgical / Denmark) ranging from 40 microliters for single embryo culturing, for grouping, droplets of 120 microliters (Global total one step, Cooper Surgical / Denmark) were added (Paffoni *et al.* 2019). There were four droplets of culture media in the culture dish, with the following design: three droplets for cultured a single embryo and one droplet for a group of embryos. All droplets were covered by oil (LifeGuard® Oil/ Cooper Surgical, Denmark).

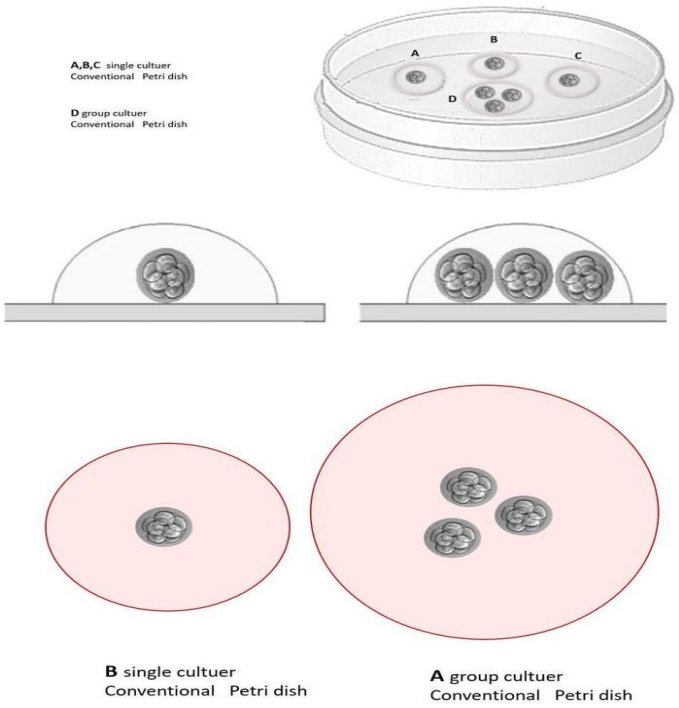

Figure 1: Culture Dish Design

4. Fertilization Assessment

Approximately 16-18 hours following microinjection, oocytes were considered fertilized if they exhibited two pronuclei (PN) and two polar bodies. If there were one or three PN, it indicated that they were abnormal. If there were no PN, they were considered unfertilized (Chuang *et al.* 2003). After reaching this step, embryo grading and observation in day 3, embryo transfer or froze, and pregnancy test were performed in a sequential time frame.

5. Collection of culturing media for embryos and measurement of human chorionic gonadotropin beta hormone quantities found in single and group embryo culture media

The culture media of the transferred embryos were removed from the four-well dish, and then transferred into Eppendorf tubes. They were then preserved in deep freezing until the day of β-hCG assessment. This step was done by applying culture media samples into full automated machine (Cobas E411/ Roche company, Germany). This devise analyses the samples by using electrochemiluminescence (ECL) technology. ECL systems have found extensive application in the identification and tracking of several disease-related biomarkers (Yoo et al. 2022).

6. Statistical analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft office 2010.The descriptive statistics including frequency, range, mean and standard error were measured to describe the data. The groups were compared by applying independent sample t-test (Unpaired t-test compare between two groups), analysis of variance ANOVA, and post hoc test paired groups' comparison. The results were considered statistically significant when p value was equal to or less than 0.05.

RESULTS

1. Baseline characteristics of patients enrolled in the present study

The baseline demographic futures of patients enrolled in this study were expressed in mean plus minus standard error of the mean (SEM). The baseline mean females ages were 33.63 ± 0.59 years and mean body mass indices (BMI) were 25.46 ± 0.32 (Kg/m²). On the other hand, the mean males ages were 38.40 ± 1.16 years with body mass indices equal to 28.09 ± 0.37 (Kg/m²) (Table 1).

Table 1: Baseline characteristics of females and males age and BMI

Parameters	Range	Mean ± SE
Female age (years)	27 - 44	33.63 ± 0.59
Female BMI (Kg/m²)	21.51 – 29.38	25.46 ± 0.32
Male age (years)	29 - 62	38.40 ± 1.16
Male BMI (Kg/m²)	25.25 – 37.92	28.09 ± 0.37

BMI: Body mass index; SE: Standard error

2 Comparison of ICSI parameters between single and group embryos cultures

Group cultures showed significantly higher total fertilized oocytes (p=0.009), fertilization rates (p=0.009), grade I embryos (p=0.001) and total numbers of freezing embryos (p=0.015), there was also insignificantly higher-grade II embryos (p=0.498), numbers of blastocysts (p=0.197), numbers of transferred embryos (p=0.512) and embryos utilization rates (p=0.070). Single embryos cultures solely showed insignificantly higher-grade III embryos (p=0.070) as presented in Table 2.

Table 2: Comparison of ICSI parameters between single and group cultures

ICSI parameters	Single culture	Group culture	p value
Total fertilized oocytes	1.95 ± 0.10	2.33 ± 0.09	0.009 Ƥ S
Fertilization rate	64.54 ± 3.38	77.10 ± 3.06	0.009 Ƥ S
Grade I embryos	1.2 ± 0.11	1.7 ± 0.11	0.001 Ƥ S
Grade II embryos	0.48 ± 0.08	0.55 ± 0.08	0.498 Ƥ NS
Grade III embryos	0.25 ± 0.07	0.08 ± 0.04	0.070 Ƥ NS
Numbers of blastocysts	1.25 ± 0.16	1.63 ± 0.18	0.197 Ƥ NS
Numbers of freezing embryos	1.58 ± 0.16	2.00 ± 0.16	0.015 Ƥ S
Numbers of transferred embryos	0.38 ± 0.11	0.48 ± 0.91	0.512 Ƥ NS
Embryo's utilization rates	91.53 ± 2.64	97.45 ± 1.43	0.070 Ƥ NS

Ƥ: Paired sample t test; S: Significant ($p \leq 0.05$); NS: Not significant ($p > 0.05$)

3 Comparison of β -HCG levels between single and group cultures embryos

β -HCG levels in group cultures embryos were also significantly higher than single grade I, grade II and grade III ($p < 0.001$) as presented in tables 3 and 4.

Table 3: Comparison of β -HCG levels between single and group cultures embryos

Parameter	Group culture embryos	Single grade I embryos	Single grade II embryos	Single grade III embryos	p value
HCG level (mIU/ml)	1.49 ± 0.05	0.91 ± 0.05	0.68 ± 0.03	0.26 ± 0.05	< 0.001 Ⱶ S

Ⱶ: ANOVA test; S: Significant ($p \leq 0.05$)

Table 4: Post hoc test of paired groups' comparison

Paired groups comparison		p value
Group culture embryos	Single grade I embryos	< 0.001 S
Group culture embryos	Single grade II embryos	< 0.001 S
Group culture embryos	Single grade III embryos	< 0.001 S

S: Significant ($p \leq 0.05$)

DISCUSSION

1 Demographic characteristic of the patients included in this study

The present study aimed to assess the effect of the embryo culture system on embryo quality. In the culture design, two systems were indicated: single embryo culturing and group embryo culturing. The couples involved in present study were of reproductive age. The total number of oocytes used in present study in both groups was 240. This study involved 40 married individuals, who were often primarily infertile. The average age of male patients was (38.40 ± 1.16) years, whereas the average age of female patients was (33.63 ± 0.59) years. Body mass index was also calculated for both sexes, and the results indicated to a large variation between males and females, ranging from 25.25 to 37.92 Kg/m² for males and from 21.51 to 29.38 Kg/m² to the females. In addition, full hormonal profile was taken for female's patients and complete study of semen parameters were done by doing semen analysis for males.

2 Comparison of ICSI parameters between single and group embryos cultures for all females enrolled in the present study

The assessment of comparison of ICSI parameters between single and group embryos cultures for all females enrolled in the present study results that appear in Table (2) agreed with Ebner et al. (2016), which demonstrate that the embryos cultured in groups are believed to foster optimal growth conditions by secreting autocrine and paracrine substances in a coordinated manner.

These factors can boost the developmental potential of individual embryos in the group, for example, Ruíz et al. (2020) found that embryos cultured in groups had a significantly higher blastocyst formation rate than those cultured singly. Also, Patel et al. (2023) discovered that group embryo cultures had fewer embryonic and chromosomal abnormalities than single embryo culture system. This suggests that having multiple embryos in the same environment may promote embryonic development while decreasing the incidence of genetic abnormalities.

3 Parallel analysis of β hCG levels in embryos cultured individually versus those cultured in a group setting

Based on tables 3 and 4, the levels of β HCG in embryos from group cultures were noticeably greater than those from single grade I, grade II, and grade III (p <0.001). Embryos in a group culture setting gain an advantage from the paracrine and autocrine components released by embryos in close proximity to them. An improvement in embryo quality and an increase in β hCG synthesis can be achieved through the use of these signaling molecules, which can improve cell proliferation, differentiation, and survival (Ebner et al. 2010). The latest data, plus another documented piece of information by Yuan et al. (2020) revealed that group culture methods have the ability to establish a microenvironment that is more supportive and closely resembles the conditions seen in living organisms compared to individual cultures. This enhanced environment has the potential to decrease stress and facilitate healthy embryonic development, all of which agreement with the outcome of this study, which shows β hCG levels are higher in grouped culture embryo than in single culture.

CONCLUSIONS

It is concluded that group cultured embryos significantly healthier than single cultured embryos. This suggests that having multiple embryos in the same environment may promote embryonic development while decreasing the incidence of genetic abnormalities. The β HCG level in grade one embryo secreted higher than grade 2 and grad 3 embryo.

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Author Contribution:
Omer Nazar Ramzi performed the study, Dr. Amal Abdulwahid Mohammed and Dr. Wassan Adnan supervised the work.

Conflict of Interest:
The authors declare no conflict of interest.

Ethical Clearance:
The study was approved by the Ethical Approval Committee.

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There is no financial disclosure

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