RBMO

SHORT COMMUNICATION

Embryos are exposed to a significant drop in temperature during the embryo transfer procedure: a pilot study

BIOGRAPHY

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KEY MESSAGE

Current embryo transfer procedures subject embryos to a rapid and profound drop in temperature between loading the catheter in the laboratory and transferring the embryo into the uterus.

ABSTRACT

Research question: During the embryo transfer procedure, to what degree of temperature drop are embryos exposed to between loading the transfer catheter and placing it into the uterus?

Design: Twenty-nine simulated embryo transfer procedures were carried out across five clinics. A thermocouple probe was used for standardized measurements inside the embryo transfer catheter to investigate the change in temperature that occurred in the time period between loading and placing the catheter in the uterus.

Results: In all cases, the temperature at the loaded catheter tip fell rapidly to ambient temperature during transit from the embryo transfer workstation in the laboratory to the procedure room, even though embryo transfer procedures, ambient temperatures and embryo transfer catheter temperatures at loading varied between clinics.

Conclusions: Given the sensitivity of the pre-implantation embryo to its immediate environment, the rapid and profound drop in temperature observed at the catheter tip that houses the embryo during transit from laboratory to the uterus may affect embryo viability and health. This issue should be addressed to ensure that the tight temperature control aimed for by IVF laboratories continues throughout the embryo transfer procedure, and could improve clinical outcomes.

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KEYWORDS

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INTRODUCTION

The culmination of all clinical and laboratory efforts during an assisted reproductive technology (ART) cycle is the transfer of an embryo from IVF laboratory to the uterine cavity. In the IVF laboratory, this procedure starts with embryo selection and the preparation of an embryo transfer catheter to load the embryo. Upon aspiration of the embryo into the embryo transfer catheter, an embryologist usually transports the loaded catheter in the open ambient environment of the laboratory and the procedure room, in which it is passed into the patient's uterus so that the embryo can be deposited into the endometrial cavity.

IVF laboratory procedures and processes have undergone vast improvements over the past decades, but the embryo transfer equipment, consumables and procedure have undergone minimal scrutiny or change. Although the transfer technique has been somewhat refined and standardized in recent years (*ASRM, 2017*), many significant factors remain. These include numerous permutations of laboratory culture systems, environment, design and layout. Importantly, the embryo transfer procedure remains operator-dependent (*Cirillo et al., 2020*), which adds to the complexity of what many practitioners consider to be one of the most critical steps of an ART cycle.

Guidelines for best practices in IVF laboratories emphasize some general principles that all IVF clinics seek to adhere to: temperature control, maintaining osmolarity and pH, and protection from oxidative stress and toxic substances (*Cairo 2018 Consensus Group, 2020*). This is based on the generally accepted requirement for an optimized and stable physicochemical environment, which includes temperature control, maintaining normal homeostasis, metabolism and spindle stability and minimizing embryonic cell stress. During preimplantation embryo development, epigenetic reprogramming occurs and aberrant environmental stress factors can disrupt this critical process and potentially damage embryos (*Ventura-Junca et al., 2015*). It is, therefore, vital to maintain stable and optimized temperatures throughout all stages of gamete and embryo processing within the IVF laboratory: this is the reason why all IVF laboratories use heated stages, warming blocks and incubators to control and maintain temperature within set control limits. During the embryo transfer procedure, no such temperature control measures are, to our knowledge, routinely in use.

MATERIALS AND METHODS

To evaluate temperature changes during the embryo transfer procedure, the same measurement system was used for all experiments. This consisted of a data-logging thermometer (Fluke 54 II B) and an insulated thermocouple probe type k with a sheath diameter of 0.25 mm (TC Ltd, Uxbridge, UK) placed at a standardized distance (3 mm) from the tip of an embryo transfer catheter (Wallace Sure View®). Calibration was carried out by an independent third party (Metrology.ch ISO/CEI 17025:2017 accredited). The maximum measurement uncertainty was 0.6°C, which is the sum of the uncertainty of the thermometer connected to the thermocouple probe (0.4°C) and the uncertainty of the calibration equipment (0.2°C). The temperature measurement system for the embryo transfer catheter is shown in the Supplementary Figure.

Twenty-nine simulated embryo transfer procedures were carried out in five IVF clinics with the calibrated measurement system. It was not felt appropriate to dictate the methodology for catheter loading. The simulations were carried out under real-life conditions, reproducing the standard routine embryo transfer procedure in place at each clinic, without embryos. The procedural differences are shown in the Supplementary Table. The embryo loading temperature (start) was defined as the temperature within the medium-primed catheter at the start of the embryo transfer procedure, when embryo loading into the catheter was simulated. As no embryos were used and no patients were involved, no ethical committee approval was required.

RESULTS

Considerable variations were found to exist in embryo transfer operating procedures, ambient temperatures and embryo transfer catheter temperatures during the procedure (Supplementary Table). The start temperature at the time of embryo loading ranged from 27.0°C to 35.9°C. Moreover, in all cases, a profound temperature drop was recorded within 20 s of loading the catheter (illustrative data presented in FIGURE 1).

In most cases, the temperature of the medium within the catheter was found to fall below room temperature. The data showed the same pattern in all five clinics, i.e a rapid decline in temperature down to ambient temperature or below (possibly due to an evaporation effect), regardless of ambient environment, type of workstation or embryo transfer operating procedures in use. Therefore, the embryo is exposed to non-physiological conditions inside the embryo transfer catheter during transit from the IVF laboratory to the patient.

DISCUSSION

This pilot study demonstrates that the embryo transfer procedure represents a 'weak link' in temperature control during the complete IVF laboratory process. Although the embryo's journey from the IVF laboratory to the patient may be short in some settings, any significant temperature decline may induce cell, mitotic stress, or both, before the embryo is safely deposited into its physiological environment, the uterine cavity. The level of stress imposed on the embryo, caused by cooling during embryo transfer, is procedure- and time-dependent. For example, suboptimal handling of the embryo in preparation for loading of the embry transfer catheter, low ambient laboratory and procedure room temperatures, airflow on the catheter, and challenges introducing the catheter into the uterus (*Kava-Braverman et al., 2017*) may all contribute. Prolonging the duration of embryo transfer will aggravate the problem and could potentially compromise the viability of the embryo. The full biological consequences of cooling during embryo transfer are unknown, but it can be postulated that temperature stress during embryo transfer could influence non-disjunction events during mitotic division, resulting in aneuploidy or mosaicism. The potential effect of local environmental factors on euploidy rates has been previously demonstrated (*Munné et al. 2017*). Because of existing variations in IVF–embryo transfer procedures, it is important to consider the accumulated stress imposed on gametes and embryos throughout the entire process, e.g.

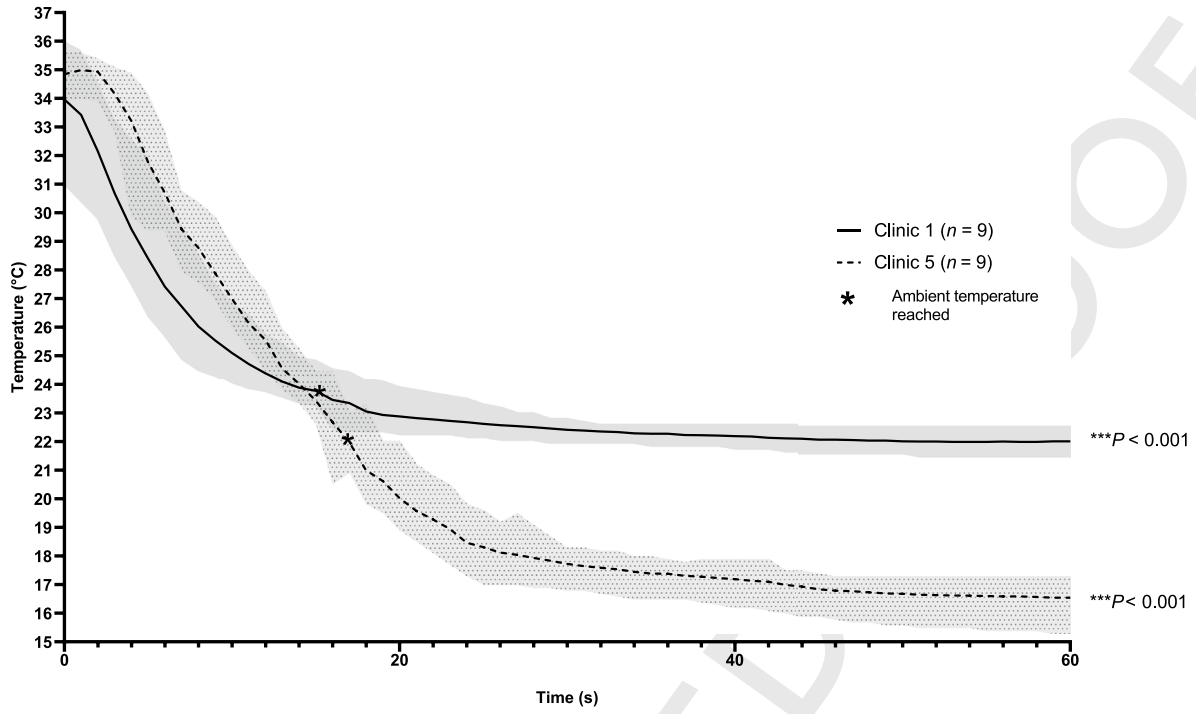

FIGURE 1 Representative data of the drop in temperature during simulated embryo transfer procedures in clinic 1 and 5. Shaded area = SD. Ambient temperatures in the procedure rooms, indicated by an asterisk, were reached inside the embryo transfer catheter in less than 20 s. Statistical significance (loading temperature versus temperature 60 s after loading) was calculated using the Mann–Whitney U test.

during oocyte aspiration, denudation, intracytoplasmic sperm injection, embryo handling, trophectoderm biopsy, vitrification and embryo transfer. Given the exquisite sensitivity of the pre-implantation embryo to its immediate environment, it is reasonable to infer that the additional stress imposed during embryo transfer could significantly affect embryo viability and negatively affect clinical outcomes.

For ethical and pragmatic reasons, determining the effect of different durations of embryo transport and degrees of cooling during embryo transfer on the live birth rate by controlled clinical trials is challenging. However, considering the general principles of minimizing environmental stress to maintain embryo viability *in vitro*, and as a measure of precaution, there may be merit in addressing this previously neglected aspect of the embryo transfer procedure. Embryo transfer represents a critical interface between laboratory and clinical procedures and is possibly the least standardized process in ART. Awareness of this previously unquantified phenomenon of embryo cooling is yet another argument for working towards standardization of the embryo transfer procedure (*ASRM, 2017*). Simple mitigating actions could include loading embryos in a temperature controlled-chamber and minimizing the duration of transport from the laboratory to the patient; however, the presented findings indicate that more consideration needs to be given to understanding the factors that lead to the acute temperature drops observed, including those below ambient temperature, and to developing strategies for maintaining the temperature of the embryo during the embryo transfer procedure.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2021.05.014.

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