Ovofast: preliminary results of a successful application of a shortened warming protocol for oocytes cryopreserved using standard vitrification, while the ultra-shortened protocol showed cytoskeletal alterations

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Introduction

Recent studies on Shortened oocyte Warming (SW) protocols have shown promising results, with survival rates and developmental competence comparable to or even superior to the standard protocol. These methods improve IVF efficiency by minimizing exposure to suboptimal temperatures, reducing osmolarity-induced stress, and mitigating the harmful effects of cryoprotectants [1]. However, research on SW protocols has predominantly focused on oocytes vitrified using Rapid Vitrification (RV) techniques, while data on their effectiveness for oocytes cryopreserved with Standard Vitrification (SV) remains limited. With millions of oocytes already vitrified using conventional methods, further research is needed to evaluate the effectiveness of SW protocols for these oocytes.

Objectives

To evaluate the efficiency of SW and Ultra-Shortened Warming (USW) protocols for oocytes cryopreserved using SV.

Results

This prospective sibling study included 390 mature human oocytes. Experiment-1 compared the standard warming (Group A) and the SW protocols (Group B), and Experiment-2 compared the SW and USW protocols (Group C). Sub-analyses focused on spindle/chromosomal configurations and oocyte quality scores (range:0-10) using an Artificial Intelligence (AI) model. Group A followed the standard warming protocol (10-minutes), Group B the SW protocol (1-minute in 1.0 M trehalose, and 1-minute in 0.5 M trehalose at 37°C), and Group C the USW protocol (1-minute in 1.0 M trehalose at 37°C). Survival rates were evaluated two hours post-warming. Spindle configurations were classified as normal, slightly aberrant, or aberrant, while chromosomal distributions as normal, misaligned, or displaced. In Experiment-1 (Group A n=150 oocytes, Group B n=148 oocytes) no significant differences were observed in survival rates (Group A:89.33% vs. Group B:93.24%, p=0.23), chromosomal [Group A (n=21): normal 72%, misaligned 14%, displaced 14% vs. Group B (n=21): normal 67%, misaligned 19%, displaced 14%, p=0.73] and spindle configurations [Group A (n=13): normal 85%, aberrant 15% vs. Group B (n=8): normal 75%, aberrant 25%, p=0.58), or AI-derived quality scores (n=57, Group A: 5.88±2.41 vs. Group B: 5.58±2.42, p=0.67). The SW protocol reduced workflow time by 80%. In Experiment-2 (Group B n=46 oocytes, Group B n=46 oocytes) no significant differences were observed in survival rates (Group B: 93.47% vs. Group C: 97.82%, p=0.30) and AI-derived quality scores (n=22, Group B: 4.58±2.84 vs. Group C: 4.85±2.66, p=0.79). We found significant differences in chromosomal [Group B (n=14): normal 64%, misaligned 14%, displaced 21% vs. Group C (n=16): normal 13%, misaligned 25%, displaced 63%, p=0.003) and spindle configurations [Group B (n=14): normal 79%, slightly aberrant 7%, aberrant 14% vs. Group C (n=14): normal 14%, slightly aberrant 14%, aberrant 72%, p<0.001].

Conclusions

Our results highlight the potential of the SW protocol, maintaining efficiency comparable to standard methods while reducing workflow time and minimizing exposure to suboptimal conditions. In contrast, the USW protocol demonstrated similar survival rates but it induced cytoskeletal alterations, particularly in chromosome and spindle configurations. If confirmed by independent studies on oocytes cryopreserved using RV, this protocol could demonstrate its universal applicability.

Bibliography

1 *Liebermann J, Brohammer R, Wagner Y, et al. Fast and furious: successful survival and resumption of meiosis in immature human oocytes vitrified and warmed using a short protocol. Reprod Biomed Online. 2024;49(1):103976.*