Relationship between development rate of supernumerary embryos and pregnancy rate with fresh embryos

	Development rate			
	0–20%	21–60%	61–80%	81–100%
Pregnancy rate with fresh embryos (%)	28	36	41	47

Conclusion: Further culture of supernumerary embryos to select viable embryos before freezing is an interesting technique. It provided a significant improvement in our results after the transfer of thawed embryos. In addition, the ability of embryos to develop appears to be a good indicator of the quality of all the embryos in a single cycle.

14.15–14.30

O-198. Pregnancies after vitrification of human day 5 embryos

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Introduction: To eliminate all physical injuries caused by the formation of extracellular and intracellular crystal ice occurring in a controlled slow freezing procedure, vitrification was proposed as another cryopreservation approach. The concentration of cryoprotectant solution was so high that during ultrarapid freezing to –196°C the solution did not crystallize but became increasingly viscous and formed a glass-like solid. Recently, vitrification had been applied successfully with bovine blastocysts produced *in vitro*. The aim of this study was to report whether morulae–blastocysts can be cryopreserved using a vitrification protocol.

Materials and methods: Out of 24 patients, morulae–blastocysts were obtained after 5 days of culture in IVF-50 and Hatch-100 medium (Scandinavian Science). The vitrification procedure was performed in two steps. Selected embryos were equilibrated at room temperature in 20% (v/v) ethylene glycol (EG20) for 3 min. Then they were placed in a second solution (EFS) containing 40% (v/v) ethylene glycol, 18% (w/v) Ficoll and 0.3 M sucrose. After a short exposure of 15 s, they were loaded into 0.25 ml straws containing EFS and then plunged into liquid nitrogen. The time between morulae–blastocyst addition to EFS and storage in liquid nitrogen was minimized (maximum 60 s). Thawing was performed at 40°C for 10 s and the contents of the straws were transferred into 0.25 M sucrose. After 3 min equilibration, embryos were washed in PBS and cultured in Hatch 100 medium for 24 h or transferred directly into the uterus.

Results: A total of 67 embryos (31 blastocysts, 15 early blastocysts, 21 morulae) were thawed after vitrification. Of these, 80% were morphologically intact after thawing (27 blastocysts, 12 early blastocysts, 15 morulae). Out of 24 transfers, four pregnancies (two after vitrification of ICSI embryos) are still ongoing (16.6 and 18.1% per vitrification attempt and per transfer respectively).

Conclusion: Vitrification is a rapid and simple method for the cryopreservation of human morula–blastocyst embryos. However, during exposure to the high concentration of cryoprotectant, embryos are susceptible to damage by solution toxicity. Therefore further investigations into the vitrification of morulae–blastocysts and also the early stages of embryo development are necessary. Factors affecting embryo survival will be discussed.

14.30–14.45

O-199. Cryopreservation of all embryos in women at risk of OHSS: efficacy and safety

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Introduction: It is well known that conception is strictly related to the development and severity of OHSS. According to our data, when a pregnancy does not occur, the incidence of OHSS is 0.3% and its duration is 6.2 ± 1.7 days. Conversely, if a pregnancy does occur, the incidence is consistently higher (2% if fewer than three embryos implant, 20% if three or more embryos implant) and its resolution is prolonged to 17.5 ± 3.0 days (Gianaroli *et al.*, 1997). Over the last 2 years we have evaluated the efficacy and safety of cryopreserving all embryos in patients considered to be at high risk of developing OHSS.

Materials and methods: In the years 1995–1996, 962 IVF cycles were performed. Cycles were cancelled because of the risk of OHSS only if oestradiol concentrations were ≥1000 pg/ml on cycle day 7. Patients with >15 oocytes recovered following retrieval and with oestradiol concentrations ≥1500 pg/ml on the day of HCG administration were randomly allocated to two groups. Group A patients had fresh embryos transferred on day 3, and group B patients had all their embryos cryopreserved and transferred with replacement therapy. Cryopreservation was performed at either the pronuclear or 2- to 4-cell stage using 1.5 M 1,2-propanediol as the cryoprotectant.

Results: In this study 102 cycles (11% of the initiated cycles) were included. In group A (mean patient age 31.4 ± 2.4 years), a median of 3.4 ± 0.5 fresh embryos were transferred per cycle. In group B (mean patient age 31.0 ± 2.9 years), a median of 8.7 ± 3.0 embryos were cryopreserved (68% 2PN stage, 32% 24-cell stage). Frozen–thawed embryos were transferred in 75% of group B patients. The embryo survival rate post-thaw was 73%, and a median of 3.2 ± 1.0 embryos were transferred.