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ARTICLE

Computer-assisted embryo selection: a benefit in the evaluation of embryo quality?

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Goedele Paternot obtained her Masters in biomedical sciences in 2007 at the Catholic University of Leuven, Belgium. In 2007 she started her PhD in medical sciences entitled 'Selecting the embryo with the highest implantation potential regarding the morphology and developmental potential in a clinical setting' at the Leuven University Fertility Centre (ISO 9001:2008 certified). The main research topics of the centre are embryo scoring and the optimization of in-vitro culture conditions, quality of patient care and endometriosis.

Abstract Embryo selection is based on embryo developmental and morphological characteristics. Standard embryo evaluation has some disadvantages. New technology using multilevel images combined with a computer-assisted scoring system (CASS) has the potential to overcome these disadvantages. The aim of this study was to compare the value of a computer-assisted scoring system (CASS) versus a standard scoring system (SSS) in predicting implantation and live birth. This prospective study included 3185 embryos obtained during 502 IVF/intracytoplasmic sperm injection cycles with single-embryo transfer on day 3. Embryos were evaluated with two scoring systems: SSS and CASS. Logistic regression analyses were performed using implantation and live birth as outcomes. According to multiple regression analysis, implantation was influenced by number and size of blastomeres on day 3 using CASS and by all embryo parameters on day 3 using SSS. Combined analysis of both scoring systems revealed that implantation was affected by number and size of blastomeres using CASS and by the degree of embryo fragmentation using SSS. Using live birth as outcome, only the number of blastomeres on day 3, evaluated by SSS and CASS, was predictive. Prediction of implantation and live birth may be superior using CASS when compared with SSS.

KEYWORDS: computer-assisted scoring system, embryo selection, implantation, live-birth rate, single-embryo transfer

Introduction

One of the major problems in reproductive medicine is the high incidence of multiple pregnancies. Multiple pregnancies lead to a higher incidence of medical complications, both perinatal and neonatal, and to a high socioeconomic cost (De Sutter, 2006; Garceau et al., 2002) and can be prevented by a reduction of the number of embryos transferred (Debrock et al., 2005). Single-embryo transfer (SET) is the most effective way to reduce the incidence of multiple pregnancies. Although, the overall pregnancy rate can be maintained when a SET is performed (Gerris et al., 2002), it is possible that the live-birth rate is reduced when compared with the transfer of two (or even three) embryos (Baruffi et al., 2009; Gelbaya et al., 2010; Van Montfoort et al., 2006). In view of increasing international recognition of the value of performing elective SET or double-embryo transfer in order to prevent higher-order multiple pregnancies,

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it becomes increasingly important to select the embryo(s) with the highest potential to implant and result in a live birth. Up until now, embryo selection has been routinely based on embryo developmental and morphological characteristics, using a variety of classification and scoring systems, to evaluate embryo quality (Desai et al., 2000; Fisch et al., 2001; Holte et al., 2007).

Standard scoring of cleavage-stage embryos using direct microscopic evaluation by an embryologist is limited by a number of disadvantages. Firstly, inter-observer variability in the evaluation of morphological parameters has been reported (Arce et al., 2006; Paternot et al., 2009). Secondly, the absence of a clearly defined standard method to measure specific characteristics can lead to a loss of important information. Regarding the evaluation of the degree of fragmentation, most scoring systems evaluate this characteristic by using categories. More accurate measurements can give additional information. Thirdly, the evaluation time has to be kept as short as possible to prevent its exposure to suboptimal conditions since fluctuations in pH and temperature have deleterious effects on the embryo development and quality (Garrisi et al., 1993). Therefore, a short evaluation time is essential. In contrast, a real-time complete evaluation of all morphological characteristics is time consuming.

New technology using multilevel images combined with a computer-assisted scoring system (CASS) has the potential to overcome most of these disadvantages associated with a standard scoring system (SSS). Firstly, CASS can store multilevel images of the embryos during development, in a fast way, limiting the exposure time to less optimal conditions. Secondly, these multilevel images allow embryologists to assess embryo quality in the same way as an exploration using the inverted microscope. This indicates that an evaluation can be performed in more detail without any time restrictions. Thirdly, embryo characteristics can be analysed semi-automatically resulting in a better and more precise way of scoring (Hnida et al., 2004) that may reduce the inter-observer variability during embryo scoring (Paternot et al., 2009).

Since there is no study comparing the use of a CASS and a SSS in the prediction of implantation, the aim of this study was to analyse which type of scoring system is the better to predict implantation and live birth.

Materials and methods

Embryo selection

This study included a total of 3185 embryos obtained during 502 IVF or intracytoplasmic sperm injection (ICSI) cycles with SET on day 3, from 463 patients younger than 36 years participating in the IVF/ICSI programme for the first or second time. The study was approved by the Institutional Review Board of the University Hospitals Leuven (ML4564). An overview of patient and cycle characteristics is given in **Table 1**. The stimulation protocol used is described in detail reported by Debrock et al. (2010).

After oocyte retrieval, the oocytes were washed through four wells each containing 500 μl fertilization medium (COOK medium, Sydney IVF fertilization, Sydney IVF, Queensland, Australia or GM501 medium, Gynemed Lensahn Germany) (37°C, pH 7.25–7.35) under mineral oil. Spermatozoa used for the IVF procedure were prepared using standard density-gradient procedures (Isolate, Irvine Scientific, USA). Sperm samples used for ICSI were diluted and were centrifuged twice for 10 min at 300 ***g***. Standard IVF/ICSI procedures were performed 2–6 h after oocyte retrieval. During the IVF procedure, oocytes were inseminated with 200,000 progressively motile spermatozoa per well (0.5 ml). 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) oocytes were checked for fertilization. Only normal fertilized oocytes (two pronuclei) were cultured individually in a 20 μl droplet of culture medium covered with mineral oil.

In the first part of this study, all the embryos were assessed by a CASS (FertiMorph, Image House, Copenhagen, Denmark) using multilevel images and by the SSS of the Leuven University Fertility Centre in order to compare the assessment of embryo characteristics. In the second part of this study, both scoring systems were compared in the 502 transferred embryos with respect to the prediction of implantation and live birth.

Table 1 Patient and cycle characteristics and clinical outcome of the study subjects.

Characteristic	*Study*
Patients (*n* = 463)	
Female age (years)	30.35 ± 3.47
Cause of subfertility	
Tubal factor	62 (13)
Ovulation	100 (22)
Endometriosis	69 (15)
Implantation	12 (3)
Other	13 (3)
Male factor	327 (71)
Cycles (*n* = 502)	
ICSI cycles	216
IVF cycles	286
Oocytes per retrieval	10.6 ± 4.8
Mature oocytes per retrieval	9.3 ± 4.2
Fertilization rate per oocyte (%)	64 ± 22
Fertilization rate per mature oocyte (%)	73 ± 21
Clinical outcomes (*n* = 502)	
Positive HCG value	183 (36)
Biochemical pregnancies	20 (4)
Implanted embryos	163 (32)
Extra-uterine sacs	4 (1)
Intrauterine sacs	159 (32)
Early miscarriages[a]	19 (4)
Late miscarriages[b]	7 (1)
Live births	133 (26)

Values are mean ± SD or *n* (%).
HCG = human chorionic gonadotrophin; ICSI = intracytoplasmic sperm injection.
[a]Defined as a miscarriage up to 12 weeks after embryo transfer.
[b]Defined as a miscarriage between 12 and 20 weeks after embryo transfer.

Embryo evaluation

The computer system was used to record image sequences of the embryos 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) of their development. This system allowed the recording of 26 sequential images of the same oocyte or embryo by automatically focusing through the complete embryo at 5 μm intervals. Using these image sequences, each embryo was evaluated semi-automatically using the morphology analysis software of the FertiMorph computer system. On day 1, the diameters of the zygotes were drawn manually. Based on these drawn diameters, the total volume of the zygote was calculated by the computer. The same was done for each individual blastomere of the day-2 and day-3 embryos. 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 $\geq$45 μm on day 2 and >40 μm on day 3. Based on the principle that the total volume does not change during the first days of development (Hnida and Ziebe, 2004), the total cytoplasmic reduction was calculated by the computer system based on the difference between the volume of the zygote and the summed volumes of the individual blastomeres. This total cytoplasmic reduction can be interpreted as fragmentation: therefore, this total cytoplasmic reduction will be called fragmentation in the following sections. In addition, the system compared the volumes of the blastomeres within the embryo and defined them as equal (<25% difference) or unequal (>25% difference) in size.

During the evaluation with SSS, one embryologist evaluated each embryo on day 2 and day 3 by scoring the number of blastomeres, the degree of fragmentation (0 = no fragmentation; 1 = <10% fragmentation; 2 = 11—25% fragmentation; 3 = 26—50% fragmentation; and 4 = >50% fragmentation) and the size of the blastomeres (0 = equally sized blastomeres; 1 = slightly unequal blastomeres (25—50% difference); 2 = unequal blastomeres (>50% difference)). To compare both scoring systems based on the latter characteristic, the 'slightly unequal' and 'unequal' group of embryos were combined and defined as 'unequal blastomeres'. On day 3, the best embryo was chosen for transfer based on SSS.

Comparison of both scoring systems: practical aspects

In the last step of the evaluation of both scoring systems, some practical aspects were evaluated. First of all, the duration of exposure to suboptimal conditions was measured. Three dishes containing four embryos cultured in individual drops were used. The time of exposure to suboptimal conditions was defined as the time interval between the removal of embryos out of the incubator and their replacement into the incubator. The embryos were evaluated using either CASS or SSS. The reproducibility of CASS was evaluated by measuring (n = 3) the diameters of 10 different embryos at three different time points on day 1, day 2 and day 3 of their development. Finally, the time needed to evaluate 10 oocytes on day 1, 10 embryos on day 2 and 10 embryos on day 3 using the CASS was evaluated.

Statistics

All embryos (n = 3185) were evaluated. To compare both embryo-scoring systems, the chi-squared test was used with a significance level of 0.05.

For the logistic regression analyses to compare both systems in the prediction of implantation and live birth, the 502 transferred embryos were used. The implantation rate, measured by the number of gestational sacs divided by the number of embryos transferred, was chosen as the endpoint (Zegers-Hochschild et al., 2009). In total, three regression analyses were performed to predict implantation: one for the characteristics measured by CASS, one for the characteristics measured by SSS and one for the combination of the characteristics evaluated with both systems. In this way, each analysis calculated the characteristics with a significant influence on implantation. The same analyses were performed using live birth as outcome. Next, regression analyses were compared to establish which model was the best in predicting the chance of implantation and live birth by calculating the log-likelihood ratio. This value gives information about the fitness of a model and can be used to evaluate which model fits significantly better and thus must be preferred compared with other models. This can be done by deriving the probability or P-value of the obtained difference (D) between the log-likelihood ratio of each model (using the following equation: $D = -2 \times$ log-likelihood ratio) using a chi-squared distribution with degrees of freedom (df1—df2) (df1 and df2 are the number of degrees of freedom for model 1 and model 2, respectively). In summary, the absolute value of this characteristic allows comparison of the regression equations in order to establish which equation gives the best prediction. All analyses were performed using Statistica software version 9.0 (StatSoft, Tulsa, Oklahoma) with a significance level <0.05.

For the evaluation of the practical aspects, a t-test was used to evaluate the mean time of exposure. All statistical analyses were done in co-operation with the Biostatistical Centre of the University of Leuven.

Results

Descriptive statistics

Descriptive analyses were performed on the total embryo population (n = 3185) to compare both scoring systems in the evaluation of the morphological characteristics. Firstly, the number of blastomeres on day 2 and day 3 were similar using both systems (**Table 2**). Secondly, embryos evaluated on day 2 ($P < 0.0001$) and day 3 ($P < 0.0001$) with CASS were identified as embryos with a higher degree of fragmentation for all categories (**Figure 1**). It is important to note that the number of blastomeres was not assessed fully automatically in CASS, since the embryologist had to define which structure was a blastomere or fragment, as outlined in Materials and Methods. Thirdly, equally sized blastomeres were observed more frequently on day 2 using CASS compared

Table 2 Embryos ($n = 3185$) according to number of blastomeres on day 2 and day 3 according to both embryo-scoring systems.

No. of blastomeres	*CASS*	*SSS*
Day 2		
1	4 (120)	4 (132)
2	23 (748)	24 (772)
3	16 (505)	15 (466)
4	45 (1429)	45 (1432)
5	10 (304)	9 (296)
≥6	2 (79)	3 (87)
Day 3		
≤5	40 (1272)	38 (1205)
6	14 (461)	15 (483)
7	18 (580)	17 (552)
8	22 (689)	23 (737)
9	4 (122)	4 (133)
≥10	2 (61)	2 (75)

Values are % (n). No statistical differences were found in the evaluation of the number of blastomeres on day 2 and day 3 by both scoring systems.
CASS = computer-assisted scoring system; SSS = standard scoring system.

with SSS ($P < 0.001$). However, on day 3, equally sized blastomeres were observed less frequently using CASS when compared with SSS ($P = 0.002$) (**Table 3**).

Logistic regression analyses to predict implantation

The primary outcome of this study was implantation rate. A total of 140 transferred embryos resulted in an intrauterine pregnancy and were considered as implanted embryos. Since no difference was found between the implantation rate of an embryo of the first (26%, 97/371) or second (33%, 43/131) IVF/ICSI cycle, both groups were analysed as one group in the following analyses.

Computer-assisted scoring system

Using simple regression analysis, the implantation rate was significantly affected by the number of blastomeres on day 2 ($P = 0.029$) and the number of blastomeres on day 3 ($P < 0.001$) (**Table 4**). Multiple regression analysis (backward selection) using the number, size and degree of fragmentation of blastomeres on day 3, showed that the implantation rate was significantly affected by the number ($P < 0.001$) and size ($P = 0.018$) of the blastomeres on day 3 (**Table 4**).

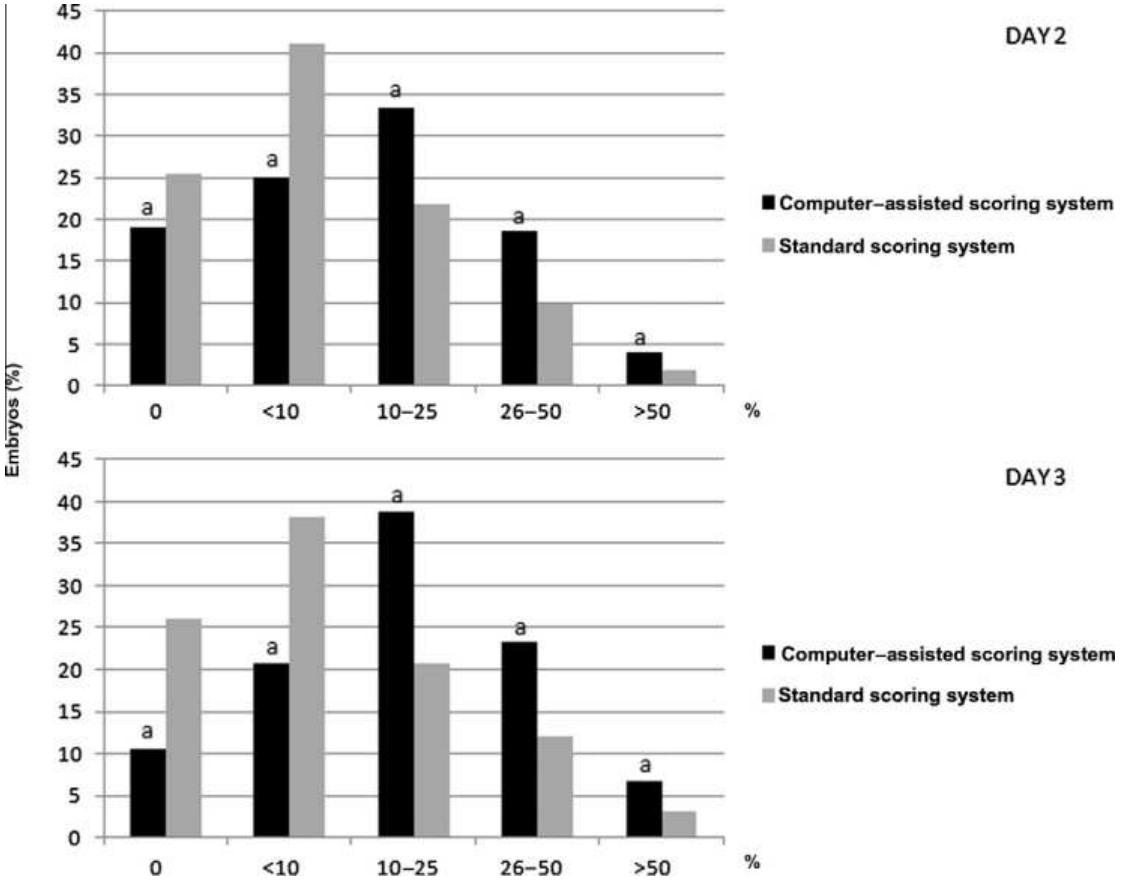

Figure 1 Evaluation of the degree of fragmentation on day 2 and day 3 by both scoring systems. *P*-values for both day 2 and day 3 are <0.0001.

Table 3 Embryos ($n = 3185$) with equal or unequal size of blastomeres on day 2 and day 3 according to both embryo-scoring systems.

Size of blastomeres	*CASS*	*SSS*
Day 2		
Equal[a]	60 (1917)	49 (1560)
Unequal	40 (1268)	51 (1625)
Day 3		
Equal[b]	37 (1179)	42 (1322)
Unequal	63 (2006)	58 (1863)

Values are % (*n*).
CASS = computer-assisted scoring system; SSS = standard scoring system.
[a]Statistically significantly different, $P < 0.001$.
[b]Statistically significantly different, $P = 0.002$.

Standard scoring system

Using simple regression analysis, the implantation rate was significantly affected by the number of blastomeres on day 3 ($P < 0.001$), the degree of fragmentation on day 3 ($P = 0.019$) and the size of blastomeres on day 3 ($P = 0.017$) and was affected in a borderline, non-significant way by the size of blastomeres on day 2 (**Table 4**). Multiple regression analysis (backward selection) using these three characteristics on day 3, showed that the implantation rate was significantly affected by the following variables: blastomere number ($P < 0.001$), degree of fragmentation ($P = 0.004$) and blastomere size ($P = 0.04$) (**Table 4**).

Multiple regression analysis integrating both CASS and SSS

Next, a regression analysis was performed integrating the characteristics measured by both systems. After backward selection, the number ($P < 0.001$) and size ($P = 0.009$) of blastomeres on day 3 measured with CASS and the degree of fragmentation on day 3 ($P = 0.001$) evaluated with SSS had a significant impact on the implantation rate (**Table 4**).

Comparison of CASS, SSS and combined CASS/SSS using log-likelihood ratios

In a next step, the log-likelihood ratio was calculated for each model to establish the best model for predicting the chance of implantation. The log-likelihood ratio of the model for CASS was −2053.74, for SSS −2047.78 and for the CASS/SSS combination −2044.61 (**Table 4**). First, a comparison was made between the CASS and SSS. To compare both log-likelihood ratios, the difference (11.92) between $-2 \times$ log-likelihood ratio of each model was taken. Since the difference follows the chi-squared distribution with five degrees of freedoms, the log-likelihood ratio of the model for CASS was significantly higher ($P < 0.05$). Next, the model given by the CASS was compared with the combined model. The difference between both models was 18.26. In this case, the difference follows the chi-squared distribution with four degrees of freedom, which implies that the CASS is better in predicting the chance of implantation.

Logistic regression analyses to predict live birth

Live-birth rate was significantly affected by the number of blastomeres on day 3 in both the CASS and SSS (both $P < 0.001$) using simple regression analysis.

Comparison of CASS and SSS using log-likelihood ratios

Since both scoring systems measured the same characteristic, no multiple regression analyses could be performed. In a next step, the log-likelihood ratio was calculated for each

Table 4 Overview of the results of logistic regression analyses (simple and multiple) for both scoring systems and for the combination (multiple) of both scoring systems using implantation rate as the outcome.

Variable	*CASS*	*SSS*	*CASS + SSS*	*P-value*
Simple logistic regression				
Day 2				
No. of blastomeres	0.029	NS	–	–
Degree of fragmentation	NS	NS	–	–
Size of blastomeres	NS	NS	–	–
Day 3				
No. of blastomeres	<0.001	<0.001	–	–
Degree of fragmentation	NS	0.019	–	–
Size of blastomeres	NS	0.017	–	–
Multiple logistic regression				
Day 3				
No. of blastomeres	<0.001	<0.001	<0.001 (CASS)	–
Degree of fragmentation	NS	0.004	0.001 (SSS)	–
Size of blastomeres	0.018	0.04	0.009 (CASS)	–
Log-likelihood ratio	−2053.74	−2047.78	−2044.61	<0.05

Values are *P*-values unless otherwise stated.
CASS = computer-assisted scoring system; NS = not statistically significant; SSS = standard scoring system.

Table 5 Overview of the results of logistic regression analyses (simple) using live-birth rate as the outcome.

Variable	*CASS*	*SSS*	*P-value*
Day 2			
No. of blastomeres day 2	NS	NS	–
Degree of fragmentation	NS	NS	–
Size of blastomeres day 2	NS	NS	–
Day 3			
No. of blastomeres day 3	<0.0001	<0.0001	–
Degree of fragmentation	NS	NS	–
Size of blastomeres day 3	NS	NS	–
Log-likelihood ratio	377.29	333.92	<0.05

Values are *P*-values unless otherwise stated.
NS = not statistically significant.

model to establish the best model for predicting the live-birth rate. The log-likelihood ratio of the model for CASS was −377.29 and for SSS −333.92 (**Table 5**). To compare both log-likelihood ratios, the difference between −2 × log-likelihood ratio of each model was taken (86.74). The log-likelihood ratio of the model for the CASS was significantly higher ($P < 0.05$) implicating that the CASS is better in predicting the live-birth rate.

Comparison of both scoring systems: practical aspects

The mean time interval between removal of embryos out of the incubator and their replacement into the incubator was significantly lower after evaluation with CASS than after evaluation with SSS (74.33 s versus 88.33 s, $P = 0.007$). As a result, embryos were exposed 1.2 times longer to suboptimal conditions when they were evaluated with SSS. Regarding the reproducibility of CASS, a very low variation in the total cytoplasmic volume (mean variation coefficient 0.031) was observed. The mean ± SD time needed to evaluate 10 oocytes on day 1 was 2.73 ± 0.12 minutes, the time needed to evaluate 10 day-2 embryos was 5.83 ± 0.42 minutes and 9.17 ± 0.15 minutes for the evaluation of 10 day-3 embryos.

A manuscript reporting further descriptive biological data is currently in preparation.

Discussion

This study shows for the first time that the use of a CASS is better in predicting implantation and live birth than a SSS. The results of the descriptive statistics show no differences in the evaluation of the number of blastomeres.

It is important to note that the CASS was not a fully automatic system: the number of blastomeres was still evaluated manually. Indeed, it was still the embryologist who had to outline the boundaries of the cells and, based on the predefined definition, a distinction could be made between a fragment and a blastomere. This limitation will continue to exist until the CASS has been optimized allowing a fully automatic detection and recognition of the blastomeres by a software programme.

Significant differences between both scoring systems were seen in the evaluation of the degree of fragmentation and the size of blastomeres on day 2 and day 3 of embryo development. The CASS identified more embryos with a higher degree of fragmentation, equally sized blastomeres on day 2 and fewer embryos with equally sized blastomeres on day 3 compared with the SSS. The regression analyses showed that characteristics measured by this CASS give more information about the chance of implantation and live birth than the use of a SSS. Using the number and size of blastomeres measured by the CASS predicted the chance of implantation better compared with the SSS or a combination of both. Using the number of blastomeres on day 3 the CASS allowed a better prediction of the live-birth rate. This might be explained by the fact that in CASS a lower limit was defined for the blastomere size based on the study by Johansson *et al.* (2003). Differentiating between a blastomere and a fragment using a SSS is totally dependent on the embryologist and clearly not based an objective measurement of the size.

The CASS has some important advantages over the SSS. Firstly, due to the use of the multilevel images, there is no limitation on analysis time. Once the multilevel image is made, the evaluation can be performed over an unlimited time period that allows a more detailed evaluation of the embryo. The detection of embryo characteristics like nuclear structures can be improved using a CASS (Hnida et al., 2005). Moreover, the present study showed that no extra exposure time to suboptimal conditions is needed with the CASS when compared with SSS. Secondly, automatic mathematical calculations of embryo characteristics, such as the degree of fragmentation and the size of blastomeres, are possible using CASS software. This added value may provide a more mathematical and accurate way to reduce reported intra- and inter-observer variability in the evaluation of morphological characteristics of human embryos (Arce et al., 2006; Paternot et al., 2009) and may contribute to a validated method to assess embryo morphology.

One major disadvantage of CASS is the time needed to analyse multilevel images since the diameter of every individual blastomere has to be outlined manually. An automatic blastomere identification and counting system would largely reduce the time of analysis and result in a more user-friendly system and should be developed.

In this study, a good-prognosis population group of patients (<36 years old, first or second IVF/ICSI cycle) was evaluated. More studies are needed to confirm these results in the total population of IVF patients. A major disadvantage of studies evaluating embryo-scoring systems is the absence of a gold standard making it difficult to extrapolate results to all scoring systems used in different laboratories. Therefore, the present conclusions are limited to the comparison between the CASS and a specific conventional scoring system (in this case SSS, the one used at the Leuven University). Clearly, these results need to be confirmed in larger multicentre studies. In addition, no conclusions can be made on other morphological characteristics described by other groups (De Neubourg et al., 2004; Guérif et al., 2010; Hesters et al., 2008; Wong et al., 2010;) since CASS allows the evaluation of only three characteristics: the number and size of blastomeres and the degree of fragmentation.

A cost-effectiveness analysis was not performed regarding the investment in the CASS software and the cost linked to the image analysis. Future work is needed to define if the additive value of the CASS in the prediction of live-birth rate is in balance with the total cost. However, interest in computer-assisted systems is increasing in several disciplines and will be part of future daily practice. As mentioned before, optimization of the software can be one of the solutions to reduce the time needed for the image analysis.

In conclusion, this study shows, as far as is known for the first time, that a CASS allows a better prediction of implantation rate based on the number and size of blastomeres on day 3 when compared with a SSS. Evaluating the number of blastomeres using the CASS results in a better prediction of live-birth rate. Future research is needed to confirm these findings in a multicentre prospective trial predicting embryo implantation based on multilevel computer-generated images and mathematical engineering.

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References

Arce, J.C., Ziebe, S., Lundin, K., Janssens, R., Holmgaard, L., Sorensen, P., 2006. Interobserver agreement and intra-observer reproducibility of embryo quality assessments. Hum. Reprod. 21, 2141—2148.

Baruffi, R.L.R., Mauri, A.L., Petersen, C.G., Nicoletti, A., Pontes, A., Oliviera, J.B.A., Franco, J.G., 2009. Single-embryo transfer reduces clinical pregnancy rates and live births in fresh IVF and Intracytoplasmic Sperm Injection (ICSI) cycles: a meta-analysis. Reprod. Biol. Endocrinol. 7, 36.

Debrock, S., Spiessens, C., Meuleman, C., Segal, L., De Loecker, P., Meeuwis, L., D'Hooghe, T.M., 2005. New Belgian legislation regarding the limitation of transferable embryos in in vitro fertilization cycles does not significantly influence the pregnancy rate but reduces the multiple pregnancy rate in a threefold way in the Leuven University Fertility Center. Fertil. Steril. 83, 1572—1574.

Debrock, S., Melotte, C., Spiessens, C., Peeraer, K., Vanneste, E., Meeuwis, L., Meuleman, C., Frijns, J.P., Vermeersch, J.R., D'Hooghe, T.M., 2010. 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. 93, 364—373.

Desai, N.N., Goldstein, J., Rowland, D.Y., Goldfarb, J.M., 2000. Morphological evaluation of human embryos and derivation of an embryo quality scoring system specific for day 3 embryos: a preliminary study. Hum. Reprod. 15, 2190—2196.

De Neubourg, D., Gerris, J., Mangelschots, K., Van Royen, E., Vercruyssen, M., Elseviers, M., 2004. Sinlge top quality embryo transfer as a model for prediction of early pregnancy outcome. Hum. Reprod. 19, 1476—1479.

De Sutter, P., 2006. Single embryo transfer (SET) not only leads to a reduction in twinning rates after IVF/ICSI, but also improves obstetrical and perinatal outcome of singletons. Verh. K. Acad. Geneeskd. Belg. 68, 319—327.

Fisch, J.D., Rodriguez, H., Ross, R., Overby, G., Geoffrey, Sher, 2001. The graduated embryo score (GES) predicts blastocyst formation and pregnancy rate from cleavage-stage embryos. Hum. Reprod. 16, 1970—1975.

Garceau, L., Henderson, J., Davis, L.J., Petrou, S., Henderson, L.R., McVeigh, E., Barlow, D.H., Davidson, L.L., 2002. Economic implications of assisted reproductive techniques: a systematic review. Hum. Reprod. 17, 3090—3099.

Garrisi, G.J., Chin, A.J., Dolan, P.M., Nagler, H.M., Vasquez-levin, M., Navot, D., Gordon, J.W., 1993. Analysis of factors contributing to success in a program of micro-manipulation-assisted fertilization. Fertil. Steril. 59, 366—374.

Gelbaya, T.A., Tsoumpou, I., Nardo, L.G., 2010. The likelihood of live birth and multiple birth after single versus double embryo transfer at the cleavage stage: a systematic review and meta-analysis. Fertil. Steril. 94, 936—945.

Gerris, J., De Neubourg, D., Mangelschots, K., Van Royen, E., Vercruyssen, M., Barudy-Vasquez, J., Valkenburg, M., Ryckaert, G., 2002. Elective single day 3 embryo transfer halves the twinning rate without decrease in the ongoing pregnancy rate of an IVF/ICSI programme. Hum. Reprod. 17, 2626—2631.

Guérif, F., Lemseffer, M., Leger, J., Bidault, R., Cadoret, V., Chavez, C., Gasnier, O., Saussereau, M.H., Royere, D., 2010. Does early morphology provide additional selection power to blastocyst selection for transfer? Reprod. Biomed. Online 21, 510—519.

Hesters, L., Prisant, N., Franchin, R., Mendez Lozane, D.H., Feyereisen, E., Frydman, R., Tachdijan, G., Frydman, N., 2008. Impact of early cleaved zygote morphology on embryo development and in vitro fertilization — embryo transfer outcome: a prospective study. Fertil. Steril. 89, 1677—1684.

Hnida, C., Ziebe, S., 2004. Total cytoplasmic volume as biomarker of fragmentation in human embryos. J. Assist. Repord. Genet. 21, 335—340.

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

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

Holte, J., Berglund, L., Milton, K., Garello, C., Gennarelli, G., Revelli, A., Bergh, T., 2007. Construction of an evidence-based integrated morphology cleavage embryo score for implantation potential of embryos scored and transferred on day 2 after oocyte retrieval. Hum. Reprod. 22, 548—557.

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

Paternot, G., Devroe, J., Debrock, S., D'Hooghe, T.M., Spiessens, C., 2009. Intra- and interobserver analysis in the morphological assessment of early-stage embryos. Reprod. Biol. Endocrinol. 7, 105.

Van Montfoort, A.P.A., Fiddelers, A.A.A., Janssen, J.M., Derhaag, J.G., Dirksen, C.D., Dunselman, G.A.J., Land, J.A., Geraerdts, J.P., Evers, J.L., Dumoulin, J.C., 2006. In unselected patients, elective single embryo transfer prevents all multiples, but results in significantly lower pregnancy rates compared with double embryo transfer: a randomized controlled trial. Hum. Reprod. 21, 338—343.

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. Nat. Biotechnol. 28, 1115—1121.

Zegers-Hochschild, F., Adamson, G.D., de Mouzon, J., Ishihara, O., Mansour, R., Nygren, K., Sullivan, E., van der Poel, S., 2009. The

international committee for monitoring assisted reproductive technology (ICMART) and the World Health Organization (WHO) revised glossary on ART technology. Hum. Reprod. 24, 2683—2687.

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