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The negative influence of sperm cryopreservation on the quality and development of the embryo depends on the morphology of the oocyte

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SUMMARY

The present case–control study aimed to identify the effect of sperm cryopreservation on the quality of the embryo and on the probability of blastocyst formation when oocytes free of dimorphisms are injected and when at least one dymorphism is present. The study included 22 186 zygotes, obtained from 2802 patients undergoing intracytoplasmic sperm injection cycles, in a private assisted reproduction center, using either fresh or cryopreserved sperm. The effect of sperm cryopreservation on the embryo quality on cleavage stage and blastocyst formation chance were evaluated when oocytes free of dimorphisms are injected and when at least one dymorphism is present. The quality of the embryo on cleavage stage as well as the chance for blastocyst formation was not influenced by the origin of the spermatozoa when the quality of the oocyte was not considered. When at least one oocyte defect was present, a negative influence of sperm cryopreservation on cleavage stage embryo quality and the chance for blastocyst formation was noted. In oocytes with extra-cytoplasmic dimorphisms, the injection of cryopreserved sperm did not affect the quality of the embryo during the cleavage stage, but did affect the chance for blastocyst formation. Conversely, in oocytes with intracytoplasmic defects, the quality of the embryos on cleavage stage and the chance of blastocyst formation were negatively influenced by the injection of cryopreserved sperm. The results suggest an oocyte quality-dependent negative effect of sperm cryopreservation on embryo quality and on the probability of blastocyst formation.

INTRODUCTION

The introduction of sophisticated assisted reproduction techniques (ART) during the last few decades, such as in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI), overcame severe problems with sperm concentration and motility (Steptoe & Edwards, 1978; Palermo *et al.*, 1992). One of the most significant achievements in ART was the development of the cryopreservation technique (Cohen *et al.*, 2012; Edgar & Gook, 2012). Since then, the use of sperm cryopreservation has evolved rapidly and has become a vital instrument in ART (Quaas *et al.*, 2013).

Sperm cryopreservation represents a valuable therapeutic option in the management of infertility that has become particularly important in cases of preservation of male fertility before cancer therapy (Hallak *et al.*, 2000).Other indications for sperm cryopreservation include: (i) mandatory use in donor semen programmes; (ii) patient's convenience (i.e. partner's absence where the ART is performed in the presence of normal sperm parameters); and (iii) fertility preservation for patients undergoing vasectomy, when 'banking' may provide a future spermatozoa source for possible use in ART. Although the cryopreservation of spermatozoa is an important routine technique, during the process of cooling, freezing and thawing, spermatozoa are subjected to a series of drastic changes with respect to their environment (Gandini *et al.*, 2006), which may cause cell damage. The cryopreservation of human spermatozoa is known to result in diminished motility and morphology (Nallella *et al.*, 2004). A possible link between sperm cryoinjury and the early events of fertilisation has been proposed (Nishizono *et al.*, 2004; Lewis & Aitken, 2005), however no correlation with the cleavage rate and blastocyst formation was observed.

Despite that sperm survival and ICSI outcomes following the cryopreservation/thawing of donor sperm samples are favourable, the use of frozen/thawed spermatozoa from infertile men results in parameters that have been shown to be poor. Although

the ICSI may circumvent some of the problems with sperm quality, whether ICSI can overcome the effect of the cryo-damage of spermatozoa after the freezing/thawing processes and, therefore, avoid detrimental effects on the quality and development of the embryo, has yet to be elucidated.

The quality of the oocyte has been described as a variable that influences the quality and development of derived embryos. To date, many published reports have focused on the impact of oocyte morphology on the quality of the embryo (Wilding *et al.*, 2007; Braga *et al.*, 2013). Various factors may be responsible for the variation in the quality of the embryo and in the rate of formation of the blastocyst. Considering the vital role played by the oocyte in the developmental process, the non-invasive identification of oocyte dimorphisms before fertilisation is extremely useful for the prediction of the rate of blastocyst formation (Braga *et al.*, 2013).

Therefore, the goal of the present study was to identify the effect of sperm cryopreservation on embryo quality and the chance of blastocyst formation when oocytes free of dimorphisms are injected and when at least one oocyte dimorphism is present.

MATERIALS AND METHODS

Study design

This study included 22 186 normally fertilised zygotes, which were obtained from 2802 patients who underwent ICSI cycles with either fresh or cryopreserved sperm. The oocytes were evaluated immediately before the sperm injection, and the embryos were evaluated 16–18 h post-ICSI and on days 2, 3 and 5 of development. The effect of sperm cryopreservation on embryo quality on days 2 and 3 and on the chance of blastocyst formation was evaluated. In addition, the effect of sperm cryopreservation on embryo quality and blastocyst formation, when at least one oocyte dimorphism was present was investigated.

Moreover, the embryos were split into four different experimental groups: (i) fresh spermatozoa with no oocyte defects (FSNOD); fresh spermatozoa with oocyte defects (FSOD); cryopreserved sperm with no oocyte defects (CSNOD); and cryopreserved sperm with oocyte defects (CSOD). The embryo quality on days 2 and 3 and the chance of blastocyst formation were compared among the experimental groups.

To elucidate whether the oocyte quality-dependent influence of the injection of cryopreserved sperm was because of an extracytoplasmic or intracytoplasmic oocyte defect, two more groups were formed: Embryos derived from oocytes with at least one intracytoplasmic defect and embryos derived from oocytes with at least one extracytoplasmic defect. All cases of surgically retrieved spermatozoa were excluded from the study. Written informed consent, in which patients agreed to share the outcomes of their cycles for research purposes, was obtained, and the local institutional review board approved the study.

Controlled ovarian stimulation

Controlled ovarian stimulation was achieved with a daily dose of recombinant FSH (Gonal-F; Serono, Geneva, Switzerland), starting on day 3 of the cycle. Pituitary blockage was performed with a GnRH antagonist (Cetrotide; Serono, Geneva, Switzerland), starting when at least one follicle ≥14 mm in diameter was visualised.

Follicular growth was monitored using transvaginal ultrasound examination starting on day 4 of gonadotropin administration. When adequate follicular growth and serum E2 levels were observed, recombinant hCG (Ovidrel; Serono, Geneva, Switzerland) was administered in order to trigger the final follicular maturation. The oocytes were collected 35 h after the administration of hCG through transvaginal ultrasound-guided ovum pick-up.

Preparation of oocytes

The retrieved oocytes were maintained in the culture medium (Global for fertilisation; LifeGlobal, CT, USA LifeGlobal, Connecticut, USA) supplemented with 10% protein supplement (LGPS; LifeGlobal) and covered with paraffin oil (Paraffin oil P.G.; LifeGlobal) for 2–3 h before the removal of cumulus cells. The surrounding cumulus cells were removed after exposure to a HEPES-buffered medium that contained hyaluronidase (80 IU/mL; LifeGlobal). The remaining cumulus cells were mechanically removed by gently pipetting with a hand-drawn Pasteur pipette (Humagen Fertility Diagnostics, Charlottesville, VA, USA).

The oocyte morphology was assessed just before the injection of sperm (4 h after retrieval), using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon, Tokyo, Japan) with a Hoffmann modulation contrast system under 400× magnification. The following oocyte dysmorphisms were recorded: (i) cytoplasmic granularity, (ii) cytoplasmic colour, (iii) vacuoles in the ooplasm, (iv) aggregates of smooth endoplasmic reticulum clusters in the ooplasm, (v) large perivitelline space (PVS), (vi) PVS granularity, (vii) fragmented polar body (PB), (viii) abnormalities in the zona pellucida (ZP) and (ix) abnormalities in the oocyte shape.

Oocytes that had released the first PB were considered mature and were used for ICSI.

Intracytoplasmic sperm injection

Intracytoplasmic sperm injection was performed in a microinjection dish prepared with 4-μL droplets of buffered medium (Global w/HEPES; LifeGlobal) and covered with paraffin oil on the heated stage of an inverted microscope (37.0 ± 0.5 °C). Approximately 16 h after ICSI, fertilisation was confirmed by the presence of two pronuclei and the extrusion of the second PB. Embryos were maintained in a 50-μL drop of culture medium (Global; LifeGlobal) supplemented with 10% protein supplement and covered with paraffin oil in a humidified atmosphere under 6% CO_2 at 37 °C for 3 days.

Evaluation of the morphology of the embryo

The morphology of the embryo was assessed at the zygote stage (16–18 h post-ICSI) and on the mornings of days 2, 3, and 5 of embryonic development using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon) with a Hoffmann modulation contrast system under 400× magnification. The morphology of the embryo was also assessed immediately before embryo transfer.

To evaluate the morphology at the cleavage-stage, the following parameters were recorded: the number of blastomeres, the degree of fragmentation, the variation in blastomere symmetry, the presence of multinucleation and defects in the ZP and cytoplasm. The high-quality embryos in the cleavage stage were defined as those with all of the following characteristics: 4 cells

on day 2 or 8–10 cells on day 3, <15% fragmentation, symmetric blastomeres, the absence of multinucleation, colourless cytoplasm with moderate granulation and no inclusions, the absence of granularity of the PVS, and the absence of dysmorphism of the ZP. Embryos that lacked any of these characteristics were considered to be of low quality.

Embryos that reached the blastocyst status were considered when: (i) the blastocoel was greater than half the volume of the embryo; (ii) the blastocoel completely filled the embryo; (iii) the blastocysts were expanded; (iv) blastocyst hatching occurred; and (v), blastocysts hatched.

Statistical analyses

The characteristics of the patients and the cycles were compared between the groups using either chi-squared test or ANOVA. Dichotomous variables were evaluated using chi-squared test, and the results were expressed as percentages. Continuous variables were evaluated by ANOVA, and the results were expressed as the mean ± standard deviation.

A multiple logistic regression analysis was performed to evaluate the influence of sperm cryopreservation on embryo quality on days 2 and 3 and during blastocyst formation. All regression analyses were adjusted for maternal age, the number of retrieved oocytes, the total dose of FSH used for ovarian stimulation and the fertilisation rate, as these variables were considered potential confounders in the association between the factors evaluated and ICSI outcomes. Results were expressed as OR, regression coefficients, 95% confidence intervals and *p* values.

Chi-squared analyses were used to compare the embryo quality and the chance of blastocyst formation in the four different experimental groups: (i) FSNOD, FSOD, CSNOD and CSOD. Results were expressed as percentages.

The results were considered to be significant at the 5% critical level ($p < 0.05$). Data analysis was performed using the Minitab Statistical Software (version 16) Minitab Inc., Pennsylvania, USA.

RESULTS

The characteristics of the patients and the cycles were equally distributed among the groups (Table 1).

A total of 22 186 oocytes/embryos were evaluated. In all, 15 621 (70.4%) embryos were derived from oocytes that had at least one morphological defect while 2741 (12.3%) were derived from oocytes that were injected with the cryopreserved sperm.

The binary regression model showed that the embryo quality on days 2 and 3 and the chance of blastocyst formation were not influenced by the origin of the spermatozoa (i.e. fresh or cryopreserved) when the quality of the oocyte was not considered. However, when at least one oocyte defect was present, a negative influence of the sperm cryopreservation on the quality of the embryo with respect to the cleavage stage and blastocyst formation chance was noted (Table 2).

The injection of cryopreserved sperm into oocytes with extracytoplasmic dimorphisms did not affect the quality of the embryo at the cleavage stage, but did affect the chance of blastocyst formation. Conversely, the quality of the embryo on days 2 and 3 and the chance of blastocyst formation were negatively influenced by the injection of the cryopreserved sperm in oocytes with intracytoplasmic defects (Table 2).

The influence of sperm cryopreservation on the quality of the embryo, in the presence of oocyte dimorphisms, was not exclusively because of the morphological abnormality of the oocyte but rather, was a result of the association of both factors. A comparison was made between embryos derived from oocytes that were free of defects and those with at least one defect that were injected with the fresh or cryopreserved sperm.

The quality of the embryo during the cleavage stage and during blastocyst formation was higher among the embryos that were derived from oocytes free of defects and injected with fresh sperm; however, the quality was lower among the embryos that were derived from oocytes with at least one defect and injected with the cryopreserved sperm. When evaluated separately, a significant difference was observed between the quality of the embryo at the cleavage stage and during blastocyst formation in embryos derived from oocytes with at least one defect and those injected with fresh or cryopreserved sperm (Table 3).

In a further analysis, the cycles were split according to age: Patients ≤35 years old and patients >35 years. No significant differences among the groups were observed when the blastocyst formation was evaluated, however, a significant difference between the quality of the embryo at the cleavage stage was still noted. For younger patients the difference between groups was not as evident as that observed in the whole group, however, embryos derived from defective oocytes injected with cryopreserved sperm had a significantly lower quality than those derived from oocytes free of defects infected with fresh sperm. The same was observed for patients >35 years old for day 2 embryo quality, but not for day 3 embryo quality (Tables 4 and 5).

DISCUSSION

Despite its routine use in ART, it has been suggested that sperm cryopreservation may cause cell injury and reduce the proportion of fully functional sperm in a sample (Gandini *et al.*, 2006). The most important parameter that may be affected by sperm freezing is the motility (Nallella *et al.*, 2004), which is

Table 1 Comparison of the characteristics of the patients and the cycles among the four experimental groups

Variable	FSNOD (*n* = 720)	FSOD (*n* = 1715)	CSNOD (*n* = 115)	CSOD (*n* = 252)	*p*
Female age (years-old)	35.4 ± 4.4	35.7 ± 5.9	34.8 ± 4.2	35.1 ± 4.8	0.642
Male age (years-old)	37.2 ± 7.3	38.4 ± 3.2	37.8 ± 3.4	37.9 ± 2.3	0.532
BMI (kg/m^2)	24.1 ± 3.6	24.3 ± 3.3	24.2 ± 3.8	24.1 ± 3.7	0.546
FSH dose (IU)	2154.3 ± 533.5	2212.8 ± 570.5	2241.2 ± 589.4	2214.0 ± 556.6	0.234
No. of follicles	20.15 ± 15.2	20.2 ± 14.3	21.2 ± 14.9	20.1 ± 14.7	0.091
No. of oocytes	14.6 ± 12.2	15.6 ± 12.6	14.8 ± 10.4	16.9 ± 10.9	0.098
No. of MII oocytes	11.2 ± 6.2	11.6 ± 6.1	11.0 ± 6.7	11.3 ± 7.3	0.205
MII oocyte rate (%)	74.8 ± 19.9	72.4 ± 20.4	74.2 ± 18.4	75.9 ± 18.1	0.243

FSNOD, fresh sperm with no oocyte defects; FSOD, fresh sperm with oocyte defects; CSNOD, cryopreserved sperm with no oocyte defects; CSOD, cryopreserved sperm with oocyte defects; BMI, body mass index; MII, Metaphase.

Table 2 Multiple logistic regression analysis of factors that may affect the quality of the embryo and the chance of blastocyst formation during the cleavage stage, with the following predictive variables: (i) cryopreserved sperm used for sperm injection; (ii) fresh spermatozoa and the presence of at least one oocyte defect; (iii) cryopreserved sperm injection and the presence of at least one oocyte defect, (iv) cryopreserved sperm injection and the presence of at least one intracytoplasmic oocyte defect; and (v) cryopreserved sperm injection and the presence of at least one extracytoplasmic oocyte defect

Response variable	Predictor variable	*p*	OR	CI: lower	CI: upper
Embryo quality on day 2	Cryopreserved sperm	0.177	0.96	0.92	1.15
	Fresh spermatozoa and general oocyte defect	0.008	0.73	0.62	0.93
	Cryo sperm and general oocyte defect	>0.001	0.90	0.85	0.96
	Cryo sperm and extracytoplasmic oocyte defect	0.143	0.98	0.93	1.13
	Cryo sperm and intracytoplasmic oocyte defect	>0.001	0.58	0.32	0.76
Embryo quality on day 3	Cryopreserved sperm	0.277	0.95	0.90	1.13
	Fresh spermatozoa and general oocyte defect	0.039	0.94	0.89	0.99
	Cryo sperm and general oocyte defect	0.003	0.91	0.86	0.97
	Cryo sperm and extracytoplasmic oocyte defect	0.187	0.97	0.91	1.35
	Cryo sperm and intracytoplasmic oocyte defect	0.045	0.87	0.75	0.98
Chance of blastocyst formation	Cryopreserved sperm	0.341	0.97	0.93	1.23
	Fresh sperm and general oocyte defect	0.045	0.93	0.87	0.98
	Cryo sperm and general oocyte defect	>0.001	0.83	0.76	0.92
	Cryo sperm and extracytoplasmic oocyte defect	0.015	0.79	0.59	0.95
	Cryo sperm and intracytoplasmic oocyte defect	0.046	0.95	0.92	0.99

Table 3 The percentage of high-quality embryos on days 2 and 3 and percentage of embryos that reached the blastocyst stage, when ICSI was performed in patients including all ages using: (i) fresh spermatozoa and oocytes free of defects; (ii) fresh spermatozoa and oocytes with defects; (iii) cryopreserved sperm and oocytes free of defects and; (iv) cryopreserved sperm and oocytes with defects

Variable	FSNOD (*n* = 5715)	FSOD (*n* = 13 730)	CSNOD (*n* = 850)	CSOD (*n* = 1891)	*p*
Day 2 high-quality embryos	56.55%[a] (3232/5715)	54.00%[b] (7414/13730)	52.71%[b] (448/850)	48.23%[c] (912/1891)	<0.001
Day 3 high-quality embryos	51.19%[a] (2926/5715)	49.50%[b] (6797/13730)	48.94%[b] (416/850)	44.84[c] (848/1891)	<0.001
Blastocyst formation	50.70%[a] (1014/2000)	50.29%[a] (2508/4987)	47.50%[a] (133/280)	45.38%[c] (295/650)	0.019

Different subscripts in the same line are significantly different. ICSI, intracytoplasmic sperm injection; FSNOD, fresh sperm with no oocyte defects; FSOD, fresh sperm with oocyte defects; CSNOD, cryopreserved sperm with no oocyte defects; CSOD, cryopreserved sperm with oocyte defects.

Table 4 The percentage of high-quality embryos on days 2 and 3 and percentage of embryos that reached the blastocyst stage, when ICSI was performed in patients ≤35 years old using: (i) fresh sperm and oocytes free of defects; (ii) fresh sperm and oocytes with defects; (iii) cryopreserved sperm and oocytes free of defects and; (iv) cryopreserved sperm and oocytes with defects

Variable	FSNOD (*n* = 2400)	FSOD (*n* = 5752)	CSNOD (*n* = 364)	CSOD (*n* = 784)	*p*
Day 2 high-quality embryos	65.0%[a] (1560/2400)	63.0%[a] (3623/5752)	61.0%[a] (222/364)	58.9%[b] (462/784)	0.016
Day 3 high-quality embryos	55.5%[a] (1331/2400)	53.0%[b,c] (3048/5752)	50.5%[a,c] (184/364)	49.5%[d] (388/784)	0.016
Blastocyst formation	54.0% (691/1280)	51.5% (1540/2992)	48.8% (78/160)	47.3% (175/370)	0.104

Different subscripts in the same line are significantly different. ICSI, intracytoplasmic sperm injection; FSNOD, fresh sperm with no oocyte defects; FSOD, fresh sperm with oocyte defects; CSNOD, cryopreserved sperm with no oocyte defects; CSOD, cryopreserved sperm with oocyte defects.

Table 5 The percentage of high-quality embryos on days 2 and 3 and percentage of embryos that reached the blastocyst stage, when ICSI was performed in patients ≤35 years old using: (i) fresh sperm and oocytes free of defects; (ii) fresh sperm and oocytes with defects; (iii) cryopreserved sperm and oocytes free of defects and; (iv) cryopreserved sperm and oocytes with defects

Variable	FSNOD (*n* = 3315)	FSOD (*n* = 7938)	CSNOD (*n* = 486)	CSOD (*n* = 1107)	*p*
Day 2 high-quality embryos	50.4%[a] (1672/3315)	47.8%[b] (3791/7938)	46.5%[a,b] (226/486)	40.7%[c] (450/1107)	<0.001
Day 3 high-quality embryos	39.1%[a] (1295/3315)	47.2%[b] (3749/7938)	47.7%[b] (232/486)	41.6%[a] (460/1107)	<0.001
Blastocyst formation	44.9% (323/720)	48.5% (968/1995)	48.8% (55/120)	42.9% (120/280)	0.160

Different subscripts in the same line are significantly different. ICSI, intracytoplasmic sperm injection; FSNOD, fresh sperm with no oocyte defects; FSOD, fresh sperm with oocyte defects; CSNOD, cryopreserved sperm with no oocyte defects; CSOD, cryopreserved sperm with oocyte defects.

highly correlated with IVF success. Since its introduction, ICSI has been widely used to treat severe male infertility and may overcome the detrimental effects associated with freezing/thawing because only one viable spermatozoa is needed for fertilisation.

Although the success rates of ICSI were thought to be independent of basic sperm parameters (Nagy *et al.*, 1995; Donnelly *et al.*, 1998), more recent reports have suggested that repeated failures after ICSI may be caused by the effect of sperm-derived factors on the development of the preimplantation embryo

which are referred to as 'paternal effects' (Tesarik, 2005; Tesarik *et al.*, 2006).

Human sperm cells have a highly dynamic and essential participation in embryogenesis that clearly goes beyond the fertilisation process. The first divisions of the newly formed embryo depend on the machinery of the oocyte. Activation of the embryonic genome occurs at the stage of 4–8 cells. At this stage, sperm-derived genes that influence the viability of the embryo is also activated, which allows for the selection of a genetically normal embryo (Langley *et al.*, 2001; Wilson *et al.*, 2002).

This study evaluated the effect of sperm cryopreservation on embryo quality at the cleavage stage and on the rate of blastocyst formation when oocyte defects are present or absent. Our results showed that embryo quality on days 2 and 3 and the probability of blastocyst formation are not influenced by sperm cryopreservation. This is in agreement with previous reports that have shown that even for patients with poor sperm quality, cryopreservation does not affect the rates of fertilisation and pregnancy after ICSI (Kuczynski *et al.*, 2001).

However, in the presence of oocyte defects, both the embryo quality and the chance of blastocyst formation are influenced by the injection of cryopreserved/thawed sperm. Cryopreservation has well-established effects on spermatozoa. In addition to the decrease in the velocity of sperm movement (Donnelly *et al.*, 2001), spermatozoa exposed to physical and chemical stressors express adverse changes in membrane lipid composition and acrosome status (Ozkavukcu *et al.*, 2008). Sperm cryopreservation is also associated with damage from oxidative stress and reactive oxygen species, which leads to lipid peroxidation and DNA damage (Agarwal *et al.*, 2008). Recently, Wang *et al.* (Wang *et al.*, 2014) described marked differences in protein degradation and protein phosphorylation between fresh spermatozoa and freeze-thawed spermatozoa. Nevertheless, when injected into morphologically normal oocytes, no impact of sperm cryopreservation on the quality and development of the embryo was noted.

It has been described that the human oocyte is able to repair some of the abnormalities within the paternal DNA (Wells *et al.*, 2005; Gasca *et al.*, 2007). The mammalian oocyte is equipped with machinery that is responsible for the repair of DNA damage in both parental genomes after fertilisation (Menezo *et al.*, 2010). The ability to repair, however, depends not only on the type and extent of the DNA damage, but mainly on the quality of the oocyte (Marchetti *et al.*, 2007; Meseguer *et al.*, 2011).

There is little data as to whether it is possible to increase DNA repair capacity in oocytes, and the mechanism by which the oocyte DNA repair capacity may be diminished in specific conditions, is still not well understood. One fact is clear, the poor efficiency of DNA repair may depend on age (Hamatani *et al.*, 2004), ovarian environment, and maternal genotype (Marchetti *et al.*, 2007). There is little data as to whether it is possible to increase DNA repair capacity in oocytes, and the mechanism by which the oocyte DNA repair capacity may be diminished in specific conditions, is still not well understood. One fact is clear, the poor efficiency of DNA repair may depend on age (Hamatani *et al.*, 2004), ovarian environment, and maternal genotype (Marchetti *et al.*, 2007). Thus, embryo development failure may occur as a result of DNA misrepair in the oocyte.

Recently, Meseguer *et al.* (2011) analysed the effect of sperm fragmentation on two different populations of patients: patients whose autologous oocytes were used in fertility treatments and patients who received oocytes from young and fertile donors. Sperm DNA fragmentation was not found to have any clinical significance in fertile women with high-quality oocytes. It was therefore concluded that although some paternal factors may adversely affect the ART outcomes, this issue concerns the combination of male (i.e. damaged DNA) and female (i.e. capacity to repair DNA) factors. In this way, the problem of reduced spermatozoa DNA integrity is a result of infertility of mixed origin.

After consideration, we hypothesised that oocytes with dimorphisms, particularly intracytoplasmic defects, possess a reduced ability to repair cryo-injuries of spermatozoa that may compromise embryonic development.

One could argue that our findings may represent merely the effect of oocyte quality on ICSI outcomes rather than the association of both oocyte quality and sperm cryopreservation. To exclude a possible bias, in addition to the regression analyses, we also performed chi squared analyses in order to compare embryos derived from four different groups: FSNOD, FSOD, CSNOD, and CSOD. Lower embryo quality and blastocyst formation were observed among embryos derived from defective oocytes that were injected with either fresh or cryopreserved/thawed sperm. This proves that the presence of oocyte defects enhances the negative effect of sperm cryopreservation on the quality and development of the embryo.

A limitation of the study is that no results on clinical pregnancy were described. However, the main goal for the present study was to evaluate the effect of sperm cryopreservation on the embryo developmental competence, and because in many cycles, more than one embryo was transferred, these embryos could be derived from oocytes either with or without defects and therefore the analyses of pregnancy, implantation and even miscarriage was impossible.

In summary, the results suggest an oocyte quality-dependent negative effect of sperm cryopreservation on embryo quality and the chance of blastocyst formation. Apparently, when at least one morphological defect is present, the oocyte is not able to repair a possible negative effect of sperm cryopreservation; therefore, the embryo quality is negatively affected at the cleavage stage and during blastocyst formation. Therefore, for patients undergoing ICSI cycles, in which oocyte defects are detected, the injection of fresh spermatozoa, if possible, would be a better approach.

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