Can temperature stability be improved during micromanipulation procedures by introducing a novel air warming system?

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Introduction

Dish temperature stability during micromanipulation procedures relies upon a heated stage, usually a metal plate with an aperture, or a glass - Indium Tin Oxide (ITO) platform. Both present different challenges in maintaining temperature stability over extended periods of time. Introducing an air warming system (AWS) may overcome these issues and improve temperature stability.

Materials and Methods

This study compared three micromanipulation workstations;

- **Workstation 1 (WS1): heated stage with aperture** (Integra Ti™ 2011 plus WIS metal stage, *Research Instruments (RI), Falmouth*).
- **Workstation 2 (WS2): heated stage with glass platform** (Integra Ti™ 2011 plus ITO, *RI, Falmouth*).
- **Workstation 3 (WS3): heated stage with aperture and AWS** (Integra 3™ 2013 plus Thermosafe, *RI, Falmouth*).

ICSI dishes were prepared, equilibrated and placed on the heated workstation. Temperature readings were recorded in 5µl media drops positioned centrally in the field of view of the microscope. Measurements were taken immediately and at five minute intervals for one hour on each working objective (x20, x40 and x60). Measurements were repeated five times for each objective on each workstation. Thermal images were taken in order to identify potential 'hot-spots' or 'cold-spots' on each workstation using different objectives.

Results

Comparison of mean objective temperatures on the same workstation

Mean droplet temperature did not differ significantly between the 3 different objectives on WS1 and WS3, however, a significant difference was observed on WS2 as determined by one-way ANOVA ($F(2,36)=48.92$, $p<0.0001$), figure 1. Post hoc analysis using Bonferroni corrected t-tests indicated a significant difference in mean temperature readings between each objective where the x40 objective read 1.3°C lower than the x20 (35.4 vs. 36.7, $p<0.0001$) and 0.8°C lower than the x60 objective (35.4 vs. 36.2, $p<0.0001$); further to this the x60 objective read 0.5°C lower than the x20 objective (36.2 vs. 36.7, $p<0.01$).

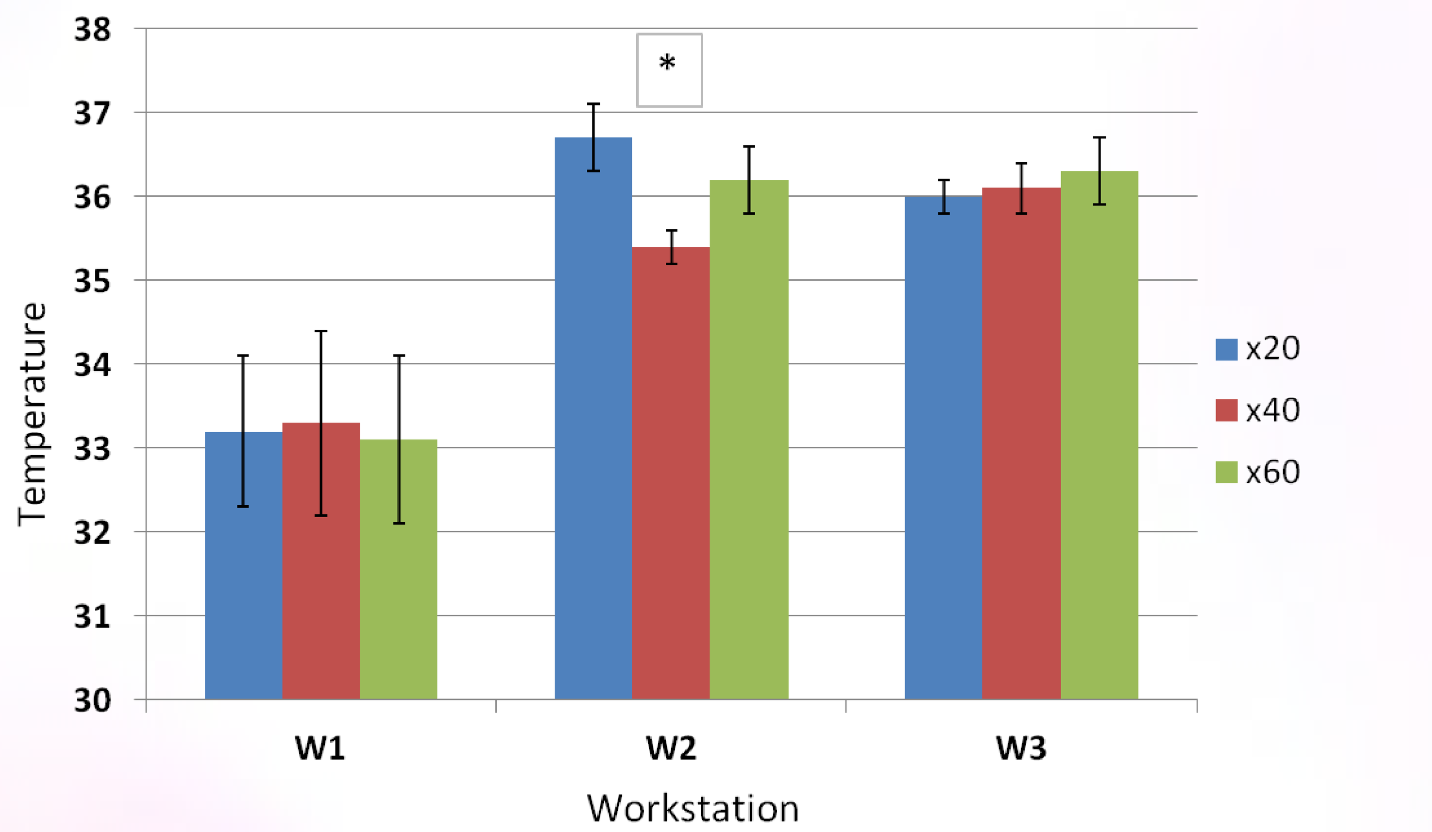
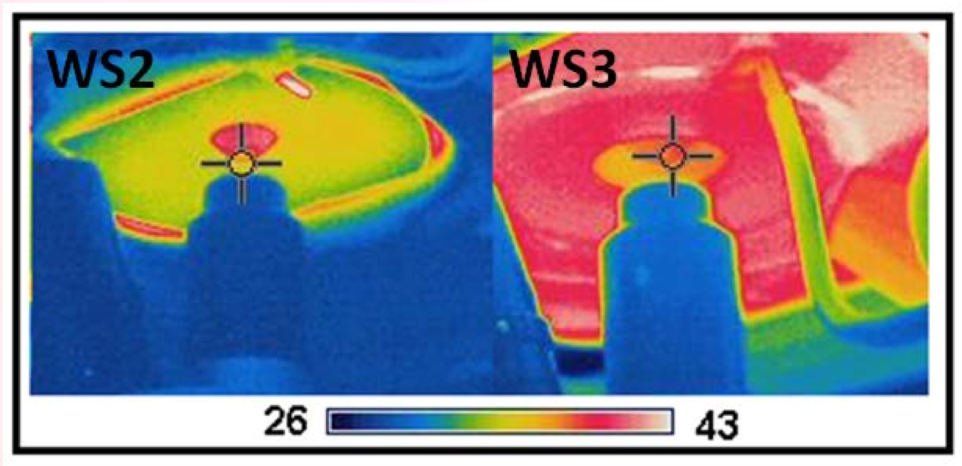


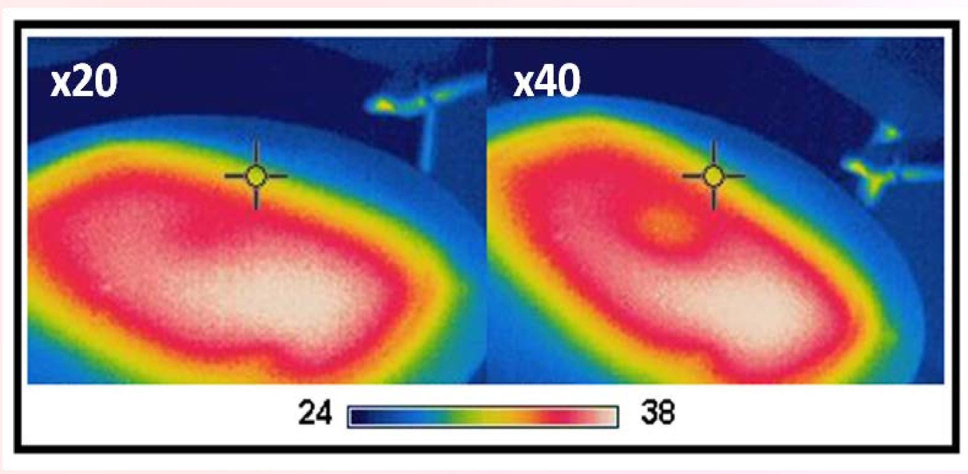
Figure 1. Mean objective temperature over a 1 hour period for each workstation, *p<0.01.

Thermal Imaging



Thermal imaging of WS2 & WS3 demonstrated that the objective was the coldest area of the working environment, figure 3.

Figure 3. View from the objective turret to the bottom of the dish on WS2 and WS3 (x20 objective).



Comparing objectives on WS2 showed a reduced temperature in the critical work area when using the x40 objective compared to the x20, figure 4.

Figure 4. Comparative thermal images of x20 and x40 objective on glass platform of WS2 .

Comparison of temperature stability over time

The temperature stability over the 1 hour test period was lowest on WS1 for all objectives (x20 = 33.2 ± 0.9; x40 = 33.3 ± 1.1; x60 = 33.1 ± 1.0), figure 2. Both WS2 and WS3 demonstrated high temperature stability for all 3 objectives (WS2: x20 = 36.7 ± 0.4; x40 = 35.4 ± 0.2; x60 = 36.2 ± 0.4 and WS3: x20 = 36.0 ± 0.2; x40 = 36.1 ± 0.3; x60 = 36.3 ± 0.4), figure 2.

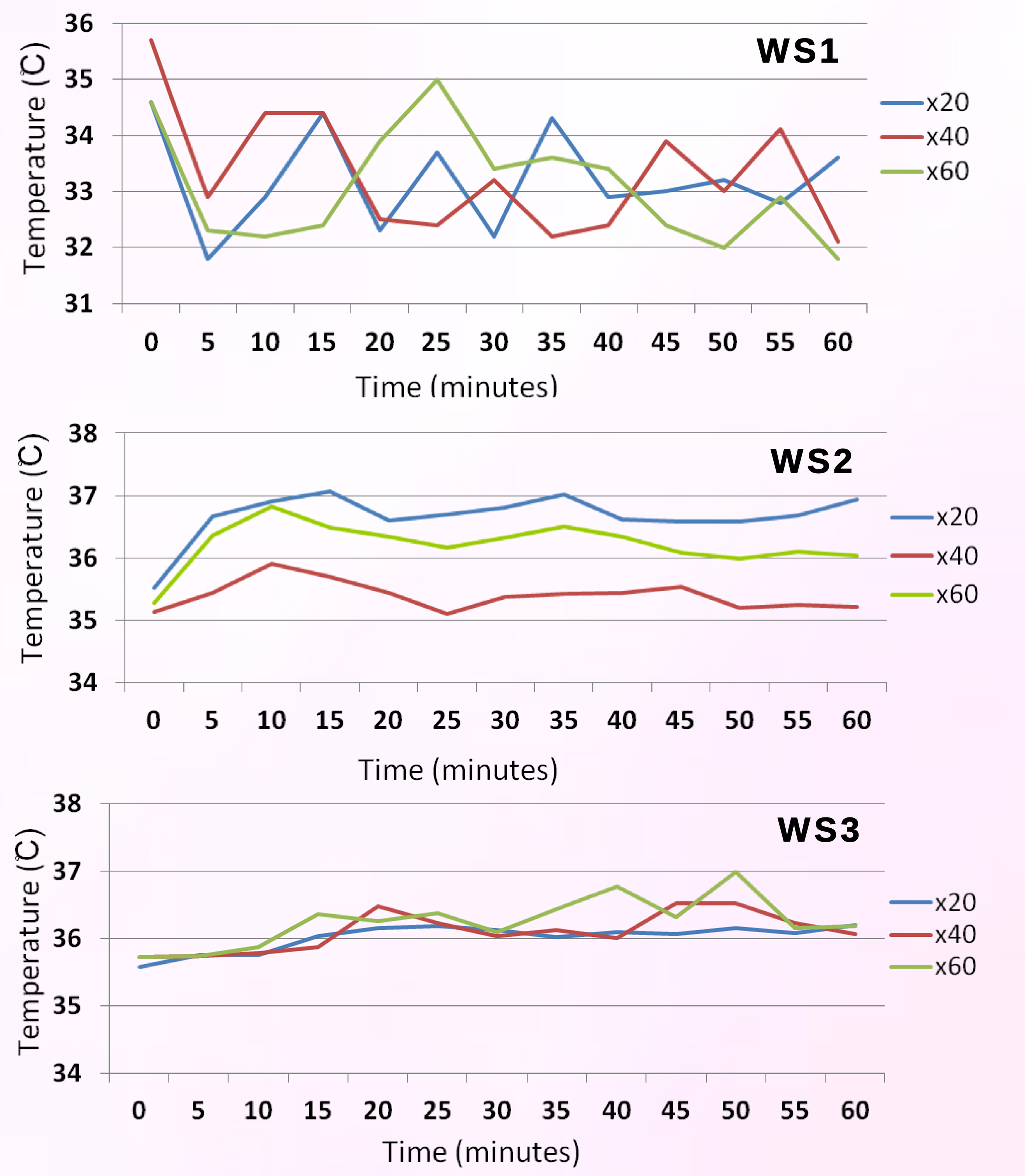


Figure 2. Mean temperature readings over a 1 hour period for WS1, WS2 and WS3.

Discussion

Temperature profiling has demonstrated that culture media drops undergo fluctuations when a workstation with aperture (WS1) is used for micromanipulation procedures. This problem can be partially overcome by replacing the aperture with an ITO glass platform (WS2); however, our data demonstrate that the culture drops are still susceptible to significant changes in temperature caused by different working objectives. This is concerning as studies have demonstrated subtle changes in oocyte temperature can impact on meiotic spindle integrity, which can affect fertilisation and subsequent embryo development[1,2].

It is postulated that the thermal mass of the objectives and differing working distances causes a variable heat sink effect which can be overcome using an AWS, figure 3. The relationship between these inconstant conditions is the subject of ongoing investigation.

An AWS maintains culture dish temperature stability over extended periods of time and is not significantly affected by the choice of objective.

Conclusion: The AWS provides optimum temperature control during extended micromanipulation procedures such as ICSI, IMSI and embryo biopsy when compared to alternatives currently available.

1. Wei-Hua Wang, Li Meng et al Human Reproduction Vol.16, No11 pp. 2374-2378, 2001
2. Wei-Hua Wang, Li Meng et al Fertility and Sterility Vol 77, No 6, pp. 1274–p1277, 2002

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