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Non-invasive embryo assessment in IVF

Vergouw, C.G.

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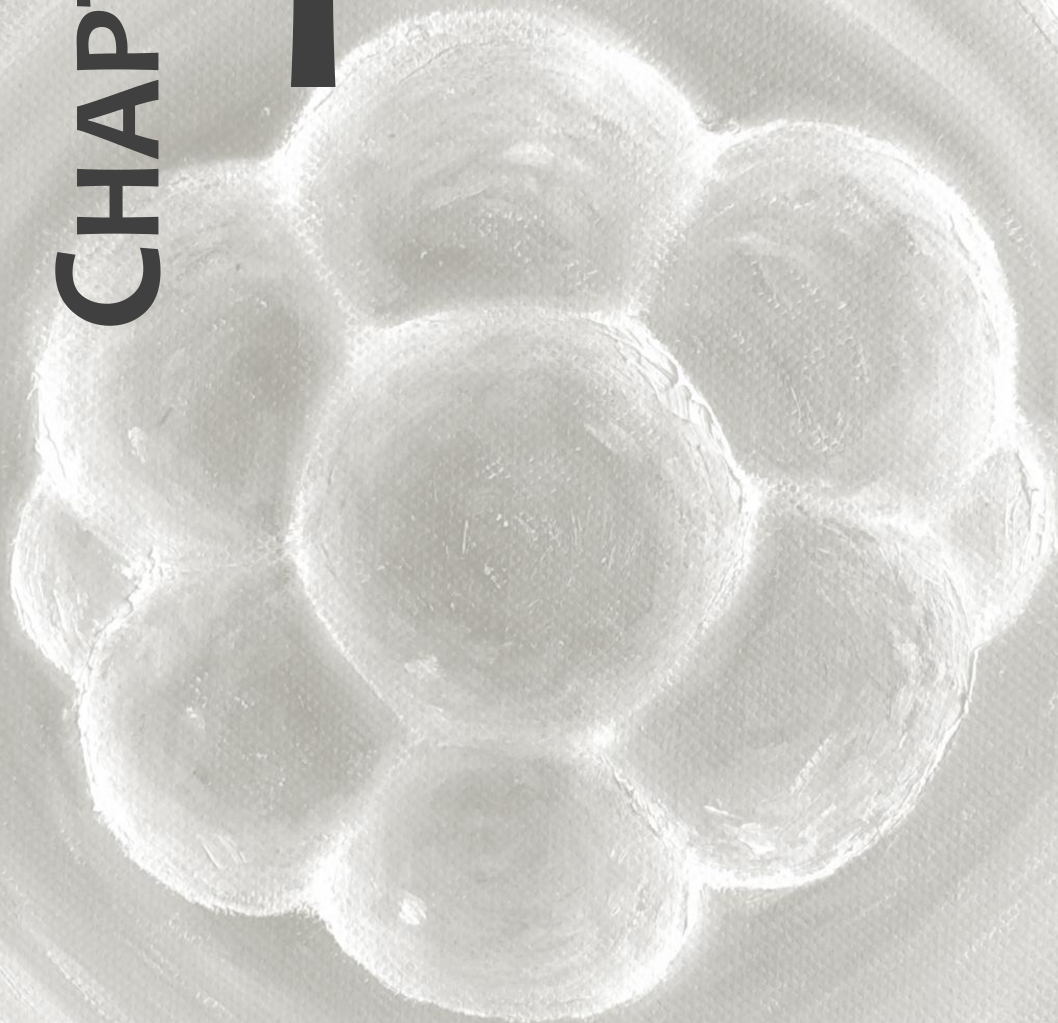
CHAPTER 1



General introduction

GENERAL INTRODUCTION

Currently, the assessment of an embryo's viability is predominantly based on morphological appearance. The most commonly used criteria for embryo selection of cleavage stage embryos are based on cleavage rate (number of blastomeres at certain time points), uniformity of blastomere size, degree of fragmentation and presence of multinucleation in a single blastomere[1].

CLEAVAGE RATE

The single most important parameter for embryo viability is the cleavage rate[2]. There appears to be an optimal cleavage pattern for early embryos: cleaving too fast or too slow has a negative impact on implantation rates[3,4]. Cell division arrest, slow and fast cleaving have been associated with chromosomal abnormalities such as mosaicism and aneuploidies (reviewed by Rienzi[1]).
Day 2 and day 3 embryo assessment should be performed 44h ± 1h and 68h ± 1h post insemination respectively[2]. A high quality embryo should have four or five blastomeres on day 2 and at least seven blastomeres on day 3[4].
Furthermore, it has been suggested that the timing of the first mitosis of the zygote is a parameter of embryo viability. These so called early cleavage embryos are embryos that have cleaved to the two cell stage 25-27h post insemination[5]. Early cleavage embryos develop more often into good quality embryos[6,7] and their transfer leads to higher implantation rates[5-8].

BLASTOMERE SIZE

Several studies have shown that unequally sized blastomeres are associated with a higher degree of aneuploidy and multinuclear rate which have a negative impact on implantation rates[9,10]. This impairment is probably instigated by the uneven distribution of proteins, mRNA, mitochondria and different cell organelles between the resulting sister cells of an unequally divided blastomere[9]. However, from a biological point of view, during the division from one to eight cells, some sort of asynchrony should be expected in three, five, six and seven cell embryos[11]. Asymmetry of blastomeres in these stages rather reflects cleavage asynchrony than uneven cytoplasmic distribution[1].

FRAGMENTATION

Fragments are anuclear, membrane-bound, extracellular structures[2]. Fragmentation is a dynamic process. Studies have shown that some fragments can be reincorporated into

blastomeres[9,12]. The assessment of fragmentation is difficult, as it is important to differentiate between blastomeres and fragments. Johansson et al.[13] defined fragments as cells with a diameter of <45 μm on day two of embryo development and cells with a diameter of <40 μm on day three of embryo development.

The influence of fragmentation on clinical outcomes has been frequently studied (reviewed by Fujimoto et al.[14]). The commonly acknowledged negative influence of fragmentation on embryo implantation is mainly attributed to the degree and pattern of fragmentation[14,15].

Highly fragmented embryos implant less frequently than embryos with moderate or no fragmentation[14]. The release of large fragments at an early stage of embryo development leads to a considerable loss of cytoplasmic volume of the portion of cells that contains the nucleus. This results in the depletion of essential organelles such as proteins, mitochondria and mRNA, and may therefore cause the cell to arrest[13]. The presence of minimal fragmentation, however, may be part of normal embryo development and does not have to impair implantation[13,14]. Several studies report a threshold under which embryo fragmentation does not influence pregnancy outcomes. This threshold varies from <10% fragmentation[16], to <15% fragmentation[15] and <20% fragmentation[3,17].

The pattern of fragmentation influences implantation rates as well[13-15]. Basic patterns of fragmentation have been described[15]. Type 1 fragmentation is minimal in volume and fragments are associated typically with one blastomere. Type 2 fragmentation are localized fragments, predominantly occupying the perivitelline space and associated with one or more blastomeres. Type 3 fragmentation represents small, scattered fragments, positioned peripherally or in the cleavage cavity. Type 4 fragments are randomly distributed, large and are often associated with uneven blastomeres. They resemble sometimes whole blastomeres. Large, asymmetrical fragments that cause loss of large volumes of cytoplasm (designated as type 4) lead to a considerable loss of implantation potential[15].

Fragments can be removed microsurgically. The complete removal of fragmentation seems to have a beneficial effect on embryo development and implantation, indicating that fragments have a harmful effect on their neighbouring blastomeres[15,18].

MULTINUCLEATION

Multinucleation is defined as the presence of two or more nuclei present in a single blastomere at a given time. Multinucleated blastomeres have been identified in 14.3% to 74% of examined IVF embryos and up to 87% of the patients have at least one embryo with multinucleated blastomeres[19]. Possible explanations for multinucleation include

karyokinesis in absence of cytokinesis[20], partial fragmentation of nuclei[21], abnormal sperm centrosome[22], changes in temperature and suboptimal culture conditions[23]; and defective migration of chromosomes at the mitotic anaphase[1].
Multinucleation has been correlated to an elevated degree of fragmentation and suboptimal cleavage rates[24]; and with increased rates of aneuploidy and chromosomal abnormalities[25-28]. Consequently, a low implantation rate of multinucleated embryos has been reported in several studies (reviewed by Rienzi[1]).

The combination of several different morphological criteria, each predictive of embryo viability, may result in a more accurate embryo selection[1]. However, there are claims that, although very helpful, morphological assessment is relatively unreliable in predicting an embryo's viability[29,30], because some of the most important aspects of embryo viability remain invisible to such analysis[31]. Embryos with the highest morphological scores often fail to achieve implantation and embryos with a poor morphological score sometimes result in a live birth. In addition to morphology, other embryo assessment methods may be useful to select the most viable embryo to transfer. Especially with the current trend of moving more and more towards single embryo transfer, it is essential to have a rapid, non-invasive, cost effective, reproducible and accurate embryo selection tool available. In this context, during the last few years, new non-invasive embryo selection methods that analyse culture medium constituents have been studied. An embryo uses nutrients and generates metabolites, which means that the embryo changes the composition of its culture medium. These changes reflect the activity and efficiency of embryo metabolism, which may be used as a predictor of embryo viability: viable embryos modify their culture medium differently than non-viable embryos. One of these methods, analysing the changes in culture medium made by the embryo, is a screening technology using near-infrared (NIR) spectroscopy.

METABOLOMIC PROFILING OF EMBRYO CULTURE MEDIUM BY NEAR-INFRARED (NIR) SPECTROSCOPY

The metabolome represents the complete inventory of small molecules (<1 kDa), such as metabolic intermediates (amino acids, lipids, nucleotides), adenosine triphosphate (ATP), hormones, other signalling molecules and secondary metabolites that are found within a biological milieu[32]. Metabolomics is the study of the inventory of small-molecule biomarkers that represents the functional phenotype at the cellular level[32,33]. To analyse complex metabolomic profiles, spectroscopic (optical and non-optical) and chromatographic techniques are specifically used as analytical technologies[33]. Nuclear

magnetic resonance (NMR) and Mass Spectrometry (including high-performance liquid chromatography [HPLC]) are examples of non-optical spectroscopies. NIR spectroscopy and Raman spectroscopy are examples of optical spectroscopies[33].
This thesis is mainly focused on the use of an optical spectroscopic technology called NIR spectroscopy. This technique is especially applicable for clinical use because of the small volumes (< 15-µl) that can be measured, the quick analysis time (<1 min) and the relatively simple procedure[34].
A NIR spectrometer simultaneously measures the quantitative absorption of key functional groups such as -CH, -OH and -NH in a fluid (embryo culture medium) and generates a correlating spectrum (Figure 1).

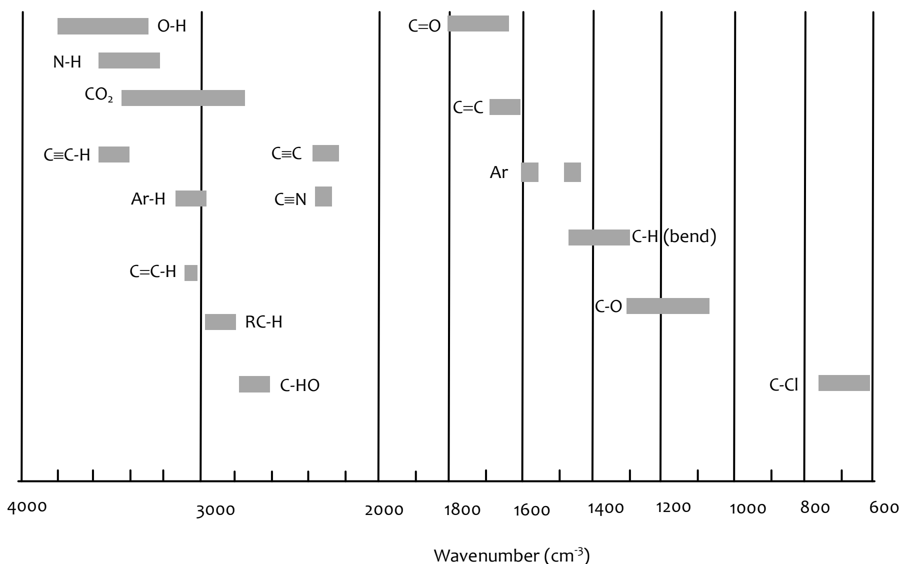


Figure 1. Infrared absorption bands.

These key functional groups are associated with culture medium constituents such as glucose, pyruvate, albumin, glutamate and lactate. By analysing a blank medium sample which is cultured alongside the embryo medium drops, and comparing it to the spectrum of the spent embryo culture medium, it is possible to detect what the embryo has altered in the medium.
The unique spectral profiles can be quantified and expressed as a viability score. This is done by analysing culture media from a large number of single transferred embryos

with known implantation outcome. NIR spectra of medium from embryos that resulted in an ongoing implantation can be compared to NIR spectra of medium from embryos that did not implant. Combinations of wavelength regions that are discriminative of ability to implant are determined by inverse least-squares regression and a genetic algorithm optimization. According to their importance, they are weighted. This results in the equation:

viability score= $\alpha(W\alpha) + \beta(W\beta) + \gamma(W\gamma) + \delta(W\delta)$,

where α, β,γ and δ are the spectral regions used to determine the viability score and W is the weighting of their importance (Figure 2).

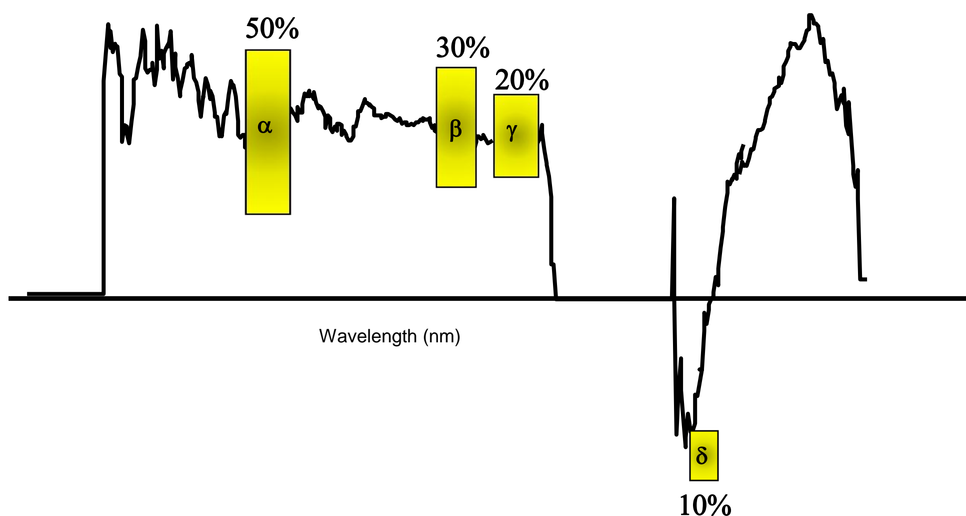


Figure 2. Weighting of wavelength regions that are discriminative of ability to implant.

An on-site instrument can be loaded with previously established predictive algorithms. This way, viability scores can be generated for embryo cohorts of patients in IVF laboratories. The NIR spectrometer adapted to use in IVF laboratories for generating viability scores has been developed by Molecular Biometrics Inc. The instrument was marketed as the ViaMetrics-E™.

The process of generating viability scores is shown in Figure 3. First, the embryos are removed from their culture medium droplet in which they were cultured for at least

24h. Then 10-µl of spent embryo culture medium of one embryo is aspirated and loaded in a special developed sample cell. The sample cell is subsequently loaded in a temperature stabilizer, in which it is incubated for at least four minutes. Then the sample cell is put into the ViaMetrics-E™ and scanned three times. After this, the spent embryo culture medium is removed and replaced by control medium. This is culture medium from the same batch that has been equilibrated in an incubator, but no embryo has been cultured in it. The whole process is then repeated with the control medium and after the third scan of the control medium, the ViaMetrics-E™ presents the viability score of that specific embryo that was cultured in the analysed culture medium drop. When all individual medium drops are analysed, the embryos can be ranked according to their viability score. The method is developed to be used to select the most viable embryo within a group of good quality embryos. Therefore, the embryo with the highest viability score within the group of good quality embryos is the embryo that has the preference to be transferred.

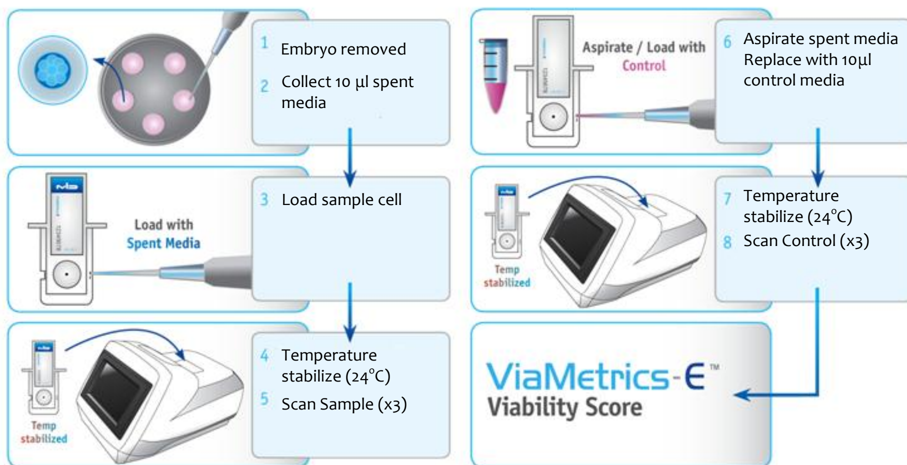


Figure 3. The process of generating a viability score.
(Extracted with permission from the Molecular Biometrics User Guide).

AIMS AND OUTLINE OF THE THESIS

In this thesis, we study novel, non-invasive embryo assessment and selection tools. Our main goal is to find a non-invasive embryo assessment tool that can be used as an

additional marker of embryo viability to supplement current criteria for embryo selection. The studies we present in this thesis are part of a clinical validation process. For this purpose we investigated a new tool that analyses constituents of spent embryo culture medium (metabolomic profiling of spent culture medium by NIR spectroscopy). Furthermore, we present new objective variables that measure instead of estimate familiar morphological parameters such as fragmentation, (dis)similarities between blastomeres and the presence of non-spherical blastomeres. We also describe a study which was part of a validation process of vital changes in our routine laboratory culture system.

In **chapter 2** we present a proof of principle study in which we retrospectively investigated if metabolomic profiling of spent culture medium by NIR spectroscopy correlates with ongoing pregnancy when the single transferred embryos were selected by conventional morphological selection criteria.

In **chapter 3** we further investigate the data of the proof of principle study (described in **chapter 2**) with an addition of data of the Kato Ladies Clinic in Japan. A validation of the prediction model is performed and the possible benefit that may derive from combining non-invasive metabolomic analysis of embryo culture medium with morphologic assessment to determine reproductive potential of embryos in IVF is assessed.

In **chapter 4** we investigate if metabolomic profiling of culture medium by NIR spectroscopy is related to live birth rates in a retrospective analysis of 127 frozen-thawed single embryo transfers.

In **chapter 5** we investigate if the selection of a single day 3 embryo by metabolomic profiling of culture medium with NIR spectroscopy technology as an adjunct to morphology is able to improve live birth rates in IVF, compared to embryo selection by morphology alone. For this purpose we conducted a double blind randomized controlled trial (RCT) in which 417 couples undergoing IVF with a single embryo transfer (SET) were included.

In **chapter 6** we report the results of a meta-analysis with individual patient data (IPD) in which relevant papers that reported the results of an RCT comparing embryo selection by morphology with embryo selection by morphology and the use of NIR spectroscopy of spent embryo culture medium by the ViaMetrics-E™ were included.

In **chapter 7** we introduce novel, objective variables that resemble embryo quality and analyse their relation with ongoing implantation in a retrospective analysis, using multilevel imaging of 659 day 3 single-transferred embryos.

In **chapter 8** we describe the results of a retrospective analysis of birthweights of singleton newborns after a fresh (day 3) or frozen-thawed (day 5) SET cycle, who were cultured as an embryo in two different types of commercially available culture media.

In **chapter 9** we discuss the implications of our findings for clinical practice and we elaborate on the importance of a validation of new laboratory technologies in light of practical application in a clinical setting. Furthermore, we elaborate on future perspectives with regard to the focus of our research activities.

In **chapter 10**, a summary of this thesis is presented.

REFERENCES

1. Rienzi L. Significance of morphological attributes of the early embryo. Reprod Biomed Online 2005; 10: 669-681.

2. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group in Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod 2011; 26: 1270-1283.

3. 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.

4. Van Royen E, Mangelschots K, de Neubourg D, Valkenburg M, van de Meerssche M, Ryckaert G, Eestermans W, Gerris J. Characterization of a top quality embryo, a step towards single-embryo transfer. Hum Reprod 1999; 14: 2345-2349.

5. Sakkas D, Percival G, D'Arcy Y, Sharif K, Afnan M. Assessment of early cleaving in in-vitro fertilized human embryos at the 2-cell stage before transfer improves embryo selection. Fert Steril 2001; 76: 1150-1156.

6. Fu J, Wang XJ, Wang YW, Sun J, Gemzell-Danielsson K, Sun XX. The influence of early cleavage on embryo developmental potential and IVF/ICSI outcome. J Assist Reprod Genet 2009; 26: 437-441.

7. Lee MJ, Lee RK, Lin MH, Hwu YM. Cleavage speed and implantation potential of early-cleavage embryos in IVF or ICSI cycles. J Assist Reprod Genet 2012; 29: 745-750.

8. Van Montfoort AP, Dumoulin JC, Kester AD, Evers JL. Early cleavage is a valuable addition to existing embryo selection parameters: a study using single embryo transfers. Hum Reprod 2004; 19: 2103-2108.

9. Hardarson T, Löfman C, Coull G, Sjögren A, Hamberger L, Edwards R. Internalization of cellular fragments in a human embryo: time lapse recordings. Reprod Biomed Online 2002; 5: 36-38.

10. Ziebe S, Lundin K, Loft A, Bergh C, Nyboe Andersen A, Selleskog U, Nielsen D, Grøndahl C, Kim H, Acre JC, CEMAS II and study group. FISH analysis for chromosomes 13, 16, 18, 21, 22, X and Y in all blastomeres of IVF pre-embryos from 144 randomly selected donated human embryos and impact on pre-embryo morphology. Hum Reprod 2003; 12: 2575-2581.

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

12. Van Blerkom J, Davis P, Alexander S. A microscopic and biochemical study of fragmentation phenotypes in stage-appropriate human embryos. Hum Reprod 2001; 16: 719-729.

13. 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.

14. Fujimoto VY, Browne RW, Bloom MS, Sakkas D, Alikani M. Pathogenesis, developmental consequences, and clinical correlations of human embryo fragmentation. Fert Steril 2011; 95: 1197-1204.

15. Alikani M, Cohen J, Tomkin G, Garrisi GJ, Mack C, Scott RT. Human embryo fragmentation in vitro and its implications for pregnancy and implantation. Fert Steril 1999; 71: 836-842.

16. Van Royen E, Mangelschots K, de Neubourg D, Laureys I, Ryckaert G, Gerris J. Calculating the implantation potential of day 3 embryos in women younger than 38 years of age: a new model. Hum Reprod 2001; 16: 326–332.

17. Staessen C, Camus M, Bollen N, Devroey P, van Steirteghem AG. The relationship between embryo quality and the occurrence of multiple pregnancies. Fert Steril 1992; 57: 626-630.

18. Chi HJ, Koo JJ, Choi SY, Jeong HJ, Roh SI. Fragmentation of embryos is associated with both necrosis and apoptosis. Fert Steril 2011; 96: 187-192.

19. Gil M, D'Ommar G, Póo ME, Sosa A, Piras M, Piras R, Rísquez F. Insights on blastomere nuclearity. J Assist Reprod Genet 2007; 24: 17-22.

20. Hardy K, Winston RM, Handyside AH. Binucleate blastomeres in preimplantation human embryos in vitro: failure of cytokinesis during early cleavage. J Reprod Fert 1993; 98: 549-558.

21. Tesarik J, Kopency V, Planchot M, Mandelbaum J. Ultrastructural and autoradiographic observations on multinucleated blastomeres of human cleaving embryos obtained by in-vitro fertilization. Hum Reprod 1987; 2: 127-136.

22. Palermo G, Munne S, Cohen J. The human zygote inherits its mitotic potential from the male gamete. Hum Reprod 1994; 9: 1220-1225.

23. Winston NJ, Baude PR, Pickering SJ, George MA, Cant A, Currie J, Johnson MH. The incidence of abnormal morphology and nucleocytoplasmic ratios in 2-, 3-, and 5-day human pre-embryos. Hum Reprod 1991; 6: 17-24.

24. Van Royen E, Mangelschots K, Vercruyssen M, de Neubourg D, Valkenburg M, Ryckaert G, Gerris J. Multinucleation in cleavage stage embryos. Hum Reprod 2003; 18: 1062-1069.

25. Munné S, Cohen J. Unsuitability of multinucleated human blastomeres for preimplantation genetic diagnosis. Hum Reprod 1993; 8: 1120-1125.

26. Kligman I, Benadiva C, Alokani M, Munné S. The presence of multinucleated blastomeres in human embryos is correlated with chromosomal abnormalities. Hum Reprod 1996; 11: 1492-1498.

27. Laverge H, de Sutter P, Verschraegen-Spae MR, de Paepe A, Dhont M. Triple colour fluorescent in-situ hybridization for chromosomes X, Y and 1 on spare human embryos. Hum Reprod 1997; 12: 809-814.

28. 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.

29. Brison D, Houghton F, Falconer D, Roberts S, Hawkhead J, Humpherson P, Lieberman B, Leese H. Identification of viable embryos in IVF by non-invasive measurement of amino acid turnover. Hum Reprod 2004; 19: 2319-2324.

30. Sturmey R, Hawkhead J, Barker E, Leese H. DNA damage and metabolic activity in the preimplantation embryo. Hum Reprod 2009; 24: 81-91.

31. Wells D, Alfarawati S, Fragouli E. Use of comprehensive chromosomal screening for embryo assessment: microarrays and CGH. Mol Hum Reprod 2008; 14: 703-710.

32. Botros L, Sakkas D, Seli E. Metabolomics and its application for non-invasive embryo assessment in IVF. Mol Hum Reprod 2008; 14: 679-690.

33. Nel-Themaat L, Nagy ZP. A review of the promises and pitfalls of oocyte and embryo metabolomics. Placenta 2011; 32: S257-S263.

34. Seli E, Robert C, Sirard MA. OMICS in assisted reproduction: possibilities and pitfalls. Mol Hum Reprod 2010; 16: 513-530.