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Qualitative assessment of warmed pronuclear stage zygotes after slow-rate freezing and Cryotech vitrification

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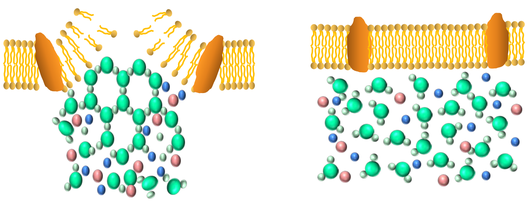
Introduction

Based on legal restrictions, cryopreserved 2PN zygotes are used to perform frozen embryo transfers in Switzerland. The cryopreservation of 2PNs by slow-rate freezing is not just time consuming and uses a lot of LN2, it also yields only a limited survival of competent zygotes. Furthermore, non-inseminated oocytes for emergency freezing (e.g. where no sperm were available) show poor success when cryopreserved by the slow-rate freezing method. However, the very high cryosurvival rates that can now be achieved using vitrification have seen its rapid adoption in many centres. Consequently, the aim of this study was to evaluate both the practical utility and clinical relevance of frozen/warmed 2PN zygotes employing from two different freezing principles.

Materials and Methods

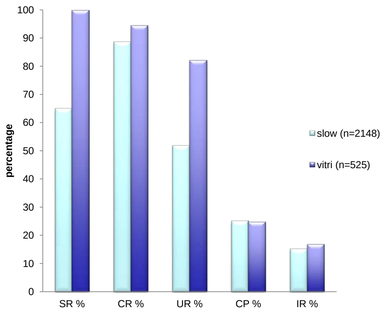
2PN zygotes were cryopreserved at 16–20 h post-insemination using either the standard propanediol/glucose slow-rate freezing method (Group A) or Kuwayama's new Cryotec vitrification method (Cryotech, Tokyo, Japan; Group B). Zygotes were thawed/warmed using the appropriate protocol and cultured to either Day 3 or Day 5 according to clinical preference. The two methods were compared in terms of the cryosurvival rate (SR), cleavage rate (CR), embryo utilization rate (UR), clinical pregnancy rate (CP), and the implantation rate (IR).

slow freezing **vitrification**



The prevention of ice cristal formation is crucial in cryopreservation

Comparison of slow rate freezing (Group A) with Cryotech vitrification (Group B)



Results

The ages of the patients in the two groups were similar 35.0 ± 4.6 yrs *c.f.* 35.5 ± 4.1 yrs for Groups A and B respectively; $P>0.05$).

The cryosurvival rate was significantly higher following vitrification (99.8% [524/525] *c.f.* 65.0% [1396/2148], $P<0.0001$), as was the embryo utilization rate (82.1% [431/525] *c.f.* 51.9% [1115/2148], $P<0.0001$).

However, the cleavage rates, clinical pregnancy rates and implantation rates were not significantly different between the two methods: 88.6 % [1237/1396] *c.f.* 94.5% [495/524], 25.1% [154/614] *c.f.* 24.6% [62/252], and 15.2% [170/1115] *c.f.* 16.7% [72/43], for Groups A and B respectively.

On average, achieving a clinical pregnancy required more slow-frozen zygotes than vitrified zygotes (3.5 *vs* 2.1).

Discussion

Although this comparison of the two zygote cryopreservation methods did not reveal any significant differences in clinical pregnancy outcomes, a significantly higher cryosurvival rate – and thus embryo utilization rate – could be achieved by vitrification. Furthermore, more slow-frozen zygotes had to be thawed in order to achieve a clinical pregnancy compared to vitrified zygotes needing to be warmed.

Long-term follow-up studies to consolidate the beneficial impact of the Cryotec vitrification method are being undertaken.