Original Article

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Randomized Controlled Trial Comparing Single Embryo Culture with Group Culture in a Micro-Well Dish: Impact on Embryo Development and Clinical Outcomes

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ABSTRACT

Purpose: The optimal culture environment for embryos *in vitro* remains a topic of ongoing debate. Group embryo culture using a micro-well dish has been suggested to enhance embryo development by facilitating autocrine and paracrine signaling, but its effect on clinical outcomes in human in vitro fertilization (IVF) remains unclear. The aim of this study was to compare embryo development and clinical outcomes between single embryo culture and group embryo culture in micro-well dishes in human IVF cycles.

Methods: In this prospective, randomized controlled trial, patients undergoing IVF at the Eurofertil IVF Center were allocated to either the single embryo culture group or the group culture (GC) arm. Patients under 40 years old with a minimum of five normally fertilized oocytes were included. Primary outcomes were blastocyst formation rates, while secondary outcomes included embryo development, clinical pregnancy rates, implantation rates, and live birth rates.

Results: A total of 160 patients participated, split equally between the two study arms. GC significantly increased the rate of top-quality blastocysts compared to single culture ($p<0.05$), with no significant differences in clinical pregnancy and live birth rates between the two groups ($p>0.05$). Fresh embryo transfer was performed in all cycles, and no preimplantation genetic testing was applied to the embryos.

Conclusion: GC using a micro-well dish led to a higher yield of top-quality blastocysts, although it did not significantly improve clinical outcomes. These results suggest that GC may be advantageous for cycles requiring cryopreservation or biopsy of multiple blastocysts.

Keywords: Embryo development, embryo culture, micro-well dish, pregnancy rate

INTRODUCTION

The quest to establish optimal culture conditions for human embryos *in vitro* remains a central focus in assisted reproductive technologies (ART). Different methods, such as single embryo culture and group culture (GC), have been extensively studied, yet no consensus has been reached regarding which technique yields the best clinical outcomes (Dai et al.[1], Ieda et al.[2]). Recent advances have suggested that GC, particularly when using micro-well dishes, may enhance embryo development due to autocrine and paracrine signaling effects, which may positively impact embryo quality (Contramaestre et al.[3]). Studies in animal models have provided significant evidence supporting these mechanisms, though data in humans remain inconsistent (Hoelker et al.[4]). Despite encouraging results from these various animal studies, there is limited and conflicting evidence regarding the impact of GC on clinical outcomes in human ART, particularly regarding blastocyst formation and live birth rates (Herreros et al.[5]). In addition, recent innovations like the well-of-the-well dish have

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shown promise, but their application to human embryos has yet to be thoroughly explored (Rebollar-Lazaro and Matson[6]). The aim of this study was to attempt to address the gap in the literature by comparing single and group embryo culture in a micro-well dish system, focusing on blastocyst development, clinical pregnancy, and live birth rates in human in vitro fertilization (IVF) cycles.

METHODS

Study Design

This study was a prospective, randomized controlled trial conducted at Eurofertil IVF Center between May 2013 and April 2014. Patients were randomly assigned to either the single embryo culture group or the group embryo culture; all culturing was carried out in micro-well dishes. Randomization was performed using a computer-generated list, with allocation occurring after the fertilization check.

The ethics approval for the study was obtained from Kocaeli University Faculty of Medicine Ethics Committee (approval number: KOU KAEK 2013179, date: 19.09.2023).

Participants

A total of 215 patients were initially screened, and those who met the inclusion criteria were invited to participate. Inclusion criteria were: female age <40 years, male age <60 years, and a minimum of five normally fertilized oocytes at the time of fertilization check. Patients with failed fertilization, use of surgically retrieved sperm, or those undergoing preimplantation genetic testing (PGT) were excluded. The study population consisted exclusively of antagonist protocol IVF cycles with high fertilization rates. The participants were allocated to the GC arm or the single culture (SC) arm, randomly.

Data Collection

Embryo development was assessed on days 2, 3, and 5 post-fertilization, following the ESHRE/alpha consensus timeline. Blastocysts were graded using Gardner's criteria (Zou et al.[7]). Fresh embryo transfer was performed on day 5, and all embryo transfers were performed by the same clinician using the same type of catheter.

Statistical Analysis

Statistical analyses were performed using SPSS, version 20.0 (IBM Inc., Armonk, NY, USA). Continuous variables were tested for normality using the Kolmogorov-Smirnov test and compared using the Student's t-test, while categorical variables were analyzed using the chi-square test. A p-value of <0.05 was considered statistically significant. Adjustments were made for multiple comparisons using the Bonferroni correction method where applicable.

RESULTS

A total of 160 patients were included in the study, with 80 patients allocated to the SC arm and 80 to the GC arm (Figure 1). The review of study as shown on CONSORT diagram (Diagram 1). Baseline characteristics, such as age, body mass index, and duration of infertility were similar between the two groups (p>0.05), ensuring comparability (Table 1). The total blastocyst development rate was significantly higher in the GC arm compared to the SC arm (GC: 62.5% vs. SC: 51.8%, p=0.04). Furthermore, the number of top-quality blastocysts was significantly greater in the GC arm (GC: 40.2% vs. SC: 27.5%, p<0.05). Furthermore, a significantly higher number of cryopreserved blastocysts were obtained from the GC arm (GC: 15.3% vs. SC: 8.6%, p=0.04) (Table 2). In terms of clinical outcomes, clinical pregnancy rates were higher in the GC arm, although this difference was not significant (GC: 48.1% vs. SC: 44.7%, p>0.05). Similarly, no

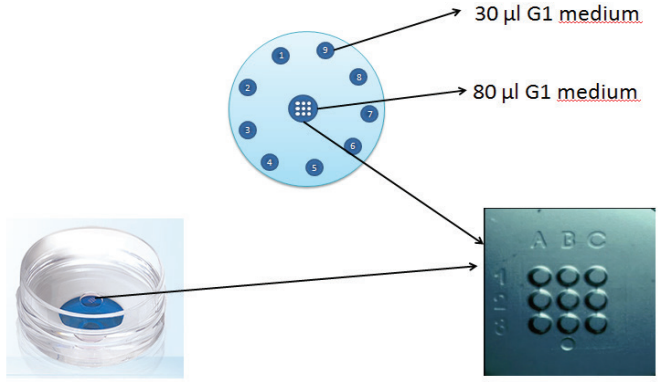

Figure 1. Preparation of WOW dish
WOW: Well-of-the-well

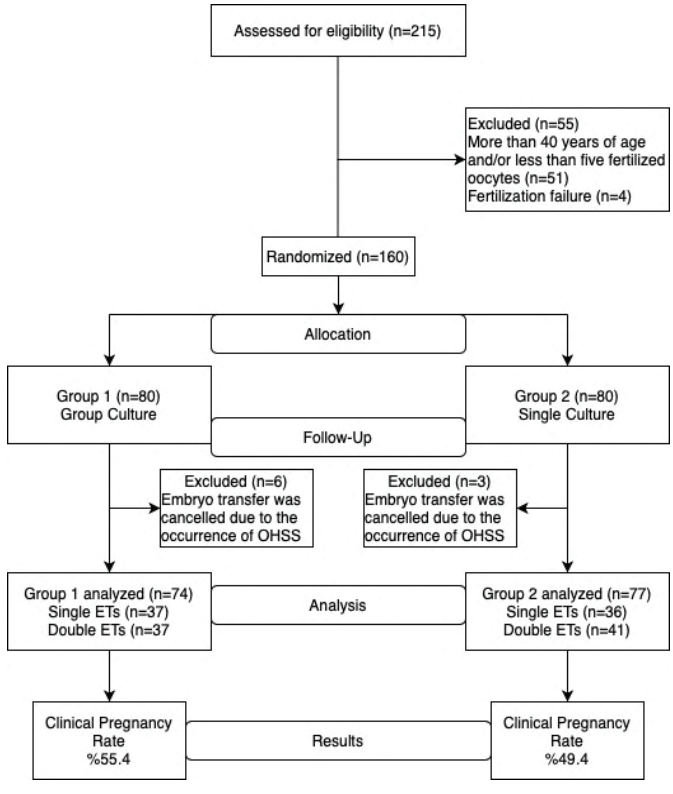

Diagram 1. The CONSORT 2010 flowchart
ETs: OHSS: Ovarian hyperstimulation syndrome

significant differences were found in implantation rates or live birth rates between the two groups (p>0.05) (Table 3). Of note, all embryo transfers in this study were fresh, with no frozen embryo transfers performed during the study period. Moreover, none of the embryos underwent PGT.

Table 1. Demographic characteristics of the patients in both groups

Variable	Group 1, (n=80)	Group 2, (n=80)	p-value
Female age (y)	29±4.8	29.3±4.4	0.7
Male age (y)	33±4.7	33.2±4.8	0.7
BMI (kg/m^2)	25.3±4.2	25.2±4.9	0.9
Duration of infertility (y)	4.86±3.2	4.94±3.5	0.88

BMI: Body mass index, y: Years

Table 2. Cycle characteristics of the groups

Variable	Group 1, (n=80)	Group 2, (n=80)	p-value
Number of oocytes retrieved (n)	14.1±6.7	14.4±6.2	0.76
Number of 2PN (n)	8.9±4.2	8.7±3.6	0.79
Fertilization rate (%)	81.6±14.5	79±14.5	0.26
Number of cleaved embryos (n)	8.7±4.2	8.5±3.6	0.79
Cleavage rate (%)	97.5±5.7	97.9±6.04	0.58
Day 2 good quality embryos (n)	5.84±3.3	5.35±2.9	0.32
Rate of day 2 good quality embryos (%)	66.3±22.2	62.1±23.1	0.25
Day 3 good quality embryos (n)	5.35±3.5	4.72±3.15	0.23
Rate of day 3 good quality embryos (%)	59.6±23.9	53.9±25.8	0.15
Total blastocysts (n)	6.47±3.7	5.85±3.1	0.24
Rate of total blastocysts (%)	72.2±19.8	67.4±21.0	0.02*
Top quality blasts (n)	3.6±2.9	2.6±2.1	0.005*
Rate of top quality blasts (%)	38.5±22.2	31.1±23.3	0.04*
Cryopreserved blasts (n)	3.16±2.9	2.43±2.3	

*p<0.05 statistically significant; 95% confidence interval

Table 3. Clinical and cumulative outcomes of both groups

Clinical outcomes (all transfers)	GC group, (n=74)	SC group, (n=77)	p-value
Single ETs (n/%)	37/46	36/45	0.56
Double ETs (n/%)	37/46	41/51	0.72
Clinical pregnancy rate (%)	55.4	49.4	0.46
Implantation rate (%)	36.9	32.2	0.5
Live birth rate (%)	45.3	40.3	0.52
Cumulative live birth rate (%) (including fresh and all thaw cycles)	53.2	47.5	0.68

ETs: GC: Group culture, SC: Single culture

These results suggest that while GC improves blastocyst quality and the number of cryopreserved embryos, it does not significantly impact clinical pregnancy or live birth rates.

DISCUSSION

The findings from the present study support previous research, indicating that group embryo culture enhances blastocyst development and improves the yield of top-quality blastocysts (Herreros et al.[5]). Our results also align with those of Herreros et al.[5], who found that micro-well GC systems promote better embryo development, likely due to enhanced autocrine and paracrine signaling within the microenvironment. Despite these promising findings, we did not observe significant differences in clinical pregnancy or live birth rates between the SC and GC groups. This is again consistent with earlier human studies (Hoelker et al.[4], Tao et al.[8]), which also failed to find significant improvements in clinical outcomes, despite enhanced blastocyst quality. One potential explanation for the lack of significant differences in clinical outcomes may be related to the patient population. All patients in this study were under 40 years old and had favorable prognostic factors, which may have minimized the potential impact of culture conditions on clinical outcomes. Future studies should investigate the effects of GC in a broader population, including older patients and those with poorer prognosis. Moreover, the present study was limited to fresh embryo transfers, and the potential impact of GC on frozen-thawed embryo transfers remains unexplored. Further research should focus on examining whether the benefits observed in blastocyst development translate to improved outcomes in frozen-thawed cycles.

Study Limitations

One limitation of this study was the exclusion of patients with poor prognosis or male factor infertility, which limits the generalizability of the findings. In addition, the exclusive use of fresh embryo transfers may have influenced the results, as frozen embryo transfers could yield different outcomes. The study also lacks long-term follow-up data on live birth outcomes.

CONCLUSION

The use of a micro-well dish for GC provides a practical method to improve blastocyst quality without additional costs, making it a viable option for IVF laboratories aiming to maximize embryo yield. However, the lack of significant clinical outcome differences suggests that more research is needed to determine whether these improvements in blastocyst quality can consistently lead to better pregnancy outcomes, particularly in specific patient subgroups.

Ethics

Ethics Committee Approval: The ethics approval for the study was obtained from Kocaeli University Faculty of Medicine Ethics Committee (approval number: KOU KAEK 2013179, date: 19.09.2023).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Authorship Contributions

Surgical and Medical Practices: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö., Concept: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö., Design: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö., Data Collection or Processing: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö., Analysis or Interpretation: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö., Literature Search: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö., Writing: E.E., A.İ.T., Z.Ö., E.Y.K., H.M.Ö.

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