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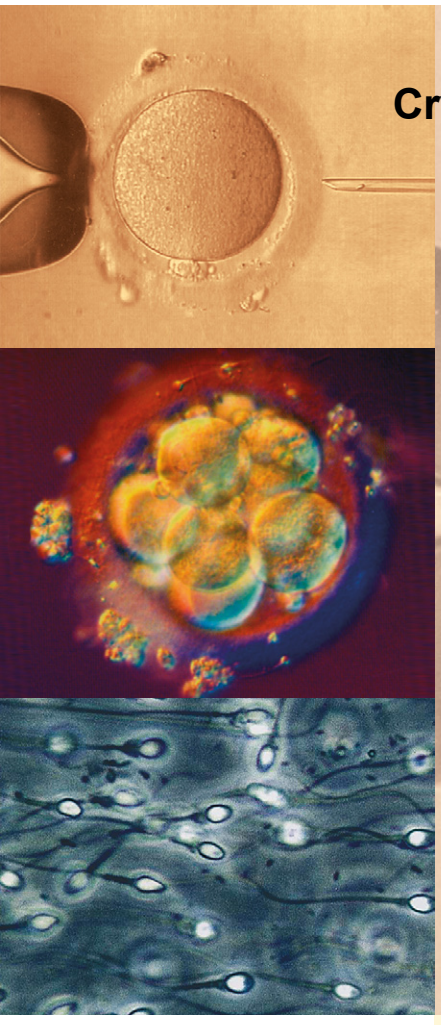

Cryopreservation of Unfertilized and Pronuclear Human Oocytes

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Cryopreservation of Unfertilized and Pronuclear Human Oocytes

M. Kalff-Suske

Unlike the cryopreservation of male gametes, cryopreserved female gametes have found little entry into clinical reproductive practice. Although potential applications in the preservation of fertility and treatment of infertility are numerous, the efficacy of the oocyte freezing process is still low due to specific properties of mature oocytes. The freezing of earlier stages has been hampered in the past by difficulties in the in vitro maturation of germinal vesicle oocytes.

In contrast, cryopreservation of fertilized oocytes at the pronuclear stage and of embryos has become a routine procedure in human assisted reproduction. The use of controlled ovarian stimulation protocols initiates the maturation of large numbers of oocytes, of whom more may become fertilized than medically reasonable and/or legally permitted to be transferred into the uterine cavity for implantation. In case of surplus fertilization, cryopreservation provides a means of achieving pregnancies beyond the initial cycle (using fresh embryos) without recurring to another oocyte retrieval. ***J Reproduktionsmed Endokrinol 2007; 4 (2): 92–3.***

Key words: *oocyte, pronuclear stage, embryo, cryopreservation, assisted reproduction*

Cryopreservation of Oocytes at the Pronuclear Stage

In view of achieving pregnancies, human assisted reproduction relies heavily on controlled ovarian stimulation protocols for the maturation of sufficient numbers of oocytes. Consequently, stimulated in vitro fertilization (IVF) cycles often yield more zygotes and/or embryos than intended for later embryo transfer. Single embryo transfer is desirable in order to reduce the risk of multiples but in practice, two or more embryos are often transferred [1]. Surplus fertilized oocytes are suitable for cryopreservation (CP) either at the pronuclear stage with still separate female and male genomes or at different embryo stages.

Embryo CP has become common practice in many IVF clinics to augment the success of assisted conception treatment for infertile couples. However, in certain countries, embryo selection is prohibited by law. In Germany and Switzerland, no more than 3 zygotes are to be developed to cleavage stage embryos for later transfer and are consequently not available for CP at these stages. According to the German Embryo Protection Act, however, surplus zygotes may be frozen at the pronuclear stage in view of a subsequent homologous embryo transfer.

Both, CP of zygotes and embryos, unquestionably contribute to raise the cumulative ongoing pregnancy rates though success rates reported in the literature vary considerably [2]. Additional benefits include the possibility of avoiding waste of fertilized oocytes/embryos in case transfer is not possible for medical reasons in the fresh cycle. Transfer of fertilized oocytes following the freezing-thawing procedure does not involve the risk of ovarian hyperstimulation due to the absence of or only mild stimulation in comparatively low-cost cryocycles. The variables controlling the success of cryopreservation programs in human assisted reproduction include (i) the incidence of cycles of CP in the IVF program, (ii) the criteria for selection of fertilized oocytes and/or embryos, (iii) the result of fresh embryo transfers and (iv) the efficacy of the freezing method. CP programs are then determined both by components intrinsic to the centre offering them like patient collective, choice of stimulation and freezing protocols as well as by external components such as national legislation. The legal restriction of CP to a certain stage excludes concerned centers from the debate about the best stage for freezing [3, 4]. The evaluation of stage-specific criteria may be particularly beneficial to obtain higher pregnancy rates. In Germany, this holds for the morphological characterization of pronuclear stages in terms of number and distribution of nucleolar precursor bodies to determine the fertilized oocytes for the fresh transfer [5]. Similarly, consideration of pronuclear scoring data for oocytes undergoing cryopreservation is likely to raise cumulative pregnancy rates.

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Cryopreservation of Unfertilized Oocytes

Despite its potential beneficial application, cryopreservation of unfertilized oocytes is far less widespread compared with CP of fertilized oocytes/embryos. Italy, where freezing of post-fertilization stages is no longer allowed by the new legislation, presents an exception. Adaptations in the slow-freezing protocols have raised the oocyte post-thaw survival as well as fertilization and embryo cleavage rates [6].

With the recent use of in vitro maturation protocols, immature oocytes may progress in culture to metaphase II (MII) oocytes [7]. Fertilization of frozen-thawed in vitro matured oocytes and their transfer in later cycles resulting in live-births has been reported [8]. Until further improvement is achieved, however, deleterious effects noted on the organization of the meiotic spindle and chromosome alignment of human oocytes upon in vitro maturation still disfavor the routine use of cryopreservation of matured oocytes in human assisted reproduction [9].

The availability of frozen oocytes, however, would eliminate donor-recipient synchronisation problems in assisted reproduction. They occur if the male partner is unable to produce sperm or male gametes cannot be found in the available testis material at the time of oocyte retrieval. Generally, the technique of freezing unfertilized

MII oocytes opens the possibility of establishing "egg banks", thus offering potential benefits to various groups. In countries with oocyte donation programs, the majority of women involved would very clearly benefit. By the same token, oocyte CP would enable women to postpone reproduction until later in life – for whatever personal reasons. In addition, oocyte freezing may present an alternative in case of ethical objections to embryo freezing.

There are few options available to women to preserve fertility before undergoing cancer treatment or facing loss of ovarian function. In addition to cryopreservation of ovarian tissue [10, 11], cryostorage of oocytes obtained after ovarian stimulation or retrieved during tissue dissection may present yet another possibility to preserve female fertility prior to chemotherapy and/or radiotherapy protocols.

The reluctance of including oocyte freezing techniques in IVF programs may eventually be overcome by the introduction of improved freezing protocols yielding reproducibly high viability scores after thawing.

Technical Considerations Regarding Cryopreservation of Oocytes, Zygotes and Embryos

The basic principles of cellular cryobiology reviewed in previous contributions [12] apply to the freezing of unfertilized and fertilized oocytes as well as embryos. Protocols have been designed to meet specific requirements of a wide variety of reproductive cells in order to achieve satisfactory viability rates following the freeze-thaw procedure [13, 14]. Advances in the cryopreservation of animal gametes and embryos have frequently been adapted to the human species. The traditional method of slow freezing referred to as "equilibrium cooling" with controlled ice crystal formation (by seeding close to the eutectic point prior to slow cooling to –30 °C/–40 °C) in the presence of low concentrations of cryoprotective agents, pioneered by Lassalle and coworkers for embryo freezing, is widely used [15]. Differences in permeability to cryoprotective agents are taken into account. The membrane-permeating 1,2 propanediol for zygotes and cleavage stage embryos and glycerol for blastocyst stage embryos each, in the presence of stepwise elevated concentrations of osmotically active membrane non-permeating sucrose, are used to avoid lethal intracellular ice formation due to refractive dehydration. The success of the method lies in its simplicity, reproducibility and the high rate of cellular integrity of both pronuclear stage oocytes and embryos compatible with embryo development and acceptable pregnancy rates.

More recently, the focus of interest, especially with the cryopreservation of human oocytes and blastocysts has been the vitrification or "rapid non-equilibrium cooling" method. Biological material exposed to high concentrations of cryoprotectants will transit into a glassy solid state without ice crystal formation immediately upon fast cooling together with the extracellular fraction. This is thought to alleviate some of the inherent problems of oocyte CP. The human oocyte is a large cell with a low surface-to-volume ratio – properties adverse to the dehydration process. In the MII oocyte, chromosomes are aligned across the metaphase plate of the temperature-sensitive meiotic spindle which makes them prone to injury. Vitrification methods are being modified such as to satisfy sterility demands [16].

The European Directive 2004/23/EC is intended to warrant a minimum standard of quality for the donation and long-term storage of different types of reproductive cells among member states. In addition to technical considerations, the entry of cryopreserved gametes, zygotes and embryos into therapeutic concepts in reproductive medicine, however, requires the debate about the legal, moral and ethical issues associated with their use and long-term storage.

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