AFRICAN JOURNAL OF MEDICINE and medical sciences

Volume 36 Number 4 December 2007

ISSN 1116—4077

Afr. J. Med. Med. Sci. (2007) 36, 325-334

An evaluation of embryo, zygote and oocyte cryopreservation in assisted reproductive technology

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Summary

The study compared (i) the outcome between cryopreserved ICSI fertilized embryo and cryopreserved ICSI fertilized pronuclear stage zygotes and (ii) the outcome between cryopreserved fertilized mature oocytes with cryopreserved immature oocytes, fertilized by ICSI following thawing. Comparative retrospective review of relevant studies satisfying study criteria. Studies were identified through MEDLINE literature search. Study outcomes were cryosurvival, fertilization, implantation, pregnancy, and delivery rates. Pronucleate zygotes had better cryosurvival rates than the cleaving embryo (90.7% versus 59.9%) and almost double the clinical pregnancy rates for cleaving embryo (12.8% versus 7.0%). There was an almost three-fold higher delivery rate for the pronucleate zygote compared with cleaving embryo (11.6% versus 4.3%). Immature oocytes had lower cryosurvival rates than mature oocytes (34.5% versus 52.3%). Mature and immature oocytes showed good fertilization rates (59.3% versus 50%) and satisfactory embryonic development (90.3% versus 100.0%). Immature oocytes had higher delivery rate per embryo transferred than the mature oocyte (40.0% versus 2.7%). Pronucleate zygote cryopreservation has an advantage over the cleaving embryo. The low survival rate following thawing is still an obstacle to the integration of oocyte cryopreservation into ART.

Keywords: *Oocyte cryopreservation, embryo cryopreservation, zygote cryopreservation, intracytoplasmic sperm injection*

Résumé

Pour comparer les résultats entre cryopréservation ICSI des embryons fertilisés et cryopréservation ICSI des zygotes en stage pro nucléaire fertilisés avec (ii) les résultats entre cryopreservé des oocytes matures fertilisés et cryopreservé des oocytes immatures, fertilisés par ICSI de degivrement. La revue rétrospective comparative des études appropriées satisfaisant les critères d'étude. Les études étaient identifiées à travers les recherches de littérature MEDLINE. Les résultats de l'étude étaient documentés la cryosurvie, de la fertilisation, implantation, grossesse et le taux d'accouchement. Les zygotes pronuclués avait un meilleur taux de cryosurvie que les embryon se divisant (90.7% contre) et presque le double du taux de grossesse clinique pour les divisions embryonnaires (12.8% contre 7.0%). il y'avait presque un taux d'accouchement de trois fois plus élevés pour les zygotes pronuclués comparés avec les embryons divisés (11.6%contre4.3%). Les oocytes immatures avaient un taux cryosurvival plus bas que les oocytes matures (34.5% contre 52.3%). Les oocytes matures et immatures montraient un bon taux de fertilisation. Le faible taux de survie après degivrement reste un obstacle a l'intégration de l'ovocyte cryopreservé dans l'ART.

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Introduction

Introduction of cryopreservation into clinical practice marked a turning point in the development of assisted reproductive technology (ART). Sperm, oocyte, and embryo cryopreservation have opened new vistas in the range of solutions to various infertility problems. Embryo cryopreservation has been integrated into ART. With refinement of the technical procedure, the clinical outcome is comparable with figures for fresh embryo transfer [1] An analysis of the birth characteristics of babies born after embryo cryopreservation by Wada *et al* [2] revealed a significantly lower incidence of malformations compared with babies born following conventional in vitro fertilization (IVF) technology, thus lending support to the safety of embryo cryopreservation.

However, ethical and moral considerations have always been strong impediments to research and services with respect to human embryo cryopreservation [3]. In Germany and Switzerland, protective legislation restricts the number of embryos to be developed in vitro to a maximum of three which are to be transferred during the same cycle [4,5]. In these countries, freezing of cleaved embryo is forbidden; however it is permitted to freeze at the pronuclear stage. Thus cryopreservation of pronuclear zygotes evolved out of these legal and ethical concerns. Cryopreservation at the pronucleate zygote stage has been shown to have a similar chance of establishing pregnancy to those that were replaced fresh during the retrieval cycle [6]. Freezing at the pronucleate stage has also been reported to be more effective at establishing pregnancy than cleaving embryos [7,8,9]. Further work has shown similar outcome of pregnancy for cryopreservation at the pronuclear stage after intracytoplasmic sperm injection (ICSI) in comparison with conventional IVF [4].

Whereas embryo cryopreservation has been incorporated into routine clinical practice, some technical aspects remain unclear with respect to oocyte cryopreservation. The human oocyte has been observed to tolerate cryopreservation poorly, being susceptible to various forms of cryo-injury including damage to the meiotic spindle [10,11,12], breaching and hardening of the zona pellucida [13,14] and spontaneous discharge of the cortical granules leading to a premature cortical reaction [15,16]. These defects may account for the increased incidence of parthenogenesis, reduced fertilization rate and the increased incidence of arrest of cleavage [12] and aneuploidy [17]. Generally the outcome of unfertilized oocyte cryopreservation has been poor, casting doubt on its overall safety; and no reliable protocol has been established [18] thus accounting for the reluctance to integrate this procedure into routine IVF programmes.

However, other investigators have questioned this pessimism on the grounds of their observation that the human oocyte could survive cryopreservation without compromising cell integrity [15]. The cryoprotectants generally used in oocyte cryopreservation are 1, 2-propanediol (PrOH) and sucrose using slow freeze-rapid thaw protocol. Limited success has been recorded with other cryoprotectants such as ethylene glycol [18] and dimethylsulphoxide (DMSO) [20,21].

Introduction of ICSI [22] has brightened the potential of oocyte cryopreservation. Studies have established that the outcome of cryopreservation following ICSI is comparable with conventional IVF [23,24]. ICSI has been reported to yield better outcome for frozen-thawed oocytes than the conventional insemination of cryopreserved oocytes in IVF [25,26]. Both studies showed that frozen-thawed oocytes fertilized by ICSI could undergo normal pre-implantation development. Pregnancy rates acceptable for an oocyte cryopreservation programme have also been reported with the use of ICSI [27].

Given the multiple advantages inherent in oocyte cryopreservation such as circumvention of ethical, legal, moral and religious problems, diminished severity of ovarian hyperstimulation, conservation of fertility for medical and social reasons among others, the development and introduction of this technology into clinical practice will improve the efficacy of the ART programme.

This study was designed to evaluate the current status of embryo, zygote and oocyte cryopreservation in ART. Its first objective was to compare the clinical outcome of thawed cryopreserved cleaving embryos that have been fertilized by ICSI with thawed pronuclear stage zygotes that have been fertilized by ICSI. It also aimed to compare the clinical outcome of cryopreserved mature (metaphase II) oocytes fertilized by ICSI following thawing with thawed immature (prophase I) oocytes subsequently injected with spermatozoa in an ICSI programme.

Materials and methods

This retrospective comparative study conducted between September and November 2001 involved an analysis of all studies utilizing cryopreserved embryos, cryopreserved zygotes, and cryopreserved mature and immature oocytes subsequently fertilized by ICSI. Studies were included if they met the following criteria: cryopreserved thawed embryos fertilized by ICSI, cryopreserved thawed pronuclear zygotes fertilized by ICSI, cryopreserved mature oocytes thawed and fertilized by ICSI, cryopreserved immature oocytes thawed and fertilized by ICSI, use of ICSI as the only method of insemination, and complete report of outcome for all embryos and oocytes. Studies that utilized mixed methods of insemination and/or mixed analysis of techniques were excluded from this review.

Selection of studies

Studies were identified through computer literature search of Medline augmented with hand search. Relevant studies were identified using the search terms: human oocyte cryopreservation, embryo cryopreservation, zygote cryopreservation and ICSI.

Table 1: Selected studies and their characteristics

Study	Study type	Cell type	Cryoprotectant	Freeze-thaw protocol
Damario *et al*[28]	Clinical trial	Zygote	PrOH	Slow freeze-rapid thaw
Van den Abbeel *et al*[29]	Clinical trial	Multicellular embryo	DMSO	Slow freeze-rapid thaw
Van Steirteghem *et al*[30]	Clinical trial	Multicellular embryo	DMSO	Slow freeze-rapid thaw
Edirisinghe *et al*[31]	Case report	Zygote	PrOH	Slow freeze-rapid thaw
Tucker *et al*[32]	Case report	Immature oocyte	PrOH	Slow freeze-rapid thaw
Porcu *et al*[33]	Case report	Mature oocyte	PrOH	Slow freeze-rapid thaw
Kuleshova *et al*[19]	Case report	Mature oocyte	Ethylene glycol	Vitrification
Polak de Fried *et al*[34]	Case report	Mature oocyte	PrOH	Slow freeze-rapid thaw
Nawroth and Kissing[35]	Case report	Mature oocyte	PrOH	Slow freeze-rapid thaw
Tucker *et al*[36]	Clinical trial	Mature oocyte	PrOH	Slow freeze-rapid thaw
Tucker *et al*[36]	Clinical trial	Immature oocyte	PrOH	Slow freeze-rapid thaw
Young *et al*[37]	Case report	Mature oocyte	PrOH	Slow freeze-rapid thaw
Tucker *et al*[38]	Clinical trial	Matureoocyte	PrOH	Slow freeze-rapid thaw
Porcu *et al*[39]	Case report	Mature oocyte	PrOH	Slow freeze-rapid thaw

The references of extracted articles were also searched. Relevant studies acceptable for the study included randomised clinical trials, clinical trials and case reports.

Outcome measures

The primary outcome measures were: oocyte/embryo/zygote cryosurvival rate, fertilization rate, implantation rate, chemical pregnancy rate, preclinical abortion rate, clinical pregnancy rate, clinical abortion rate, delivery rate and live birth rate.

Data were recorded on a data sheet which sought to identify the cell type, method of insemination, number of embryos/oocytes thawed, number of oocytes/embryos maturing, number of oocytes/embryos fertilized and the number transferred, number of implantations, pregnancies and the outcome.

Definition of terms

The following terms were employed in this study: *immature oocyte* - unfertilized human oocyte at the prophase stage of the first meiotic division (prophase I); *mature oocyte* - unfertilized human oocyte at the metaphase stage of the second meiotic division (metaphase II); *cleaving embryo* - fertilized human embryo which is in the early phase of development (2-8 cells); *pronuclear zygote* - fertilized unicellular human embryo with two pronuclei; *implantation* rate - the number of intra-uterine gestation sacs with detectable cardiac activity at sonography as a percentage of the total number of embryos/zygotes transferred; *chemical pregnancy rate* - the number of transfers in which serial serum hCG testing increases at least twice as a percentage of total transfers; *cryosurvival* rate - the number of embryos/oocytes that are morphologically intact post-thaw as a percentage of the total cryopreserved; *pre-clinical abortion* - when chemical pregnancy has been confirmed but there is no obvious clinical pregnancy at sonography.

Results

Only two trials on cryopreserved cleaving embryo [29,30] satisfied the inclusion criteria; while two publications on cryopreserved pronucleate zygote [28,31] met the inclusion criteria for the present study. Nine publications on cryopreservation of mature and immature oocytes [19,32,33,34,35,36,37,38,39] satisfied the inclusion criteria and were included. The selected studies and case reports are listed in Table 1.

Cleaving embryos and Pronucleate zygotes

The two studies on cleaving embryo employed the slow freeze-rapid thaw protocol utilizing DMSO as cryoprotectant. The case report on pronucleate zygote employed the rapid freeze protocol while the pronucleate zygote trial employed a slow freeze protocol. The cryoprotectant used in both reports was 1, 2-propanediol. A total of 8872 cleaving embryos were thawed while 365 pronucleate zygotes were thawed yielding 5313 and 331 morphologically intact embryos and pronucleate zygotes respectively (Table 2). One thousand four hundred and eighty two embryo transfers produced 257 clinical pregnancies which resulted in 63 clinical abortions and 159 deliveries.

Ninety four pronucleate zygote transfer procedures yielded 42 clinical pregnancies, and four clinical abortions. The cryosurvival, abortion (pre-clinical and clinical), pregnancy and delivery rates are shown in Table 3. Pronucleate zygotes demonstrated a higher capability of surviving the

Table 2: Cryosurvival, pregnancy, and pregnancy outcome in cleaving embryos and pronucleate zygotes

	Cleaving embryo (n)	Pronucleate zygote(n)
No. of embryo thawed	8872	365
No. of embryos morphologically intact	5313	331
No. of thaw transfers	1482	94
No. of embryos transferred	3690	329
No. of positive Hcg measurements	280	-
No. of pre-clinical abortions	51	3
No. of clinical pregnancies	257	42
Outcome of clinical pregnancies :		
Abortions	63	4
Extra-uterine pregnancies	-	-
Termination of pregnancy	1	-
No. of deliveries	159	38
Singletons	126	21
Multiple	33	17
Total no. of children born	196	66

freezing procedure than cleaving embryos (90.7% versus 59.9%). Pronucleate zygotes also showed almost double the clinical pregnancy rate per zygote transferred in comparison with cleaving embryos (12.8% versus 7.0%); and had a reduced tendency to abort (9.5% versus 24.5).

Mature oocytes and immature oocytes

All reports except one employed the slow freeze-rapid protocol and 1,2-propanediol as cryoprotectant. Only one case report utilized the rapid freeze-rapid thaw protocol with ethylene glycol as cryoprotectant. Table 4 shows the distribution of the cryosurvival, pregnancy, implantation and the pregnancy outcome between the mature and immature oocytes. Two thousand and seventy one and 29 mature and immature oocytes were thawed with 1084 mature oocytes and 10 immature oocytes respectively surviving the cryopreservation procedure. Five hundred and eighty nine embryos derived from mature oocytes produced 26 implantations, 19 clinical pregnancies, and 16 deliveries with a total of 19 children.

All surviving immature oocytes were normally fertilized and showed good embryonic development. They produced two clinical pregnancies and two deliveries. The cryosurvival rate, implantation, pregnancy and delivery rates for mature and immature oocytes are compared in Table 5. Immature oocytes had a reduced ability to survive the cryopreservation procedure compared with mature oocytes (34.5% versus 52.3%). They also showed a slightly lower normal fertilization rate compared with mature oocytes (50.0% versus 59.2%). However, it appears that once mature and immature oocytes overcome the

Table 3: Cryosurvival rate, pregnancy and delivery rates in cleaving embryos and pronucleate zygotes

	Cleaving Embryo		Pronucleate Zygote	
	n	(%)	n	(%)
Cryosurvival rate	5313/8872	(59.9)	331/365	(90.7)
Pre-clinical abortion rate	51/280	(18.2)	-	-
Clinical abortion rate	63/257	(24.5)	4/42	(9.5)
Clinical pregnancy rate per transfer	257/1482	(17.3)	42/94	(44.7)
Clinical pregnancy rate per embryo transferred	257/3690	(7.0)	42/329	(12.8)
Delivery rate per transfer	159/1482	(10.7)	38/94	(40.4)
Delivery rate per embryo transferred	159/3690	(4.3)	38/329	(11.6)
Live birth per transfer	196/1482	(13.2)	66/94	(70.2)
Live birth per embryo transferred	196/3690	(5.3)	66/329	(20.1)

For every transfer procedure, there was a higher clinical pregnancy rate for the pronucleate zygote (44.7% versus 17.3%). For each embryo transferred, the pronucleate zygote had almost a three-fold chance of ending in a delivery compared with the cleaving embryo (11.6% versus 4.3%) ; and almost a four-fold chance of delivery for each transfer procedure (40.4% versus 10.7%).

obstacles of the freezing and fertilization processes, they demonstrate good and comparable embryonic development (90.3% and 100.0% respectively).

Beyond fertilization, immature oocytes produced better outcome for all outcome measures: almost 10-fold higher clinical implantation rate per embryo transferred (40.0% versus 4.4%); higher

Table 4: Cryosurvival, pregnancy, implantation and pregnancy outcome in mature (metaphase II) and immature (prophase I) oocytes

	Mature oocytes (n)	Immature oocytes (n)
No. of oocytes thawed	2071	29
No. of oocytes morphorlogically Intact	1084	10
No. of oocytes inseminated	1084	9
No. of oocytes normally fertilized	642	5
No. showing cleavage	580	5
No. of thaw-transfers	16	3
No. of embryos transferred	589	5
No. of clinical pregnancies	19	2
Type of pregnancy :		
Singleton	15	2
Twins	3	-
Triplets	1	-
Pregnancy outcome :		
First trimester abortions	5	-
Termination of pregnancy	1	-
Number of deliveries	16	2
Singleton	13	2
Twins	3	-
Total no. of children born	19	2

cleaved embryo. Mandelbaum *et al* [40] attributed this to the tendency for clinics to use either zygote or cleaved embryo thus making comparisons difficult. Thus there remains an on-going debate regarding the better stage of embryonic development at which freezing achieves maximal cryosurvival and pregnancy rates.

Extrapolating data for retrospective analysis of the outcome of cryopreservation suffers from the multiple disadvantages of different stimulation and freezing protocols, cryoprotectants and embryo culture condition, expression and interpretation of results.

Nonetheless, the present study demonstrates that cryopreservation of pronuclear stage zygote has a better clinical outcome in comparison with cleaving embryo. Many reports confirm a consistently high cryosurvival ability of the pronucleate zygote: 76.6% [4], 74.4% [9], 93.2% [41], 75.9% [42], 78% [43], 87.7% [44]. Owens *et al* [23] however reported a lower rate (39.8%). A wider variation of cryosurvival rates is reported for cleaving embryo: 77.4% [9], 60.2% [29] and 53% [30]. Hu *et al* [24] reported 88% post-thaw survival rate for ICSI fertilized embryos. However, the study involved both pronuclear zygotes and cleaving embryos. In an appraisal of cumulative experience during a 10-year period in which 14,222 stored embryos were thawed, Mandelbaum *et al* [40], recorded a 73%

Table 5: Cryosurvival rate, implantation, pregnancy and delivery rates in mature and immature oocytes

	Mature oocyte		Immature oocyte	
	n	(%)	n	(%)
Cryosurvival rate	1084/2071	(52.3)	10/29	(34.5)
Normal fertilization rate	642/1084	(59.2)	5/10	(50.0)
Cleavage rate	580/642	(90.3)	5/5	(100.0)
Clinical abortion rate	5/19	(26.3)	-	-
Clinical implantation rate per embryo transferred	26/589	(4.4)	2/5	(40.0)
Clinical pregnancy rate per embryo transferred	19/589	(3.2)	2/5	(40.0)
Delivery rate per embryo transferred	16/589	(2.7)	2/5	(40.0)
Live birth per embryo transferred	19/589	(3.2)	2/5	(40.0)

clinical pregnancy rate per embryo transferred (40.0% versus 3.2%) and a higher delivery rate per embryo transferred (40.0% versus 2.7%).

Discussion

Cleaving embryo and Pronucleate zygotes

Relatively few prospective studies have compared the clinical outcome between cryopreserved zygote and cryosurvival rate for cleaved embryos, which is probably a more representative value.

The clinical pregnancy rate per transfer for the cleaving embryo in the current study (17.3%) is within the range found in literature: 12.9% [30], 16.0% [40] and 24.4% [45]. The clinical pregnancy rate of 44.7% for pronuclear stage zygote reported in this study is higher than most figures reported in literature: 17.0% [4], 14.0% [41], 15.8% [45]. Damario *et al* [28] were

the only investigators to record a similar high rate of 44.1%. Small numbers is a possible explanation for this wide disparity. In literature, the pronucleate stage zygote is associated with higher pregnancy losses than the 9.5% reported in this study (18% [4] and 25% [41]). The clinical abortion rate of 24.5% observed for cleaving embryos is similar to 23% reported by Mandelbaum *et al* [40].

According to published data, the clinical outcome is generally similar for the pronucleate stage zygote and cleaving embryo [40]. However, findings in this study appear to tip the scale in favour of the pronucleate stage zygote by virtue of its higher delivery rate per embryo transferred and lower clinical abortion rates. However, for each laboratory, the stage at which to freeze will ultimately be influenced by the law. Specifically, decisions will invariably be determined by the availability of adequate in-vitro culture facilities, the type of cryoprotectant and the freezing protocol and lastly by the issue of embryo selection [46].

Multicellular embryos have the inherent advantage over pronuclear zygote in allowing for embryo selection. However, research is beginning to evaluate the prospects of predicting zygote morphology [47] with a view to facilitating zygote selection. Finally, the true difference between zygote and embryo cryopreservation resides in the freezing of all zygotes obtained in vitro and the use of the best quality embryos. Subsequent comparison of the cumulative pregnancy rates will resolve the current debate. In view of the remarkable success achieved with human embryo cryopreservation, it has been established as an integral component of the ART programme. Human embryo cryopreservation makes substantial contribution to the ART programme as reflected in its involvement in over 40% of every component of the treatment cycle: during cycle initiation, in cycles reaching oocyte collection, and of patients reaching embryo replacement producing sufficient embryo for cryopreservation [9].

Overall, embryo/zygote cryopreservation is safe and efficient with birth rates comparable with figures for fresh embryos [40]. Embryo and zygote cryopreservation will reduce costs, the incidence of ovarian hyperstimulation syndrome and higher order multiple pregnancies. The cumulative pregnancy rate for all embryos resulting from each retrieval cycle will increase significantly [6]. Embryo cryopreservation may also reduce the potential risk of ovarian cancer associated with multiple ovarian stimulation.

Mature and immature oocytes

Failure to reproduce the initial successes with oocyte cryopreservation [20,21] dampened enthusiasm about the technique. Regardless of this failure, the potential advantages of an oocyte cryopreservation programme have propelled an extensive investigation into the various aspects of oocyte cryobiology.

Survival after thawing is currently the major obstacle to oocyte cryopreservation. The oocyte in general is more sensitive to freeze/thaw damage than later embryogenic stages [48]. The extreme sensitivity of the mature oocyte to temperature and toxic shocks during the freezing and thawing process is a consequence of its cellular architecture and physiology [49]. The small surface-to-volume ratio combined with the loose binding of the chromosomes to the spindle in the absence of a nuclear membrane exposes the oocyte to the risk of damage to the zona pellucida, meiotic spindle, cytoskeleton, and cortical granules [50]. Changes during the freeze-thaw process can induce depolarisation of the spindle microtubules, causing disruption of chromatid separation at the moment of fertilization; which may cause the spread of chromosomes and potential induction of aneuploidy [48,49]. However, Gook *et al* [51] reported normal karyotypes and the absence of stray chromosomes in cryopreserved human oocytes. The oocyte quality in terms of age, maturity and size is believed to play a role in oocyte cryosurvival.

A role for the oocyte quality is supported by the observation that most oocyte derived pregnancies have been achieved with metaphase II oocytes [50]. This observation is confirmed by this study. The poor results obtained with the mature oocyte shifted attention to prophase I oocyte which was thought would be less sensitive to cryoinjury; being in a state of arrested meiosis at the dictyate stage with its chromosomes located in the membrane-bound nucleus. Furthermore, the prophase I oocyte from stimulated and unstimulated ovarian tissue had been shown to be capable of meiotic maturation after cryopreservation [52,53,54].

The present study shows that the immature oocyte has poor cryosurvival rates. Its cryosurvival rate observed in this study (34.5%) is lower when compared with the survival rates reported by Son *et al* (55.1%) [54]. The cryosurvival rate of the mature oocyte in this study is higher than the low survival rates (25-40%) reported from the analysis of combined data of four studies [40]. These findings support the poor survival of the human oocyte although an increasing trend is being observed with the increasing use of 1,2-propanediol as the cryoprotectant [55]. A number of other biological and technical factors may influence oocyte cryosurvival including the cumulus oophorus, the cryoprotectant and the freezing protocol [56]. The role of the cumulus oophorus remains controversial with investigators

arguing for [57] and against [15]. Yet others reported that it has no impact on oocyte survival [40,56,58]. The slow freeze-rapid thaw is reported to be the most successful technique [50]. However, given the generally poor cryosurvival rates reported in the literature, further development of cryoprotectants and the freeze/thaw procedure is imperative. Cha *et al* [59] reported a new vitrification method which is capable of improving the cryosurvival rate. They achieved an 83% cryosurvival rate, 68% fertilization rate and 90% cleavage rate for the immature oocyte. However, no pregnancy was recorded.

Having survived cryopreservation, both immature and mature oocytes demonstrated satisfactory embryonic development potentials. The good fertilization rates in both mature and immature oocytes observed in this study ($\geq$ 50%) are comparable with reports in the literature [25,56]. The survival, fertilization and cleavage rates noted in this study are similar to the 37%, 45.4% and 86.3% respectively in 68 women treated in 86 thawing cycles by Borini *et al* [60]. The observation of higher implantation and pregnancy rates reported for the immature oocyte may be an indication of an inherent valuable potential which requires further investigations. Overall, the study findings are in agreement with the suggestion that at present, and until the efficiency of in vitro maturation improves, cryopreservation of prophase I oocytes offers little advantage over freeze-storage of metaphase II oocytes.

Though the numbers are small, this study has demonstrated that oocyte cryopreservation is becoming a reproducible procedure, and makes a strong case for the need to initiate clinical trials. Enthusiasm with this technology seems justified given the observation that it continues to improve [61]. Cryopreservation is apparently the last hurdle in the process leading to the utilization of oocytes. Coticchio *et al* [62] attributed the limitations of oocyte cryopreservation to the fact that current techniques employ methods originally designed for cleaving embryos. With further development of the cryopreservation technique, its introduction into clinical practice will become feasible. Reassuringly, the few children born after oocyte cryopreservation and ICSI are healthy and normal [33].

Oocyte cryopreservation will contribute directly to the establishment of an oocyte donation system which is a feasible system to treat a number of congenital infertility disorders such as hypoplastic ovaries, premature ovarian failure and conserves fertility for patients who receive anti-cancer treatment [59,63,64,65]. A recent meta-analysis comparing the outcome of IVF-ICSI cycles between frozen and unfrozen oocytes demonstrated significantly lower success rates with cryopreserved oocytes [66]. The authors concluded that oocyte cryopreservation was justified for preserving fertility in the presence of a medical indication. Cryopreservation will increase the flexibility of the ART programme, allowing the possibility of postponement of the initial treatment cycle in the event that the patient develops hyperstimulation or the inability of the partner to produce a viable sperm sample [18,63].

ICSI and cryopreservation

ICSI is well established in ART. It is reported to account for about 27% of assisted conception treatment for severe male factor infertility in the UK; and achieving higher success rates than IVF [67]. However, concern is being raised on the potential risk of congenital malformations and genetic defects in the offspring after ICSI, given that ICSI, by direct injection of spermatozoa into the oocyte bypasses the natural barrier of sperm selection. This concern becomes heightened with the application of ICSI in cryopreservation, raising the fear of the possibility of combined assault on the genetic material.

Available evidence on the short-term health of ICSI offspring is generally reassuring [67]. Detailed follow-up studies of 1987 ICSI children including 79 children from cryopreserved ICSI embryo in Belgium did not demonstrate increased chromosomal or structural aberrations [68].

Conclusion

Cryopreservation is well established in ART. Pronucleate zygote and embryo cryopreservation give satisfactory clinical outcome and are currently the most widely-used cryopreservation techniques [69]. While ICSI has improved the prospects of oocyte cryopreservation, making it a reproducible technique, the low survival rates militate against its routine clinical application at the present time. However, optimization of cryotechnology will hopefully enhance its clinical utility. The short term health of infants born after cryopreservation has been satisfactory. Cryopreservation has broadened the scope and the clinical utility of ART. Ovarian cryopreservation when it becomes clinically available will resolve the dilemma of fertility conservation for children and young women [69,70] thus increasing the overall efficiency of the ART programme.

Acknowledgements

This study was undertaken under the auspices of the Geneva Foundation for Medical Education and Research at the Clinic of Infertility and Gynaecologic Endocrinology, Department of Obstetrics and Gynaecology, University of Geneva, Geneva.

Financial support by the Bertarelli Foundation and supervision by Drs. Herve Lucas and Corrine de Vantery are gratefully acknowledged.

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Received: 17/10/06
Accepted: 21/08/07