Cryopreservation of the human female gamete: current and future issues

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OPINION

The potential advantages associated with the ability to freeze and store human oocytes successfully have been well established for some time. Not only would it allow the circumvention of moral, ethical and legal problems which arise as an inevitable consequence of embryo freezing, but also it would offer the prospect of broadening the fertility options available to women who, for a variety of medical reasons, are likely to lose ovarian function prematurely. Banks of frozen donated oocytes would facilitate the donation process, which is often complicated by a requirement for donor–recipient synchrony. More controversially, oocyte storage would also open the door to the possibility of women, with no medical indications and no immediate plans to conceive, being able to store ‘young eggs’ for potential use at a later date.

Technically, the possibility of offering oocyte cryopreservation as a routine procedure had seemed somewhat remote after failure to reproduce early reports of success with a dimethylsulphoxide-based method in the 1980s (Chen, 1986; Al-Hasani et al., 1987; Van Uem et al., 1987). The difficulties encountered in achieving success with human mature (metaphase II) oocytes were postulated to arise mainly from inherent problems associated with the susceptibility of the mammalian spindle to freezing induced damage. Such problems were highlighted by reports of elevated rates of post-thaw aneuploidy in cryopreserved mouse oocytes (Kola et al., 1988). However, work by our group using propanediol and sucrose as cryoprotectants in conjunction with a slow freeze/rapid thaw method based on that successfully used for post-fertilization stages in the human, suggested that this pessimism was not justified. We showed that oocytes could survive cryopreservation without compromising spindle integrity (Gook et al., 1993), that post-thaw fertilization was characterized by normal karyotypes and an absence of stray chromosomes (Gook et al., 1994), and that frozen/thawed oocytes fertilized by intracytoplasmic sperm injection (ICSI) could undergo normal preimplantation development (Gook et al., 1995). This validation of the feasibility of the method culminated in the first reported live birth (Porcu et al., 1997). Subsequent reports of series of pregnancies and live births (Porcu et al., 1998; Porcu et al., 1998; Young et al., 1998) have confirmed that mature oocyte cryopreservation is now a viable option in appropriate circumstances. Cautious optimism for the future of the clinical application of this technology has been expressed (Tucker et al., 1998a); these authors have also demonstrated that cryopreservation of human immature oocytes with subsequent in-vitro maturation can be compatible with fertilization, pregnancy and live birth (Tucker et al., 1998b).

Two important issues, however, remain to be resolved before the role of this technology in current practice can be fully elucidated. Firstly, it would be preferable if continuing research could determine whether modifications to the above method could improve outcome. A tendency to adopt techniques widely when they show promise often occurs at the expense of further refinement. For example, a recent study has suggested that optimal survival of human oocytes may be achieved at an ice-seeding temperature which is higher than that used in current clinical oocyte cryopreservation protocols (Trad et al., 1998).

This is particularly important when we consider the second issue, which concerns the relative efficiency of oocyte and embryo freezing. If we are to consider mature oocyte freezing as an alternative to embryo freezing then we need to be aware of the likely return from the available material in each case. In other words we need to ask ‘How many implantations can we expect per 100 fresh oocytes available?’. Figures from our own unit suggest that ~90% of collected oocytes may be expected to be mature, that ICSI would be expected to result in normal (dipronucleate) fertilization in ~60% of injected mature oocytes, and that ~90% of early cleavage stage (day 2) embryos generated would be considered suitable for cryopreservation. This means that just under 50 embryos would be frozen per 100 oocytes collected in a unit where embryo utilization/cryopreservation rates are high. Our post-thaw results suggest that, although only 15% of all embryos frozen fail to survive cryopreservation, only 55% of frozen embryos will survive with all blastomeres intact after thawing. The percentage of fully intact thawed embryos which develop to the fetal heart (FH) stage following transfer in our unit is ~12%, a figure which is almost double the value for embryos which have suffered blastomere loss as a result of cryopreservation. Taking all the above into account would result in an estimated four FHs per 100 collected oocytes subjected to the process of in-vitro fertilization (IVF) and embryo cryopreservation. While a more selective approach to the choice of embryos for cryopreservation and for post-thaw transfer may increase the apparent implantation rate, the overall attrition...
rate would remain unaltered. Although the application of mature oocyte cryopreservation is at an early stage, it would not be unreasonable to expect that technical refinement and more widespread adoption may result in a similar return. Early clinical results (Borini et al., 1998; Porcu et al., 1998) suggest that this may be so although, for comparisons to be valid, all stages at which attrition occurs (i.e. pre- and post-freeze) must be included in the calculations for both processes.

Even if, however, the technique does not fully match the expectations from embryo cryopreservation it will be an important option in cases where moral, ethical, religious and even psychological issues are considered to be paramount. We cannot accurately estimate the psychological effects on individuals or couples of having surplus embryos in storage. In many cases, disposal of such embryos may be a legal requirement following an elapsed period of time thereby providing an additional dilemma. In addition, we may expect that the demand for oocyte freezing will grow in response to the trend towards deferring conception until later in the female reproductive life.

It is for another group of patients, however, that the existing technology and its future development is of crucial importance. The ability to detect many cancers at relatively early stages and the use of aggressive cytotoxic therapy have greatly improved the long term survival prospects for many patients. Among them are young women who will be rendered infertile by the loss of ovarian function which is a consequence of the therapy. Many of these women will wish to explore the option of preserving their gametes prior to therapy for potential use at a later date. In such circumstances, although mature oocyte cryopreservation would seem to offer some hope, the available time before commencing cytotoxic treatment often precludes the administration of ovarian stimulation and therefore the hope of harvesting multiple oocytes for storage. An alternative approach involves freezing ovarian tissue which contains multiple immature oocytes contained within predominantly primordial follicles. This approach has been employed with some success in other species (Gosden et al., 1994; Candy et al., 1995; Gunasena et al., 1997) and preliminary data on human tissue (Zhang et al., 1995; Newton et al., 1996; Hovatta et al., 1997; Oktay et al., 1997) suggest that it may offer a clinical option in the future. However, there are specific prerequisites before application of this technology that can be considered with any degree of confidence.

Firstly, our initial work with propanediol/sucrose (Gook et al., 1999) suggests that it is important to establish a cryopreservation regimen which allows survival of not only the oocyte but also the associated somatic cell compartments of the tissue. This will be of crucial importance in determining the resumption of follicle growth and eventual oocyte maturation. These developmental stages may, of course, take place in vitro or in vivo following thawing of the tissue. Considering the fact that much of the stored tissue is likely to be derived from patients with malignant disease, an earlier report (Shaw et al., 1996), which demonstrated the transmission of malignant cells following transplantation of mouse ovarian tissue, points to a potential problem with the latter option in many, if not all, cases. This highlights the second important factor in applying the technology, the need to develop in-vitro methods for human primordial follicle development and oocyte maturation which result in oocytes which are suitable for IVF and can demonstrate subsequent developmental competence. This poses a significant challenge both at present and in the future. It has been stressed that hasty clinical application of this technology should be discouraged until the outcome of ongoing clinical research trials is known (Gosden et al., 1999).

Storage of human oocytes, either in the mature state or as immature oocytes in follicles within ovarian tissue, will inevitably be subject to similar considerations as those applying to sperm storage. For example, the question of whether different guidelines should apply to donated material or material intended for an individual’s own use has been raised. In the state of Victoria in Australia, legislation allows storage of gametes for a maximum period of 10 years although extensions may be granted by the statutory body in response to applications. Clinics and legislators will also be faced with decisions on what action to take if a patient dies with oocytes/ovarian tissue in storage, and whether such material can be used to form embryos for transfer to a surrogate uterus. As the technology moves forward the need to reach a consensus on the above issues will become more urgent.

References


