Human Reproduction, Vol.0, No.0 pp. 1–7, 2013

doi:10.1093/humrep/des427

human reproduction

ORIGINAL ARTICLE *Embryology*

Semi-automated morphometric analysis of human embryos can reveal correlations between total embryo volume and clinical pregnancy

G. Paternot*, S. Debrock, D. De Neubourg, T.M. D'Hooghe, and C. Spiessens

Leuven University Fertility Center, UZ Leuven Campus Gasthuisberg, Leuven, Belgium

*Correspondence address. E-mail: goedele.paternot@uzleuven.be

Submitted on September 26, 2011; resubmitted on November 8, 2012; accepted on November 19, 2012

STUDY QUESTION: Is there a link between morphometric characteristics measured by a computer-assisted scoring system and clinical pregnancy outcome?

SUMMARY ANSWER: The results confirm that computer-assisted assessment of the total embryo volume is associated with clinical pregnancy outcome and can be used to complement current procedures of embryo selection.

WHAT IS KNOWN ALREADY: Morphometric analysis of a large group of embryos has revealed the potential to optimize algorithms for image-analysis systems for the grading of embryos and predicting pregnancy outcomes.

STUDY DESIGN, SIZE, DURATION: Oocytes and embryos were obtained from 458 patients who underwent single embryo transfer on Day 3 after IVF/ICSI, between September 2006 and December 2010 at the Leuven University Fertility Center, Belgium. In total, the data set contained 2796 embryos including 458 embryos that were transferred on Day 3. Ongoing pregnancy was defined as the presence of at least one intrauterine gestational sac at 20 weeks.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Patients included in this study were younger than 36 years, entering their first ($n = 375$) or second ($n = 83$) IVF/ICSI cycle and were only included once. Patients were excluded if the cycle included biopsy for PGD or if donor sperm/donor oocytes were used. Based on the 26 sequential images of the same embryo taken at one time point in different planes, the software calculates the total cytoplasmic volume for each time point, from which any reduction or change in the volume with time can be assessed (which helps interpret the degree of fragmentation) and the size of blastomeres. The diameter of the smallest and largest blastomere and the total volume of each embryo were extracted from the computer-assisted scoring system database and the coefficient of diversity was calculated for Days 1, 2 and 3. A logistic regression analysis was performed to determine the range of embryo volume associated with an increased chance of pregnancy.

MAIN RESULTS AND THE ROLE OF CHANCE: On Day 3, blastomeres of 8-cell stage embryos were less divergent in size than those of 6-, 7-, 9-cell stage embryos. Although, the coefficients of diversity (ratio of the largest:smallest blastomeres) of implanted embryos tended to be lower than for non-implanted embryos, the difference was only significant for 6-cell stage embryos ($P = 0.02$). After logistic regression, an association between total embryo volume and pregnancy was observed which had a quadratic nature: both lower and higher volumes were associated with a lower probability of successful pregnancy. A significant association was identified between total embryo volume and pregnancy rate on both Days 2 ($P = 0.003$) and 3 ($P = 0.0003$). Diagnostic measures (sensitivity, specificity, positive predictive value, accuracy and c-statistics) of the defined volume range were relatively poor. However, results showed a good negative predictive value [76.86% (95% confidence interval 71.03–82.02) on Day 3].

LIMITATIONS, REASONS FOR CAUTION: A general disadvantage of studies evaluating the impact of a characteristic on the implantation potential of an embryo is the fact that the best embryo is chosen for transfer. No comparisons can therefore be made with the other embryos. Moreover, the decision process is currently based on a non-automated, standard scoring system, which means that a 'bias' in the selection process is always present.

WIDER IMPLICATIONS OF THE FINDINGS: Our results are an important step towards the development of an automated computer-assisted scoring system for the morphological characteristics of human embryos to improve embryo selection for optimizing implantation potential. Total embryo volume appears to be one of the objective characteristics that should be included.

STUDY FUNDING/COMPETING INTEREST(S): None.

TRIAL REGISTRATION NUMBER: Not applicable.

Key words: blastomere / morphometrics / embryo volume / ongoing pregnancy / implantation

Introduction

A variety of evaluation techniques have been described to assess the viability of embryos in assisted reproduction techniques (ARTs). These evaluations are mostly based on the morphological characteristics of the embryos (Backzkowski *et al.*, 2004), which are basically evaluated by an embryologist in a fast but subjective way (Paternot *et al.*, 2009). Although many studies have been published on different morphological embryo characteristics (Backzkowski *et al.*, 2004), there is no 'golden standard' which reliably predicts embryo viability.

Computer-assisted scoring systems in combination with automation of embryo visualization can improve embryo assessment (Paternot *et al.*, 2011). These systems give additional information on embryo characteristics that cannot be evaluated by a manual scoring. Multilevel imaging of the embryo is very helpful for embryo evaluation. In one study, using computer-controlled multilevel analyses, the mean blastomere size was correlated with the degree of fragmentation and multinucleation (Hnida *et al.*, 2004). In another study, multilevel images were analyzed to evaluate if nuclear size and number are indicators of aberrant chromosome content (Agerholm *et al.*, 2008): these researchers concluded that the mean nuclear size may be a marker of abnormal chromosomal status in multinucleated but not in mononucleated blastomeres. Advanced imaging methods now allow automatic detection of blastomeres of day 2 embryos (Filho *et al.*, 2004) and morphological assessment of human 2PN zygotes (Beuchat *et al.*, 2008). Recently, our group reported that computer-assisted scoring gave a better prediction of the implantation potential and live birth rate than manual scoring (Paternot *et al.*, 2011). In addition, computer-assisted scoring may overcome the subjectivity of currently used grading systems and images can be evaluated without time restriction (Paternot *et al.*, 2011). Finally, a large database of images can be developed (Paternot *et al.*, 2011).

In order to have an efficient and accurate morphometric scoring system, it is necessary to evaluate how closely morphometric characteristics of the embryo correlate with the implantation rate. In the early 1980's, some studies correlated morphometric characteristics with embryo viability. In mouse embryos, nutrient uptake was correlated with the surface area to volume ratio (Brower and Schultz, 1982). In human embryos, the surface to volume ratio of the internal and external limits of the zona pellucida remained unchanged during the first three divisions (Maneiro *et al.*, 1991).

Other morphometric characteristics that have been evaluated include the absolute volumes, coefficients of form and the coefficient of diversity of human oocytes and/or embryos (Goyanes *et al.*, 1990). No significant changes in the total volume occurred during successive cleavages nor were there significant differences in the coefficient of form for normal/abnormal embryos or for implanted/non-implanted embryos (Goyanes *et al.*, 1990): in this study, blastomeres started as irregular spheres at the 2-cell stage embryo and became ellipsoid by the 8-cell stage. Roux *et al.* (1995) described morphometric characteristics of human embryos and stated that further experiments on larger groups and multiple regression analysis of morphometric parameters would be needed to be certain that these characteristics correlate with a successful implantation. Optimization of algorithms for image analysis to improve the embryo grading systems and embryo selection still needs the evaluation of morphometric characteristics in a large group of embryos plus the monitoring of pregnancy outcome.

The primary aim of this study was to evaluate morphometric variables in a large data set and to determine a possible link between the total volume of a transferred human embryo (calculated by computer) and pregnancy rate. As a secondary aim, we wanted to determine if there was an optimal range of embryo volume associated with an increased pregnancy rate.

Materials and Methods

Patients

Oocytes and embryos examined in this study were obtained from 458 patients who received a single embryo transfer on Day 3 between September 2006 and December 2010 at the Leuven University Fertility Center. Patients were younger than 36 years, entered their first ($n = 375$) or second ($n = 83$) IVF/ICSI cycle and were only included once. The stimulation protocol used in this study has been described before (Debrock *et al.*, 2010). Patients were excluded if the cycle included biopsy for PGD or if donor sperm/donor oocytes were used. In total, the data set contained 2796 embryos including 458 embryos that were transferred on Day 3. Patients and cycle characteristics are listed in Table I.

ART procedure

After oocyte retrieval, the oocytes were washed four times prior to fertilization in order to minimize the amount of blood/follicular fluid and placed in a four-well dish containing 500 µl fertilization medium (sequential medium: COOK medium, Sydney IVF fertilization, Sydney IVF, QLD, Australia or single medium GM501 medium, Gynemed, Lensahn, Germany) (37°C, pH 7.25–7.35) per well, under mineral oil (Gynemed). Spermatozoa for the IVF procedure were prepared using standard density gradient procedures (Isolate, Irvine Scientific, USA). Sperm samples for ICSI were diluted and centrifuged twice at 300*g* for 10 min. Standard IVF/ICSI procedures were performed 2–6 h after oocyte retrieval. In the IVF procedure, oocytes were inseminated with 300 000 progressively motile spermatozoa per well (maximum of five oocytes in 0.5 ml per patient). In the case of an ICSI cycle, injected oocytes were incubated together in a 20 µl culture medium droplet under oil. On Day 1 (16–20 h after insemination/injection) fertilization was evaluated. Only normally fertilized oocytes (2PN) were cultured individually in a 20 µl droplet of culture

Table I Characteristics of the patients and IVF/ICSI cycles.

Patients	*n* = 458
Female age (years)	30.55 ± 3.44
Causes of subfertility	
Tubal factor (%)	61 (13)
Ovulation (%)	100 (22)
Endometriosis (%)	67 (15)
Implantation (%)	9 (2)
Other (%)	13 (3)
Male factor (%)	318 (69)
Cycles (*n* = 458)	
ICSI cycles (%)	277 (60)
IVF cycles (%)	181 (40)
Oocytes per retrieval	10.47 ± 4.72
Mature oocytes per retrieval	9.18 ± 4.21
Fertilization rate per oocyte (%)	65 ± 21
Fertilization rate per mature oocyte (%)	75 ± 20
Clinical outcome	
Positive hCG (%)	173 (38)
Biochemical pregnancies (%)	21 (5)
Implanted embryos (%)	152 (33)
Extra-uterine sacs (%)	3 (1)
Intrauterine sacs (%)	149 (33)
Early spontaneous abortion (%)	2 (0)
Late spontaneous abortion (%)	16 (3)
Ongoing pregnancy (%)	131 (29)

Values are the mean ± SD or *n* (%).

medium (COOK medium, Sydney IVF fertilization, Sydney IVF or GM501 medium, Gynemed) covered with mineral oil.

Embryo evaluation

Image sequences for each embryo were recorded on Day 1 (16–20 h after insemination/injection), Day 2 (41–44 h after insemination/injection) and Day 3 (66–71 h after insemination/injection, the day of transfer) using a computer-assisted scoring system (FertiMorph, Image House, Copenhagen, Denmark). This semi-automatic embryo quality assessment system has been described recently by Paternot *et al.* (2011). Based on the 26 sequential images of the same embryo taken at one time point in different planes, the software calculates the total cytoplasmic volume for each time point, from which any change in the volume with time can be assessed (which helps interpret the degree of fragmentation) and the size of blastomeres. The criteria for distinguishing between a blastomere and a fragment were based on the findings by Hnida *et al.* (2005) and Johansson *et al.* (2003) who reported that the diameter of a blastomere should be ≥45 μm on Day 2 and ≥40 μm on Day 3. For this study, the diameters and the volume of each blastomere were extracted from the database. In addition, the embryos were evaluated using the manual scoring system of the Leuven University Fertility Center: this embryo evaluation was based on the assessment by an embryologist who visually evaluated the number and size of blastomeres and the degree of fragmentation. On Day 3 the best embryo available was chosen for transfer based on the manual standard scoring system. An ongoing pregnancy was defined as the presence of at least one intrauterine gestational sac after 20 weeks of pregnancy.

Morphometric characteristics of the embryos

The diameter of the smallest and largest blastomere and the volume of each embryo were extracted from the database. The size ratio of the largest/smallest blastomeres, here called the coefficient of diversity, was calculated. This characteristic is based only on the blastomeres present in the embryo and excludes the fragments.

As only 26 5-cell stage embryos and 12 10-cell stage embryos were transferred of which only 3 and 2 embryos, respectively, implanted, these embryos were excluded from the coefficient of diversity analysis.

Statistics

The analyses were performed using SAS software, version 9.2 (SAS System for Windows Copyright 2002 SAS Institute, Inc., SAS, Cary, NC, USA).

Descriptive statistics were performed by summarizing the measurements by Day (days 1, 2 or 3) and according to the final decision (embryos transferred, cryopreserved or discarded). In addition, for those embryos transferred on Day 3, the measurements were also evaluated according to pregnancy outcome and plotted with 95% confidence interval (CI). A logistic regression was performed using total volume as a continuous variable to test the association between the total volume and pregnancy outcome. The functional shape of the association was investigated by fitting lines and polynomials and by evaluating the results using Akaike's Information Criterion (AIC), whereby lower values indicate a better fit. For each of the three days (1, 2 and 3) of embryo development, the best fit of the data was obtained by modeling the association between the volume and pregnancy outcome as a quadratic curve, with lower chances of pregnancy towards both lower and higher values of total volume.

When a quadratic shape for the association was obtained, the range of values with an increased probability of pregnancy was estimated by cutting off the low and high volumes where the predicted probability was lower than a certain pre-chosen limit. The cut-off values and their 95% CIs were estimated by means of bootstrapping whereby the above procedure was repeated on 1000 bootstrap samples, meaning that 1000 lower and higher cut-off points were obtained. The final estimate of the lower and higher cut-off was obtained by averaging the bootstrap results.

To evaluate how well the obtained ranges estimated the outcomes, we assessed the sensitivity (proportion of embryos that resulted in a positive clinical outcome and had a volume within the range), specificity (proportion of embryos that resulted in a negative clinical outcome and had a volume out of the range), positive predictive value (probability that embryos with a volume within the range result in a positive clinical outcome), negative predictive value (probability that embryos with a volume out of the range result in a negative clinical outcome), accuracy (degree of veracity) and c-statistic. The c-statistic was used as a measure of discrimination to determine how accurately total blastomere volume could differentiate between embryos that would lead to pregnancy and embryos that would not.

Models with a c-statistic >0.80 are generally assumed to provide sufficient discrimination for predictive use (Harrell , 2001). A *P* value of <0.05 was considered statistically significant.

Results

Morphometric characteristics

In total, 2796 embryos were evaluated on Days 1, 2 and 3 of development. A total of 458 embryos were transferred (single embryo

transfer), 1000 embryos were cryopreserved and 1338 embryos were discarded on Day 3. The total volume of the embryo and the diameter of the smallest and largest blastomere of embryos on Days 2 and 3 are summarized in Table II.

In Table III, the data of the transferred embryos are summarized based on the implantation outcome. A total of 131 transferred embryos resulted in an ongoing pregnancy. As no difference was found between the pregnancy rates in the first [28% (104/375)] or second [33% (27/83)] ($P = 0.386$) IVF/ICSI cycle, both groups were combined and analyzed as one group.

In Table IV, the coefficients of diversity (= ratio of largest/smallest blastomere) are listed for the total group of embryos on Day 3, and separately for implanted and non-implanted embryos. Analyzing the total group of embryos on Day 3, the lowest coefficient of diversity was found for the 8-cell stage embryos (1.29 ± 0.14). Although the coefficients of diversity of implanted embryos were lower than those of non-implanted embryos, this difference was only significant for 6-cell stage embryos ($P = 0.02$).

Impact of culture medium on embryo volume

Embryos were cultured in two types of culture media. In total 1472 embryos were cultured in the sequential medium and 1324 embryos in the single culture medium and no difference was found in total volume ($P = 0.86$). The pregnancy rate was not influenced by the embryo culture media [sequential medium: 28% (78/276); single medium: 29% (53/182); $P = 0.92$], indicating that the embryos can be evaluated as one group.

Impact of volume of the embryo on pregnancy

The logistic regression analysis included pregnancy as the dependent variable and total volume as a continuous independent variable. Investigation of the shape of the association indicated that the best fit was provided when using a quadratic function. The results of the logistic regression analysis are presented in Table V. An overall test of the effect of volume, simultaneously testing the linear and quadratic term of the association, was performed by means of a likelihood ratio. In addition, the resulting AIC value was added to the table. Inspection of the results showed no significant association between the total volume on Day 1 and pregnancy. However, a significant association was identified between total embryo volume and pregnancy rate on both Day 2 ($P = 0.003$) and Day 3 ($P = 0.0003$). Inspection of the AIC values revealed that the total volume measured on Day 3 is the best predictor of pregnancy outcome. Despite statistical significance, discrimination of the models at each of the days was relatively poor, as measured by the c-statistic (<0.80) (Table V).

As the overall chance of pregnancy in the data set was 29%, we determined the range of volumes that could predict a pregnancy rate of least 30%. The resulting estimates of the lower and upper limit of these ranges (and their 95% CIs) together with the observed pregnancy rates for total volumes within and outside the estimated range are presented in Table VI. Once the ranges were determined,

Table II Morphometric data (mean ± SD) for human embryos on Days 2 and 3 of development after IVF/ICSI, obtained using computer-assisted analysis.

	Total cytoplasmic embryo volume (μm^3)	Minimal blastomere diameter (μm)	Maximal blastomere diameter (μm)
Day 2			
Embryo transfer ($n = 458$)	672 555 (± 116 933)	63.11 (± 8.88)	76.57 (± 7.61)
Cryopreservation ($n = 1000$)	674 019 (± 124 822)	62.56 (± 10.34)	77.17 (± 8.18)
Discard ($n = 1338$)	577 730 (± 150 750)	66.09 (± 16.72)	80.72 (± 11.97)
Day 3			
Embryo transfer ($n = 458$)	644 503 (± 115 023)	48.28 (± 4.54)	61.92 (± 5.64)
Cryopreservation ($n = 1000$)	636 942 (± 102 756)	47.48 (± 3.99)	62.68 (± 5.44)
Discard ($n = 1338$)	539 429 (± 147 749)	53.93 (± 12.63)	70.93 (± 10.82)

Table III Summary of measurements (mean ± SD) over time for human embryos which did or did not implant.

	Total cytoplasmic volume (μm^3)	Minimal diameter (μm)	Maximal diameter (μm)
Day 2			
No implantation ($n = 327$)	664 713 (± 122 243)	62.88 (± 8.95)	76.61 (± 7.83)
Implantation ($n = 131$)	692 128 (± 100 265)	63.71 (± 8.70)	76.49 (± 7.04)
Day 3			
No implantation ($n = 327$)	633 218 (± 118 773)	48.09 (± 4.79)	62.36 (± 5.95)
Implantation ($n = 131$)	672 670 (± 100 066)	48.76 (± 3.81)	60.84 (± 4.61)

Table IV Coefficients of diversity (ratio of largest/smallest blastomere: mean ± SD) for the human embryos.

Blastomeres at Day 3 (*n*)	*n* embryos total group	Coefficient of diversity total group	*n* embryos transferred	*n* embryos implanted	Coefficient of diversity implanted embryos	Coefficient of diversity non-implanted embryos
6	409	1.38 (±0.16)	30	6	1.23 (±0.09)*	1.43 (±0.18)*
7	490	1.34 (±0.15)	103	20	1.28 (±0.12)	1.34 (±0.15)
8	595	1.29 (±0.14)	238	86	1.23 (±0.09)	1.26 (±0.13)
9	134	1.37 (±0.17)	35	14	1.30 (±0.09)	1.34 (±0.17)

*$P = 0.02$.

Table V Results of the logistic regression for association between total human embryo volume and outcome and c-statistic.

Day	Coefficient	Estimate	95% CI	*P*-value	Overall *P*-value	AIC value	C-statistic
Day 1	Intercept	−5.175	(−11.46; 1.113)	0.1067		551.00	0.556
	Linear	0.922	(−0.634; 2.477)	0.2454	0.1945		
	Quadratic	−0.046	(−0.142; 0.050)	0.3503			
Day 2	Intercept	−8.021	(−13.48; −2.563)	0.0040		542.66	0.594
	Linear	1.831	(0.299; 3.364)	0.0192	0.0030		
	Quadratic	−0.113	(−0.220; −0.006)	0.0389			
Day 3	Intercept	−7.715	(−12.69; −2.740)	0.0024		538.20	0.611
	Linear	1.741	(0.298; 3.184)	0.0180	0.0003		
	Quadratic	−0.104	(−0.209; −0.000)	0.0494			

AIC, Akaike's Information Criterion; CI, confidence interval.

Table VI Estimates of lower and upper limits of the ranges for total human embryo volume.

Measure	Day 1		Day 2		Day 3	
	n/*N*	Estimate (95% CI)	*n*/*N*	Estimate (95% CI)	*n*/*N*	Estimate (95% CI)
Sensitivity (%)	69/131	52.67 (43.77; 61.45)	80/131	61.07 (52.16; 69.46)	75/131	52.25 (48.32; 65.85)
Specificity (%)	177/327	54.13 (48.56; 59.62)	166/327	50.76 (45.21; 56.31)	186/327	56.88 (51.32; 62.32)
PPV (%)	69/219	31.51 (25.41; 38.11)	80/241	33.20 (27.28; 39.53)	75/216	34.72 (28.39; 41.48)
NPV (%)	177/239	74.06 (68.01; 79.49)	166/217	76.50 (70.28; 81.97)	186/242	76.86 (71.03; 82.02)
Accuracy (%)	246/458	53.71 (49.02; 58.35)	246/458	53.71 (49.02; 58.35)	261/458	56.99 (52.31; 61.57)
C-statistic	0.53		0.56		0.57	

Diagnostic measurements used to assess the usefulness of the ranges for predicting pregnancy.
PPV, positive predictive value; NPV, negative predictive value.

the usefulness of these ranges in predicting pregnancy was assessed. The results showed a poor sensitivity and specificity. The positive predictive value was just >30% and a discriminatory power (c-statistic) of <0.6 for Days 1, 2 and 3. However, the results also indicate a fairly high negative predictive value on Day 3: 76.86% (95% CI: 71.03–82.02) (Table VI).

Discussion

The aim of this study was to describe a number of morphometric characteristics in a large data set. A total of 2796 embryos were included of which 458 embryos were transferred on Day 3 and their implantation potential examined, as assessed by a pregnancy at 20 weeks.

When investigating the influence of the difference in blastomere size, the lowest coefficient of diversity was found for 8-cell stage embryos on Day 3. This finding confirmed the results described by Goyanes *et al.* (1990) who found a higher coefficient of diversity for asynchronous divisions (6-cell stage embryos). In contrast, Goyanes *et al.* (1990) reported that successfully implanted embryos had a higher coefficient of diversity between sister blastomeres. It should be mentioned that the study of Goyanes *et al.* (1990) included a

very low number of subjects (*n* total group = 110, *n* implanted embryos = 7). In our study, implanted embryos tended to have more uniform blastomere size than non-implanted embryos, although this was only significant for the 6-cell stage embryos. Since the coefficient of diversity is calculated as the ratio of the largest and smallest blastomere diameter, this finding indicates that 6-cell stage embryos with more equally sized blastomeres have a higher implantation potential. This is in line with current scoring systems using blastomere uniformity as the characteristic for selection of embryos (Backzkowski *et al.*, 2004). The presence of unevenly sized blastomeres has been reported to be negatively correlated with implantation (Ziebe *et al.*, 1997) and pregnancy rates (Hardarson *et al.*, 2001). However, this finding is in contrast to the division model described by Roux *et al.* (1995) who suggested that during the intermediate steps (5, 6, 7-cell stages), the population of blastomeres have a bimodal distribution and therefore can be divided into two subpopulations. This means that, theoretically, a 6-cell stage embryo has unequally sized blastomeres and, as a consequence, a higher coefficient of diversity between blastomeres. The results of our study regarding the 6-cell embryos, however, have to be confirmed in future studies because only 30 embryos at the 6-cell stage were transferred, of which 6 embryos implanted.

Another aim of this study was to investigate the influence of total embryo volume on pregnancy outcome. The total volume was measured on Days 1, 2 and 3, and logistic regression analysis showed that the total volume on Day 3 has an influence on the implantation potential of the embryo: a high negative predictive value was found, indicating that embryos with a total volume beyond the optimal range have a lower chance of resulting in an ongoing pregnancy. It should be stressed that these results only pertain to transferred embryos and cannot be generalized to the whole population of embryos. To our knowledge, this is the first study evaluating the impact of total volume on pregnancy rate, and in order to assess whether the total embryo volume could be used as a factor in the decision-making process, further studies are needed.

A general disadvantage of studies evaluating the impact of a characteristic on the implantation potential is the fact that the best embryos are chosen for transfer, and therefore no conclusions can be reached concerning the other embryos. Moreover, the decision process is currently based on a standard non-automated scoring system. This means that there is always a bias in the selection process. Based on the current data, a trial could be set up in which patients are randomized to one of the two decision procedures for embryo transfer, whereby one is the standard method and the other procedure is the standard method supplemented with the total volume.

The biological relevance of the embryo volume as a characteristic which is important for implantation potential can be explained by the fact that volume regulation is an essential process in the embryo development, as a failure in volume regulation can result in blocked embryos (Baltz, 2010). Early embryos use unique mechanisms to maintain cell volume. The major mechanism before compaction is based on the accumulation of glycine by the GLYT1 transporter. In addition, betaine or proline transport plays a role in cell homeostasis. We found an association which is of a quadratic nature, whereby lower and higher volumes have a lower probability of successful pregnancy, which hypothetically could be explained by failure in some of these biological mechanisms. As mentioned before, the type of culture medium can have an impact on the volume of an embryo (Baltz, 2010); however, this observation was not confirmed in our study. In conclusion, the results of this study are an important step towards the development of an automated computer-assisted scoring system to improve human embryo selection. Such an automated system may assist in determining which embryo has the highest implantation potential, based on different characteristics of the embryos, and total embryo volume could be one of the objective characteristics that should be included.

Acknowledgements

The authors thank the Biostatistical Centre of the University of Leuven for the help and advice on the statistics.

Authors' roles

P.G. and S.C. contributed to the paper by defining the design of the study, the analysis and the interpretation of the data. Both authors draft the paper and approved the final version. D.S., D.N. and D'H.T.M. interpreted the data and revised the paper critically for important intellectual content and approved the final version.

Funding

No external funding was either sought or obtained for this study.

Conflict of interest

None declared.

References

Agerholm IE, Hnida C, Crüger DB, Berg C, Bruun-Petersen G, Kolvraa S, Ziebe S. Nuclei size in relation to nuclear status and aneuploidy rate for 13 chromosomes in donated four cells embryos. *J Assist Reprod Genet* 2008;**25**:95–102.

Backzkowski T, Kurzawa R, Gabowski W. Methods of embryo scoring in *in vitro* fertilization. *Reprod Biol* 2004;**4**:5–22.

Baltz JM, Tartia AP. Cell volume regulation in oocytes and early embryos: connecting physiology to successful culture media. *Hum Reprod Update* 2010;**16**:166–176.

Beuchat A, Thévenaz P, Unser M, Ebner T, Senn A, Urner F, Germond M, Sorzano COS. Quantitative morphometrical characterization of human pronuclear zygotes. *Hum Reprod* 2008;**23**:1983–1992.

Brower PT, Schultz RM. Intercellular communication between granulose cells and mouse oocytes: existence and possible nutritional role during oocyte growth. *Dev Biol* 1982;**90**:144–153.

Debrock S, Melotte C, Spiessens C, Peeraer K, Vanneste E, Meeuwis L, Meuleman C, Frijns JP, Vermeersch JR, D'Hooghe TM. Pre-implantation genetic screening for aneuploidy of embryos after *in vitro* fertilization in women aged at least 35 years: a prospective randomized trial. *Fertil Steril* 2010;**93**:364–373.

Filho ES, Noble JA, Wells D. A review on automatic analysis of human embryo microscope images. Open Biomed Eng J, 2004;**4**:170–177.

Goyanes VJ, Ron-Corzo A, Costas E, Maneiro E. Morphometric categorization of the human oocyte and early conceptus. *Hum Reprod* 1990;**5**:613–618.

Hardarson T, Hanson C, Sjögren A, Lundin K. Human embryos with unevenly sized blastomeres have lower pregnancy and implantation rates: indications for aneuploidy and multinucleation. *Hum Reprod* 2001;**16**:313–318.

Harrell FEJ. *Regression Modeling Strategies with Applications to Linear Models, Logistic Regression Survival Analysis*. New York, NY: Springer, 2001.

Hnida C, Engenheiro E, Ziebe S. Computer-controlled, multilevel, morphometric analysis of blastomere size as biomarker of fragmentation and multinuclearity in human embryos. *Hum Reprod* 2004;**19**:288–293.

Hnida C, Agerholm I, Ziebe S. Traditional detection versus computer-controlled multilevel analysis of nuclear structures from donated human embryos. *Hum Reprod* 2005;**20**:665–671.

Johansson M, Hardarson T, Lundin K. There is a cutoff limit in diameter between a blastomere and a small anucleate fragment. *J Assist Reprod Genet* 2003;**20**:309–313.

Maneiro E, Ron-Corzo A, Julve J, Goyanes VJ. Surface area/volume ratio and growth equation of the human early embryo. *Int J Dev Biol* 1991; **35**:139–143.

Paternot G, Devroe J, Debrock S, D'Hooghe TM, Spiessens C. Intra- and interobserver analysis in the morphological assessment of early-stage embryos. *Reprod Biol Endocrinol* 2009;**7**:105.

Paternot G, Debrock S, D'Hooghe TM, Spiessens C. Computer-assisted embryo selection: a benefit in the evaluation of embryo quality? *Reprod Biomed Online* 2011;**23**:347–354.

Roux C, Joanne C, Angnani G, Fromm M, Clavequin MC, Bresson JL. Morphometric parameters of living human in-vitro fertilization embryos; importance of the asynchronous division process. *Hum Reprod* 1995;**10**:1201–1207.

Ziebe S, Petersen K, Lindenberg S, Andersen AG, Gabrielsen A, Andersen AN. Embryo morphology or cleavage stage: how to select the best embryos for transfer after in-vitro fertilization. *Hum Reprod* 1997;**12**:1545–1549.