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ARTICLE

Analysis of oocyte cryopreservation in assisted reproduction: the Italian National Register data from 2005 to 2007

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Abstract This paper reports on oocyte cryopreservation efficacy in Italy with respect to successful IVF from 2005 to 2007, presenting data from 193 centres collected by the Italian National Register. Post-thawing survival rates, number of transferred embryos, implantation rates and clinical pregnancy rates per transfer with respect to frozen/vitrified oocytes (FVO) were analysed. These numbers were compared with those obtained using frozen embryos or fresh oocytes. A total of 121,708 cycles were initiated, of which, 7.1% (8682) were FVO cycles and 2.4% (2952) were frozen embryo cycles. Of the 81,786 FVO, 52.5% (42,917) were thawed and 26.9% (22,005) inseminated. Of those inseminated, 68.0% (14,966) yielded good embryos. These numbers were significantly lower than those using fresh oocytes in which 77.9% (197,242; fresh oocytes versus FVO $P < 0.001$) of inseminated oocytes generated good embryos. Implantation rate using FVO was 6.9%, which was significantly lower than that using fresh oocytes (13.5%; $P < 0.001$) and frozen embryos (8.8%; $P < 0.001$). Pregnancy rate per transfer using FVO was 12.5% and significantly lower than that using fresh oocytes (24.9%; $P < 0.001$) or frozen embryos (16.4%; $P < 0.001$). There were 505 deliveries after IVF with FVO and 582 babies.

KEYWORDS: national register, oocyte cryopreservation, slow freezing, vitrification

Introduction

Since zygote and embryo cryopreservation was banned by law in Italy in 2004, gamete cryopreservation became the only option for the storage of reproductive material. Freezing of human spermatozoa has been successfully performed for several decades. Conversely, oocyte cryopreservation has been quite slow to evolve due to several known physical characteristics of this cell. First, the human oocyte has a critical size characterized by a low surface-to-volume ratio which limits the penetration of water and cryoprotectants across the cell plasma membrane, thus making it difficult

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to protect from intracellular ice formation (Coticchio et al., 2004). Second, mature oocytes contain the spindle apparatus which is known to be susceptible to the deleterious effects of hypothermia (Chen et al., 2003). Finally, freezing and thawing processes are thought to alter the zona pellucida, impairing sperm penetration or attachment (Kazem et al., 1995). Development of oocyte freezing procedures is ongoing and the introduction of elevated dehydrating sucrose concentrations in the slow-cooling protocol increased survival and fertilization rates (Fabbri et al., 2001). More recently the application of vitrification, a super-rapid cooling technique using liquid nitrogen which prevents ice crystal formation, resulted in a promising protocol for oocyte cryopreservation (Liebermann et al., 2002). It has been proposed that vitrification may be less shocking to the meiotic spindle than slow freezing and may also have fewer consequences for cell functioning (Chen and Yang, 2009). However, in Italy, the slow-freezing protocol was by far the most used method to freeze oocytes, at least in the studied period. In order to evaluate the efficacy of oocyte cryopreservation in Italy, the data of 121,708 cycles collected from 193 IVF centres from 2005 to 2007 were analysed.

Materials and methods

Aggregate data concerning IVF procedures performed from 2005 to 2007 were collected starting from January 2006 until December 2008 from public and private clinics offering assisted reproduction procedures. Data were collected using www.iss.it/rpma, a resource set up by the assisted reproduction National Registry at the National Centre for Epidemiology, Surveillance and Health Promotion, Istituto Superiore di Sanità. Records were stored on a secure, password-protected server. Data sharing and data protection were evaluated and maintained by the registry.

Assisted reproduction clinics may use different protocols to cryopreserve (slow freezing or vitrification) and thaw/warm oocytes. No more than three embryos were generated after oocyte thawing/warming because this was the maximum number allowed by the Italian law. Records did not include a specific request to report the cryopreservation protocol. Only in 2007 was a new variable included in the question grid in order to record the freezing procedure (slow cooling or vitrification). Implantation rates were calculated by dividing the number of gestational sacs with the number of transferred embryos. Clinical pregnancy was defined, according to the International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) (Zegers-Hochschild et al., 2009), as the presence of at least a single gestational sac visualized on ultrasound or as the presence of definitive clinical signs of pregnancy. Ectopic pregnancy was included. Cumulative pregnancy rate (CPR) was calculated as the number of pregnancies from frozen/vitrified oocyte (FVO) cycles plus the number of pregnancies from fresh cycles per retrievals. However, it should be noted that all the cryopreserved eggs were not always thawed for successful treatment. To calculate CPR, only centres that had performed freeze–thaw oocyte cycles (98 clinics; 50.8% out of the total) were included. A live birth was defined as a viable infant born at 25 weeks of gestation or later. Any malformed baby was recorded and described in the paper.

Statistical analysis

Data were analysed with the Statistical Package for Social Sciences version 17.0 (SPSS, USA). Percentages of transferred embryos per inseminated oocytes, implantation rate, pregnancy rate, delivery rate, negative outcome per monitored pregnancy and malformed babies per live birth were calculated. These parameters were statistically compared between fresh and cryopreserved cycles using crude odds ratio (OR) of exposure to oocyte cryopreservation and 95% confidence intervals (CI).

Results

During the 3-year study period, 193 Italian assisted reproduction centres performed 121,708 cycles, of which 2.4% (2952) were frozen embryo cycles and 7.1% (8682) FVO cycles. The number of oocytes retrieved was 666,599, of which 12.3% (81,786) were cryopreserved (**Table 1**). Egg cryopreservation was performed by 54.0% of centres (105 clinics). Patients decided to cryopreserve oocytes as result of a surplus yield after stimulation or when they were at high risk of ovarian hyperstimulation syndrome. In 2007, the requirement to report on the cryopreservation methods was introduced. Based on these accounts, 81.0% (2426) of the cryopreservation cycles were performed using slow freezing–thawing and 19.0% (568) with vitrification–warming. In the same period, 42,917 oocytes were thawed of which 51.3% (22,005) yielded viable cells suitable for insemination (**Table 2**).

To evaluate cryopreservation efficacy, data regarding three crucial steps of the IVF procedure (number of embryos transferred, implantation and pregnancy rates) were analysed. Since the Italian National Register does not collect data on cryopreserved eggs successfully fertilized, these data could not be presented and the number of transferable embryos per number of inseminated oocytes are presented (**Table 3**). The results show that the percentage of transferable embryos per inseminated oocytes generated from fresh oocyte cycles was 9.9% higher than those obtained from FVO. Moreover, the probability of obtaining a transferable embryo using fresh oocytes or FVO was calculated as the OR (**Table 3**). Given an OR relative to FVO cycles of 1, the comparison yielded an OR of 1.65 (95% CI

Table 1 Numbers and percentages of cryopreserved oocytes with respect to the total retrievals from 2005 to 2007.

Year	*No. of oocytes retrieved*	*No. of frozen oocytes*	*Frozen oocytes (%)*
2005	209,236	25,489	12.2
2006	223,359	28,784	12.9
2007	234,004	27,513	11.8
Total	666,599	81,786	12.3