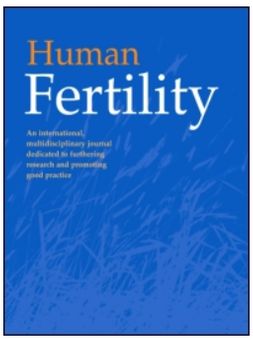

Human Fertility

an international, multidisciplinary journal dedicated to furthering research and promoting good practice

ISSN: 1464-7273 (Print) 1742-8149 (Online) Journal homepage: http://www.tandfonline.com/loi/ihuf20

Intracytoplasmic sperm injection using hyaluronic acid or polyvinylpyrrolidone: a time-lapse sibling oocyte study

Yanhe Liu, Katie Feenan, Vincent Chapple, Peter Roberts & Phillip Matson

To cite this article: Yanhe Liu, Katie Feenan, Vincent Chapple, Peter Roberts & Phillip Matson (2017): Intracytoplasmic sperm injection using hyaluronic acid or polyvinylpyrrolidone: a time-lapse sibling oocyte study, Human Fertility

To link to this article: http://dx.doi.org/10.1080/14647273.2017.1366077

Published online: 17 Aug 2017.

HUMAN FERTILITY, 2017
https://doi.org/10.1080/14647273.2017.1366077

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Intracytoplasmic sperm injection using hyaluronic acid or polyvinylpyrrolidone: a time-lapse sibling oocyte study

Yanhe Liu[a,b], Katie Feenan[a], Vincent Chapple[a], Peter Roberts[b] and Phillip Matson[a,b]

[a]Fertility North, Joondalup, Australia; [b]Systems and Intervention Research Centre for Health (SIRCH), School of Medical and Health Sciences, Edith Cowan University, Joondalup, Australia

ABSTRACT

This study evaluated the effect of sperm selection and intracytoplasmic sperm injection (ICSI) on subsequent fertilization and embryo development using the hyaluronic acid-based SpermSlow™ (HA-ICSI) compared to injection with polyvinylpyrrolidone (PVP-ICSI). A total of 206 metaphase II oocytes were collected from 21 prospectively enrolled ICSI cycles at Fertility North between July 2014 and March 2015. Sibling oocytes were randomized into HA-ICSI and PVP-ICSI ($n = 103$ per group). Subsequent fertilization outcomes and embryo development in terms of qualitative and quantitative time-lapse measures following three-day culture in the Embryoscope™ were compared. HA-ICSI resulted in significantly lower abnormal fertilization rates (1.9% vs 9.7%, $p = 0.017$), and a trend towards increased normal fertilization rates (73.8% vs 62.1%, $p = 0.073$) with increased injection time (2.5 vs 2.1 min, $p = 0.001$). No differences between HA-ICSI and PVP-ICSI were observed in (a) the proportion of good conventional morphology embryos (50% vs 53.1%, $p = 0.712$), (b) time-lapse qualitative measures ($p > 0.05$) and (c) time-lapse quantitative measures ($p > 0.05$). In conclusion, HA-ICSI improves fertilization outcomes although sperm injection takes longer to complete. Subsequent embryo development up to day 3 is not affected.

ARTICLE HISTORY
Received 29 March 2017
Accepted 1 July 2017

KEYWORDS
Intracytoplasmic sperm injection (ICSI); embryo culture; spermatozoa

Introduction

Intracytoplasmic sperm injection (ICSI) is widely used in assisted reproduction technology (ART) clinics for fertility treatment, in particular in the treatment of male factor infertility (Mansour et al., 2014; Palermo, Joris, Devroey, & Van Steirteghem, 1992). However, the technique bypasses natural physiological barriers that occur during the *in vivo* fertilization process and conventional *in vitro* fertilization (IVF), such as sperm-cumulus interaction (Carrell, Middleton, Peterson, Jones, & Urry, 1993) and sperm-zona penetration (Overstreet, Yanagimachi, Katz, Hayashi, & Hanson, 1980). In conventional ICSI, a single spermatozoon is artificially selected by the embryologist for injection, based mainly on motility and morphology. The deviation from *in vivo* biological process has led to concerns over the potential adverse impact of ICSI on subsequent fertilization, embryo development and on the health of the resulting offspring (Lewis & Kumar, 2015; Said & Land, 2011; Sakkas, Manicardi, Bizzaro, & Bianchi, 2000). To facilitate the ICSI process, polyvinylpyrrolidone (PVP) is used to reduce sperm velocity and assist with the smooth injection of oocytes. However, PVP is potentially toxic to human gametes and embryos, due to sperm membrane integrity damage (Esfahani et al., 2008) and the inability of human oocytes to degrade it (Jean, Mirallie, Boudineau, Tatin, & Barriere, 2001). As such, efforts have been made to discover a physiologically more friendly alternative sperm handling/injection medium (Butler & Masson, 1997; Ebner, Filicori, Tews, & Parmegiani, 2012). Hyaluronic acid (HA) is a natural component of the extracellular matrix (ECM) surrounding human oocytes and has been proposed as an alternative to PVP (Parmegiani, Cognigni, Bernardi, et al., 2010; Parmegiani, Cognigni, Ciampaglia, et al., 2010). Mature motile human sperm have HA receptors on the cell membrane on the head region enabling them to pass through the ECM, thus reaching the oocyte faster (Huszar et al., 2003). *In vivo*, sperm without the HA receptors may be deselected against, resulting in a reduced chance of reaching the zona. However, *in vitro*, sperm without such HA receptors could be selected and injected during the ICSI process.

A number of commercial products use binding capacity to HA as an indicator of sperm integrity.

CONTACT Yanhe Liu gift0409@yahoo.com.au Reproductive Medicine Center, Tianjin United Family Hospital, 22 Tianxiaoyuan, Tanjiang Road, Hexi District, Tianjin 300221, China

Furthermore an increasing number of reports have documented the practical benefits of such products, including HA coated dishes (PICSI® dish) and HA containing media (SpermSlow™ and SpermCatch™) (Balaban et al., 2003; Beck-Fruchter, Shalev, & Weiss, 2016; Majumdar & Majumdar, 2013; McDowell et al., 2014; Nasr-Esfahani, Razavi, Vahdati, Fathi, & Tavalaee, 2008; Parmegiani et al., 2012; Parmegiani, Cognigni, Bernardi, et al., 2010; Parmegiani, Cognigni, Ciampaglia, et al., 2010; Pregl Breznik, Kovacic, & Vlaisavljevic, 2013; Worrilow et al., 2013). Randomized controlled trials (RCTs) have compared the use of PICSI® dishes to conventional ICSI using PVP in terms of fertilization, embryo quality and pregnancy outcomes, however, conclusions have not been consistent (Beck-Fruchter et al., 2016; Majumdar & Majumdar, 2013; McDowell et al., 2014; Worrilow et al., 2013). Worrilow et al. (2013) have proposed that HA-ICSI is only beneficial within selected patient population groups, however, a study on couples with unexplained infertility reported improvements using HA-ICSI compared to PVP-ICSI (Majumdar & Majumdar, 2013).

With the introduction of time-lapse technology in human ART laboratories, a greater level of detail can be observed in embryo development with the advantage of uninterrupted culture condition (Gardner, Meseguer, Rubio, & Treff, 2015). Information obtained via time-lapse culture has potential benefits for embryo selection, using embryo morphokinetics (Meseguer et al., 2011; Wong et al., 2010) and abnormal cleavage patterns (Liu, Chapple, Feenan, Roberts, & Matson, 2015; Liu, Chapple, Roberts, & Matson, 2014; Rubio et al., 2012). With such measures, selection and deselection models have been reported which have claimed to improve the prediction of treatment outcomes (Liu, Chapple, Feenan, Roberts, & Matson, 2016; Petersen, Boel, Montag, & Gardner, 2016). The present study aims to compare the fertilization outcome and time-lapse measures of randomized groups of sibling embryos created via PVP-ICSI or HA-ICSI (SpermSlow™).

Materials and methods

Patient recruitment

This prospective randomized sibling oocyte study was a registered clinical trial (reference number ACTRN12613000957796 at www.anzctr.org). Data collection and analysis were approved by the Ethics Committees at both Joondalup Health Campus (Ref 1319) and Edith Cowan University (Ref 9975). Recruitment of participants occurred between July 2014 and March 2015. Inclusion criteria comprised: (1) patients undergoing routine ICSI treatment at Fertility North; (2) patients who had elected to allow the use of the Embryoscope™ for embryo culture; (3) couples in which semen sample was an ejaculated sample with an initial motile sperm count $\geq$1 M/ml on the day of oocyte collection (fresh samples) or $\geq$1 M/ml post thaw (frozen samples); and (4) cycles in which at least 4 M II oocytes were available for injection after denudation. Twenty-two couples were recruited and consented, but one couple was then excluded due to poor sperm motility (motile sperm count $<$1 M/ml) on the day of treatment.

Patient characteristics

Table 1 summarizes the characteristics of the 21 ICSI treatment cycles. All 21 couples were diagnosed with male factor infertility, five of which (23.8%) also had female factors – two with tubal obstruction and three with endometriosis. The median age of the women was 35 years (inter quartile range: 32–37 years) with two previous IVF attempts (inter quartile range: 1–3). Ovarian stimulation protocols involved agonist 33.3% (7/21) or antagonist 66.7% (14/21) cycles. The median age of the male partners was 35 years (inter quartile range: 28–40 years), with sperm concentration on the day of treatment having a median value of 10.5 M/ml (inter quartile range: 4.7–27.5 M/ml) and progressive sperm motility 42% (inter quartile range: 25–60%).

Ovarian stimulation and gamete collection

Ovarian stimulation was administrated as previously described (Liu et al., 2014), and oocyte collection performed 36 hours post human chronic gonadotrophin (HCG) trigger injection. Semen samples were collected within two hours post oocyte collection, either via masturbation or by thawing of a previously frozen sample. Sperm for treatment were isolated using 95%:50% density gradients (Puresperm, Nidacon, Mölndal, Sweden) centrifugation. Cumulus cells were removed at least one hour post collection by brief incubation with Synvitro Hyadase (Origio, Måløv, Denmark) and mechanical denudation. After assessing the maturity of all oocytes within the same cohort, metaphase II (M II) oocytes were divided equally between wells 1 and 2 in a 4-well dish (Nunc, Roskilde, Denmark). If an odd number of M II oocytes occurred, the extra oocyte was allocated to well 1. Oocytes at metaphase I (M I) and germinal vesicle (GV) stages were placed in a separate well. Randomization was used to allocate M II oocytes into HA-ICSI or

Table 1. Cycle characteristics.

Parameters	
No. ICSI cycles included	21
Female age (years, median/interquartile range)	35 (32–37)
No. previous attempts (median/interquartile range)	2 (1–3)
Aetiology	
Male factor only	76.2% (16/21)
Combined male/female factors	23.8% (5/21)
Ovarian stimulating protocol	
GnRH agonist	33.3% (7/21)
GnRH antagonist	66.7% (14/21)
Male age (years, median/interquartile range)	35 (28–40)
Sperm count (M/ml, median/interquartile range)	10.5 (4.7–27.5)
Sperm progressive motility (median/interquartile range)	42% (25–60%)
No. oocytes collected (median/interquartile range)	11 (8–15)
No. M II oocytes for injection (median/interquartile range)	9 (7–12)
No. oocytes normally fertilized (median/interquartile range)	5 (4–7)
No. good quality embryos (median/interquartile range)	3 (2–4)
No. cycles with embryo transfer	20
Clinical pregnancy rate	30% (6/20)
Live birth rate	30% (6/20)
Live birth involving HA-ICSI only	33.3% (3/9)
Live birth involving PVP-ICSI only	16.7% (1/6)
Live birth involving both	40% (2/5)

ICSI: intracytoplasmic sperm injection; M II: metaphase II; HA-ICSI: ICSI using hyaluronic acid; PVP-ICSI: ICSI using polyvinylpyrrolidone.

PVP-ICSI using the list randomizer at https://www.random.org/lists/. M I oocytes that became M II stage by the time of ICSI were allocated to either or both groups to achieve a balanced distribution.

HA-ICSI and PVP-ICSI

The ICSI procedure was performed between 4 and 6 hours post oocyte collection. In the PVP-ICSI group, sperm were selected for injection based upon good motility and morphology, and immobilized in a 10-µl drop of PVP (Origio, Måløv, Denmark) immediately before injection. In the HA-ICSI group, additional sperm selection was performed based upon the presence of sperm binding to HA with the use of SpermSlow™ medium (Origio, Måløv, Denmark). Binding was indicated by the presence of a hyperactive tail movement without progressive motility, as originally described by Parmegiani et al. (2012). The time taken to complete injection was recorded for each cohort of M II oocytes and used to calculate the average time of injection per oocyte. Injected oocytes were placed in a MINC incubator (Cook, Brisbane, Australia) for overnight culture at 37 °C under 6% CO_2, 5% O_2 and 89% N_2. The laboratory had a quality control programme which compared the performance of all ICSI operators on a quarterly basis, with indicators including normal/abnormal fertilization rate, degeneration rate and subsequent pregnancy rate. Any operator consistently underperforming underwent retraining and supervision to improve their results.

Embryo culture and annotation

Fertilization was assessed at 16–18 hours post injection by observing two pronuclei (PN) under an inverted microscope (Olympus, Tokyo, Japan). Other patterns of PN structure (e.g. 1PN, 3PN, 4PN, or fragmented PN) were classified as abnormal fertilization. Fertilized oocytes were transferred to an Embryoslide® and cultured individually in the Embryoscope™ incubator (both Vitrolife, Göteborg, Sweden) at 37 °C under 6% CO_2, 5% O_2 and 89% N_2 for three days before transfer or cryopreservation. Embryo annotations were performed using the EmbryoViewer™ software (Vitrolife, Göteborg, Sweden) and a range of time-lapse measurements taken. Qualitative measures included multinucleation (MN) at 2- and 4-cell stages (Ergin et al., 2014), direct cleavage (DC) (Rubio et al., 2012), reverse cleavage (RC) (Liu et al., 2014), and fewer than six intercellular contacts at the end of the 4-cell stage (<6 ICCP) (Liu et al., 2015). Quantitative measures included the time from pronuclear fading to the 5-cell stage (T5_PNF), duration of the 2-cell stage (CC2), duration of the 3-cell stage (S2), time from the 4-cell to 5-cell stage (T5_T4), from pronuclear fading to the 8-cell stage (T8_PNF), and from the 5-cell to 8-cell stage (S3).

Embryo transfer and clinical outcome

One or two embryos were selected and transferred on day 3, depending on the female age and treatment history as judged by the clinical practitioner. Clinical pregnancy was confirmed by detection of foetal heart

Table 2. Comparison of conventional embryology parameters between oocytes inseminated via HA-ICSI and PVP-ICSI.

	HA-ICSI	PVP-ICSI	*p* value
No. sibling M II oocytes allocated	103	103	–
Time spent on injection per oocyte (min)	2.5 (2.0–2.9)	2.1 (1.6–2.5)	0.001
Normal fertilization rate (2PN)	73.8% (76/103)	62.1% (64/103)	0.073
Abnormal fertilization rate (1PN or 3PN)	1.9% (2/103)	9.7% (10/103)	0.017
1PN	0/103	5/103	0.024
3PN	2/103	5/103	0.249
Good quality embryo rate per 2PN	50% (38/76)	53.1% (34/64)	0.712
Good quality embryo rate per injected oocyte	36.9% (38/103)	33.0% (34/103)	0.559

HA-ICSI: intracytoplasmic sperm injection using hyaluronic acid; PVP-ICSI: intracytoplasmic sperm injection using polyvinylpyrrolidone; M II: metaphase II; PN: pronucleus.

beat under ultrasound at 7 week pregnancy. All pregnancies were followed up until birth. Twenty out of the 21 cycles led to embryo transfer with pregnancy and birth outcomes summarized in Table 1, while one cycle had all embryos frozen due to the risk of ovarian overstimulation.

Statistical analysis

Continuous data were expressed as median (interquartile range), and tested for significance using the Mann–Whitney *U* test. Proportional data were tested using Chi-squared analysis. All statistical analyses were carried out using the Statistical Package for the Social Sciences 20.0 (SPSS, Chicago, IL), with $p < 0.05$ considered significant.

Results

Conventional embryology outcomes

Table 2 provides a comparison in fertilization outcomes and conventional embryology markers between HA-ICSI and PVP-ICSI groups after randomization of sibling oocytes. Injection of oocytes in the HA-ICSI group (2.5, 2.0–2.9 min per oocyte) took significantly longer ($p = 0.001$) than oocytes in the PVP-ICSI group (2.1, 1.6–2.5 min per oocyte). There was an increased incidence of abnormal fertilization in the PVP-ICSI group (9.7% vs 1.9%, $p = 0.017$), with a trend towards an increased normal fertilization rate in the HA-ICSI group (73.8% vs 62.1%, $p = 0.073$). The proportion of good quality embryos was similar in the HA-ICSI and PVP-ICSI groups (50% vs 53.1% per fertilized oocyte, $p = 0.712$; 36.9% vs 33.0% per injected oocyte, $p = 0.559$; respectively).

Time-lapse markers

Table 3 shows abnormal biological events during three-day time-lapse culture of embryos in the HA-ICSI and PVP-ICSI groups. No significant differences were detected between the two groups, for DC (14.5% vs 7.8%, $p = 0.217$), RC (32.9% vs 32.8%, $p = 1$), <6 ICCP (16.1% vs 22.2%, $p = 0.432$), and MN at 2- (44.7% vs 45.2%, $p = 0.964$) and 4-cell stages (14.3% vs 8.8%, $p = 0.347$).

Table 3. Qualitative time-lapse parameters of embryos generated from HA-ICSI and PVP-ICSI.

	HA-ICSI	PVP-ICSI	*p* value
DC	14.5% (11/76)	7.8% (5/64)	0.217
RC	32.9% (25/76)	32.8% (21/64)	1
<6ICCP at the end of 4-cell stage	16.1% (9/56)	22.2% (10/45)	0.432
MN @ 2-cell stage	44.7% (34/76)	45.2% (28/62)	0.964
MN @ 4-cell stage	14.3% (9/63)	8.8% (5/57)	0.347

HA-ICSI: intracytoplasmic sperm injection using hyaluronic acid; PVP-ICSI: intracytoplasmic sperm injection using polyvinylpyrrolidone; DC: direct cleavage; RC: reverse cleavage; ICCP: intercellular contact points; MN: multinucleation.
<6ICCP at the end of 4-cell stage was assessed on embryos that reached at least the 5-cell stage on day 3.
MN @ 2-cell stage was assessed on embryos that reached at least the 2-cell stage on day 3.
MN @ 4-cell stage was assessed on embryos that reached at least the 4-cell stage on day 3.

Table 4 compares the embryo morphokinetic data in the HA-ICSI and PVP-ICSI groups. Note that embryos displaying DC (embryos dividing directly from 1 to 3 cells, or 2 to 5 cells, etc.) were excluded from this analysis since DC can skew morphokinetic cleavage data (Liu et al., 2016). HA-ICSI embryos had slightly faster cleavage rates compared to their PVP-ICSI counterparts; however, the differences were not statistically significant, expressed as medians in hours (HA-ICSI vs PVP-ICSI), T5_PNF (26.5 vs 27.2, $p = 0.398$), CC2 (11.0 vs 11.4, $p = 0.163$), S2 (0.7 vs 0.7. $p = 0.953$), T5_T4 (12.2 vs 13.4, $p = 0.099$), T8_PNF (30.9 vs 30.5, $p = 0.406$) and S3 (3.8 vs 3.6, $p = 0.245$).

Discussion

Following the extensive application of ICSI in ART clinics around the world, increasing concerns have been raised about the safety of the technique (Davies et al., 2012). Concerns have mainly related to the bypass of natural sperm selection barriers during the ICSI process (Jean et al., 2001; Overstreet et al., 1980). Although efforts have been made to improve sperm selection during ICSI, such as intracytoplasmic morphologically

Table 4. Morphokinetics of embryos following HA-ICSI and PVP-ICSI.

	HA-ICSI			PVP-ICSI			
Parameters (h)	*n*	Median	Interquartile range	*n*	Median	Interquartile range	*p* value
T5_PNF	49	26.5	25.9–28.8	40	27.2	26.2–29.3	0.398
CC2	49	11.0	10.5–11.3	40	11.4	10.5–12.2	0.163
S2	49	0.7	0.2–1.8	40	0.7	0.3–1.2	0.953
T5_T4	49	12.2	11.0–13.2	40	13.4	11.8–13.9	0.099
T8_PNF	25	30.9	28.1–38.4	22	30.5	27.9–31.9	0.406
S3	25	3.8	2.3–8.8	22	3.6	1.7–6.5	0.245

HA-ICSI: intracytoplasmic sperm injection using hyaluronic acid; PVP-ICSI: intracytoplasmic sperm injection using polyvinylpyrrolidone; T5_PNF: duration between pronuclear fading and 5-cell stage; CC2: duration of 2-cell stage; S2: duration of 3-cell stage; T5_T4: duration of 4-cell stage; T8_PNF: duration between pronuclear fading and 8-cell stage; S3: duration between 5- and 8-cell stage.
Comparisons include fully annotated embryos that reached the 5-cell stage at 68 hours post insemination, excluding those displaying direct cleavage. Embryos with direct cleavage can skew morphokinetic data, as embryos appears to have cleavage faster.

selected sperm injection (IMSI) (Teixeira et al., 2013), sperm surface charge (Ainsworth, Nixon, & Aitken, 2005), sperm apoptosis (Grunewald, Paasch, & Glander, 2001) and HA binding based selection; there is still insufficient evidence to support the introduction of such techniques into routine practice (Beck-Fruchter et al., 2016; McDowell et al., 2014). Previously HA binding of sperm was associated with improved DNA integrity (Parmegiani, Cognigni, Bernardi, et al., 2010; Yagci, Murk, Stronk, & Huszar, 2010) and the ploidy status of sperm (Jakab et al., 2005), thus having the potential to be a beneficial non-invasive sperm selection method. Different types of HA binding products have been investigated including the PICSI® dish and SpermSlow™ medium (Beck-Fruchter et al., 2016). However, the latter is a liquid, which is potentially more versatile, cost effective and easier to handle compared to the PICSI® dish and may have contributed to reduced injection times observed within a SpermSlow™ group compared to a PICSI® dish group in a recent sibling oocyte RCT (Parmegiani et al., 2012). In addition, the liquid form of HA can be used as a sperm handling medium for manipulations and smooth injections, replacing the potentially toxic PVP (Jean et al., 2001).

HA binding capability is related to the membrane, cytoplasmic and nuclear integrity of human sperm (Huszar et al., 2003; Pregl Breznik et al., 2013; Yagci et al., 2010), thus providing a possible means of optimizing outcomes in ART such as fertilization and embryo quality. Findings in the literature however are inconsistent (Beck-Fruchter et al., 2016; McDowell et al., 2014). In the current study, the two sperm selection methods tested resulted in different rates of abnormal fertilization; and a trend towards, but not statistically significant, higher normal fertilization rates (in the HA-ICSI). Similarly Pregl Breznik et al. (2013) reported a positive correlation between the HA binding capability of sperm and subsequent fertilization rates after IVF/ICSI, although another group did not (Majumdar & Majumdar, 2013). Differences in these data could be due to different patient populations (Beck-Fruchter et al., 2016; Majumdar & Majumdar, 2013; McDowell et al., 2014).

In the current study, a longer injection time was observed in the SpermSlow™ group compared to the PVP-ICSI group possibly due to the additional sperm selection step in the HA-ICSI group. Thus, new users would need to be mindful of this increase; a practical solution could be to limit the number of oocytes for injection per dish, thereby limiting the time oocytes spend outside a controlled incubator. Improvement in HA-ICSI operator rate might occur with experience. However, the benefits of improved fertilization results may outweigh the additional time oocytes spend outside a controlled environment.

Time-lapse technology is a recent introduction in IVF laboratories and has provided increased opportunities to observe embryo development in detail without interrupting culture conditions (Gardner et al., 2015). Recent research has shown the potential of this method to predict implantation using morphokinetic data or by identifying abnormal cleavage patterns, both of which are not possible in conventional culture (Liu et al., 2014, 2015, 2016; Petersen et al., 2016; Rubio et al., 2012). The present study differs from previous studies investigating HA-ICSI, which only used conventional morphological score to assess embryo quality. In addition, we used time-lapse parameters to evaluate qualitative (Table 3) and quantitative parameters (Table 4) of embryo development. Results showed no significant differences in the incidences of MN, abnormal cleavage patterns (DC, RC and <6 ICCP at the end of 4-cell stage) nor in the timing markers between PVP-ICSI and HA-ICSI embryos. Previously, time-lapse technology enabled the identification of groups of embryos with very low implantation potential, one of which was DC. Such embryos can have a confounding impact when analysing embryo cleavage rates by skewing results towards faster cleavage rates

(Liu et al., 2016). In the present study, DC embryos were removed from the cohort before analysing the timing parameters. Interestingly, in a recent meta-analysis of conventionally cultured embryos comparing HA-ICSI vs conventional ICSI, faster cleavage rates were seen in the conventional ICSI group, but the proportion of top quality embryos was higher in the HA-ICSI group (Beck-Fruchter et al., 2016). The inclusion of DC embryos may explain the conflicting conclusions drawn from previous studies that used only conventional static morphological assessment.

It is accepted that embryonic genome is not activated until the 4–8-cell stage of human embryo development (Tesarik, 2005). In the present study, qualitative and quantitative time-lapse markers were similar up to the 8-cell stage of development between the HA-ICSI and PVP-ICSI groups (Table 4). Differences in the morphokinetics between these two groups might become evident if embryos were analysed up to the blastocyst stage of development. However, this was not possible in the current study as the clinic operated a day 3 cleavage stage programme to avoid extended culture (Liu et al., 2016). Since the majority of embryos were transferred or stored on day 3, the blastocyst data were not available in the current dataset. Another limitation was the small sample size. We originally aimed for 60 cases however, after 21 participants had been enrolled, recruitment for more participants became very difficult due to a change of financial situation at the clinic. Additionally, due to the sibling oocyte design, live birth outcomes were difficult to compare owing to the potential mixture of embryos originating from different groups in double embryo transfers. Live birth outcome appeared to be favoured in the HA-ICSI group as shown in Table 1, but the difference did not reach statistical significance due to small sample size. However, randomizing at the oocyte level enabled an unbiased comparison of subsequent embryo development, which was further carried out using time-lapse analysis. Further, large scale RCTs using live birth as an end point are required to validate potential benefits of HA-ICSI over PVP-ICSI before routine practice can be advised. Finally, further information on early stage biological events post ICSI, such as the timing of 2nd polar body extrusion or PN appearance, were unavailable since fertilized oocytes were only placed into the Embryoscope™ after fertilization check on day 1.

Acknowledgements

The authors would like to thank the clinical, nursing and embryology teams at Fertility North for their technical input during this study.

Disclosure statement

There are no conflicts of interest to declare.

References

Ainsworth, C., Nixon, B., & Aitken, R.J. (2005). Development of a novel electrophoretic system for the isolation of human spermatozoa. *Human Reproduction*, *20*, 2261–2270. doi: 10.1093/humrep/dei024.

Balaban, B., Lundin, K., Morrell, J.M., Tjellstrom, H., Urman, B., & Holmes, P.V. (2003). An alternative to PVP for slowing sperm prior to ICSI. *Human Reproduction (Oxford, England)*, *18*, 1887–1889. doi: 10.1093/humrep/deg385.

Beck-Fruchter, R., Shalev, E., & Weiss, A. (2016). Clinical benefit using sperm hyaluronic acid binding technique in ICSI cycles: A systematic review and meta-analysis. *Reproductive Biomedicine Online*, *32*, 286–298. doi: 10.1016/j.rbmo.2015.12.001.

Butler, E.A., & Masson, G.M. (1997). Development of a successful ICSI programme without the use of PVP. *Human Reproduction (Oxford, England)*, *12*, 1115–1116. doi: 10.1093/oxfordjournals.humrep.a019581.

Carrell, D.T., Middleton, R.G., Peterson, C.M., Jones, K.P., & Urry, R.L. (1993). Role of the cumulus in the selection of morphologically normal sperm and induction of the acrosome reaction during human in vitro fertilization. *Archives of Andrology*, *31*, 133–137. doi: 10.3109/01485019308988391.

Davies, M.J., Moore, V.M., Willson, K.J., Van Essen, P., Priest, K., Scott, H., … Chan, A. (2012). Reproductive technologies and the risk of birth defects. *The New England Journal of Medicine*, *366*, 1803–1813. doi: 10.1056/NEJMoa1008095.

Ebner, T., Filicori, M., Tews, G., & Parmegiani, L. (2012). A plea for a more physiological ICSI. *Andrologia*, *44 Suppl 1*, 2–19. doi: 10.1111/j.1439-0272.2011.01266.x.

Ergin, E.G., Caliskan, E., Yalcinkaya, E., Oztel, Z., Cokelez, K., Ozay, A., & Ozornek, H.M. (2014). Frequency of embryo multinucleation detected by time-lapse system and its impact on pregnancy outcome. *Fertility and Sterility*, *102*, 1029–1033 e1021. doi: 10.1016/j.fertnstert.2014.06.030.

Esfahani, M.H.N., Razavi, S., Mardani, M., Aboutorabi, R., Sheikhi, A., & Mohammadpour, M. (2008). Effect of polyvinyl pyrrolidone on sperm membrane integrity and chromatin status. *International Journal of Fertility and Sterility*, *1*, 155–158. Retrieved from https://doaj.org/article/e5febfca50264e0485ecfd156a8c7158.

Gardner, D.K., Meseguer, M., Rubio, C., & Treff, N.R. (2015). Diagnosis of human preimplantation embryo viability. *Human Reproduction Update*, *21*, 727–747. doi: 10.1093/humupd/dmu064.

Grunewald, S., Paasch, U., & Glander, H.J. (2001). Enrichment of non-apoptotic human spermatozoa after cryopreservation by immunomagnetic cell sorting. *Cell and Tissue Banking*, *2*, 127–133. doi: 10.1023/A:1020188913551.

Huszar, G., Ozenci, C.C., Cayli, S., Zavaczki, Z., Hansch, E., & Vigue, L. (2003). Hyaluronic acid binding by human sperm indicates cellular maturity, viability, and unreacted acrosomal status. *Fertility and Sterility*, *79 Suppl 3*, 1616–1624. doi: 10.1016/S0015-0282(03)00402-3.

Jakab, A., Sakkas, D., Delpiano, E., Cayli, S., Kovanci, E., Ward, D., … Huszar, G. (2005). Intracytoplasmic sperm injection: A novel selection method for sperm with normal frequency of chromosomal aneuploidies. *Fertility and Sterility*, *84*, 1665–1673. doi: 10.1016/j.fertnstert.2005.05.068.

Jean, M., Mirallie, S., Boudineau, M., Tatin, C., & Barriere, P. (2001). Intracytoplasmic sperm injection with polyvinylpyrrolidone: A potential risk. *Fertility and Sterility*, *76*, 419–420. doi: 10.1016/S0015-0282(01)01874-X.

Lewis, S.E., & Kumar, K. (2015). The paternal genome and the health of the assisted reproductive technology child. *Asian Journal of Andrology*, *17*, 616–622. doi: 10.4103/1008-682X.153301.

Liu, Y., Chapple, V., Feenan, K., Roberts, P., & Matson, P. (2015). 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. *Fertility and Sterility*, *103*, 1485–1491. e1. doi: 10.1016/j.fertnstert.2015.03.017.

Liu, Y., Chapple, V., Feenan, K., Roberts, P., & Matson, P. (2016). Time-lapse deselection model for human day 3 in vitro fertilization embryos: The combination of qualitative and quantitative measures of embryo growth. *Fertility and Sterility*, *105*, 656–662. e1. doi: 10.1016/j.fertnstert.2015.11.003.

Liu, Y., Chapple, V., Roberts, P., & Matson, P. (2014). Prevalence, consequence, and significance of reverse cleavage by human embryos viewed with the use of the Embryoscope time-lapse video system. *Fertility and Sterility*, *102*, 1295–1300.e2. doi: 10.1016/j.fertnstert.2014.07.1235.

Majumdar, G., & Majumdar, A. (2013). A prospective randomized study to evaluate the effect of hyaluronic acid sperm selection on the intracytoplasmic sperm injection outcome of patients with unexplained infertility having normal semen parameters. *Journal of Assisted Reproduction and Genetics*, *30*, 1471–1475. doi: 10.1007/s10815-013-0108-9.

Mansour, R., Ishihara, O., Adamson, G.D., Dyer, S., de Mouzon, J., Nygren, K.G., … Zegers-Hochschild, F. (2014). International Committee for Monitoring Assisted Reproductive Technologies world report: Assisted Reproductive Technology 2006. *Human Reproduction*, *29*, 1536–1551. doi: 10.1093/humrep/deu084.

McDowell, S., Kroon, B., Ford, E., Hook, Y., Glujovsky, D., & Yazdani, A. (2014). Advanced sperm selection techniques for assisted reproduction. *Cochrane Database of Systematic Reviews*, *10*, CD010461. doi: 10.1002/14651858.CD010461.pub2.

Meseguer, M., Herrero, J., Tejera, A., Hilligsoe, K.M., Ramsing, N.B., & Remohi, J. (2011). The use of morphokinetics as a predictor of embryo implantation. *Human Reproduction (Oxford, England)*, *26*, 2658–2671. doi: 10.1093/humrep/der256.

Nasr-Esfahani, M.H., Razavi, S., Vahdati, A.A., Fathi, F., & Tavalaee, M. (2008). Evaluation of sperm selection procedure based on hyaluronic acid binding ability on ICSI outcome. *Journal of Assisted Reproduction and Genetics*, *25*, 197–203. doi: 10.1007/s10815-008-9223-4.

Overstreet, J.W., Yanagimachi, R., Katz, D.F., Hayashi, K., & Hanson, F.W. (1980). Penetration of human spermatozoa into the human zona pellucida and the zona-free hamster egg: A study of fertile donors and infertile patients. *Fertility and Sterility*, *33*, 534–542. doi: 10.1016/S0015-0282(16)44720-5.

Palermo, G., Joris, H., Devroey, P., & Van Steirteghem, A.C. (1992). Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*, *340*, 17–18. doi: 10.1016/0140-6736(92)92425-F.

Parmegiani, L., Cognigni, G.E., Bernardi, S., Troilo, E., Ciampaglia, W., & Filicori, M. (2010). "Physiologic ICSI": Hyaluronic acid (HA) favors selection of spermatozoa without DNA fragmentation and with normal nucleus, resulting in improvement of embryo quality. *Fertility and Sterility*, *93*, 598–604. doi: 10.1016/j.fertnstert.2009.03.033.

Parmegiani, L., Cognigni, G.E., Bernardi, S., Troilo, E., Taraborrelli, S., Arnone, A., … Filicori, M. (2012). Comparison of two ready-to-use systems designed for sperm-hyaluronic acid binding selection before intracytoplasmic sperm injection: PICSI vs. sperm slow: A prospective, randomized trial. *Fertility and Sterility*, *98*, 632–637. doi: 10.1016/j.fertnstert.2012.05.043.

Parmegiani, L., Cognigni, G.E., Ciampaglia, W., Pocognoli, P., Marchi, F., & Filicori, M. (2010). Efficiency of hyaluronic acid (HA) sperm selection. *Journal of Assisted Reproduction and Genetics*, *27*, 13–16. doi: 10.1007/s10815-009-9380-0.

Petersen, B.M., Boel, M., Montag, M., & Gardner, D.K. (2016). Development of a generally applicable morphokinetic algorithm capable of predicting the implantation potential of embryos transferred on Day 3. *Human Reproduction*, *31*, 2231–2244. doi: 10.1093/humrep/dew188.

Pregl Breznik, B., Kovacic, B., & Vlaisavljevic, V. (2013). Are sperm DNA fragmentation, hyperactivation, and hyaluronan-binding ability predictive for fertilization and embryo development in in vitro fertilization and intracytoplasmic sperm injection? *Fertility and Sterility*, *99*, 1233–1241. doi: 10.1016/j.fertnstert.2012.11.048.

Rubio, I., Kuhlmann, R., Agerholm, I., Kirk, J., Herrero, J., Escriba, M.J., … Meseguer, M. (2012). Limited implantation success of direct-cleaved human zygotes: A time-lapse study. *Fertility and Sterility*, *98*, 1458–1463. doi: 10.1016/j.fertnstert.2012.07.1135.

Said, T.M., & Land, J.A. (2011). Effects of advanced selection methods on sperm quality and ART outcome: A systematic review. *Human Reproduction Update*, *17*, 719–733. doi: 10.1093/humupd/dmr032.

Sakkas, D., Manicardi, G., Bizzaro, D., & Bianchi, P.G. (2000). Possible consequences of performing intracytoplasmic sperm injection (ICSI) with sperm possessing nuclear DNA damage. *Human Fertility*, *3*, 26–30. doi: 10.1080/1464772002000198651.

Teixeira, D.M., Barbosa, M.A., Ferriani, R.A., Navarro, P.A., Raine-Fenning, N., Nastri, C.O., & Martins, W.P. (2013). Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction. *Cochrane Database of Systematic Reviews*, *7*, CD010167. doi: 10.1002/14651858.CD010167.pub2.

Tesarik, J. (2005). Paternal effects on cell division in the human preimplantation embryo. *Reproductive Biomedicine Online*, *10*, 370–375. doi: 10.1016/S1472-6483(10)61798-1.

Wong, C.C., Loewke, K.E., Bossert, N.L., Behr, B., De Jonge, C.J., Baer, T.M., & Reijo Pera, R.A. (2010). Non-invasive imaging of human embryos before embryonic genome activation

predicts development to the blastocyst stage. *Nature Biotechnology*, *28*, 1115–1121. doi: 10.1038/nbt.1686.

Worrilow, K.C., Eid, S., Woodhouse, D., Perloe, M., Smith, S., Witmyer, J., … Lieberman, J. (2013). Use of hyaluronan in the selection of sperm for intracytoplasmic sperm injection (ICSI): Significant improvement in clinical outcomes – Multicenter, double-blinded and randomized controlled trial. *Human Reproduction*, *28*, 306–314. doi: 10.1093/humrep/des417.

Yagci, A., Murk, W., Stronk, J., & Huszar, G. (2010). Spermatozoa bound to solid state hyaluronic acid show chromatin structure with high DNA chain integrity: An acridine orange fluorescence study. *Journal of Andrology*, *31*, 566–572. doi: 10.2164/jandrol.109.008912.