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Severe cytoplasmic abnormalities of the oocyte decrease cryosurvival and subsequent embryonic development of cryopreserved embryos

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BACKGROUND: Abnormalities of oocyte morphology affect embryo quality and viability. Whether morphological abnormalities of the oocyte influence cryosurvival and further development of derived embryos is not known. The aim of this study was to compare cryosurvival and progression to the blastocyst stage of frozen–thawed embryos derived from normal and abnormal oocytes. METHODS: A total of 5292 Grade 1 and 2 embryos from 964 women were frozen, thawed and subsequently cultured up to the blastocyst stage. The study was performed on excess embryos from patients who did not opt for cryopreservation. Cryosurvival, progression to the blastocyst stage and hatching were correlated with morphological characteristics of the oocytes that embryos were derived from. RESULTS: Presence of a cytoplasmic abnormality of the oocyte significantly decreased cryosurvival. This detrimental effect was more pronounced in embryos derived from oocytes with vacuolar cytoplasm or with central granulation. Furthermore, these embryos did not have the potential to develop into good quality blastocysts or reach the hatching stage. On the other hand, presence of a single extracytoplasmic abnormality of the oocyte did not affect cryosurvival and the potential to develop into good quality blastocysts. Grade 2 embryos derived from oocytes with irregular shape or a large perivitelline space had decreased cryosurvival. However when these embryos survived cryopreservation, their potential to develop good quality blastocysts or to reach hatching stage was unaffected. CONCLUSIONS: Embryos derived from oocytes with vacuolar cytoplasm or central granulation do not seem to bear the potential to develop good quality blastocysts or to reach hatching stage after cryopreservation. The presence of extracytoplasmic abnormalities alone does not affect blastocyst development despite decreasing cryosurvival.

Clinicaltrials.gov Trial registration number NCT00521443.

Keywords: oocyte morphology; cryopreservation; cytoplasmic abnormality; blastocyst quality; hatching blastocyst

Introduction

Assessment of oocyte morphology is an important task since deviations from normal may affect embryo development and its potential for implantation and healthy pregnancy/delivery (Serhal *et al*., 1997; Ebner *et al*., 2008a,b). Of the oocytes that were not fertilized, 13% were shown to be morphologically abnormal (Van Blerkom and Henry, 1992). When cumulus cells are denuded for ICSI, approximately 60–70% of the oocytes retrieved following controlled ovarian stimulation show abnormal morphological characteristics (Veeck, 1998). Morphologic abnormalities may either be extracytoplasmic or cytoplasmic (Van Blerkom, 1990). Oocyte morphology, particularly in its severe forms that involve the cytoplasm, has been shown to affect embryo quality (Balaban and Urman, 2006). Although it is generally accepted that extracytoplasmic abnormalities of the oocyte do not affect embryo quality, Ebner *et al*. recently reported that the preimplantation development of embryos derived from ovoid oocytes with abnormal cleavage pattern was delayed (Balaban and Urman, 2006; Ebner *et al*., 2008a,b). Cytoplasmic abnormalities such as central granulations and vacuoles may indicate genetic, epigenetic or metabolic defects and thus give rise to morphologically and/or genetically abnormal embryos (Kahraman *et al*., 2000; Yakin *et al*., 2007).

The effect of cleavage stage morphology on cryosurvival and further embryo development has been previously studied (Karlström *et al*., 1997; Salumets *et al*., 2003; Veeck, 2003; Anderson *et al*., 2004). However, it is not known whether cryosurvival and blastocyst formation of cryopreserved and thawed embryos are affected by the morphology of the oocyte they were derived from.

The aim of this study was to compare survival, blastocyst formation and hatching rates of frozen–thawed Day 3 cleavage

stage embryos derived from morphologically abnormal or normal metaphase II (MII) oocytes.

Material and Methods

The study was performed at the assisted reproduction unit of a private tertiary care hospital between 2004 and 2007, on Day 3 cleavage stage embryos obtained from 964 patients. A total of 5292 embryos were frozen and subsequently cultured up to the blastocyst stage after thawing. The study was performed on excess embryos from patients who did not accept cryopreservation and subsequent transfer for financial or other reasons. All patients gave informed consent for embryo cryopreservation and subsequent thaw and *in vitro* culture. Following a maximum of 6 day *in vitro* culture period all embryos were discarded. None was transferred.

The women were stimulated with recombinant FSH in either a long GnRH agonist or a GnRH antagonist protocol. Controlled ovarian stimulation, oocyte recovery, *in vitro* culture and embryo transfer were performed as previously described (Balaban and Urman, 2005; Urman *et al*., 2007). Fertilization was affected by ICSI due to the presence of male factor infertility in the majority of cases. Treatment cycles with testicular or epididymal spermatozoa were excluded. Following the transfer of fresh embryos on Day 3, all couples were offered the option to cryopreserve their excess good quality embryos when available. Only good quality embryos having five or more equal and homogeneous blastomeres with $<20\%$ fragmentation on Day 3 were deemed suitable for cryopreservation. Embryos that had evenly shaped, equal sized blastomeres and no fragmentation were defined as Grade 1 embryos, whereas embryos that had evenly shaped and equal sized blastomeres, and 1–20% fragmentation were defined as Grade 2 embryos. Slow freezing method was used as previously described (Balaban *et al*., 2007; Urman *et al*., 2007). Cryosurvival was assessed according to Rienzi *et al*., (2002). Frozen–thawed embryos were considered to have survived if more than 50% of the blastomeres were intact or had at least three viable cells present at thawing, while showing at least one blastomere divided by 18 h of post-thaw culture. Blastomeres that were degenerated upon thawing were not removed.

Each embryo was cultured and followed individually. Characteristics of the oocyte from which the embryo was derived were recorded and correlated with cryosurvival, progression to the blastocyst stage, blastocyst quality and the occurrence of *in vitro* hatching. Blastocysts were graded according to a three part scoring system based on blastocyst expansion, inner cell mass and trophoectoderm development (Gardner and Schoolcraft, 1999). Embryos were studied under four groups (Table I), further divided into subgroups as the following; Group 1: embryos derived from morphologically normal MII oocytes, Group 2: embryos derived from MII oocytes with a single extracytoplasmic abnormality, Group 2.1 with irregular shape; Group 2.2 with large perivitelline space; Group 2.3, dark zona pellucida; Group 3: embryos derived from MII oocytes with a single cytoplasmic abnormality, Group 3.1, with dark cytoplasm with slight granulation; Group 3.2, with vacuolar cytoplasm; Group 3.3, central granulation. Vacuolar cytoplasm and central granulation were regarded as severe cytoplasmic abnormalities, Group 4: embryos derived from MII oocytes with multiple morphological abnormalities (extracytoplasmic $\pm$ cytoplasmic abnormalities), Group 4.1, MII oocytes with any two extracytoplasmic abnormalities; Group 4.2, with two cytoplasmic abnormalities; Group 4.3, with one extracytoplasmic and one cytoplasmic abnormality; Group 4.4, with three abnormalities confined to the extracytoplasmic components; Group 4.5, with three abnormalities belonging to both extracytoplasmic and cytoplasmic components. Each group is presented with a separate column in Tables II and III.

Statistical methods

The data were analysed in 12 groups under three categories. Four parameters were evaluated in each group. Significance testing was omitted in order to avoid false-positive results due to multiple comparisons. Relative risks and 95% confidence intervals (CIs) were calculated for each variable as compared to the corresponding values in the reference group, i.e. embryos derived from morphologically normal MII oocytes. The difference was considered statistically significant when the 95% CI excluded one.

Results

A total of 5292 embryos from 964 treatment cycles were analysed. All embryos were cryopreserved on Day 3 and subsequently thawed and observed under *in vitro* culture. The mean age of the women from which the embryos were derived was 32.4 years (range: 22–37).

Baseline characteristics of frozen embryos

The majority of the cryopreserved Grade 1 and 2 embryos were derived from normal MII oocytes or oocytes with only a single extracytoplasmic abnormality (26.4 and 26.8%, respectively). Embryos derived from oocytes with severe cytoplasmic or multiple cytoplasmic abnormalities (3.5 and 3.3%, respectively) constituted only 6.8% of the total. Day 3 embryos with eight blastomeres were also more frequently derived from normal oocytes or oocytes with extracytoplasmic abnormalities (Table II).

Cryosurvival and blastocyst development of cryopreserved embryos derived from oocytes with a single extracytoplasmic abnormality

Although the presence of a single extracytoplasmic abnormality did not affect the cryosurvival rate of Grade 1 or 8 cell embryos, cryosurvival rate of Grade 2 embryos derived from oocytes with irregular shapes or with a large perivitelline space was significantly decreased when compared with embryos derived from morphologically normal MII oocytes. However subsequent to cryosurvival, blastocyst development was not affected. Likewise, blastocyst quality and rate of hatching blastocysts were similar to that of embryos derived from normal MII oocytes. Dark appearance of zona pellucida did not have any effect on cryosurvival or blastocyst development rates (Table II; columns 3, 4 and 5).

Cryosurvival and blastocyst development of cryopreserved embryos derived from oocytes with a single cytoplasmic abnormality

Presence of a single cytoplasmic abnormality in the oocyte significantly decreased cryosurvival of embryos. Cryosurvival rate was almost halved in embryos derived from oocytes with vacuolar cytoplasm or central granulation. The effect of dark cytoplasm with slight granulation was not as pronounced (Table II; columns 6, 7 and 8). Although a smaller proportion of embryos derived from oocytes with vacuolar cytoplasm or central granulation reached to the blastocyst stage after thawing as compared to embryos derived from normal MII oocytes, the differences reached statistical significance only for Grade 2 embryos. However, other than only one 8-cell

Table I. Characteristics of the study groups.

Group	Origin of embryos	Subgroup	Morphologic abnormalities of the oocytes
1	Morphologically normal MII oocytes	None	Morphologically normal MII oocytes
2	MII oocytes with a single extracytoplasmic abnormality	Group 2.1	Irregular shape
		Group 2.2	Large perivitelline space
		Group 2.3	Dark zona pellucida
3	MII oocytes with a single cytoplasmic abnormality	Group 3.1	Dark cytoplasm with slight granulation
		Group 3.2	Vacuolar cytoplasm
		Group 3.3	Central granulation
4	MII oocytes with multiple morphological abnormalities	Group 4.1	Any two extracytoplasmic abnormalities
		Group 4.2	Two cytoplasmic abnormalities
		Group 4.3	One extracytoplasmic and one cytoplasmic abnormality
		Group 4.4	Three abnormalities confined to the extracytoplasmic components
		Group 4.5	Three abnormalities belonging to both extracytoplasmic and cytoplasmic components

MII, metaphase II.

Grade 2 embryo, none of these embryos, developed into a good quality blastocyst. No embryo in these groups reached the hatching stage either (Table II; columns 7 and 8). On the other hand, blastocyst development rate of embryos derived from oocytes with dark cytoplasm with slight granulation, and the incidence of good quality and hatching blastocysts were not significantly decreased as compared to embryos derived from morphologically normal oocytes (Table II; column 6).

Progression to the blastocyst stage subsequent to thawing was severely affected in embryos derived from oocytes with vacuolar cytoplasm or central granulation. Only 8.3% of the thawed embryos derived from these oocytes eventually resulted in good quality blastocysts, however, none of these hatched (Table II; columns 7 and 8).

Cryosurvival and blastocyst development of cryopreserved embryos derived from oocytes with multiple morphological abnormalities

Presence of multiple morphological abnormalities of the oocyte significantly decreased cryosurvival rates of embryos derived from these oocytes (Table III; rows 4, 10 and 16). This effect was more prominent in embryos derived from oocytes with multiple cytoplasmic anomalies as compared to those with only extracytoplasmic anomalies (Table III, columns 3, 5 and 6).

Blastocyst development rate of cryosurvived embryos, incidence of good quality and hatching blastocysts were not significantly decreased for embryos derived from oocytes with multiple abnormalities confined to the extracellular compartment (Table III; columns 3 and 6). The only exception was the significant decrease in the blastocyst development rate of Grade 2 embryos derived from oocytes with triple extracytoplasmic abnormalities (Table III; column 6).

Blastocyst development rate of embryos derived from oocytes with two cytoplasmic abnormalities were decreased as compared to embryos derived from normal MII oocytes; however, differences were not statistically significant for Grade I embryos or embryos with eight blastomeres. None of the embryos derived from such oocytes developed into a good quality blastocyst or reached the hatching stage (Table III; column 4).

Blastocyst development rate of embryos derived from oocytes with an extracytoplasmic abnormality combined with a cytoplasmic abnormality was significantly lower as compared to embryos developed from normal MII oocytes. The incidence of good quality blastocysts was significantly lower in this group, with the exception of Grade 1 embryos. Moreover, none of these embryos hatched *in vitro* (Table III; column 5).

Cryosurvival and blastocyst development rate of embryos derived from oocytes with three morphological abnormalities at least one being cytoplasmic was significantly decreased as compared to embryos derived from normal oocytes. None of the embryos derived from such oocytes developed into a good quality or hatched *in vitro* (Table III; column 7).

Discussion

Our results indicate that oocyte morphology affects cryosurvival and blastocyst development of derived embryos that were cryopreserved, subsequently thawed and cultured up to the blastocyst stage.

It is very difficult to estimate the impact of oocyte morphology on the implantation potential of the derived embryo when multiple embryos are transferred. Furthermore, given the fact that transition from the gamete to the embryo is a continuum, it is difficult to evaluate the contribution of the oocyte on implantation potential of the derived embryo. Even if clinical outcome of frozen–thawed embryo transfer cycles in relation to oocyte morphology cannot be inferred from the results of this study we showed that the developmental behavior of frozen–thawed embryos derived from dysmorphic oocytes was similar when compared to results from the literature reported for fresh embryos (Balaban and Urman, 2006; Ebner *et al*., 2006).

It can be stated that cryosurvival and subsequent development of embryos derived from oocytes with a single extracytoplasmic defect are not affected. Although absolute differences in cryosurvival rates have reached statistical significance in a relatively large sample, clinical significance of these differences is doubtful. On the other hand, severe cytoplasmic defects of the oocytes significantly hamper cryosurvival and progression to the blastocyst stage. This detrimental effect is

Table II. Outcome of embryos developed from oocytes bearing a single morphologic anomaly.

	Normal MII Oocytes	Irregular shape	Large perivitelline space	Dark zona pellucida	Dark cytoplasm with slight granulation	Vacuolar cytoplasm	Central granulation
Grade 1 Embryos							
No	352 (6.7)	117 (2.2)	122 (2.3)	109 (2.1)	100 (1.9)	12 (0.2)	14 (0.3)
Cryosurvival	317 (90.0)	99 (84.6) (0.94, 0.86–1.02)	100 (81.9) (0.91, 0.83–1.0)	93 (85.3) (0.95, 0.87–1.03)	80 (80)[a] (0.89, 0.8–0.99)	6 (50)[a] (0.56, 0.31–0.98)	7 (50)[a] (0.56, 0.33–0.94)
Blastocyst development	170 (53.6)	50 (50.5) (0.94, 0.76–1.17)	49 (49) (0.91, 0.73–1.14)	46 (49.4) (0.92, 0.73–1.16)	39 (48.8) (0.91, 0.71–1.16)	2 (33.3) (0.62, 0.2–1.94)	2 (28.5) (0.53, 0.16–1.73)
Good quality blastocyst	106 (62.3)	30 (60) (0.96, 0.75–1.24)	29 (59.1) (0.95, 0.73–1.23)	29 (63.0) (1.01, 0.79–1.30)	22 (56.4) (0.90, 0.67–1.22)	0	0
Hatching blastocyst	48 (28.2)	14 (28.0) (1.03, 0.67–1.59)	13 (26.5) (0.99, 0.63–1.56)	12 (26)(0.91, 0.56–1.48)	10 (25.6) (1.0, 0.61–1.66)	0	0
Grade 2 Embryos							
No	1047 (19.8)	363 (6.9)	393 (7.4)	318 (6.0)	321 (6.1)	96 (1.8)	68 (1.3)
Cryosurvival	914 (87.2)	297 (81.8)[a] (0.94, 0.89–0.99)	318 (80.9)[a] (0.93, 0.88–0.98)	266 (83.6) (0.96, 0.91–1.01)	258 (80.3)[a](0.92, 0.87–0.98)	43 (44.7)[a] (0.51, 0.41–0.64)	29 (42.6)[a] (0.49, 0.37–0.64)
Blastocyst development	482 (52.7)	151 (50.8) (0.96, 0.85–1.10)	155 (48.7) (0.92, 0.81–1.05)	133 (50) (0.95, 0.83–1.09)	126 (48.8) (0.93, 0.81–1.06)	10 (23.2)[a] (0.44, 0.26–0.76)	6 (20.6)[a] (0.39, 0.19–0.80)
Good quality blastocyst	296 (61.4)	89 (58.9) (0.96, 0.83–1.12)	93 (60.0) (0.98, 0.84–1.13)	81 (60.9) (0.99, 0.85–1.16)	72 (57.1) (0.93, 0.79–1.10)	1 (10.0) (0.16, 0.03–1.05)	0
Hatching blastocyst	137 (28.4)	43 (28.4) (1.04, 0.82–1.34)	40 (25.8) (0.93, 0.71–1.21)	35 (26.3) (0.93, 0.71–1.23)	30 (23.8) (0.90, 0.67–1.21)	0	0
8 cell embryos							
No	570 (29.5)	180 (9.3)	199 (10.3)	166 (8.6)	168 (8.7)	21 (1.0)	22 (1.1)
Cryosurvival	514 (90.1)	152 (84.4) (0.94, 0.87–1.00)	168 (84.4) (0.94, 0.88–1.00)	142 (85.5) (0.95, 0.89–1.02)	138 (82.1)[a] (0.91, 0.84–0.98)	10 (47.6)[a] (0.53, 0.34–0.83)	10 (45.4)[a] (0.50, 0.32–0.80)
Blastocyst development	280 (54.4)	80 (52.6) (0.97, 0.81–1.15)	84 (50)(0.92, 0.77–1.09)	73 (51.4) (0.94, 0.79–1.13)	68 (49.2) (0.90, 0.75–1.09)	3 (30) (0.55, 0.21–1.42)	3 (30) (0.55, 0.21–1.42)
Good quality blastocyst	180 (64.2)	48 (60.0) (0.93, 0.76–1.14)	51 (60.7) (0.94, 0.78–1.15)	46 (63.0) (0.98, 0.81–1.19)	39 (57.3) (0.89, 0.71–1.11)	1 (31.3) (0.52, 0.10–2.58)	0
Hatching blastocyst	82 (29.2)	24 (30) (1.10, 0.79–1.52)	22 (26.1) (0.95, 0.67–1.35)	20 (27.3) (0.95, 0.66–1.38)	17 (25) (0.96, 0.65–1.41)	0	0

Values are *N* (%) (relative risk, 95% confidence interval as compared to embryos derived from morphologically normal metaphase two oocytes); [a]significantly different as compared to embryos derived from morphologically normal metaphase two oocytes.

Table III. Outcome of embryos developed from oocytes bearing multiple morphologic anomalies.

	Normal MII Oocytes	Double extracytoplasmic anomalies	Double cytoplasmic anomalies	Double combined anomalies	Triple extracytoplasmic anomalies	Triple combined anomalies
Grade 1 Embryos						
No	352 (6.7)	153 (2.9)	16 (0.3)	84 (1.6)	79 (1.5)	48 (0.9)
Cryosurvival	317 (90.0)	121 (79)[a] (0.88, 0.80–0.96)	5 (31.2)[a] (0.38, 0.17–0.72)	39 (46.4)[a] (0.52, 0.41–0.65)	56 (70.8)[a] (0.79, 0.68–0.91)	18 (37.5)[a] (0.42, 0.29–0.60)
Blastocyst development	170 (53.6)	59 (48.7) (0.91, 0.74–1.13)	1 (20.0)(0.37, 0.06–2.16)	11 (28.2)[a] (0.53, 0.32–0.88)	26 (46.4) (0.87, 0.64–1.17)	4 (22.2)[a] (0.41, 0.17–0.99)
Good quality blastocyst	106 (62.3)	33 (56.0) (0.90, 0.67–1.16)	0	2 (18.1) (0.29, 0.08–1.03)	14 (53.8)(0.86, 0.59–1.26)	0
Hatching blastocyst	48 (28.2)	14 (23.7) (0.94, 0.60–1.47)	0	0	6 (23) (0.95, 0.50–1.79)	0
Grade 2 Embryos						
No	1047 (19.8)	477 (9.0)	161 (3.0)	334 (6.3)	315 (6.0)	193 (3.6)
Cryosurvival	914 (87.2)	375 (78.6)[a] (0.90, 0.85–0.95)	50 (31.0)[a] (0.36, 0.28–0.45)	155 (46.4)[a] (0.53, 0.47–0.60)	226 (71.7)[a] (0.82, 0.76–0.88)	72 (37.3)[a] (0.43, 0.36–0.51)
Blastocyst development	482 (52.7)	182 (49.5) (0.92, 0.82–1.04)	7 (14.0)[a] (0.27, 0.13–0.53)	41 (26.4)[a] (0.50, 0.38–0.66)	101 (44.6)[a] (0.85, 0.72–0.99)	12 (16.6)[a] (0.32, 0.19–0.53)
Good quality blastocyst	296 (61.4)	102 (56.0) (0.91, 0.79–1.06)	0	8 (19.5)[a] (0.32, 0.17–0.59)	55 (54.4) (0.89, 0.73–1.07)	0
Hatching blastocyst	137 (28.4)	42 (23) (0.90, 0.68–1.16)	0	0	22 (21.7) (0.86, 0.61–1.22)	0
8 cell embryos						
No	570 (29.5)	237 (12.2)	20 (1)	134 (6.9)	138 (7.1)	72 (3.7)
Cryosurvival	514 (90.1)	188 (79.3)[a] (0.88, 0.82–0.94)	7 (35.0)[a] (0.39, 0.21–0.71)	64 (47.7)[a] (0.53, 0.44–0.63)	101 (73.1)[a] (0.88, 0.80–0.96)	29 (40.2)[a] (0.45, 0.34–0.59)
Blastocyst development	280 (54.4)	94 (50) (0.92, 0.78–1.08)	2 (28.3) (0.52, 0.16–1.70)	18 (28.1)[a] (0.52, 0.35–0.77)	47 (46.5) (0.85, 0.68–1.07)	6 (20.6) (0.38, 0.19–0.78)
Good quality blastocyst	180 (64.2)	54 (57.4) (0.89, 0.74–1.09)	0	4 (22.2)[a] (0.36, 0.15–0.82)	26 (55.3) (0.86, 0.66–1.13)	0
Hatching blastocyst	82 (29.2)	23 (24.5) (0.93, 0.66–1.32)	0	0	11 (23.4) (0.93, 0.58–1.50)	0

Values are *N* (%) (relative risk, 95% confidence interval as compared to embryos derived from morphologically normal metaphase two oocytes); [a]significantly different as compared to embryos derived from morphologically normal metaphase two oocytes.

more pronounced in embryos derived from oocytes with vacuolar cytoplasm or with central granulation. Frozen–thawed embryos derived from such oocytes do not have the potential to develop into good quality blastocysts or to hatch *in vitro*. Further embryonic development is affected in the presence of even only one severe cytoplasmic defect. Therefore, it maybe concluded that extracytoplasmic structural changes should not be considered as abnormalities but only phenotypic deviations from the normal. Severe cytoplasmic abnormalities, however, should be considered as genuine abnormalities and patients should be informed regarding the poor developmental outcome of embryos derived from these types of oocytes. Two severe cytoplasmic defects were studied. One was the appearance of translucent vacuoles in the cytoplasm that might be also named as smooth endoplasmic reticulum clusters (SERC) (Meriano *et al*., 2001; Otsuki *et al*., 2004). These translucent vacuoles should be differentiated from membrane bound cytoplasmic inclusions filled with fluid that is virtually identical to perivitelline fluid (Van Blerkom, 1990). Membrane bound vacuoles are spherical in shape and are thought to form during the maturation period between metaphase I and MII stages (Ebner *et al*., 2005). The second cytoplasmic defect was the appearance of a large, dark, spongy granulated area in the cytoplasm, which was previously defined as centrally located granular cytoplasm (CLGC) or as organelle clustering (Kahraman *et al*., 2000; Meriano *et al*., 2001).

Different hypotheses might be introduced to explain the decreased cryosurvival and blastocyst development rates from morphologically good looking embryos that are derived from oocytes with severe cytoplasmic defects. One is the increased rate of aneuploidy found in the embryos derived from such oocytes. Van Blerkom and Henry (1992) showed that as many as half of the oocytes with dysmorphic phenotypes such as organelle clustering are aneuploid, with hypohaploidy being the predominant abnormality. MII oocytes that showed such severe cytoplasmic disorganization had a lower intracytoplasmic pH and ATP content as well as increased incidence of aneuploidy and chromosomal scattering (Van Blerkom *et al*., 1997). A more recent study by our group showed that cytoplasmic and multiple abnormalities where at least one cytoplasmic abnormality was included significantly impaired blastocyst development (Yakin *et al*., 2007). Although a 20% higher aneuploidy rate was found among embryos derived from these oocytes the difference did not reach statistical significance.

In a similar study, a very high aneuploidy rate (52.2%) was reported in embryos derived from oocytes with CLGC (Kahraman *et al*., 2000). Although the cleavage stage embryo quality and pregnancy rates were not significantly different after transfer of embryos derived from oocytes with or without granulation, implantation and ongoing pregnancy rates were lower in the presence of CLGC (Kahraman *et al*., 2000). Meriano *et al*. (2001) showed that intracytoplasmic organelle clustering is the only severe abnormality found significantly repetitive in consecutive cycles and is a negative predictor of pregnancy and implantation rates, even though the cleavage stage embryo quality was not affected.

The appearance of translucent vacuoles (SERC) is a more complex phenomenon since the mechanism responsible for these abnormalities has not been elucidated. Smooth endoplasmic reticulum (SER) is the second most common organelle in the ooplasm after mitochondria, and exists in two forms: isolated vesicular SER that is evenly distributed in the ooplasm, or peripheral aggregates of smaller elements of SER that are tubular or irregular in shape (Sundstrom and Nilsson, 1989; Sathananthan *et al*., 1993). These aggregates increase in content during preovulatory maturation and are evidently sensitive to gonadotrophin stimulation.

Otsuki *et al*. (2004) studied the relationship between the pregnancy outcome and SERC in MII human oocytes. They compared the embryonic development and clinical outcome from oocytes with and without SERC. The rate of excellent quality embryos was significantly higher in SERC(−) cycles. Although fertilization rates, cleavage stage embryo quality and cell division rates during the early developmental stages were identical in the two groups, clinical pregnancy as well as implantation rates were significantly decreased in SERC(+) cycles (Otsuki *et al*., 2004).

Recently Ebner *et al*. (2008a,b) indicated the importance of SERCs in the cytoplasm of the oocyte. They showed lower fertilization (58.9 versus 77.4%) and blastulation rates (44.0 versus 87.7%) in oocytes bearing this cytoplasmic abnormality compared to unaffected sibling oocytes. Although the clinical pregnancy rate was decreased in cycles where embryos derived from SERC positive oocytes were transferred, the difference failed to reach statistical significance as compared to transfer of embryos derived from SERC negative oocytes (26.7 versus 41.1%, respectively). Patients who had one or more gametes showing SERC had significantly higher spontaneous abortion rates. Once pregnancy was achieved, significantly higher obstetric problems as well as neonatal deaths were observed in SERC positive cycles. Birthweight was also significantly lower in the group of patients who had at least one or more gametes affected (Ebner *et al*., 2008a,b). On the basis of the results of this study, it may be concluded that embryos derived from normal or abnormal oocytes show different developmental capacities. Furthermore, morphology of the oocyte may affect pregnancy outcome. This may be related to the intrinsic oocyte-specific defects of important molecular and cellular activities that might not be detectable with conventional microscopic techniques.

Beckwith–Wiedemann syndrome was diagnosed in a newborn following the transfer of embryos derived from SERC (+) oocytes, which leads to the question whether inheritance of this disease is causally related to the application of assisted reproductive technology (Gosden *et al*., 2003; Maher *et al*., 2003). It is difficult to speculate whether the diminished potential of blastocyst formation of embryos derived from oocytes with vacuolization might be an epigenetic effect since there is no evidence linking SERCs to genomic imprinting defects.

Other defects, such as gene expression alterations in the embryos derived from oocytes with severe cytoplasmic alterations, might be a possible cause of failure in blastocyst formation. Wells *et al*. (2005) assessed a small number of embryos derived from centrally granulated oocytes for gene expression on Day 3 post-fertilization and found out that this

type of oocyte morphology is associated with altered BUB1 and BRCA1 (breast cancer) expression. BRCA1 has a central role in DNA damage repair and it may be speculated that these embryos may have received damaged maternal DNA or accumulated DNA damage during the first mitotic divisions.

An alternative hypothesis to explain reduced cryosurvival of embryos derived from oocytes with a non-homogenous cytoplasm may be prevention of dissemination of the cryoprotectant within the cell due to the presence of cytoplasmic vacuoles or centrally located granulation. Proper dissemination of the cryoprotectant within the cell is crucial for cryosurvival.

There may be downstream effects on cell function and developmental competence that arise in the oocyte and in some cases have discrete cytoplasmic phenotypes. The SERC is probably the most concerning because of the possibility of abnormal calcium regulation early on which can perturb development later. Significant mitochondrial clustering may perturb local pH that interferes with normal signal transduction in the oocyte and early embryo (J. VanBlerkom, personal communication).

In conclusion, presence of extracytoplasmic abnormalities alone does not affect blastocyst development despite decreasing cryosurvival. However, embryos derived from oocytes with vacuolar cytoplasm or central granulation do not seem to bear the potential to develop into good quality blastocysts or to reach hatching stage after cryopreservation. These cytoplasmic abnormalities may be reflections of genetic, epigenetic or metabolic defects in the oocyte. Embryos with severe cytoplasmic abnormalities comprise around 5% of all embryos suitable for cryopreservation. Only one of the 203 embryos that had originated from an oocyte with at least one severe cytoplasmic abnormality (Groups 3.2, 3.3 and 4.2) developed into a good quality blastocyst, moreover none of these embryos reached the hatching stage. Although transfer of such embryos may not affect overall success rate of a cryopreservation program, from a clinical standpoint priority should be given to the transfer of embryos developed from oocytes without cytoplasmic abnormalities whenever possible. Women who have all of their excess embryos derived from oocytes bearing severe cytoplasmic abnormalities should be counseled about the reduced chance of these embryos developing into good quality blastocysts or reaching the hatching stage. It is difficult to assume that transfer of such an embryo will result in pregnancy. Cryopreservation and subsequent transfer of embryos derived from oocytes with severe cytoplasmic abnormalities should be avoided.

Author role

B.B.—Design and institution of the study protocol, collection of data, drafting the article, and approval of the final version.

B.A.—Collection and analysis of the data, review and final preparation of the article, and approval of the final version.

A.I.—Institution of the study protocol, collection of data.

K.Y.—Reviewing the article, approval of the final version.

B.U.—Design of the study protocol, review and final preparation of the article, approval of the final version.

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