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EMBRYO BIOLOGY

Is early embryo development as observed by time-lapse microscopy dependent on whether fresh or frozen sperm was used for ICSI? A cohort study

Jessica Eastick[1,2] · **Christos Venetis**[1,2] · **Simon Cooke**[1] · **Ashleigh Storr**[1,2] · **Daisy Susetio**[1] · **Michael Chapman**[1,2]

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Abstract
Purpose The aim of this study was to compare timings of key events of embryo development from those originating from either fresh or cryopreserved ejaculate sperm using time-lapse technology.
Methods In this retrospective observational cohort study, time-lapse technology was used to monitor 1927 embryos from 234 women undergoing intracytoplasmic sperm injection (ICSI) and utilizing either fresh (n = 172 cycles) or cryopreserved ejaculate sperm (n = 62 cycles) for insemination were included in the study. Key developmental events as described in time-lapse were compared with the use of generalized estimating equations (GEE) to adjust for any autocorrelation between the observations. In addition, multivariable logit regression models were used to account for any known baseline differences between the two groups.
Results There were no differences in conventional embryo development such as number of 8-cell embryos by 72 h (p = 0.359), the number of blastocysts by 120 h (p = 0.417), and the number of top quality blastocysts (p = 0.956) between the two groups compared. There were no statistical differences in the timings of any of the key embryo developmental events (PN_t1, NEBD, cytokinesis, t2, t3, t4, t5, t6, t7, t8, tM, tSB, tEB, tHB, s1, s2, s3, cc2, and cc3) when either fresh or cryopreserved ejaculate sperm was used for ICSI. This was also confirmed with conventional morphological assessment.
Conclusions This observational cohort study has shown that there are no differences in the morphokinetic parameters of early embryo development when either fresh or frozen ejaculate sperm are used for ICSI insemination.

Keywords Time-lapse · Morphokinetic · Semen cryopreservation · Embryo development · ICSI

Introduction

Cryopreservation of human semen was first introduced in 1953 by Bunge and Sherman [1] and occurs routinely in the in vitro fertilization (IVF) laboratory for a variety of circumstances. These may involve partners being absent on oocyte collection, fertility preservation prior to the male undergoing chemotherapy/radiation treatment, or in patients with severe oligoasthenoteratospermia (OAT) as a form of "back up." It is also widely used for storage of donor semen until seronegativity is confirmed [2].

Cryopreservation of the male gamete has been suggested to have detrimental effects on its competence [3]. The most frequently reported effect is a decrease in motility [2–6] although morphology and vitality have also been shown to be negatively affected by cryopreservation [7]. In addition to this, other studies have suggested lower fertilization capacity [5, 7]. The exact mechanisms behind these observations are unclear. It has been suggested that following thawing there are various structural changes such as membrane, acrosome, and organelle damage as well as tail coiling [2, 5].

It has also been suggested that the cryopreservation process of semen samples both generates as well as increases existing deoxyribose nucleic acid (DNA) fragmentation [7]. This possible damage to the DNA is in the form of base modifications and fragmentation and could possibly arise from reactive

✉ Jessica Eastick
Jessica.eastick@hunterivf.com.au

[1] Hunter IVF, IVF Australia, PO Box 417, New Lambton, New South Wales 2305, Australia

[2] University of New South Wales, Sydney, New South Wales, Australia

oxygen species (ROS) [3] or oxidative stress [7]. DNA integrity in the male gamete is essential in achieving successful fertilization and further embryo development [8]. Whether such changes in the cryopreserved sperm DNA are associated with early embryo development events is still largely unknown [9].

There is a scarcity of studies comparing embryo development between fresh and frozen ejaculate sperm, since most of the published studies that look at fresh ejaculate compare it to cryopreserved surgically retrieved sperm [10–12]. In addition, a number of studies have compared surgically retrieved fresh and frozen sperm [13–19]. The studies that have compared fresh and frozen ejaculate sperm have produced conflicting results regarding fertilization outcomes, with one study reporting a lower fertilization rate when using cryopreserved sperm [20], while another reports no difference [21]. On the other hand, other studies have shown that embryo morphology, blastulation rates, as well as the number of cells on day 2 and day 3 [21, 22] are comparable between embryos originating from fresh and frozen ejaculate sperm.

It should be noted that in these studies, conventional morphological grading at static time points has been used. The introduction of time-lapse monitoring (TLM) systems to the IVF laboratory provides a unique opportunity for a detailed, dynamic assessment of early embryo development. TLM systems enable almost continuous monitoring of embryo's development through frequent multiple images without disrupting the microenvironment in which the embryo is cultured [23]. Traditional static embryo assessment is known to suffer from significant inter and intra-observer variability [24, 25], while TLM annotations seem to have high inter and intra-observer agreement [25]. For that reason, TLM has already been employed to study the association of early embryo development with blastocyst formation, aneuploidy, and achievement of pregnancy [26–28]. Additionally, these kinetic parameters have been implemented into some laboratory settings in the form of algorithms in an attempt to predict the embryo/s within the cohort with the highest implantation potential [29, 30]. For this reason, it is important to identify any potential confounding clinical factors that may impact morphokinetic parameters and adjust for them accordingly. Considering this, it provides a unique way to compare early embryo development after insemination with fresh and cryopreserved ejaculate sperm.

The purpose of this study was to compare timings of key developmental events of embryos originating from either fresh or cryopreserved ejaculate sperm using time-lapse technology.

Materials and methods

Study design

This was a single-center observational cohort study performed between November 2012 and September 2016. Approval for the study was obtained from the IVF Australia Human Research Ethics Committee (2014/104). One thousand nine hundred twenty-seven oocytes from 234 women undergoing intracytoplasmic sperm injection (ICSI) and utilizing either fresh or cryopreserved ejaculate sperm for insemination were included in the study. Fresh autologous and donor oocytes were included. Frozen oocytes were excluded from the study. Patients were not eligible if they had In Vitro Fertilization (IVF), preimplantation genetic diagnosis, or had any type of surgically retrieved sperm used for insemination.

Ovarian stimulation and oocyte retrieval

Controlled ovarian stimulation was achieved with the administration of gonadotrophins. The treating clinician determined the starting dose based on age, body mass index, anti-mullerian hormone, the presence of polycystic ovaries, and response to previous stimulated cycles. Prevention of a premature luteinizing hormone (LH) surge was achieved using gonadotrophin-releasing hormone (GnRH) agonists or antagonists. Transvaginal ultrasounds and serum estradiol levels were used to monitor follicular growth. Once two or more follicles of at least 18 mm were observed, final oocyte maturation was triggered with human chorionic gonadotrophin (hCG) (either 250 μg of recombinant hCG or 10,000 IU of urinary hCG) or in some cases of GnRH antagonist cycles with a GnRH agonist in the form of leuprorelin acetate (Lucrin, Abbott Australasia Pty Ltd., Sydney, Australia). Ultrasound-guided oocyte retrieval was performed 34–38 h after triggering using a single lumen 17 gauge needle (COOK, Brisbane, Australia). Oocytes were collected and transported to the IVF Laboratory in G-MOPS PLUS media (Vitrolife, Sweden) at 37 °C in a portable incubator (Biotherm, Cryologic Australia). Oocytes were cultured prior to ICSI using G-1 PLUS media (Vitrolife, Sweden) at 37 °C, 5% O_2, and 6% $C0_2$ in bench-top incubator (MINC, COOK, Australia).

Fresh ejaculate semen preparation and ICSI

For fresh ejaculate samples, male partners provided semen by masturbation on the morning of oocyte retrieval, having been requested to abstain for between 2 and 5 days. Sperm were isolated from semen using 40:80% density gradients (Puresperm, Nidacon, Sweden). The pellet was re-suspended in GMOPS media (Vitrolife, Australia) and held at 37 °C until use. Prior to ICSI, denudation of cumulus cells were performed in an EmCell (HD Scientific, Australia) at 6% $C0_2$ and at 37 °C by mechanical pipetting in hyaluronidase 30 IU/ml (Hyalase, Sanofi Aventis, Australia) in G1-PLUS media using 170 or 140-μm flexipette (COOK, Australia). All metaphase II oocytes present at 39–41 h post triggering injection were injected using standard ICSI procedures at ×200 magnification. Oocytes were injected in pre-warmed 5 ul G-MOPS PLUS droplets immersed in mineral oil (Ovoil, Vitrolife, Sweden). Prepared

sperm was placed in a separated droplet containing 7% polyvinyl prolidone (CooperSage, USA).

Semen cryopreservation and thaw

For patients using cryopreserved semen, it was frozen prior to oocyte retrieval by diluting the sample 1:1 with Quinn's Advantage Sperm Freezing Medium (Origio, Denmark) at room temperature. The sample was loaded into CBS 0.5 ml straws (Cryo Bio System, France) and frozen using either a Planer Controlled Rate Freezer (Planer, Australia) or a cryological cryo chamber (Biotherm, Cryologic Australia). Patients using frozen semen (donor or male partner frozen samples) were thawed by warming to 37 °C and then prepared using the above method once retrieval of a mature egg was confirmed.

Embryo culture

Following ICSI injection, zygotes were placed individually into a pre-equilibrated culture slide (EmbryoSlide®, Unisense Fertilitech, Aarhus, Denmark) 6% $C0_2$ and at 37 °C. The EmbryoSlide® has 12 separate wells for individual culture of embryos. Each of these wells was filled with 25 μl of G1-PLUS (Vitrolife, Sweden) and 1.4 ml of Ovoil mineral oil (Vitrolife, Sweden) to prevent evaporation. Order of injection was preserved from ICSI dish to culture slide. Air bubbles that may have formed during the pre-equilibration period were removed before zygotes were added to the wells. The EmbryoSlide® containing the zygotes was loaded into an EmbryoScope at 37 °C, 5% O_2, and 6% C02 immediately at completion of ICSI. A dish changeover was performed on day 3 of embryo culture into pre-equilibrated dishes containing G2-PLUS media (Vitrolife, Sweden) using the same method as day 0 dish preparation.

Embryo assessment

Embryo development was monitored using the image software EmbyroViewer® (EmbryoScope™, Unisense Fertilitech, Aarhus, Denmark). Fertilization was assessed 16–19 h post-insemination through the use of images provided by the time-lapse incubator software. Embryo morphology was assessed without removal from the EmbryoScope using the Gardner criteria on day 3 and 5 [31]. There were three trained scientists that performed all embryo assessments in order to limit inter-observer assessment errors.

Time-lapse monitoring

The EmbryoScope was programmed to capture photographs every 7 min at five focal planes. Key events in embryo development were annotated using the software EmbryoViewer®. The timings of each annotation were adjusted for the exact time of ICSI of each embryo [30]. The same three trained scientists that performed the morphological assessments were also trained in time-lapse assessments and annotated all embryos used in the study. Key developmental events (Table 1) as described in the time-lapse literature [30] were annotated for all embryos up until day 5 of culture or until utilized for transfer or freezing.

Statistical analysis

All continuous variables are presented as mean and standard deviation (SD). Categorical variables are presented as proportions. Due to the clustering nature of data (i.e., some oocytes and/or embryos originate from the same patient), the analysis of data was performed with the use of generalized estimating equations (GEE), which adjust for any auto-correlation between the observations. Furthermore, considering the observational, retrospective nature of this study, statistical adjustment for baseline differences identified through bivariate analyses was performed. An adjustment was also made for the embryologist performing the ICSI. This was achieved by constructing appropriate multivariable logit regression models. All statistical analyses were performed with STATA (v.14.2, StataCorp, USA) and statistical significance was set at $p \leq 0.05$.

Table 1 Time-lapse morphokinetic parameters evaluated to assess key embryo developmental events used in the current study

Parameter	Description
Pn_t1	Time of pronuclei formation
NEBD	Nuclear envelope break down
Cytokinesis	Cytokinesis
t2	Time of cleavage to a 2-cell embryo
t3	Time of cleavage to a 3-cell embryo
t4	Time of cleavage to a 4-cell embryo
t5	Time of cleavage to a 5-cell embryo
t6	Time of cleavage to a 6-cell embryo
t7	Time of cleavage to a 7-cell embryo
t8	Time of cleavage to a 8-cell embryo
tM	Time to full compaction
tSB	Time to the first signs of blastulation
tB	Time to full blastocyst
tEB	Time to expanded blastocyst
tHB	Time to hatching blastocyst
s1	Time between NEBD and subsequent division to 2-cells
s2	Time between division to 3-cells and subsequent division to 4-cells
s3	Time between division to 5-cells and subsequent division to 8-cells
cc2	Duration of the second cell cycle
cc3	Duration of the third cell cycle

Results

A total of 234 patients fulfilled the inclusion criteria. Each patient contributed only one cycle in the study sample. Out of 234 cycles, 2466 oocytes were retrieved (mean 10.5 oocytes, SD 5.2) and 1927 oocytes were injected (mean 8.3 oocytes, SD 3.9). Of these, 1784 oocytes were mature at time of denuding (mean 7.6 oocytes, SD 3.9) and 143 were late maturing MII oocytes (mean 0.6 oocytes, SD 1.1) at time of ICSI. The frozen-thawed ejaculate sperm group contained 486 embryos (mean 7.8 embryos, SD 3.4) while the fresh ejaculate group contained 1441 embryos (mean 8.4 embryos, SD 4.1). One hundred and seventy-two patients contributed to the fresh ejaculate group while the remaining fell into the frozen-thawed group (n = 62). Regarding cycles where frozen-thawed sperm was used, 87.1% (n = 54) utilized de-identified donor sperm, 8.1% (n = 5) frozen-thawed partner sperm, and 4.8% (n = 3) known donor sperm.

Female demographics and cycle characteristics

The mean female age was lower in the fresh group compared to that of the frozen-thawed group (Table 2). No differences were detected between patient BMI, serum AMH, and the prevalence of primary infertility between the two groups. There was a significant difference between the two groups for the indication for treatment ($p < 0.001$) with the majority of the cases in the frozen-thawed group falling into the social category for obvious reasons. No significant differences were observed in cycle characteristics (Table 2).

Table 3 Male baseline demographics for cycles where fresh or frozen-thawed ejaculate spermatozoa have been used for ICSI

Parameter	Fresh sperm (n = 172)	Frozen sperm (n = 62)	P value
Male age (years)	37.6 (36.6–38.6)	34.9 (32.7–37.1)	0.010
Sperm motility (%)	52.1 (49.2–55.0)	56.8 (52.4–61.1)	0.100
Seminal volume (ml)	3.0 (2.6–3.4)	3.8 (3.3–4.2)	0.044
Sperm concentration (M/ml)	59.6 (51.2–68.0)	60.8 (51.2–70.4)	0.879
Abstinence (days)	3.2 (2.9–3.5)	3.3 (2.9–3.7)	0.792

Male demographics

The two groups exhibited differences in a number of male demographic parameters (Table 3). There was a difference in male age (p = 0.010) with the frozen sperm group consisting of a younger population. There was also a difference in the

Table 2 Female baseline demographics and cycle characteristics for cycles where fresh or frozen-thawed ejaculate spermatozoa have been used for ICSI

Parameter	Fresh sperm (n = 172)	Frozen sperm (n = 62)	P value
Female age (years)	35.9 (35.2–36.6)	37.8 (36.7–38.9)	0.005[a]
BMI (kg/m^2)	24.9 (24.1–25.6)	24.9 (23.3–26.5)	0.997[a]
AMH (pmol/L)	19.9 (16.2–23.6)	15.7 (12.2–19.2)	0.166[a]
Type of infertility (%)	Primary 78.5% Secondary 21.5%	Primary 85.5% Secondary 14.5%	0.268[b]
Indication for treatment (%)	Male factor 40.7 Female factor 12.2 Combination 23.8 Unknown 23.3 Social 0	Male factor 12.9 Female factor 0 Combination 4.8 Unknown 3.2 Social 79.0	<0.001[a]
Starting FSH dose (IU)	206.6 (188.5–224.7)	227.4 (207.2–247.6)	0.207[a]
GnRH analogue (%)	Antagonist 83.7 Agonist 16.3	Antagonist 85.5 Agonist 14.5	0. 841[b]
Total FSH dose (IU)	2896 (2662–3131)	3017 (2648–3386)	0.594[a]
Duration of stimulation (days)	10.9 (10.5–11.2)	10.4 (9.8–11.0)	0.139[a]

[a] Student's t test

[b] Chi-squared or Fisher's exact test

Table 4 Embryological characteristics for cycles where fresh or frozen-thawed ejaculate spermatozoa have been used for ICSI

Parameter	Fresh sperm (n = 172)	Frozen sperm (n = 62)	P value
Oocytes retrieved/patient	10.7 (9.9–11.5)	10.1 (9.0–11.3)	0.469[a]
Oocytes injected/patient	8.4 (7.8–9.0)	7.8 (6.9–8.7)	0.304[a]
Late maturing MII's (%)	MII 92.6 Late maturing MII 7.4	MII 92.0 Late maturing MII 8.0	0.755[b]
Oocytes fertilized (%)	71.6 (68.4–74.7)	66.1 (61.1–71.1)	0.066[b]

[a] Student's t test

[b] Generalized estimating equation accounting for the non-dependents of data

seminal volume between the two groups (p = 0.044). The delay of sperm preparation to insemination time differed significantly between the two groups ($p < 0.001$). Almost 90% of the fresh ejaculate samples were prepared more than 3 h prior to ICSI insemination, while 96.8% of the frozen samples were prepared within 3 h prior to insemination.

Embryological characteristics

There was no statistical difference observed between the two groups when the embryological characteristics where examined. The number of oocytes retrieved, injected, maturity state, and fertilization rate (Table 4) where similar between embryos originating from fresh and frozen-thawed sperm.

Conventional embryo development

The two groups showed no statistical difference when observing the number and proportion of cleavage stage embryos at both day 3 (72 h post-insemination) and blastulation at day 5 (120 h post-insemination) (Table 5). The number of top quality blastocysts did not differ significantly between the two groups. In addition, the proportion of top quality blastocyst per blastocyst formed was similar between the two groups with embryo's originating from fresh sperm 23.5% and frozen-thawed sperm 25.9%.

Morphokinetic parameters

In Table 6, a comparison of the morphokinetic timings for embryos originating from both fresh and frozen-thawed ejaculate sperm. This table has been adjusted for female and male age, seminal volume, the indication for treatment, and the identification of the embryologist performing the ICSI. There was no statistical difference in the parameters for all morphokinetic markers evaluated (Table 6) between embryos originating from fresh or frozen-thawed sperm.

Discussion

This study does not support the presence of major differences in key early embryo developmental events when either fresh or cryopreserved ejaculate sperm is used for ICSI insemination. The two groups were compared in terms of widely used morphokinetic parameters, and this lack of a difference was

Table 5 Conventional embryo development for cycles where fresh or frozen-thawed ejaculate spermatozoa have been used for ICSI

Parameter	Fresh sperm (n = 172)	Frozen sperm (n = 62)	P value
Number of 8-cell embryos by 72 h	2.9	2.2	0.359
mean (95% CI)[a]	(2.4–3.5)	(1.0–3.4)	
Proportion of 8-cell embryos at 72 h after ICSI	33.1	33.4	0.969
mean (95% CI)[a]	(27.6–38.6)	(19.0–47.9)	
Number of embryos by 120 h with early signs of blastulation	3.7	3.0	0.417
mean (95% CI)[a]	(3.1–4.3)	(1.6–4.3)	
Proportion of embryos by 120 h with early signs of blastulation	41.7	43.5	0.855
mean (95% CI)[a]	(35.9–47.4)	(28.0–59.0)	
Number of top quality blastocyst	0.8	0.8	0.956
	(0.6–1.1)	(0.1–1.5)	
Proportion of top quality blastocysts/blast (%)[b]	23.5	25.9	0.880
	(15.7–31.3)	(1.0–50.7)	

[a] Proportions have been calculated for per oocyte injected

[b] Per total number of top quality blastocysts per blastocyst available on the day

[*] Cleavage rate <72 h post-insemination

[**] Blastulation rate <120 h post-insemination

Table 6 Multivariable logit regression model for the morphokinetic markers adjusted for known baseline differences in embryos originating from fresh and frozen-thawed ejaculate sperm

Parameter	Fresh sperm (n = 172)	Frozen sperm (n = 62)	P value
PN_t1	23.2 (20.7–25.7)	24.1 (15.2–33.1)	0.868
NEBD	25.2 (24.0–26.4)	28.9 (23.6–34.3)	0.260
Cytokinesis	27.7 (26.4–28.8)	31.3 (26.2–36.5)	0.250
t2	28.3 (27.3–29.3)	31.2 (27.3–35.1)	0.231
t3	38.0 (37.1–38.8)	38.4 (36.1–40.7)	0.759
t4	39.8 (38.8–40.8)	41.7 (38.5–44.9)	0.354
t5	49.9 (48.8–50.9)	52.1 (49.4–54.7)	0.183
t6	52.8 (51.7–53.9)	55.1 (51.9–58.2)	0.254
t7	57.0 (55.7–58.3)	60.0 (56.3–63.7)	0.201
t8	60.7 (59.1–62.4)	63.6 (58.7–68.5)	0.361
tM	97.9 (96.1–99.6)	98.1 (93.2–103.0)	0.946
tSB	105.9 (104.3–107.5)	106.1 (101.0–111.1)	0.946
tB	112.8 (110.7–114.8)	110.8 (103.9–117.6)	0.646
tEB	117.8 (115.9–119.6)	112.4 (106.6–118.2)	0.151
tHB	122.1 (117.8–126.4)	118.1 (107.7–128.5)	0.531
s1	2.9 (2.7–3.2)	3.8 (3.0–4.6)	0.084
s2	2.3 (1.9–2.8)	2.8 (1.1–4.4)	0.689
cc2	9.6 (9.0–10.2)	9.7 (8.0–11.4)	0.894
s3	11.4 (10.1–12.7)	11.2 (7.1–15.3)	0.937
cc3	12.4 (11.9–13.0)	13.7 (12.1–15.3)	0.200

All effect sizes and p values have been adjusted for male female and male age, seminal volume, the indication for treatment, the time delay of sperm preparation to insemination, the id of the embryologist performing the ICSI, and media type

also confirmed when using the conventional morphological assessment.

The only other study evaluating a similar question has been published previously as an abstract [32]. The authors compared the development of embryos using TLM after using frozen-thawed ejaculate sperm with fresh ejaculate sperm. Within the study, the frozen-thawed group assessed 209 embryos while in the fresh group 246 embryos were assessed. They found significant differences for PNBD, t2, t7, and t8 between the frozen group and the fresh group [32]. However, it appears that no multivariable adjustment for confounders was performed and this might explain the discrepancy with the results of the present study which statistically controlled for baseline difference between the two groups compared.

In a recent study, the authors aimed to compare fresh ejaculate sperm with frozen-thawed surgical retrieved sperm by utilizing TLM [24]. In that study, it was shown that the morphokinetic parameters between the two groups were similar except for three time points, t3, t8, and s2 [24]. The distinct differences between these results compared with the current study could be attributed to the fact that surgically retrieved sperm and not ejaculate sperm was evaluated. Hence, it could be hypothesized that the observed differences reflect not just the effect of cryopreservation but also the effect of the origin of sperm.

The strengths of the current study include the largest sample size published to date on this comparison, providing more statistical power for the detection of differences in the morphokinetic parameters. Furthermore, as in Storr et al. [30], we determined the exact injection time for each oocyte and annotation timings were adjusted in accordance to give a true representation of t0. Many studies use t0 as the average ICSI time, the ICSI start time, or pronuclear fading time [33]; however, these can vary dramatically between embryologists performing the inseminations and between cases. Also, this study takes into account the cluster effect (the fact that some embryos originate from the same patients) and has applied statistical methods that have adjusted for this correlation, hence providing more accurate estimates [34].

When compared to standard incubators, TLM provides comprehensive data on early embryo development kinetics as well as the morphological characteristics of the dynamically developing embryo. This study has utilized such a system over standard incubators to attain detailed metrics of early embryo development, such as fertilization. In a standard incubator, assessment of fertilization can contain large standard deviations due to the inability to identify precise timings. Furthermore, during analyses of these events, the embryos are disrupted from the culture environment negatively impacting the way the embryo develops. With TLM, this is overcome, making it a superior system in terms of metrics. Notably, there is a range of reports that suggests that embryos cultured in both system types show no difference between fertilization, cleavage, and blastulation rates [35–37].

This study also has some limitations that should be discussed. Although this is the largest study to date evaluating this research question, considering the sample size of the frozen sperm group, it cannot be excluded that a type II statistical error might still be present. It should be noted that systematic differences between the two groups may confound the examined associations. Although an effort was made to remove the effect of such differences by statistically adjusting with the use of multivariable regression models, the presence of residual confounding bias cannot be excluded.

This is a preliminary study and results should be interpreted with caution. An adequately powered study with a larger sample size in the frozen-thawed group may be able to detect smaller differences between embryo development events; however it remains to be seen if these would be of clinical significance.

The results of this study, in combination with the literature, may be used by clinicians to council their patients about their embryos that were made using frozen sperm and that they do not exhibit different developmental parameters compared to those created after using fresh sperm. In addition to this, the current study suggests that predictive algorithms for TLM, at this point, do not need to account for whether the male gametes were cryopreserved or not. Furthermore, based on the findings of this study, it could be in agreement with the methodology applied in most of the published literature [29, 38, 39].

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

References

1. Bunge RG, Sherman JK. Fertilizing capacity of frozen human spermatozoa. Nature. 1953;172(4382):767–8.
2. O'Connell M, McClure N, Lewis SEM. The effects of cryopreservation on sperm morphology, motility and mitochondrial function. Hum Reprod. 2002;17(3):704–9.
3. Zribi N, et al. Effects of cryopreservation on human sperm deoxyribonucleic acid integrity. Fertil Steril. 2010;93(1):159–66.
4. Donnelly ET, McClure N, Lewis SEM. Cryopreservation of human semen and prepared sperm: effects on motility parameters and DNA integrity. Fertil Steril. 2001;76(5):892–900.
5. Nallella KP, et al. Cryopreservation of human spermatozoa: comparison of two cryopreservation methods and three cryoprotectants. Fertil Steril. 2004;82(4):913–8.
6. Di Santo M, et al. Human sperm cryopreservation: update on techniques, effect on DNA integrity, and implications for ART. Advances in Urology. 2012;2012:854837.
7. Thomson LK, et al. Cryopreservation-induced human sperm DNA damage is predominantly mediated by oxidative stress rather than apoptosis. Hum Reprod. 2009;24(9):2061–70.
8. Boitrelle F, Albert M, Theillac C, Ferfouri F, Bergere M, Ois Vialard F, Wainer R, Bailly M, Selva J. Cryopreservation of human spermatozoa decreases the number of motile normal spermatozoa, induces nuclear vacuolization and chromatin decondensation. J Androl. 2012;33(6):1371–8.
9. Loutradi KE, et al. The effects of sperm quality on embryo development after intracytoplasmic sperm injection. J Assist Reprod Genet. 2006;23(2):69–74.
10. Van Steirteghem A, et al. Results of intracytoplasmic sperm injection with ejaculated, fresh and frozen–thawed epididymal and testicular spermatozoa. Hum Reprod. 1998;13(suppl 1):134–42.
11. Lu Y-h, et al. Different sperm sources and parameters can influence intracytoplasmic sperm injection outcomes before embryo implantation. Journal of Zhejiang University Science B. 2012;13(1):1–10.
12. Holden CA, et al. Frozen-thawed epididymal spermatozoa for intracytoplasmic sperm injection. Fertil Steril. 1997;67(1):81–7.
13. Tournaye H, et al. No differences in outcome after intracytoplasmic sperm injection with fresh or with frozen–thawed epididymal spermatozoa. Hum Reprod. 1999;14(1):90–5.
14. Cayan S, et al. A comparison of ICSI outcomes with fresh and cryopreserved epididymal spermatozoa from the same couples. Hum Reprod. 2001;16(3):495–9.
15. Ohlander S, et al. Impact of fresh versus cryopreserved testicular sperm upon intracytoplasmic sperm injection pregnancy outcomes in men with azoospermia due to spermatogenic dysfunction: a meta-analysis. Fertil Steril. 2014;101(2):344–9.
16. Karacan M, et al. Outcome of intracytoplasmic sperm injection cycles with fresh testicular spermatozoa obtained on the day of or the day before oocyte collection and with cryopreserved testicular sperm in patients with azoospermia. Fertil Steril. 2013;100(4):975–80.
17. Wood S, et al. Reproductive potential of fresh and cryopreserved epididymal and testicular spermatozoa in consecutive intracytoplasmic sperm injection cycles in the same patients. Fertil Steril. 2002;77(6):1162–6.
18. Park Y-S, et al. Influence of motility on the outcome of in vitro fertilization/intracytoplasmic sperm injection with fresh vs. frozen testicular sperm from men with obstructive azoospermia. Fertil Steril. 2003;80(3):526–30.
19. Friedler S, et al. The outcome of intracytoplasmic injection of fresh and cryopreserved epididymal spermatozoa from patients with obstructive azoospermia—a comparative study. Hum Reprod. 1998;13(7):1872–7.
20. Borges E Jr, et al. Fertilization and pregnancy outcome after intracytoplasmic injection with fresh or cryopreserved ejaculated spermatozoa. Fertil Steril. 2007;87(2):316–20.
21. Braga DPAF, et al. The negative influence of sperm cryopreservation on the quality and development of the embryo depends on the morphology of the oocyte. Andrology. 2015;3(4):723–8.
22. Kuczyński W, et al. The outcome of intracytoplasmic injection of fresh and cryopreserved ejaculated spermatozoa—a prospective randomized study. Hum Reprod. 2001;16(10):2109–13.
23. Ciray HN, et al. Proposed guidelines on the nomenclature and annotation of dynamic human embryo monitoring by a time-lapse user group. Hum Reprod. 2014;29(12):2650–60.
24. Lammers J, et al. Does sperm origin affect embryo morphokinetic parameters? J Assist Reprod Genet. 2015;32(9):1325–32.
25. Sundvall L, et al. Inter- and intra-observer variability of time-lapse annotations. Hum Reprod. 2013;28(12):3215–21.
26. Meseguer M, et al. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod. 2011;26(10):2658–71.
27. Kirkegaard K, et al. Time-lapse parameters as predictors of blastocyst development and pregnancy outcome in embryos from good prognosis patients: a prospective cohort study. Hum Reprod. 2013;28(10):2643–51.
28. Kirkegaard K, et al. Timing of human preimplantation embryonic development is confounded by embryo origin. Human Reproduction (Oxford, England). 2016;31(2):324–31.
29. Basile N, et al. The use of morphokinetics as a predictor of implantation: a multicentric study to define and validate an algorithm for embryo selection. Hum Reprod. 2015;30(2):276–83.
30. Storr A, et al. Morphokinetic parameters using time-lapse technology and day 5 embryo quality: a prospective cohort study. J Assist Reprod Genet. 2015;32(7):1151–60.
31. Gardner DKS, William B. Culture and transfer of human blastocysts. Current Opinion in Obstetrics and Gynaecology. 1999;11(3):307–11.
32. Muela García L, et al. Does semen cryopreservation have influence on embryo kinetics? Fertil Steril. 2012;98(3, Supplement):S164.

33. Liu Y, et al. Time-lapse videography of human embryos: using pronuclear fading rather than insemination in IVF and ICSI cycles removes inconsistencies in time to reach early cleavage milestones. Reprod Biol. 2015;15(2):122–5.
34. Hardin JW, Hilbe JM. Generalized Estimating Equations. Chapman & Hall/CRC; 2003
35. Payne D, Flaherty SP, Barry MF, Matthews CD. Preliminary observations on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography. Hum Reprod. 1997;12(3):532–41.
36. Lemmen JG, Agerholm I, Ziebe S. Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes. Reprod BioMed Online. 2008;17(3):385–91.
37. Cruz M, et al. Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging. J Assist Reprod Genet. 2011;28(7):569–73.
38. Petersen BM, et al. Development of a generally applicable morphokinetic algorithm capable of predicting the implantation potential of embryos transferred on day 3. Hum Reprod. 2016;31(10):2231–44.
39. Liu Y, et al. Clinical significance of intercellular contact at the four-cell stage of human embryos, and the use of abnormal cleavage patterns to identify embryos with low implantation potential: a time-lapse study. Fertil Steril. 2015;103(6):1485–1491.e1.