AN EVALUATION OF EMBRYO, ZYGOTE AND OOCYTE CRYOPRESERVATION IN ASSISTED REPRODUCTIVE TECHNOLOGY

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INTRODUCTION

- Cryopreservation: a turning point in ART
- Similar outcome for embryo cryopreservation and fresh embryo transfer (Society for Assisted Reproduction Tech, 1993)
- Safety : No increased malformation with embryo cryopreservation (Wada et al, 1994)
- Ethical/moral issues remain major problems with embyo cryopreservation

INTRODUCTION (CONTD)

- Embryo cryoreservation prohibited in some countries
- Evolution of pronucleate zygote cryopreservation
- Similar chance of establishing pregnancy for cryopreserved zygote/embryo (Miller & Goldberg, 1995)
- Similar outcome of pregnancy for cryopreserved zygote after ICSI vs. IVF (Al-Hassani et al, 1996)

INTRODUCTION (CONTD.)

Oocyte cryopreservation

- **Remains largely experimental**
- **Technical difficulties with oocyte cryopreservation**
- **Cryo-induced injury: meiotic spindle, zona pellucida,**
- **cortical granules**
- **Higher incidence of aneuploidy (Kola et al, 1988)**
- **Poor outcome; no reliable protocol**

INTRODUCTION (CONTD.)

- Pessimistic attitude to oocyte cryopreservation unjustified (Gook et al, 1993)
- Chances increased with introduction of ICSI
- Outcome of cryopreservation using ICSI comparable with IVF (Owens et al, 1998; Kazem et al, 1998)
- Better outcome seen in cryopreserved oocytes fertilized with ICSI than conventional IVF (Gook et al, 1995; Kazem et al, 1995)
- Multiple advantages associated with oocyte crypreservation

OBJECTIVES

Goal: evaluation of current status of embryo, zygote and oocyte cryopreservation

Specific objectives:

- **To compare the clinical outcome of thawed cryopreserved embryo fertilized by ICSI with thawed pronuclear stage zygotes fertilized by ICSI**
- **To compare the clinical outcome of cryopreserved mature (metaphase II) oocytes fertilized by ICSI following thawing with thawed immature (prophase I) oocytes subsequently fertilized by ICSI**

Study Design

Retrospective comparative review of published studies

Selection of studies:

- **computer literature search of MEDLINE/other databases,**
- **combined with hand search**

- **search terms: human oocyte cryopreservation, embryo cryopreservation, zygote cryopreservation and ICSI**

Study design (Contd.)

Inclusion 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**
- **Complete report of outcome for all embryos and oocytes**

Study design (Contd.)

- Exclusion criteria:
- Mixed methods of insemination
- Mixed analysis of outcome

- Instrument:
- data sheet

Study design (Contd.)

- Outcome measures:
- Oocyte/embryo/zygote cryosurvival rate
- Fertilization rate
- Implantation rate
- Chemical pregnancy rate
- Preclinical abortion rate
- Clinical pregnancy rate
- Clinical abortion rate
- Delivery rate
- Live birth rate

Operational definition of terms

- **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.**

Table 1. Selected studies and their characteristics

Study	Study type	Cell type	Cryoprotectant	Freeze-thaw protocol
Damario et al[27]	Clinical trial	Zygote	PrOH	Slow freeze-rapid thaw
Van den Abbeel et al[28]	Clinical trial	Multicellular embryo	DMSO	Slow freeze-rapid thaw
Van Steirte-ghem et al[29]	Clinical trial	Multicellular embryo	DMSO	Slow freeze-rapid thaw
Edirisinghe et al[30]	Case report	Zygote	PrOH	Slow freeze-rapid thaw
Tucker et al[31]	Case report	Immature oocyte	PrOH	Slow freeze-rapid thaw
Porcu et al[32]	Case report	Mature oocyte	PrOH	Slow freeze-rapid thaw
Kuleshova et al[18]	Case report	Mature oocyte	Ethylene glycol	Vitrification
Polak de Fried et al[33]	Case report	Mature oocyte	PrOH	Slow freeze-rapid thaw
Nawroth and Kissing[34]	Case report	Mature oocyte	PrOH	Slow freeze-rapid thaw
Tucker et al[35]	Clinical trial	Mature oocyte	PrOH	Slow freeze-rapid thaw
Tucker et al[35]	Clinical trial	Immature oocyte	PrOH	Slow freeze-rapid thaw
Young et al[36]	Case report	Mature oocyte	PrOH	Slow freeze-rapid thaw
Tucker et al[37]	Clinical trial	Mature oocyte	PrOH	Slow freeze-rapid thaw
Porcu et al[38]	Case report	Mature oocyte	PrOH	Slow freeze-rapid thaw

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

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)

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

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)

DISCUSSION

Cleaving embryo/Pronucleate zygote

- **Few comparative studies available**
- **Tendency of clinics to employ only one method (Mandelbaum et al, 1998)**
- **On-going debate about better stage to freeze**
- **Comparison beset with difficulties: different stimulation/freezing protocols, cryoprotectants, expression/interpretation of results**

Cryosurvival rate

Cleaving Embryo	Pronucleate zygote
59.9% (Present study)	90.7%(Present study)
53% (Van Steirteghem et al, 1994)	76.6% (Al-Hassani et al, 1996)
60.2% (Van den Abbeel et al, 2000)	93.2% (Hoover et al, 1997)
	75.9% (Kowalik et al, 1998)
	87.7% (Macas et al, 1998)
	39.8% (Owens et al, 1998)

Clinical pregnancy rate per transfer

Cleaving embryo	Pronucleate zygote
17.3% (Present study)	44.7% (Present study)
12.9% (Van Steirteghem et al, 1994)	14.0% (Hoover et al, 1997)
	15.8% (Tucker et al, 1995)
	44.1% (Damario et al, 1999)

Clinical abortion rate

Cleaving embryo	Pronucleate zygote
24.4% (Present study)	9.5% (Present study)
23% (Mandelbaum et al, 1998)	18% (Al-Hassani et al, 1996)
	25% (Hoover et al, 1997)

Cleaving embryo/pronucleate zygote (Summary)

- In present study, better outcome in pronucleate zygote
- In published data, similar outcome in cleaving embryo/zygote (Mandelbaum et al, 1998)
- Each laboratory will decide based on prevailing circumstances
- Embryo/zygote cryopreservation safe /effective
- Advantages: cost reduction, reduced incidence of OHSS/higher order pregnancies, increased cumulative pregnancy rate per retrieval cycle.
- May also reduce risk of ovarian cancer

Mature/Immature Oocytes

- Survival is the major obstacle to oocyte cryopreservation
- Oocyte more sensitive to cryoinjury than other stages
- Oocyte cellular architecture/physiology vulnerable
- Loosely bound chromosomes, no nuclear envelope
- Damage to meiotic spindle, cytoskeleton, cortical granules, zona pellucida
- Human: Normal karyotypes/no stray chromosomes (Gook et al, 1994)

Immature oocyte:

- Capable of meiotic maturation post-cryopreservation (Toth et al, 1994; Son et al, 1996)

Oocyte cryosurvival rate

Mature oocyte	Immature oocyte
52.3% (Present study)	34.5% (Present study)
25-40% (Mandelbaum et al, 1998)	55.1% (Son et al, 1996)

DISCUSSION (CONTD.)

Other biological/technical

- **variables:**
- **cumulus oophorus**
- **cryoprotectant**
- **freezing protocol**

Satisfactory embryonic development potential:

- **fertilization rate ≥ 50%**
- **cleavage rate > 90%**

Mature/Immature Oocyte (Summary)

- **Poor cryosurvival rates**
- **Reproducible procedure**
- **Need for refinement of cryotechnology**
- **Advantages:** **-avoids legal/ethical problems**
  -conserves fertility
  -treatment of congenital infertility disorders
  -increases flexibility of ART programme

ICSI and Cryopreservation

- Concern about risk of congenital malformations and genetic defects
- Short-term health of offspring generally reassuring (Porcu et al, 2000; Campbell and Irvine, 2000)
- No inceased chromosomal/structural aberrations in 79 children from cryopreserved ICSI embryo (Bonduelle et al, 1999)

CONCLUSIONS

- Cryopreservation well established in ART
- Pronucleate /embryo cryopreservation is successful; currently the cryopreservation technique of choice
- Outcome of oocyte cryopreservation improved with ICSI; however results not yet cost-effective

LIMITATIONS

- Small numbers of oocyte/embryo for research
- Tendency of clinics to utilize one method
- Tendency of clinics to adopt one protocol
- Difficulty with comparison of methods/protocols
- Non-uniformity of expression of findings
- Non-randomisation
- Medical/clinical significance of findings

ACKNOWLEDGEMENTS

- Course faculty
- Painstaking supervision by Dr. Lucas and Dr. de Vantéry
- Bertarelli Foundation