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Clinical Efficiency of Human Oocyte Cryopreservation

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Cryopreservation of human female gametes may represent a concrete solution to several problems in Assisted Reproduction.

It might be an alternative to embryo cryopreservation, which probably presents less technical problems but much more ethical implications. In addition, it may be a precious tool which permits to preserve women fertility in case of ovarian damage (Porcu et al., 2004).

Iatrogenic sterility which follows chemo- or radiotherapy in neoplastic patients and pathologies of the reproductive system (premature ovarian failure, endometriosis, cysts and pelvic infections) can be considered the main indications for preventive human oocytes cryopreservation.

Oocyte storage may contribute to increase IVF routine flexibility. Unexpected failure to produce sperm or to retrieve testicular spermatozoa are events that may find in egg freezing a possible rescue of that cycle of therapy. In particular cases, cryopreservation of both male and female gametes makes it possible to do IVF in the physical absence of patients.

The risk of developing ovarian hyperstimulation syndrome may also benefit from egg storage with insemination and embryo transfer postponement. This strategy avoid the production and cryopreservation of the huge number of embryos frequently present in these patients.

In addition, oocyte cryopreservation can be included in programs of oocyte donation and the creation of donor oocyte cryo-banks could reduce costs and improve convenience of these treatments.

Finally, a possible application of egg storage for family planning has been proposed. According to this hypothesis, young women wishing to defer reproduction because of work or personal reasons, would benefit of oocyte cryopreservation stopping the aging process of a pool of gametes which might become a sort of reproductive insurance for the future.

The first pregnancy with human frozen oocytes was reported by Chen in 1986. The years after two additional pregnancies were reported (Van Uem 1987; Chen 1988).

However, in the subsequent ten years, only a handful of pregnancies and no births were reported. Oocytes cryopreservation were then considered a low efficiency technique.

The enthusiasm for the announcement of the first pregnancies in the eighties was not followed by a rapid incorporation of the technique in the IVF clinical routine. Human oocyte cryopreservation is still relegated in the research area and is regarded as an experimental technique while other procedures underwent a rapid and early incorporation into the clinical routine even before the

unequivocal evidence of their safety.

The failure to reproduce the early clinical success occurred in the eighties with the first pregnancies led a large part of researchers to consider human egg freezing as an inefficient and unreliable procedure. A reason for these poor results may be found in the generally low number of oocytes used in the experiments and in the bad quality of the eggs, mainly excess, discharged, aged oocytes responsible for the low survival, low fertilization and cleavage.

Survival rate seems to depend on the quality of frozen oocytes. Chen (1986) reported survival rate of 76% with mature oocytes of very good quality, while a lower survival rate was obtained by Al Hasani (1987) in excess oocytes, usually of poor quality and immature.

Fertilization rate of cryopreserved oocytes with IVF is extremely variable ranging from 13% (Kazem 1995) to 71% (Chen 1986). However, in most studies, variability is between 30 and 55% being on average lower than fertilization rate obtained with fresh oocytes.

Intracytoplasmic sperm injection (ICSI) has been proposed (Kazem 1995; Gook 1995; Tucker 1996) as a solution to possible damage of the zona pellucida and of the cortical granules.

After a preliminary experience with IVF of frozen oocytes which gave 46% of fertilization rate, also our Center undertook a study associating ICSI and oocytes cryopreservation which resulted in a normal fertilization rate ranges above 70%. Our abnormal fertilization rate (7%) resembles that found in IVF and ICSI of fresh oocytes. Most embryos are of good quality and cleave regularly.

With the introduction of ICSI, the results in terms of fertilization and embryo cleavage greatly improved. In 1997 we reported the first birth of a healthy female from intracytoplasmic sperm injection of oocytes frozen with propanediol (Porcu 1997) followed by several other pregnancies (Porcu 1998, 1999a-b; 2000 a-b; 2001 a-b). Shortly after several Authors shared the same experience with good results (Polak de Fried 1998; Young 1998; Nawroth 1998; Antinori 1998; Yang 1998; Fosas 2003; Borini 2004; Chen 2005). Interestingly, in Chen (2005) experience, the same pregnancy rate could be obtained by both cryopreserved eggs and embryos.

Technical variations such as the use of low sodium content medium resulted in 2 normal children reported by Quintans (2002) and 5 healthy infants published by Boldt (2003).

The first birth after cryopreservation of immature human oocytes has been announced in 1998 by Tucker and prompt caution optimism despite concern raised by some studies about possible chromosomal abnormalities in immature frozen oocytes. Additional pregnancies with immature frozen oocytes have been published in the following years by Cha (2000).

The first birth with vitrified human eggs was announced in 1999 by Kuleshova. Other Authors chose that alternative. With vitrification Yoon (2003) obtained appreciable results: in 474 thawed oocytes, the survival rate was 68.6, the fertilization rate 71.4, the pregnancy rate was 21.4 and 7 healthy children were born. Good results were also published by Katayama (2003) and Kuwayama (2005).

The first births of normal children from frozen eggs inseminated with epididymal (Porcu 1999a) and

testicular (Porcu 1999 b) spermatozoa were published in 1999. The following year the birth of a child coinceived with frozen eggs and frozen sperms was announced (Porcu 2000 a). Recently the combination of both frozen eggs and frozen sperms either ejaculated, epididymal or testicular was successfully adopted by some authors (Azambuja 2005; Ching Ching Tjer 2005; Levi Setti 2005). Levi Setti (2005) published a case report of an extreme form of reproductive cryopreservation where the child were conceived with a frozen embryo deriving from frozen eggs and frozen testicular spermatozoa. These cases document that reproductive storage increase the flexibility of assisted reproduction routine and, even in the most complex combination, is apparently safe.

Finally, the application of oocyte cryopreservation to store the reproductive potential in cancer patients (Porcu 2004) appear promising.

In our opinion, oocytes cyopreservation is now improving its efficiency and consistency (Porcu 2005). Taking together the clinical results published in the past ten years, it possible to calculate an overall mean survival rate of about 67%. The birth rate per thawed oocyte is around 4%. No individual freezing methodology demonstrated to be superior so far.

In the past, the main concern was related to the possible damage to the meiotic spindle and the induction of aneuploidia. However, basic investigations are reassuring and show normal karyotypes and absence of stray cromosomes in cryopreserved oocytes.

The few children born twenty years ago after oocytes freezing were healthy. The children born after oocytes cryopreservation and ICSI in the past ten years are also healthy and normal.

Insemination of frozen eggs by ICSI has solved one of the major problems of oocyte freezing, that was the low fertilization rate. Survival and implantation rates should be further improved.

References

Al Hasani S, Diedrich K, Van der Ven, Reinecke A, Hartje M, Krebs D. Cryopreservation of human oocytes. Hum Reprod 1987;2:695-700.

Antinori S, Dani G, Selman HA, Vidali A, Antinori M, Cerusico C and Versaci C. Pregnancy after sperm injection into cryopreserved human oocytes. Hum. Reprod. 1998;13 (Abstract book 1) pp. 157-158

Azambuja, M Badalotti, C Teloken, J Michelon, A Petracco. Case report: Successful birth after injection of frozen human oocytes with frozen epididymal spermatozoa. Reprod BioMed Online 2005; 11, 4 pp 449-451.

Boldt J, Cline D, McLaughlin D. Human oocyte cryopreservation as an adjunct to IVF-embryo transfer cycles. Hum Reprod. 2003;18(6) pp.1250-5.

Borini A, MA Bonu, G Coticchio, V Bianchi, M Cattoli and C Flamigni. Pregnancies and births after oocyte cryopreservation. Fertil. Steril. 2004; 82, 3 pp 601-605.

Cha K.Y., H.M. Chung, J.M. Lim, J.J. Ko. S.Y. Han, D.H. Choi and T.K. Yoon. Freezing immature oocytes. Mol. Cell. Endocrinol. 2000; 169 pp 43-47.

Chen C. Pregnancies after human oocyte cryopreservation. Ann. N.Y. Acad. Sci 1987; 541: 541-549.

Chen C. Pregnancy after human oocyte cryopreservation. Lancet i 1986; 884-886.

Chen SU, YR Lien, HF Chen, LJ Chang, YY Tsai and YS Yang. Observational clinical follow up of oocyte cryopreservation using a slow- freezing method with 1,2-propanediol plus sucrose followed by ICSI. Hum. Reprod. 2005;20, 7 pp1975-1980.

Ching-Ching Tjer, T. Tak-Yu Chiu, Lai-Ping Cheung, I. H. Lok and C. J. Haines. Birth of a healthy baby after

transfer of blastocysts derived from cryopreserved human oocytes fertilized with frozen spermatozoa. Fertil. Steril. 2005; 83 pp1547.e1-e3.

Fosas N, Marina F, Torres PJ, Jove I, Martin P, Perez N, Arnedo N, Marina S. The births of five Spanish babies from cryopreserved donated oocytes. Hum Reprod. 2003;18(7) pp.1417-21.

Gook D, Schiewe MC, Osborn S, Asch RH, Jansen RPS, Johnston WIH. Intracytoplasmic sperm injection and embryo development of human oocytes cryopreserved using 1,2-propanediol. Hum Reprod 1995; 10: 2637-2641.

Katayama KP, Stehlik J, Kuwayama M, Kato O, Stehlik E. High survival rate of vitrified human oocytes results in clinical pregnancy. Fertil Steril. 2003; 80(1) pp. 223-4.

Kazem R, Thompson LA, Srikantharajah A, Laing M.A, Hamilton MPR, Templeton A. Cryopreservation of human oocytes and fertilization by two techniques: in-vitro fertilization and intracytoplasmic sperm injection. Hum. Reprod. 1995; 10: 2650-2654.

Kuleshova L, Gianaroli L, Magli C, Ferraretti A, Trounson A. Birth following vitrification of a small number of human oocytes: case report. Hum Reprod. 1999; 14(12) pp.3077-9.

Kuwayama, G. Vajta, O. Kato, s. Leibo. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod. BioMed. Online 2005; 11, 3 pp 300-308.

Levi Setti P, E. Albani, P. V. Novara, A. Cesana, S. Bianchi, L. Negri. Normal birth after transfer of cryopreserved human embryos generated by microinjection of cryopreserved testicular spermatozoa into cryopreserved human oocytes. Fertil. Steril. 2006; 83, 4 pp 1041.

Nawroth F, Kissing K. Pregnancy after intracytoplasmatic sperm injection (ICSI) of cryopreserved human oocytes. Acta Obstet Gynecol Scand 1998; 77: 462-463.

Polak De Fried E, Notrica J, Rubinstein M, Marazzi A, Gomez Gonzalez M. Pregnancy after human donor oocyte cryopreservation and thawing in association with intracytoplasmic sperm injection in patient with ovarian failure. Fertil Steril 1998; 69: 555-557.

Porcu E. Oocyte freezing. Semin Reprod Med (2001b) 19 pp. 221-230.

Porcu E, Fabbri R, Damiano G, Fratto R, Giunchi S, Venturoli S. Oocyte cryopreservation in oncological patients. Eur J Obstet Gynecol Reprod Biol. 2004; Apr 5;113 Suppl 1:S14-6.

Porcu E, Fabbri R, Seracchioli R, Ciotti PM, Magrini O, Flamigni C. Birth of a healthy female after intracytoplasmic sperm injection of cryopreserved human oocytes. Fertil Steril 1997; 4: 724-26.

Porcu E. Cryopreservation of oocytes: indications, risks and outcome. Hum. Reprod. 2005; 20, Supplement 1 pp 50.

Porcu E., Fabbri R., Ciotti PM., Giunchi S., Fratto R., Caracciolo D. Four healthy children from frozen human oocytes and frozen human sperms. Fertil Steril 2001a; Vol. 76, N°3S, S76

Porcu E., Fabbri R., Seracchioli R., De Cesare R., Giunchi S., Caracciolo D. Obsterics, perinatal outcome and follow up of children concevied from cryopreserved oocytes. Fertil Steril 2000b; Vol.74, n.3S, Suppl.1 S48.

Porcu, E., Fabbri, R., Ciotti, P.M., Frau, F., De Cesare, R. and Venturoli, S. Oocytes or embryo storage?. Fertil. Steril. 2002; 169 (Suppl. 1) S15.

Porcu, E., Fabbri, R., Ciotti, P.M., Marsella, T., Balicchia, B., Damiano, G., Caracciolo, D., Giunchi, S., De Cesare, R. and Flamigni, C. Cycles of human oocyte cryopreservation and intracytoplasmic sperm injection: results of 112 cycles. Fertil. Steril.1999a; 72 (Suppl. 1) S2.

Porcu, E., Fabbri, R., Ciotti, P.M., Petracchi, S., Seracchioli, R. and Flamigni, C. Ongoing pregnancy after intracytoplasmic sperm injection of epididymal spermatozoa into cryopreserved human oocytes. J. Assist. Reprod. Genet. 1999c; 16 pp. 283-285.

Porcu, E., Fabbri, R., Damiano, G., Giunchi, S., Fratto, R., Ciotti, P.M., Venturoli, S. and Flamigni, C. Clinical experience and applications of oocyte cryopreservation. Mol. Cell. Endocrinol. 2000a; 169 pp. 33-37.

Porcu, E., Fabbri, R., Petracchi, S., Ciotti, P.M. and Flamigni, C. Ongoing pregnancy after intracytoplasmic sperm injection of testicular spermatozoa into cryopreserved human oocytes. Am. J. Obstet. Gynecol.1999b; 180 pp. 1044-1045.

Porcu, E., Fabbri, R., Seracchioli, R., Ciotti, P.M., Petracchi, S., Savelli, L., Ghi, T. and Flamigni, C. Birth of six healthy children after intracytoplasmic sperm injection of cryopreserved human oocytes. Hum. Reprod 1998; 13 pp 124.

Quintans CJ, Donaldson MJ, Bertolino MV, Pasqualini RS. Birth of two babies using oocytes that were cryopreserved in a choline-based freezing medium. Hum Reprod. 2002;17 (12) pp.3149-52.

Tucker M, Wright G, Morton P, Shanguo L, Massey J, Kort H. Preliminary experience with human oocyte cryopreservation using 1,2 propanediol and sucrose. Hum Reprod 1996; 11: 1513-1515.

Tucker MJ, Wright G, Morton PC, Massey JB. Bith after cryopreservation of immature oocytes with subsequent *in vitro* maturation. Fertil Steril 1998; 70: 578-579.

Van Uem JFHM, Siebzehnrubl E R, Schun B, Koch R, Trotnow S, Lang N. Birth after cryopreservation of unfertilized oocytes. Lancet i 1987;752-753.

Yang DS, Winslow KL, Blohm PL. Improved survival rate after cryopreservation of human fresh and aged unfertilized oocytes using a specially developed oocyte cryopreservation regime. Fertil Steril 1998; 70 (Suppl 1) S86. Abstract O-232.

Yoon TK, Kim TJ, Park SE, Hong SW, Ko JJ, Chung HM, Cha KY. Live births after vitrification of oocytes in a stimulated *in vitro* fertilization-embryo transfer program. Fertil Steril. 2003; 79(6) pp. 1323-6.

Young E, Kenny A, Puigdomenech E, Van Thillo G, Tiveron M, Piazza A. Triplet pregnancy after intracytoplasmic sperm injection of cryopreserved oocytes: case report. Fertil Steril. 1998; 70 (2) pp.360-1.