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EDITORIAL

The first clinical nuclear transplantation in China: new information about a case reported to ASRM in 2003

Before there was public debate on mitochondrial replacement therapy (MRT) to treat mothers at risk of transmitting mitochondrial disease (Hyslop et al., 2016), and before there was a publicly traded company called OvaScience aiming to use nuclear transplantation and stem-cell technologies to treat infertility (Woods and Tilly, 2015), there was a solitary case report from China about the use of nuclear transplantation with donor oocyte-derived cytoplasm at the zygote stage to overcome cleavage arrest in a patient's embryos (Zhang et al., 2003). The landmark abstract, presented to the 2003 annual meeting of the American Society for Reproductive Medicine (ASRM), was received with excitement and concern, sentiments that promptly seeped into the lay press. This was not surprising considering the topic and the risk-sensitive audience of reproductive scientists listening to the presentation given by the lead author, Dr John Zhang.

The case report described an IVF patient with normal fertilization whose zygotes divided only once. Early development arrest affected all the embryos of this patient in two consecutive cycles. Approximately 10% of cleaving embryos arrest in culture, but the repeated arrest of an entire cohort is indeed rare, and, based on experimental evidence, could be attributable to ooplasmic deficits. Ooplasmic developmental determinants are far from understood, but one possibility is the deregulation of proteins like the stress sensor p66Shc, which may affect signaling pathways for mitochondrial function (Betts and Madan, 2008). Complete developmental arrest represents an extreme manifestation of ooplasm-associated deficits that can occur during human assisted reproduction treatment cycles. The idea that ooplasmic deficits could be overcome by cytoplasmic replacement or augmentation using donor oocytes or embryos is based on work performed nearly three decades ago by Pratt and Muggleton-Harris (1988) in the mouse. These authors injected 8 pl of cytoplasm extracted from normally dividing 2-cell mouse embryos into single blastomeres of arrested 2-cell recipient embryos. Using various combinations of blocking and non-blocking mouse strains, 2-cell embryos were injected with the aid of a membrane relaxant in order to avoid lysis. The authors' aim was to study cell-cycle dependent blockage. They found that only 4% of the total donor blastomere cytoplasmic volume was sufficient to overcome the block. The ability of a small volume of presumably normal cytoplasm to change cell cycle events in early embryos became the basis for cytoplasmic transfer to treat oocytes of patients with multiple cycles of abnormal embryo development, using ooplasm from donor eggs during ICSI (Cohen et al., 1998). A similar approach has been recently advocated using autologous transfer of mitochondria derived from egg precursor cells (AUGMENT™, OvaScience), which morphologically appear to resemble ooplasmic mitochondria (Woods and Tilly, 2015).

In a follow-up paper by Zhang and coworkers in this issue of *RBMOnline* (Zhang et al., 2016) the case performed in China in 2003 is presented in more detail. Why the 13-year hiatus between presentation of the abstract at a meeting and publication of a full paper? Perhaps the authors were discouraged by the tragic loss of the resulting triplet pregnancy and the criticism from colleagues and ethicists. Whatever the circumstances, the current paper describes the reconstitution protocol and the DNA analyses which showed that the fetuses had mitochondrial DNA from the oocyte donor and nuclear DNA from the mother. Unfortunately, other details are missing: no micrographs are provided of the nuclear transplantation procedure and there is essentially no data or micrographs from the two unsuccessful treatment attempts that led the authors to apply nuclear transplantation in the first place. None of the patient's zygotes were kept in culture as a control in parallel with the reconstituted zygotes in order to document repeated early arrest.

Other questions remain unanswered about this case. What was the review and ethics approval process at Sun-Yat Sen University where this experiment was conducted? Was the demise of one fetus following premature rupture of membranes at 24 weeks of gestation, and cord prolapse and demise

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of the last fetus at 29 weeks, related to the manipulations or to the clinical management of this high-risk pregnancy? Could the procedure be considered successful from a technical standpoint since the majority of the reconstructed zygotes developed into apparently normal diploid embryos? This was indeed an important, if limited finding, considering the developmental history. Are there details that may shed more light on the technique itself and on whether this protocol differs significantly from those used by teams advocating MRT in more recent studies (Hyslop et al., 2016; Tachibana et al., 2013)?

These and other questions should add to the ongoing debate and discussions in the literature and among legislative and regulatory bodies such as the UK parliament, UK Human Fertilisation and Embryology Authority (HFEA) and US Food and Drug Administration (FDA) on the application of nuclear transplantation technology for the purpose of preventing the transfer of mitochondrial disease (Cohen et al., 2015; Schandera and Mackey, 2016). The 2003 nuclear transfer procedure seems to be very similar to those proposed for MRT. Is this experimental approach suitable for non-mitochondrial disease applications? The recent Institute of Medicine (IOM) report distinguishes between mitochondrial replacement therapy and cytoplasmic replacement for overcoming infertility (National Academies of Sciences, Engineering, and Medicine, 2016). The HFEA seems to take a similar position, inferring that cytoplasmic replacement should not be used for treatment of infertility (Human Fertilisation and Embryology Authority, 2015). It seems that the ethical debate has focused on the risk factors. These were not discussed in depth in the current paper.

Is it morally acceptable to carry out experimental procedures such as nuclear transplantation or spindle transfer to treat infertility patients? Medical ethics involve the presentation of a moral standard to the practice of medicine. This obviously implies the responsibility to 'do no harm', but also to avoid increased risk, in both routine and experimental treatments. This serious responsibility must always be balanced with the valid search for treatment options providing significant potential benefit even in the case of non-life threatening human disease like infertility. However, this could also be argued in relation to MRT for mitochondrial disease, as the egg, embryo and fetus do not exist prior to therapy and in some cases there are alternatives like preimplantation genetic diagnosis (PGD), egg donation or adoption, which could be considered. The suggestion that reducing the risk of transmitting mitochondrial disease is not the moral equivalent of overcoming infertility and that the latter requires a different decision-making process or legal framework would benefit from further scrutiny. A parallel can be drawn with PGD where testing for genetic mutations in embryos has been considered by many reproductive specialists as different to, and somehow more justified than, testing for numeric chromosome anomalies. With reduced error and diagnosis-failure rates, both applications seem to be more acceptable now and the delineation between the two testing types is becoming blurred. The same may be on the horizon for nuclear or cytoplasmic transfer.

Biotechnology, pharmacology and genetic diagnosis are rapidly growing areas in China, but the presentation of guidelines regarding ethics and policy debates may be perceived as not exhaustive by Western standards. This is not always justified (Sipp and Pei, 2016). Modification of the embryonic genome may be allowed, but Chinese national guidelines have prohibited the transfer of such embryos since 2003. There is no national forum to which clinical experiments may be submitted for consideration, so examination of such proposals remains the purview of local hospital ethics boards. Although this is not unlike the familiar internal review board (IRB) process, in the USA, for instance, other governmental (e.g., the FDA) and non-governmental (e.g., NAS) forums representing the public interest and including professionals in diverse fields are also involved, debating the ethics of procedures such as cytoplasmic transfer, gene therapy, xenotransplantation and MRT. Observers in the West may wonder whether the clinical research climate in China encourages experimental approaches such as stem-cell therapies, nuclear transplantation, and other developing technologies without thorough discussion, but it is likely that such deliberations do take place but are screened from public view. In that context, it is not surprising that the first clinical applications of CRISPR-based technology for genome editing are reported by Chinese teams employing the process in abnormal dispermic human zygotes (Kang et al., 2016; Liang et al., 2015). The review process in 2003 of the case presented in this issue of RBMO may have been comparable with the ethics approval process in the West in the 1970s and 1980s. Views regarding fertilization and the status of the human embryo seem less complicated in China than they are in the West. In China, life is seen as a continuum, whereas in the West, many argue that conception represents a sacred moment (life and its sanctity begin at conception). Perhaps the zygote nuclear transplantation described by Zhang et al. should be viewed from this perspective, although renewed criticism is likely to follow given the experimental nature of this case, missing data, and the prolonged period between initial announcement of the work and publication of this paper.

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Jacques Cohen
E-mail address: jc@embryos.net

Henry Malter
E-mail address: hemalter@gmail.com