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Higher β-HCG concentrations and higher birthweights ensue from single vitrified embryo transfers

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Dr John Yovich graduated in Medicine at the University of Western Australia in 1970, progressing into specialist Obstetrics and Gynaecology practice in 1976. Thereafter Dr Yovich presented his MD thesis *"Human pregnancies achieved by In-Vitro Fertilisation"* following laboratory research and clinical work undertaken with Professor Ian Craft at the Royal Free Hospital in London (1976–1980). This thesis and more than 200 other refereed publications from the early years can be found online in Research Gate, the scientific online network. He established PIVET Medical Centre in his hometown of Perth in 1981, the first private independent fertility management facility in Australia.

Abstract To examine the effect of cryopreservation on developmental potential of human embryos, this study compared quantitative β-HCG concentrations at pregnancy test after IVF-fresh embryo transfer (IVF-ET) with those arising after frozen embryo transfer (FET). It also tracked outcomes of singleton pregnancies resulting from single-embryo transfers that resulted in singleton live births ($n = 869$; with 417 derived from IVF-ET and 452 from FET). The initial serum β-HCG concentration indicating successful implantation was measured along with the birthweight of the ensuing infants. With testing at equivalent luteal phase lengths, the median pregnancy test β-HCG was significantly higher following FET compared with fresh IVF-ET (844.5 IU/l versus 369 IU/l; $P < 0.001$). Despite no significant difference in the average period of gestation (38 weeks 5 days for both groups), the mean birthweight of infants born following FET was significantly heavier by 161 g (3370 g versus 3209 g; $P < 0.001$). Furthermore, more infants exceeded 4000 g ($P < 0.001$) for FET although there was no significant difference for the macrosomic category (≥4500 g). We concluded that FET programme embryos lead to infants with equivalent (if not better) developmental potential compared with IVF-ET, demonstrated by higher pregnancy β-HCG concentrations and ensuing birthweights.

KEYWORDS: β-HCG, birthweight, FET, IVF-ET, single embryo transfer, vitrification

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Introduction

Cryopreservation might compromise the cleavage-stage embryo or the trophoblast, thus affecting the ability of the embryo to implant. Any negative effect of frozen embryo transfer (FET) may be due to apoptotic damage to embryos as a result of cryopreservation, the thawing process, or both (Li et al., 2012). However, we have an optimistic view about vitrification (Vajta et al., 2009), and this is in agreement with a recent publication (Sites et al., 2015) that found that vitrification (rather than slow-freezing) had no negative effect on the initial beta-human chorionic gonadotrophin (β-HCG) concentration or developmental potential of embryos cryopreserved.

It is well known that β-HCG plays important roles in the success of implantation and establishment of early pregnancy. Its role in embryo implantation may be exerted through its corresponding receptors on the endometrium. It also stimulates adenylate cyclase and production of progesterone through action on its receptors in trophoblast cells. Additionally, β-HCG induces relaxin secretion by the corpus luteum during the luteal phase and in early pregnancy. Both the relaxin and progesterone produced are important in the maintenance of early pregnancy (Keay et al., 2004). The serum β-HCG concentration could therefore be a good indicator of how successful a pregnancy is going to be, as shown in our earlier report (Lingam and Yovich, 2007).

PIVET Medical Centre has been using the vitrification process since late 2007 (Kuwayama et al., 2005). Anecdotal evidence suggested that the β-HCG pregnancy test in patients receiving FET appeared to be higher than those receiving fresh IVF-embryo transfer (IVF-ET). This raised the question of whether FET patients will continue to have a better outcome than those with fresh transfer, especially with regards to the birthweight. This study was carried out to address this observation.

Materials and methods

Patient selection and embryology

The data for this retrospective report was extracted from the database at PIVET Medical Centre (1 April 2008 until 30 April 2014 inclusive). All single-embryo transfer (SET) procedures following IVF-ET were analysed and their pregnancy outcomes compared with those of single cryopreserved embryo transfer procedures using vitrified-only embryos. No treatment cycles were excluded due to age or patient history, but those cycles utilizing donor oocytes were excluded because of the mixed component of cryopreserved oocyte followed by fresh embryo transfer. Women who were found to carry twins after the SET were invariably monozygotic and were excluded from the study for a possible confounding effect (**Figure 1**). Most embryo transfers at PIVET were either day 3 or day 5 (blastocyst) embryos. Day 3 embryos were graded based on PIVET's clinical protocol, which has been simplified from an earlier version (Yovich and Grudzinskas, 1990). Day 5 embryos were graded using the Gardner blastocyst grading system (Gardner and Schoolcraft, 1999). Embryos graded BC or CB or less would not be cryopreserved. Yovich et al., 2015a has reclassified these into specific groups based on the implantation rates as well as live birth rate.

SET cycles, in keeping with Australian standards (Macaldowie et al., 2015), were selected to avoid any bias in the interpretation of the pregnancy test β-HCG arising from other embryos even if such failed to implant. In addition, analysis was performed only on those with the outcome of singleton live births, hence excluding biochemical (non-continuing) pregnancies, miscarriages or blighted ova, and ectopic pregnancies as well as terminations and stillbirths. No cases included embryos screened for pre-implantation genetic diagnoses so would not be likely to cause bias towards higher implantation rates and better quality pregnancies (**Figure 1**). Pregnant patients who were lost to follow-up were tabulated as "no known outcome" and were also excluded despite having a clinical pregnancy diagnosed at 7 weeks' gestation.

Embryos not transferred during a fresh cycle were cryopreserved by vitrification using the Cryotop method (Kuwayama et al., 2005; Seet et al., 2012), mostly at the blastocyst stage following culture in Sydney IVF blastocyst medium (Cook Medical) applying sequential phases for fertilization, cleavage and blastocyst stages. Follicle stimulation, oocyte recovery, transfer and cryopreservation as well as embryo culture systems have been fully described elsewhere (Stanger et al., 2012; Yovich et al., 2012; Yovich and Stanger, 2010; Yovich et al., 2015b).

Ovarian stimulation for IVF cycles

Patients were stimulated with long down-regulation, flare cycle or antagonist protocols (Yovich et al., 2012; Yovich and Stanger, 2010). The selection of the stimulation protocol was at clinician's discretion, but the antagonist regimen was usually used for younger women with higher antral follicular count (AFC), and the flare regimen for older women with low AFC.

Ovulation triggering and luteal support for IVF

Ovulation triggering was usually initiated with a single dose of recombinant HCG (rHCG; Ovidrel: Merck Serono), two ampoules equating to approximately 13,000 IU rHCG, when there were at least two leading follicles ≥18 mm in diameter. For patients with fewer than four follicles or a previous poor recovery, three ampoules (Ovidrel) approximating to 19,500 IU rHCG, was used as the trigger. In those antagonist cycles with excessive follicle recruitment (>12 follicles over 12 mm), gonadotrophin-releasing hormone agonist (Lucrin: Abbott) trigger 50 IU was used. Oocyte recovery was at 35–37 h post trigger. IVF-ET luteal support was based on the number of oocytes recovered (Yovich et al., 2012), involving rHCG injections (where oocyte numbers were ≤12). Pregnyl 1500 IU s.c. was administered on days 6, 9, 12, and 15 after trigger with or without progesterone pessaries (Wembley Pharmacy compounded for PIVET).