Title: MICROWAVE DRYING AND STORAGE OF HUMAN OOCYTES

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Background:
Dry preservation is being developed to allow storage of gametes at non-cryogenic temperatures, eliminating reliance on liquid nitrogen and decreasing expenses of storage [1,2]. Trehalose is a non-reducing disaccharide sugar and increases dehydration tolerance of cells by maintaining three-dimensional conformation of macromolecules. It prevents collapse or aggregation of cellular structures and stabilizes cellular components in amorphous trehalose glass formed after dehydration [3]. Using trehalose, it has been shown that DNA integrity can be maintained after drying and storage of domestic cat oocytes and spermatozoa [2, 4-8].

Objective:
Determine whether introduction of trehalose, microwave drying, and storage at non-freezing temperatures adversely affects the DNA integrity of immature human oocytes.

Materials and Methods:
Translational IRB-approved study. Unidentified, fresh oocytes after routine IVF treatment that would otherwise be discarded secondary to immaturity were pooled and collected 24 hours after retrieval. Oocytes were permeabilized using hemolysin, incubated in trehalose, and dehydrated in a SAM 255 microwave system for 30 minutes at 40˚C and a power of 20%. Non-dried controls were compared to drying groups of different storage times and temperatures including: immediately rehydrated (IR), 7 days (d) at 4˚C, 7d at room temperature (RT), 28d at 4˚C, and 28d at RT. DNA integrity was evaluated using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analysis. Fluorescence intensity was quantified using ImageJ. Comparisons of signal intensity were performed using Wilcoxon rank sum tests. P-values < 0.05 were considered significant.

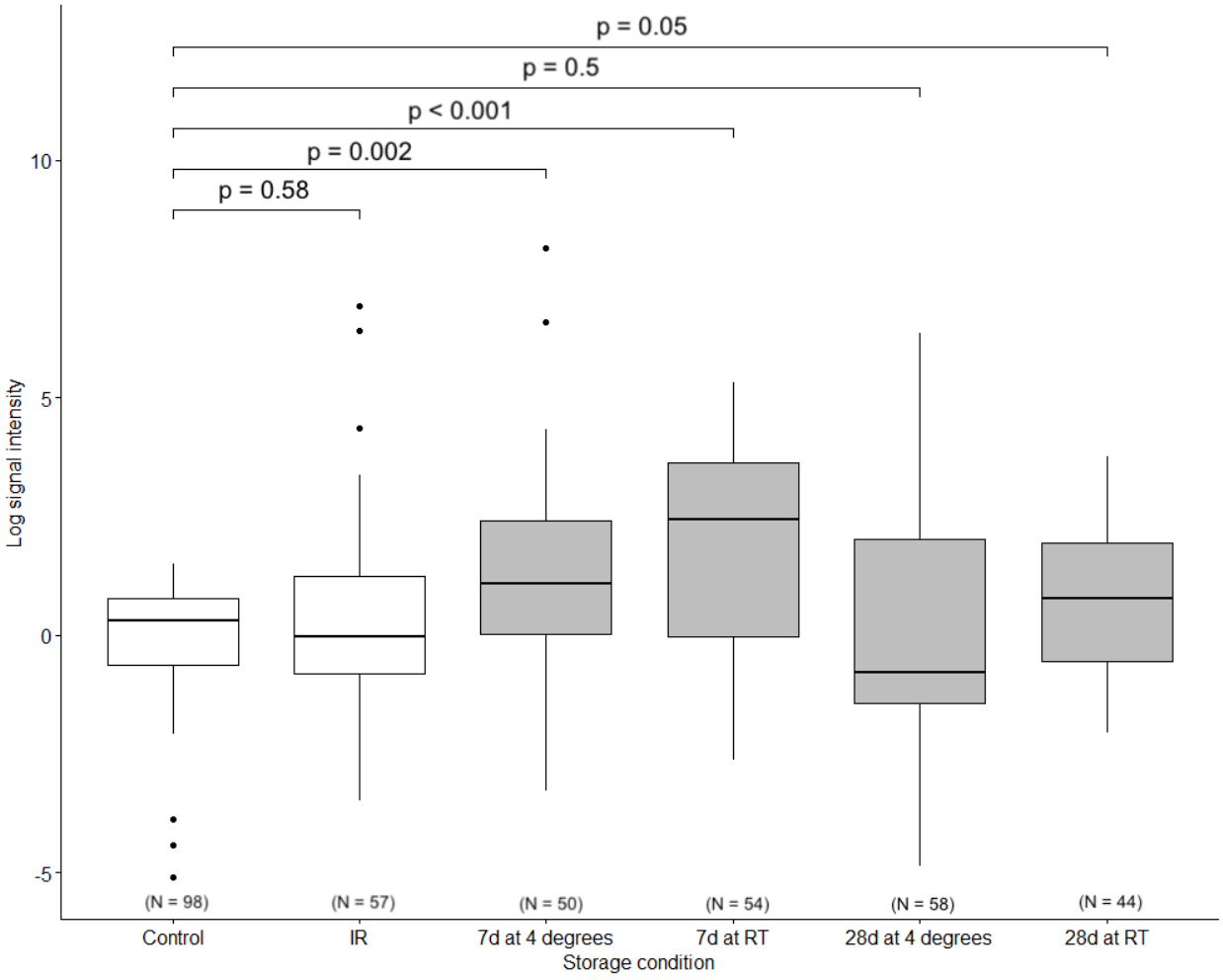
Results:
A total of 361 oocytes were analyzed (controls, n = 98; experimental groups, n = 263). Mean patient age was 35.8 years (SD 4.17; range 21-45). Percentage oocyte recovery during rehydration for all drying groups was 95.4%. There were similar levels of DNA damage comparing the control group to the IR group, 28d at 4˚C, and 28d at RT. The 7d at 4˚C and 7d at RT groups showed 2.3-fold ($p = 0.002$) and 1.6-fold ($p < 0.001$) increased DNA damage, respectively, compared to controls (Figure 1). There were no differences in comparing experimental groups by storage temperature (Figure 2). For storage duration, there was a 0.9-fold increase in DNA damage in the 7d RT group compared to 28d ($p = 0.02$) (Figure 3).

Conclusions:
DNA integrity in the oocytes was maintained after dehydration with immediate rehydration and storage for 28d at 4˚C and at RT. Interestingly, storage at both 4˚C and RT for 7d led an increase in DNA damage. The differences in DNA damage in the 7-day storage groups may be secondary to inherent characteristics of the oocytes on day of collection such as poorer quality, high variation of patient ages, and limited number of samples each day for comparison.

Conversely, the lack of differences in the 28-day groups may have been masked due to such variation in oocytes by day of collection.

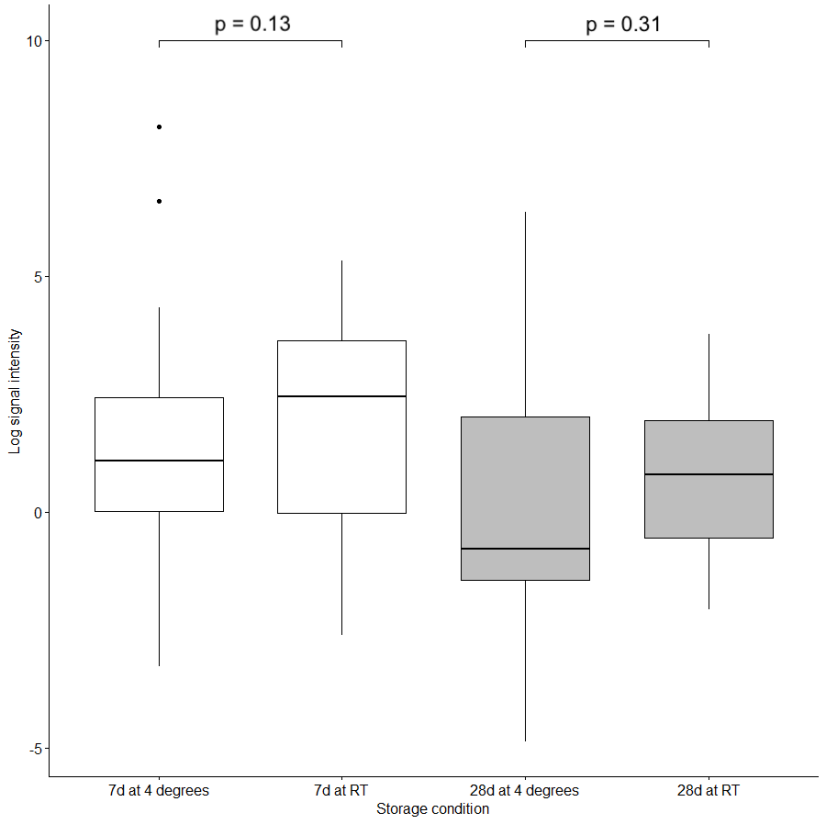
Our study provides a proof of concept for the possibility of drying and storing human oocytes at non-freezing temperatures may not affect DNA integrity of the cells. Further study is needed to assess longer storage times as well as assess oocyte competence with fertilization.

Figure 1: Comparison of DNA damage by individual storage condition



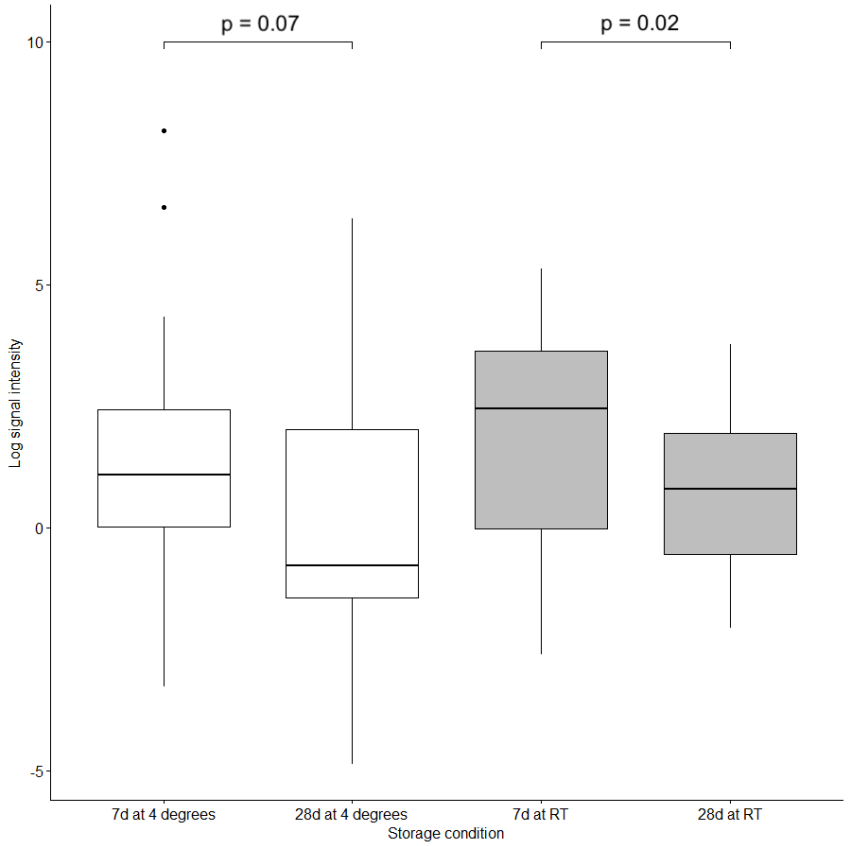
IR = immediately rehydrated, d = days, RT = room temperature

Figure 2: Comparison of DNA damage by storage temperature



IR = immediately rehydrated, d = days, RT = room temperature

Figure 3: Comparison of DNA damage by storage duration



IR = immediately rehydrated, d = days, RT = room temperature

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