Original Article

Randomized controlled trial comparing single embryo culture with group culture in a micro-well dish: impact on embryo development and clinical outcomes.

Ergin et al. Single versus group embryo culture comparison

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13.08.2024
16.10.2024

Epub: 14.05.2025

Abstract
INTRODUCTION: The optimal culture environment for embryos in vitro remains a topic of ongoing debate in assisted reproductive technologies. 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 IVF remains unclear. This study aimed to compare embryo development and clinical outcomes between single embryo culture and group embryo culture using a micro-well dish in human IVF cycles.
METHODS: In this prospective, randomized controlled trial, 160 patients undergoing IVF at Eurofertil IVF Center were allocated to either the single embryo culture group or the group culture using a micro-well dish. 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: Group culture 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: Group culture using a micro-well dish leads to a higher yield of top-quality blastocysts, although it does not significantly improve clinical outcomes. These results suggest that group culture 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, have been extensively studied, yet no consensus has been reached regarding which technique yields the best clinical outcomes (Almagor et al., 2020; Ebner et al., 2017).
Recent advances have suggested that group culture, particularly when using micro-well dishes, may enhance embryo development due to autocrine and paracrine signaling effects, which could positively impact embryo quality (Dai et al., 2018; Vajta et al., 2020). Studies in animal models have provided significant evidence supporting these mechanisms, though data in humans remain inconsistent (Hoelker et al., 2019; Matsuura et al., 2021).
Despite encouraging results from various animal studies, there is limited and conflicting evidence regarding the impact of group culture on clinical outcomes in human ART, particularly regarding blastocyst formation and live

birth rates (Herreros et al., 2019). Additionally, recent innovations like the well-of-the-well (WOW) dish have shown promise, but their application to human embryos has yet to be thoroughly explored (Fancsovits et al., 2021).

This study aims 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.

MATERİALS AND 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 using a micro-well dish. Randomization was performed using a computer-generated list, with allocation occurring after the fertilization check.

Participants: A total of 215 patients were initially screened, and 160 met the inclusion criteria. 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.

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 (Gardner et al., 2000). 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 20.0. 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 single culture group and 80 to the group culture group. Baseline characteristics such as age, BMI, and duration of infertility were similar between the two groups ($p>0.05$), ensuring comparability (Table 1).

- Blastocyst Development: The total blastocyst development rate was higher in the group culture (GC) group compared to the single culture (SC) group (GC: 62.5% vs SC: 51.8%, $p=0.04$). Furthermore, the number of top-quality blastocysts was significantly greater in the group culture (GC: 40.2% vs SC: 27.5%, $p<0.05$).
- Cryopreserved Embryos: A significantly higher number of cryopreserved blastocysts were obtained from the group culture group (GC: 15.3% vs SC: 8.6%, $p=0.04$) (Table 2).
- Clinical Outcomes: Clinical pregnancy rates were higher in the group culture, although this difference was not statistically significant (GC: 48.1% vs SC: 44.7%, $p>0.05$). Similarly, no significant differences were found in implantation rates or live birth rates between the two groups ($p>0.05$) (Table 3).
- Fresh Embryo Transfers: All embryo transfers in this study were fresh, with no frozen embryo transfers performed during the study period.
- PGT: None of the embryos underwent preimplantation genetic testing.

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

DISCUSSION

The findings from this study support previous research indicating that group embryo culture enhances blastocyst development and improves the yield of top-quality blastocysts (Vajta et al., 2020; Matsuura et al., 2021). Our results align with those of Hoelker et al. (2019), who found that micro-well group culture systems promote better embryo development, likely due to enhanced autocrine and paracrine signaling within the microenvironment.

Despite these promising findings, we did not observe statistically significant differences in clinical pregnancy or live birth rates between the single and group culture groups. This is consistent with earlier human studies (Almagor et al., 2020; Rebollar-Lazaro & Matson, 2019), 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 group culture in a broader population, including older patients and those with poorer prognosis.

Additionally, the study was limited to fresh embryo transfers, and the potential impact of group culture 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.

The use of a micro-well dish in group culture 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.
Limitations: One limitation of this study is the exclusion of patients with poor prognosis or male factor infertility, which limits the generalizability of the findings. Additionally, the use of only 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.

REFERENCES

1. Almagor M, et al. (2020). Comparison of Single and Group Culture in Human IVF. Human Reproduction, 35(4), 802-810.
2. Ebner T, et al. (2017). Group Culture and IVF Outcomes: A Review. Journal of Assisted Reproduction and Genetics, 34(5), 589-595.
3. Dai SJ, et al. (2018). The Effect of Culture Medium Volume and Embryo Density on Development of Mouse Embryos Using WOW Dish. Reproductive Biology, 18(1), 45-52.
4. Vajta G, et al. (2020). Autocrine and Paracrine Signaling in Group Culture of Embryos: A Review. Reproductive Biomedicine Online, 41(3), 402-410.
5. Hoelker M, et al. (2019). The Effects of Micro-Well Group Culture Systems on Bovine and Human Embryo Development. Reproduction in Domestic Animals, 54(8), 1564-1572.
6. Matsuura K, et al. (2021). Diffusion Effects in WOW Culture System: Implications for Human IVF. Fertility and Sterility, 116(5), 1123-1130.
7. Herreros L, et al. (2019). Group Culture vs. Individual Culture: A Comparative Study. Human Fertility, 22(2), 132-139.
8. Fancsovits P, et al. (2021). Group Culture in Human IVF: Clinical Implications and Future Directions. Journal of Reproductive Medicine, 66(2), 85-93.
9. Rebollar-Lazaro I, Matson P. (2019). Group Culture in Human IVF: A Review of Outcomes. Journal of Assisted Reproduction and Genetics, 36(6), 1001-1011.

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/m2)	25.3± 4.2	25.2± 4.9	0.9
Duration of infertility (y)	4.86± 3.2	4.94± 3.5	0.88
p<0.05 statistically significant			

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% CI			

Table 3. Clinical and cumulative outcomes of both groups

Clinical outcomes (all transfers)	**Group 1 (n=74)**	**Group 2 (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**

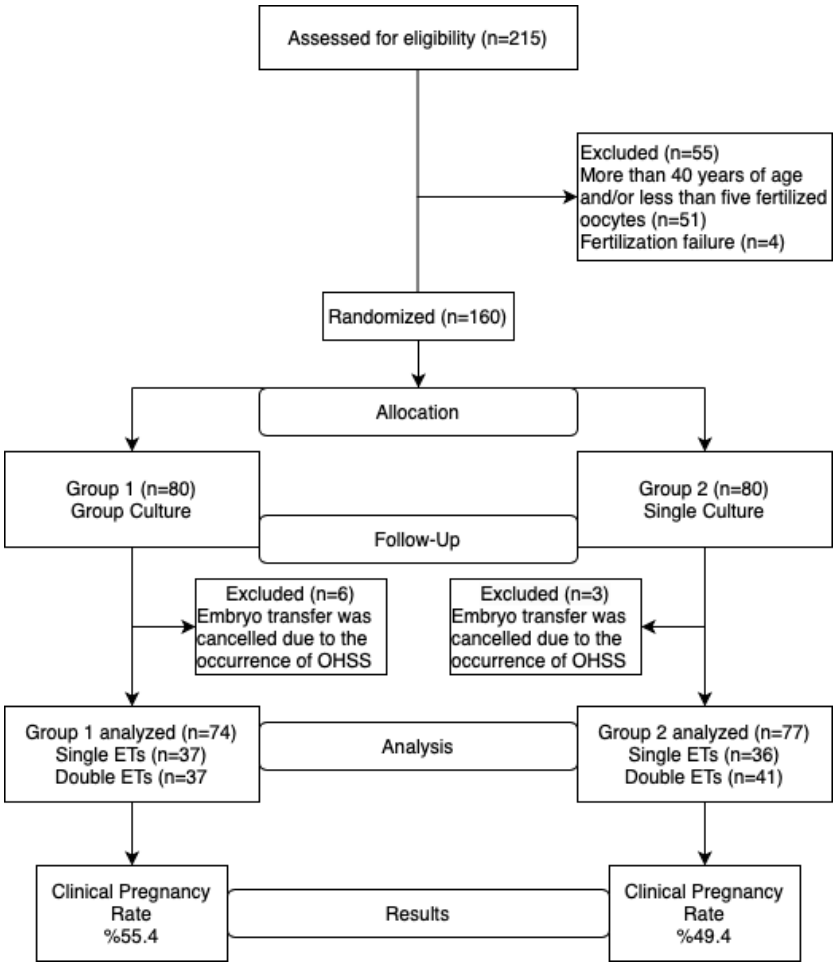

Diagram 1: The CONSORT 2010 Flowchart

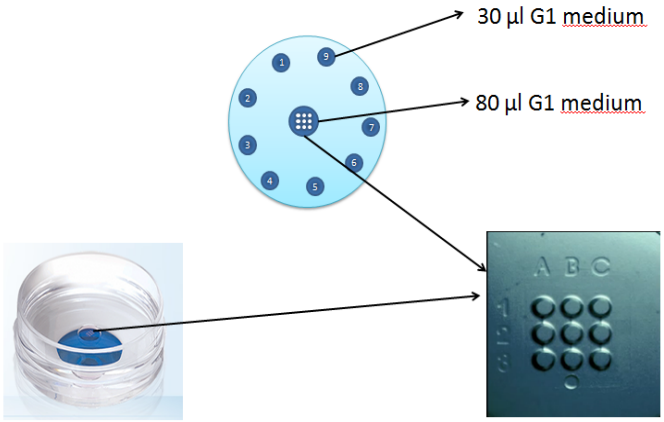

Figure 1: Preparation of WOW dish.