Zygote **25** (April), pp. 190–196. © Cambridge University Press 2017
doi:10.1017/S0967199417000041 First Published Online 7 March 2017

Oocyte morphology and embryo morphokinetics in an intra-cytoplasmic sperm injection programme. Is there a relationship?

Azita Faramarzi[2], *Mohammad Ali Khalili*[1] *and Sareh Ashourzadeh*[2,3]

Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; and Afzalipour Clinical Center for Infertility, Afzalipour Hospital, Kerman University of Medical Sciences, Kerman, Iran

Date submitted: 05.10.2016. Date revised: 28.11.2016. Date accepted: 10.01.2017

Summary

The aim was to investigate the relationship between the morphological parameters of metaphase II (MII) oocytes with morphokinetic variables of embryos following an intra-cytoplasmic sperm injection (ICSI) procedure. Morphokinetic behaviour and abnormal cleavage patterns of 334 zygotes were analyzed using time-lapse monitoring (TLM). In addition, oocyte morphology was assessed in relation to embryo morphokinetic (absolute time point, including time to second polar body (PB) extrusion (ESPB), pronuclei (PN) appearance (PNA), PN fading (PNF), time to 2-cells (t2), 3c (t3), 4c (t4), 5c (t5), 6c (t6), 7c (t7), 8c (t8) and relative timing parameters (S1, S2, CC2 and CC3). Also, cleavage patterns (uneven blastomeres, reverse, direct and arbitrary) were assessed. The data showed that 79% of the normal fertilized oocytes had at least one abnormal morphological characteristic. Intra-cytoplasmic abnormalities were observed in 12% of the oocytes. Also, extra-cytoplasmic abnormalities were noticed in 29%, while combined intra- and extra-cytoplasmic abnormalities were responsible for the remaining 38% of the oocytes. Nearly all cleavage and interval times, except extrusion of the ESPB time ($P = 0.003$), were similar between normal and abnormal morphologic oocytes ($P < 0.05$). Moreover, there was significant relationship for oocyte morphology abnormalities and cleavage patterns, including uneven blastomere ($P = 0.037$), reverse cleavage (RC) ($P = 0.0$), direct ($P = 0.001$) and arbitrary cleavages ($P = 0.001$). Using TLM, the cleavage patterns of embryos were affected by the quality of MII oocytes in the ICSI cycles. So, evaluation of oocyte morphology with subsequent embryo morphokinetics is recommended in assisted reproductive programmes.

Keywords: Embryo morphokinetics, ICSI, MII oocyte, Oocyte morphology

Introduction

Attempts have focused on the prediction of the outcomes of the assisted reproductive technique (ART) as well as improving the success rates in assisted reproduction treatment (Ceviren *et al*., 2014). The evaluation of oocyte and embryo competences to determine the best embryo(s) for transfer is a critical step in IVF treatment. In general, the ART centers perform multiple embryo transfer to enhance the implantation and pregnancy rates, which may result in a significantly higher risk of multiple gestations. Multiple gestations related to an increased risk of preterm birth and complications, such as infant death and cerebral palsy (Uyar *et al*., 2013), as well as maternal complications (Molina *et al*., 2014). However, single-embryo transfer (SET) is now the preferable practice in IVF treatment to lower the risk for damaging outcomes associated with multiple gestations (Kirkegaard *et al*., 2012).

Oocyte morphology is one of the factors that may affect the success rate of intra-cytoplasmic sperm injection (ICSI) (Halvaei *et al*., 2012). Previous studies

[1]All correspondence to: Mohammad Ali Khalili. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Tel: +98 35 38247085–6. E-mail: khalili59@hotmail.com
[2]Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[3]Afzalipour Clinical Center for Infertility, Afzalipour Hospital, Kerman University of Medical Sciences, Kerman, Iran.

https://doi.org/10.1017/S0967199417000041 Published online by Cambridge University Press

showed that 80% of metaphase II (MII) oocytes retrieved from stimulated cycles have one or more abnormal morphological characteristics (Ashrafi *et al*., 2015). The morphological parameters of oocytes were divided on the basis of the presence of extra- and intra-cytoplasmic abnormalities. Extra-cytoplasmic abnormalities include a dark or non-Hemogen zona pellucida (ZP), a wide perivitelline space (PVS), PVS debris and a fragmented polar body (PB). Intra-cytoplasmic abnormalities include dark cytoplasm, ooplasm granulation, irregular shape, refractile bodies (RF), bull eye (central aggregation of organelles and vesicles within ooplasm), smooth endoplasmic reticulum (SER) and vacuoles (Fancsovits *et al*., 2012). Oocyte morphology and its influence on embryo competence is still a controversial subject in the literature (Loutradis *et al*., 1999; Esfandiari *et al*., 2006; Nichi *et al*., 2011; Rienzi *et al*., 2011; Kaser *et al*., 2012; Braga *et al*., 2013; Sauerbrun-Cutler *et al*., 2015). Hence, the assessment of oocyte morphology remains one of the most significant purposes of ART advancement (Uyar *et al*., 2013).

The selection of the most suitable embryo(s) for transfer is an important challenge in the ART programme. The failure to properly recognize embryos with the highest implantation potential can lead to failed IVF outcomes. Despite hopeful metabolic profiling, O_2 respiration, aneuploidy screening and gene expression survey, the grading systems based on morphology still remain the preferred way of identifying embryonic development (Chamayou *et al*., 2013). However, embryo selection based on primary subjective and static morphology methods provides limited data as punctual, periodic observations (Chawla *et al*., 2015). So, more dynamic methods are needed to select the embryos with the highest implantation potential for transfer. The morphokinetic assessment of embryos at early cleavage stages improved the prediction of implantation potential. Morphokinetic information also aids the embryologists to discriminate impalpable timing diversities between developmental stages in embryos with same morphological appearance (Liu *et al*., 2014).

In recent years, continuous monitoring of embryo development throughout the culture period has been possible using TLM. This technology is considered as a secure tool in ART laboratories. It has no adverse effects on the embryo development, nevertheless maintaining stable culture conditions (Motato *et al*., 2016). However, the high expenses of this technology do not let their fulfillments in many ART laboratories (Molina *et al*., 2014). The purpose of this prospective study was to assess the relationship between oocyte morphology and morphokinetic variables and cleavage patterns of embryos in clinical setting. To the best of our knowledge, this preliminary study is the first to investigate the possible impact of oocyte morphology on the early embryo development as reflected by TLM in ICSI treatment cycles.

Materials and methods

Study design

In total, 478 MII oocytes were retrieved from 52 women (mean age 30.0 ± 3.42 years) undergoing ICSI cycles between June 2015 to June 2016. Cycles with severe male factors, frozen and surgically retrieved spermatozoa were eliminated from study due to poor recovery of motile spermatozoa. Study inclusion criteria were as follows: patients with fresh autologous oocytes and at least one normal fertilization. The ethics committee of our institution approved this study.

Ovarian stimulation and oocyte preparation

Ovarian stimulation was achieved by long pituitary down-regulation using a combination of gonadotrophin-releasing hormone (GnRH) antagonist and follicle-stimulating hormone (FSH) (Gonal-F; Serono, Geneva, Switzerland). Oocyte collection was done 36 h after human chorionic gonadotrophin (hCG; 10 000 IU) injection. Retrieved cumulus–oocyte complexes (COCs) were incubated in culture medium (G-IVF; VitroLife, Kungsbacka, Sweden) covered with mineral oil (Ovoil; VitroLife) at 37°C and 6% CO_2 for 2–3 h. After mechanic and enzymatic removal of cumulus cells (CCs) with 80 IU/ml hyaluronidase (Sigma Co, USA), MII oocytes were injected using the husband's prepared sperm.

Evaluation of oocyte morphology

Under an inverted microscope (TE300; Nikon), the morphology of oocytes was assessed after the denudation of CCs. The oocytes morphologic features were classified according to the presence of intra- and extra-cytoplasmic abnormalities. Intra-cytoplasmic abnormalities included dark oocyte, ooplasm granulation, presence of vacuoles, RF, irregular shape, bull eye and SER. Extra-cytoplasmic abnormalities included wide PVS, PVS debris, and fragmented PB (Omidi *et al*., 2014).

Embryo culture

After ICSI, each oocyte was individually placed in a pre-equilibrated culture slide (Primo Vision dish, VitroLife, Sweden). The Primo Vision dish with a central depression containing nine straight-sided circular wells filled with 40 μl of G-1+ medium, and covered with 3 ml of mineral oil. The culture slides

https://doi.org/10.1017/S0967199417000041 Published online by Cambridge University Press

were pre-equilibrated (37°C and 6% CO_2) using fresh G-$_1$+ medium and mineral oil. The culture slide was placed in a time-lapse microscope at 37°C, 5 % O_2 and 6 % CO_2 immediately after ICSI for 3 days.

Time-lapse imaging system

A Primo Vision camera was set to take a picture of the embryos every 10 min and the total scan of embryos was taken every 20 min. On day 3, embryos were morphologically assessed for timing of cell divisions and development. The following early kinetic markers were evaluated: absolute time point including time to second PB extrusion (ESPB), pronuclei (PN) appearance (PNA), PN fading (PNF), time to 2-cells (t2), 3c (t3), 4c (t4), 5c (t5), 6c (t6), 7c (t7), 8c (t8) and relative timing parameters S1, CC2, CC3, S2 and S3. Durations of the second cycle (cc2; t3-t2) and third cycle cells (cc3: t5-t3), period time of complete first synchronous division (s1:t2-tPNf), the time to complete second synchronous divisions (s2:t4-t3) were also monitored.

In addition, cleavage patterns including uneven blastomeres embryos were evaluated. Also, three cleavage anomalies specifically monitored were reverse cleavage (RC), where a blastomere was reabsorbed after cleavage. RC was defined as either: (1) two daughter blastomeres recombining after complete separation following cleavage division (type I); or (2) failed cytokinesis, the incomplete separation of blastomeres that later recombine fully (type II) (Liu *et al*., 2014). Direct cleavage (DC), in which a single blastomere divided directly from one to three cells in less than 5 h (Rubio *et al*., 2012). Arbitrary cleavage, including abnormal syngamy, (embryos with disordered PN movement in the cytoplasm, accompanied by delayed dispersion of the nuclear envelopes (AS), abnormal first cytokinesis (embryos exhibiting oolemma ruffling, and possible pseudo-furrows, before first cytokinesis (A1cyt), abnormal cleavage more than two cells during a single cell division event (AC)), and chaotic cleavage (disordered cleavage behaviour by the 4-cell stage (CC)) (Wirka *et al*., 2014).

Data analysis

Results are expressed as mean ± standard deviation (SD) for normal numeric variables, median ± interquartile range and percentage for categorical variables. The means of samples with normal distribution and of sufficient size were compared by independent samples test parametric test. In case of non-normal distribution, the medians were compared by Mann–Whitney test non-parametric test. Categorical variables were compared using chi-squared test. A P-value < 0.05 was considered as significant. Statistical analysis was performed using the Statistical Package for the Social Sciences 20 (SPSS Inc., Chicago, IL, USA).

Table 1 Evaluation of the morphological parameters in normally fertilized oocytes.

Variables	*N* (%)
Normal oocytes	84 (22.83)
Abnormal morphological oocytes	284 (77.17)
Intra-cytoplasmic abnormality	
Irregular shape	16 (4.3)
Vacuole	10 (2.7)
Refractile bodies	126 (34.2)
Smooth endoplasmic reticulum cluster	2 (0.5)
Ooplasm granulation	20 (5.4)
Dark oocytes	33 (9.0)
Bull eye	9 (2.4)
Extra-cytoplasmic abnormality	
Dark zona pellucida	16 (4.3)
Wide perivitelline space	160 (43.5)
Debris in perivitelline space	80 (21.7)
Non-Hemogen zona pellucida	44 (12.0)
Fragmented polar body	83 (22.6)

Values in parenthesis are (%).

Results

In total, 577 oocytes were retrieved, of which 19 (3.30%) were discarded after denudation because of an abnormal appearance (e.g. extensive vacuolization or degeneration). Of the remaining, 478 (85.67%) MII oocytes were injected and 368 (76.98%) of them fertilized normally. Some of the zygotes were excluded from analysis due to arresting in the early development stages or excessive fragmentation.

Incidence of the oocyte morphologic features

On assessment of normal fertilized oocytes, 284 (77.17%) had at least one abnormal morphological characteristic. From 284 abnormal MII oocytes, intra-cytoplasmic abnormalities were seen in 43 (11.7%) of the oocytes. Moreover, extra-cytoplasmic abnormalities were observed in 109 (29.6%), while combined intra- and extra-cytoplasmic abnormalities were responsible for the remaining (35.9%) oocytes (Table 1).

Correlation of oocyte morphology with embryo morphokinetic variables (absolute and relative time).

As shown in Table 2, TLM demonstrated insignificant relationship between oocytes morphology and early embryo developmental events (timing of PNA, PNF,

https://doi.org/10.1017/S0967199417000041 Published online by Cambridge University Press

Table 2 Correlation of oocyte morphology with embryo morphokinetic variables (absolute and relative time)

Kinetic parameters (h)	Oocytes with normal morphology ($N = 85$)	Oocytes with abnormal morphology ($N = 283$)	*P*-value
ESPB	2.13 ± 1.06	2.41 ± 1.00	0.003
PNA	7.30 ± 2.25	7.49 ± 2.30	0.218
PNF	21.58 ± 2.39	22.32 ± 3.26	0.570
S1	2.35 ± 1.20	2.40 ± 1.29	0.797
t2	26.13 ± 4.31	26.14 ± 5.32	0.350
t3	30.53 ± 7.18	33.08 ± 10.29	0.254
CC2	4.31 ± 9.30	9.02 ± 10.24	0.201
t4	37.01 ± 6.18	38.36 ± 8.35	0.131
S2	4.23 ± 7.52	3.11 ± 10.50	0.840
t5	43.14 ± 5.53	41.24 ± 7.58	0.249
t6	46.32 ± 6.04	47.46 ± 7.06	0.861
t7	51.17 ± 6.31	51.10 ± 7.01	0.595
t8	57.03 ± 6.21	58.03 ± 8.22	0.397

Note: Results are expressed as mean ± standard deviation (SD) for normal numeric variables, median ± interquartile range and percentage for categorical variables. Second PB extrusion (ESPB), pronuclei (PN) appearance (PNA), PN fading (PNF), t2 = first cleavage (2-cell stage); t3 = second cleavage (3-cell stage); t4 = 4-cell stage; t5 = 5-cell stage; t6 = 6-cell stage; t7 = 7-cell stage t8 = 8-cell stage. The time to complete first synchronous divisions s1 (t2-tPNf), durations of the second cycle (cc2; t3-t2), the time to complete second synchronous divisions (s2:t4-t3), durations of the third cycle (cc3:t5-t3) and the time to complete third synchronous divisions (s3:t8-t5). NS: not significant.

t2, S1, t3, CC2, t4, S2, CC3, t6, t7, t8 and S3) ($P < 0.05$), except for ESPB time. There was a significant difference between normal and abnormal oocyte morphology ($P < 0.05$) (Table 2).

Correlation of oocyte morphology with uneven blastomeres embryos

The data showed that oocytes with abnormal morphology was correlated with a higher rate of uneven blastomeres embryos compared with normal morphology oocytes (6.5% vs. 93.5%; $P = 0.037$) (Table 3).

Correlation of oocyte morphology with reverse cleavage embryos

The findings demonstrated that there was significant correlation between oocytes morphology and rate of RC embryos. Normal morphology oocytes had lower rate of RC embryos compared with abnormal morphology oocytes (7.0% vs. 93.0%) (Table 4).

Correlation of oocyte morphology with direct cleavage embryos

Also, there was significant relationship between oocyte morphology and rate of RC embryos. Embryos derived from abnormal oocyte morphology showed higher rates of direct cleavage compared with embryos derived from normal oocyte morphology (5.0% vs. 95.6%) (Table 5).

Correlation of oocyte morphology with arbitrary cleavage embryos

TLM showed that there was abnormal oocytes morphology significantly had a higher rate of arbitrary cleavage embryos compared with normal morphology oocytes (6% vs. 94%) (Table 6).

Discussion

Since the advent of ICSI, it has become possible to predict embryo viability in relation to oocyte morphological features. Also, TLM is an emerging tool which can assess the parameters that can foretell the developmental potential of cleaving embryos under stable development conditions (Faramarzi *et al*., 2015). TLM reveals abnormalities in embryo division correlated to reduce implantation potential that cannot be seen by conventional morphological assessment (Liu *et al*., 2014). With the use of continuous TLM, the present study aimed to assess oocyte dysmorphisms and their influence on the embryo morphokinetic parameters and cleavage patterns in the ICSI programme. To our knowledge, this preliminary study is the first to report the impact of oocyte morphology on early embryo development as reflected by time-lapse analysis. We found that 77.17% of the MII oocytes had at least one abnormal morphological characteristic. According to another study, at least 20% of MII oocytes from stimulated cycles had abnormal morphology and the remaining 80% exhibited one or more abnormal

https://doi.org/10.1017/S0967199417000041 Published online by Cambridge University Press

Table 3 Correlation between oocyte morphology and rate of uneven blastomeres embryos

	Uneven blastomeres			
Oocytes	Yes	No	Odds ratio (95% CI)	*P*-value
Normal morphology	2 (6.5%)	68 (22.4%)	238 (0.055–1.02)	0.037
Abnormal morphology	29 (93.5%)	235 (77.6%)		

CI, confidence interval.

Table 4 Correlation between oocyte morphology and rate of reverse cleavage embryos

	Reverse cleavage			
Oocytes	yes	No	Odds ratio (95% CI)	*P*-value
Normal morphology	9 (7.0%)	61 (30.3%)	0.174 (0.083-0.364)	0.00
Abnormal morphology	119 (93.0%)	140 (63.7%)		

CI, confidence interval.

Table 5 Correlation between oocyte morphology and rate of direct cleavage embryos

	Direct cleavage			
Oocytes	Yes	No	Odds ratio (95% CI)	*P*-value
Normal morphology	3 (5.0%)	66 (24.9%)	0.159 (0.48–0.524)	0.001
Abnormal morphology	57 (95.0%)	199 (75.1%)		

CI, confidence interval.

Table 6 Correlation between oocyte morphology and rate of arbitrary cleavage embryos

	Arbitrary cleavage			
Oocytes	Yes	No	Odds ratio (95% CI)	*P*-value
Normal morphology	4 (6.0%)	65 (25.1%)	0.189 (0.066–0.541)	0.001
Abnormal morphology oocytes	63 (94.0%)	194 (74.9%)		

CI, confidence interval.

morphological characteristics (Ashrafi *et al.*, 2015). Intrinsic factors, such as genetic defects and age, as well as extrinsic factors including controlled ovarian hyperstimulation (COH) protocols and the ovarian response to COH may result in oocyte morphological variations (Qassem *et al.*, 2015).

The relation between oocyte morphological abnormalities and ICSI outcomes have been discussed previously (Kahraman *et al.*, 2000; Plachot *et al.*, 2002; Khalili *et al.*, 2005; Yakin *et al.*, 2007; Rienzi *et al.*, 2008; Yu *et al.*, 2015). Controversies still exist on the effects of oocyte morphology on the embryo quality in ART programme (Kahraman *et al.*, 2000; Fancsovits *et al.*, 2011; Setti *et al.*, 2011; Ashrafi *et al.*, 2015, Yu *et al.*, 2015). In this study, we did not detect any effect of oocyte morphology on the morphokinetic time points of derived embryos, except for ESPB time. However, deleterious abnormal oocyte morphology influenced embryo cleavage patterns. Although, Lemmen and colleagues found that early cleavage timing was positively associated with embryo developmental potential in clinical practice (Lemmen *et al.*, 2008). During ICSI, the sperm entry time point for each oocyte may be imprecise as seen, for example, where all embryos cultured on the same slide share one single starting time point. Furthermore, mature oocytes were shown to extrude the second PB at various time intervals, indicating that MII oocytes may have different degrees of cytoplasmic maturity at the point of sperm injection (Liu *et al.*, 2016). In the other words, we assumed that since the injecting time of the oocytes is not the same, the ESPB is variable in different generated embryos.

In this work, oocyte dysmorphism significantly affected cleavage patterns, as well as increasing uneven blastomeres, RC and arbitrary cleavage in derived embryos. Significantly lower fertilization and cleavage rates were reported in dysmorphic oocytes, also reduced rates of top- or good-quality embryos formation, compared with normal morphology

https://doi.org/10.1017/S0967199417000041 Published online by Cambridge University Press

oocytes (Yu *et al*., 2015). Recently, Ashrafi *et al.* (2015) demonstrated that MII oocyte morphology had minor effects on fertilization rate, pronuclear morphology and embryo quality in women with regular menstrual cycles and normal ovarian response (Ashrafi *et al*., 2015). Whereas, Khalili and associates (2005) mentioned that normal oocyte morphology had the most significant role in producing high-grade embryos (Khalili *et al*., 2005). Higher rate of aneuploidy found in the embryos derived from abnormal morphologic oocytes and lower intra-cytoplasmic pH and adenosine triphosphate content are postulated mechanisms in order to explain the adverse effect of such oocytes on clinical outcomes (Ceviren *et al*., 2014). Embryos developed from centrally located granular ooplasm presented chromosomal abnormality in 52.2% of the biopsied blastomeres (Kahraman *et al*., 2000).

Some previous studies indicated that cleavage pattern, such as embryo with uneven blastomere size at 2-cell stage, direct cleavage, RC (types I and II) and arbitrary cleavage (abnormal first cytokinesis, abnormal cleavage and chaotic cleavage) resulted in lower embryo development and implantation potentials (Meseguer *et al*., 2011, Rubio *et al*., 2012, Liu *et al*., 2014, Wirka *et al*., 2014). The molecular mechanisms that cause abnormal cleavage are not obvious. Yet, it seems rational to suppose that mitotic errors may play a main role in this phenomenon (Almagor *et al*., 2015). Liu and colleagues also showed that RC related factors are not limited to those affecting the oocyte, but also include sperm parameters (Liu *et al*., 2014), which is not repaired by abnormal morphologic oocytes.

All earlier studies could be limited by the fact that they were performed on the basis of a static conventional observation. However, the present study was focused on oocyte morphology and its relationship with morphokinetic parameters of ICSI generated embryos and atypical cleavage with the aid of TLM. A limitation of this study was the lack of information on implantation and pregnancy outcomes. Therefore, future studies are warranted to validate findings in the present study. In conclusion, the cleavage patterns of embryos were affected by abnormal oocyte morphology. The use of TLM revealed that abnormalities of embryo division, such as uneven blastomere, RC, direct cleavage and arbitrary cleavage increased by abnormal oocyte morphology during ICSI. Therefore, evaluation of oocyte morphology followed by TLM of generated embryos in an ICSI setting is vital.

References

Almagor, M., Or, Y., Fieldust, S. & Shoham, Z. (2015). Irregular cleavage of early preimplantation human embryos: characteristics of patients and pregnancy outcomes. *J. Assist. Reprod. Genet.* **32**, 1181–5.

Ashrafi, M., Karimian, L., Eftekhari Yazdi, P., Hasani, F., Bahmanabadi, A. & Akhond, M.R. (2015). Effect of oocyte dysmorphisms on intracytoplasmic sperm injection cycle outcomes in normal ovarian responders. *J. Obstet. Gynaecol. Res.* **41**, 1912–20.

Braga, D.P.A.F., Setti, A.S., Rita de Cássia, S.F.s, Machado, R.B., Iaconelli Jr, A. & Borges Jr, E. (2013). Influence of oocyte dysmorphisms on blastocyst formation and quality. *Fertil. Steril.* **100**, 748–54.

Ceviren, A.K., Ozcelik, N.T., Urfan, A., Donmez, L. & Isikoglu, M. (2014). Characteristic cytoplasmic morphology of oocytes in endometriosis patients and its effect on the outcome of assisted reproduction treatments cycles. *IVF Lite* **1**, 88–93.

Chamayou, S., Patrizio, P., Storaci, G., Tomaselli, V., Alecci, C., Ragolia, C., Crescenzo, C. & Guglielmino, A. (2013). The use of morphokinetic parameters to select all embryos with full capacity to implant. *J. Assist. Reprod. Genet.* **30**, 703–10.

Chawla, M., Fakih, M., Shunnar, A., Bayram, A., Hellani, A., Perumal, V., Divakaranm, J. & Budak, E. (2015). Morphokinetic analysis of cleavage stage embryos and its relationship to aneuploidy in a retrospective time-lapse imaging study. *J. Assist. Reprod. Genet.* **32**, 69–75.

Esfandiari, N., Burjaq, H., Gotlieb, L. & Casper, R.F. (2006). Brown oocytes: implications for assisted reproductive technology. *Fertil. Steril.* **86**, 1522–5.

Fancsovits, P., Murber, A., Gilan, Z.T., Rigo, J. & Urbancsek, J. (2011). Human oocytes containing large cytoplasmic vacuoles can result in pregnancy and viable offspring. *Reprod. Biomed. Online* **23**, 513–6.

Fancsovits, P., Tóthné, Z., Murber, Á. & Rigó, J. & Urbancsek, J. (2012). Importance of cytoplasmic granularity of human oocytes in *in vitro* fertilization treatments. *Acta Biologica Hungarica* **63**, 189–201.

Faramarzi, A., Khalili, M.A. & Soleimani, M. (2015). First successful pregnancies following embryo selection using time-lapse technology in Iran: Case report. *Iran J. Reprod. Med.* **13**, 237.

Halvaei, I., Khalili, M.A., Razi, M.H. & Nottola, S.A. (2012). The effect of immature oocytes quantity on the rates of oocytes maturity and morphology, fertilization, and embryo development in ICSI cycles. *J. Assist. Reprod. Genet.* **29**, 803–10.

Kahraman, S., Yakın, K., Dönmez, E., Şamlı, H. & Bahce, M. (2000). Relationship between granular cytoplasm of oocytes and pregnancy outcome following intracytoplasmic sperm injection. *Hum. Reprod.* **15**, 2390–3.

Kaser, D.J., Reichman, D.E., Ginsburg, E.S. & Politch, J.A. & Racowsky, C. (2012). Developmental potential of embryos from intracytoplasmic sperm injection cycles containing fragmented oocytes. *Fertil. Steril.* **97**, 338–43.

Khalili, M.A., Mojibian, M. & Sultan, A.M. (2005). Role of oocyte morphology on fertilization and embryo formation in assisted reproductive technique. *Mid. East Fertil. Soc. J.* **10**, 72–7.

Kirkegaard, K., Agerholm, I.E. & Ingerslev, H.J. (2012). Time-lapse monitoring as a tool for clinical embryo assessment. *Hum. Reprod.* **27**, 1277–85.

https://doi.org/10.1017/S0967199417000041 Published online by Cambridge University Press

Lemmen, J., Agerholm, I. & Ziebe, S. (2008).Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes. *Reprod. Biomed. Online* **17**, 385–91.

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. *Fertil. Steril.* **102**, 1295–1300.

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. *Fertil. Steril.* **105**, 656–62.

Loutradis, D., Drakakis, P., Kallianidis, K., Milingos, S., Dendrinos, S., Michalas, S. & Stylianos, S. (1999). Oocyte morphology correlates with embryo quality and pregnancy rate after intracytoplasmic sperm injection. *Fertil. Steril.* **72**, 240–4.

Meseguer, M., Herrero, J., Tejera, A., Hilligsoe, K.M., Ramsing, N.B. & Remoh, J. (2011). The use of morphokinetics as a predictor of embryo implantation. *Hum. Reprod.* **26**, 2658–71.

Molina, I., Martínez, J.V., Pertusa, J.F., Balasch, S., Iniesta, I. & Pellicer, A. (2014). Assessment of the implantation of day-2 human embryos by morphometric nonsubjective parameters. *Fertil. Steril.* **102**, 1022–8.

Motato, Y., de los Santos, M.J., Escriba, M.J., Ruiz, B.A., Remohí, J. & Meseguer, M. (2016). Morphokinetic analysis and embryonic prediction for blastocyst formation through an integrated time-lapse system. *Fertil. Steril.* **105**, 376–84.

Nichi, M., Figueira, R.C.S., Braga, D.P.A.F., Setti, A.S., Iaconelli Jr, A. & Borges Jr, E. (2011). Decreased fertility in poor responder women is not related to oocyte morphological status. *Arch. Med. Sci*. **7**, 315–20.

Omidi, M., Khalili, M.A., Ashourzadeh, S. & Rahimipour, M. (2014). Zona pellucida birefringence and meiotic spindle visualisation of human oocytes are not influenced by IVM technology. *Reprod. Fertil. Dev.* **26**, 407–13.

Plachot, M., Selva, J., Wolf, J.P., Bastit, P. & de Mouzon, J. (2002). Consequences of oocyte dysmorphy on the fertilization rate and embryo development after intracytoplasmic sperm injection. A prospective multicenter study. *Gynecol. Obstet. Fertil.* **30**, 772–9.

Qassem, E.G., Falah, K.M., Aghaways, I.H.A. & Salih, T.A. (2015). A correlative study of oocytes morphology with fertilization, cleavage, embryo quality and implantation rates after intra cytoplasmic sperm injection. *Acta Medica Int*. **2**, 7–13.

Rienzi, L., Ubaldi, F.M., Iacobelli, M., Minasi, M.G., Romano, S., Ferrero, S., Sapienza, F., Baroni, E., Litwicka, K. & Greco, E. (2008). Significance of metaphase II human oocyte morphology on ICSI outcome. *Fertil. Steril.* **90**, 1692–700.

Rienzi, L., Vajta, G. & Ubaldi, F. (2011). Predictive value of oocyte morphology in human IVF: a systematic review of the literature. *Hum. Reprod. Update* **17**, 34–45.

Rubio, I., Kuhlmann, R., Agerholm, I., Kirk, J., Herrero, J., Escriba, M.J., Bellver, J. & Meseguer, M. (2012). Limited implantation success of direct-cleaved human zygotes: a time-lapse study. *Fertil. Steril.* **98**, 1458–63.

Sauerbrun-Cutler, M.-T., Breborowic, A., Gonzales, E., Stein, D., Lederman, M. & Keltz, M. (2015). Oocyte zona pellucida dysmorphology is associated with diminished in-vitro fertilization success. *J. Ovarian Res.* **8**, 5.

Setti, A.S., Figueira, R.C., Braga, D.P.A.F., Colturato, S.S., Iaconelli Jr, A. & Borges Jr, E. (2011). Relationship between oocyte abnormal morphology and intracytoplasmic sperm injection outcomes: a meta-analysis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **159**, 364–70.

Uyar, A., Torrealday, S. & Seli, E. (2013). Cumulus and granulosa cell markers of oocyte and embryo quality. *Fertil. Steril.* **99**, 979–97.

Wirka, K.A., Chen, A.A., Conaghan, J., Ivani, K, Gvakharia, M., Behr, B., Suraj, V. & Tan, L. (2014). Atypical embryo phenotypes identified by time-lapse microscopy: high prevalence and association with embryo development. *Fertil. Steril.* **101**, 1637–48.

Yakin, K., Balaban, B., Isiklar, A. & Urman, B. (2007). Oocyte dysmorphism is not associated with aneuploidy in the developing embryo. *Fertil. Steril.* **88**, 811–6.

Yu, E.J., Ahh, H., Lee, J.M., Jee, B.C. & Kim, S.H. (2015). Fertilization and embryo quality of mature oocytes with specific morphological abnormalities. *Clin. Exp. Reprod. Med.* **42**, 156–62.

https://doi.org/10.1017/S0967199417000041 Published online by Cambridge University Press