REVIEW ARTICLE

Successful Implantation from the Embryonic Aspect

Julia Szekeres-Bartho[1,2,3]

[1]Department of Medical Microbiology and Immunology, Medical School, Pecs University, Pecs, Hungary;
[2]MTA-PTE Human Reproduction Research Group, University of Pécs, Pecs, Hungary;
[3]János Szentágothai Research Centre, University of Pécs, Pecs, Hungary

Keywords
embryo, IVF, single transfer

Correspondence
Julia Szekeres-Bartho, Department of Medical Microbiology and Immunology, Medical School, Pecs University, 12 Szigeti Street, 7626 Pecs, Hungary.
E-mail: julia.szekeres@aok.pte.hu

Submission September 1, 2015;
accepted October 15, 2015.

Citation
Szekeres-Bartho J. Successful implantation from the embryonic aspect. Am J Reprod Immunol 2016; 75: 382–387

doi:10.1111/aji.12448

Problem
Implantation failure is common in women with advanced maternal age, partly because of the increased number of aneuploid embryos. These women constitute the majority of patients for IVF treatment. As multiple pregnancies is a major hazard of preterm delivery, the aim is to select a competent embryo for single transfer. This study reviews currently used methods for selecting the competent embryo.

Method of the study
Literature search.

Results
The clinical value of currently used tests, for example pre-implantation genetic screening for aneuploidy, embryo morphology, morphokinetic measurements, extended culture to the blastocyst stage, as well as analysis of the follicular fluid and amino acid and glucose metabolism as well as oxygen consumption in embryo culture media, are discussed.

Conclusion
Several approaches look promising, but the clinical value of these is yet to be confirmed in randomized clinical trials. Furthermore, some of the methods are too complicated for routine clinical application.

Introduction

Implantation requires an interaction between the embryo and the maternal endometrium. The efficiency of embryo implantation is surprisingly low in humans. This can either be attributed to the high rate of chromosomally abnormal embryos, or to uterine factors, if the embryo is chromosomally normal. The incidence of aneuploidy in human embryos is estimated to be ten times higher than in other mammalian species. The percentage of aneuploid embryos increases with maternal age, reaching 80% over the age of 40,[1–4] and many of these chromosomally abnormal embryos fail to implant,[5–9] which explains that according to a rather conservative estimate, only 50% of human conceptions will result in pregnancy.[10]

The role of the endometrium

Successful implantation of the embryo depends on both maternal and embryonic factors, while there is a mutual interaction between the embryonic and the endometrial side. Earlier data[11,12] suggest that embryo-derived signals influence the functioning of the maternal immune system, which might eventually lead to an altered immune balance, and enable the survival of the immunologically incompatible foetus. Indeed, a large number of cytokines and chemokines, for example IL-11, leukaemia inhibitory factor (LIF), the TGF superfamily including the colony-stimulating factors (CSF), IL-1 and IL-15, are expressed during placentation and implantation. Furthermore, the expression of these molecules is spatially and temporarily regulated. All of these have

well-established roles in the process, and dysregulation of expression or action of these cytokines results in implantation failure.[13]

In humans, implantation of a fertilized egg occurs between 6 and 12 days after ovulation during the implantation window, when the endometrium is receptive. Endometrial decidualization is characterized by the transformation of stromal fibroblasts into secretory decidual cells.[14] In humans, hormonal triggers induce decidualization during each menstrual cycle. If the fertilized ovum implants, hCG secreted by the embryo will maintain the corpus luteum, otherwise, in the absence of a continuous progesterone supply, the endometrium will shed. Therefore, those embryos, which do not produce hCG, will not be able to maintain the decidua. This observation prompted the concept of the biosensor role of the endometrium,[15] implying that the endometrium deselects incompetent embryos and allows implantation of good embryos only. This concept is well supported by the finding that co-culture of blastocysts of impaired morphology with decidualized endometrial stromal cells (but not with undifferentiated stromal cells) strongly inhibited the secretion of many important pro-implantation molecules, for example IL-1b, IL-6, IL-10, IL-17 and IL-18, as well as of C-C motif chemokine 11 (CCL11) and heparin-binding EGF-like growth factor (HB-EGF), while morphologically good embryos had little impact.[16] The above effects were caused by soluble factors, as pooled culture media from low-quality embryos downregulated 449 maternal genes, in contrast to 15 decidual genes affected by pooled media from competent embryos.[17] However, when mouse uteri were flushed with pooled culture media of competent embryos, one-third of the induced genes were found to code for known implantation factors, suggesting that the selective role of the decidua is not solely negative.[17–20]

The role of the embryo

The embryo constitutes the other side of the picture. Ideally, a competent embryo should have good chances to implant into a receptive endometrium. However, the quality of the eggs is related to the age of the mother. Women in developed countries tend to build their careers first and attempt to have a baby at a more advanced age. Because of the increased number of aneuploid embryos, implantation failure is very common in these women.

Aneuploidy originates during cell division when the chromosomes do not separate properly between the two cells. It can result from non-disjunction in meiosis I.[21,22] or premature separation of sister chromatids.

In an attempt to increase the chances of pregnancy in infertile women, many IVF centres transfer more than one embryo. This in turn also increases the hazard of twin pregnancies. As multiple pregnancies are among the most common causes of preterm birth, together with the increased risk for prematurity, it would be crucial to select the embryo that is most likely to implant and transfer that particular embryo only.

Methods for selecting competent embryos

Embryo Morphology

Many laboratories select embryos by morphological criteria, with the use of a semi-quantitative scoring system based on the number of blastomeres and embryo quality.[23–25] The results are inconsistent and do not necessarily reflect the implantation potential of the embryo. Inconsistency is due to subjectivity of the evaluation, as well as to variations in the timing of assessment.[26]

Compared with this, *morphokinetic measurements* provide more reliable data.

The major advantage of time-lapse imaging is that development is maintained in a closed system without subjecting the embryo to changes of environment, which is inevitable, when embryo morphology is assessed by conventional means.[27] Based on several parameters, algorithms have been developed for identifying competent embryos.[28]

The results seem promising. Analysis of retrospective data by Meseguer et al.[29] indicated that culturing and selecting embryos by time-lapse imaging significantly improved the relative probability of clinical pregnancy. The elevated clinical pregnancy rate was attributed to a combination of stable culture conditions and the use of morphokinetic parameters for embryo selection.[29] Similar results were reported by Campbell et al.[30].

Some studies involving a limited number of patients have even shown a correlation between aneuploidy and various morphokinetic parameters.[28,31–33] However, all of these studies used different morphokinetic parameters for predicting chromosomal abnormalities.[34]

A recent longitudinal study using 455 blastocysts from 138 patients did not confirm these results and concluded that morphokinetic features of pre-implantation development did not correlate with aneuploidy.[35]

Pre-Implantation Genetic Screening for Aneuploidy

In women of advanced maternal age who constitute the majority of IVF patients, the proportion of aneuploid embryos can exceed 60%, which explains the 60% aneuploidy rate of *in vitro*-produced embryos.[36] Pre-implantation genetic screening aims to exclude aneuploid embryos before transfer, and to improve thereby the implantation rate. Several genetic testing techniques (such as fluorescent *in situ* hybridization analysis, comparative genomic hybridization, microarray-based, multiplex real-time polymerase chain reactions, digital PCR, real-time PCR, single nucleotide polymorphism and next-generation sequencing have been developed to identify chromosomally normal embryos *in vitro*, with the aim of selecting an euploid embryo for single transfer. Invasive genetic testing involves certain risks, as biopsy might negatively influence further development of the embryo.[27] Generally, three different kinds of samples – representing different developmental stages of the embryo – are used for aneuploidy screening. All of these have their advantages and pitfalls.

Polar body biopsy can be considered non-invasive as it does not affect the embryo. Furthermore, it provides the earliest sample, consequently more time for testing. However, in the majority of polar body abnormalities, it is impossible to tell whether the embryo is euploid or aneuploid; thus, there is a high chance for misdiagnosis.[21,22]

Because *biopsy of the 3-day cleavage embryo* occurs after the completion of meiosis, meiotic errors of both parents should be detected. Embryo biopsy may also detect some mitotic errors, while major limitation of a correct diagnosis at this stage is embryonic mosaicism.[37] On the other hand, a major disadvantage of testing at this stage is that removing one of the blastomeres might affect further development of the embryo. Indeed, biopsies at day 3 cleavage stage have been shown to significantly impair implantation potential.[38]

However, a randomized controlled trial has shown that blastocyst biopsy at day 5–6 with comprehensive chromosome screening and fresh embryo transfer significantly increases *in vitro* fertilization implantation and delivery rates.[39]

Trophectoderm biopsy on day 5 to 6 has no adverse effect on the embryos. While biopsy at the cleavage stage may reduce implantation rates from 50 to 30%, biopsy at the blastocyst stage does not affect implantation potential.[21,40] However, day 5–6 is too late for performing the test before fresh transfer.

The beneficial effect of pre-implantation genetic screening has not been proved so far. Although initial studies revealed a favourable effect on implantation and pregnancy rates, recent randomized controlled trials have not been able to confirm this.[22] The negative results can be due to the fact that because of chromosomal mosaicism, the blastomere analysed does not reflect the situation in the whole embryo.[39] Indeed, most of the randomized trials were performed on cleavage-stage embryos, and aside from the fact that there is a high level of chromosomal mosaicism at this stage, cleavage-stage embryos are most vulnerable to invasive interventions. Therefore, the biopsy itself might have contributed to the failure to show positive results.

Non-Invasive Methods

If the embryo is cultured *in vitro* till it develops into blastocyst, the developmental uncertainties of cleavage-stage embryo development can be eliminated. Furthermore, the implantation potential of the blastocyst seems to be better than that of cleavage-stage embryos. A single-blastocyst transfer is much more likely to result in a singleton live birth than transfer of a single good-quality cleavage-stage embryo on day 3.[41] Therefore, simply allowing the embryo to reach the blastocyst stage might improve the implantation rate in a fresh transfer. On the other hand, blastocyst is more vulnerable during freezing; furthermore, although there is no evidence for this in humans, animal studies demonstrated epigenetic effects during an extended culture.[42,43]

Analysis of the Follicular Fluid and Embryo Culture Media

The advantage of these approaches is their non-invasive nature. Various substances in the follicular fluid have been related to pregnancy outcome.[44–46] An interesting study suggests that monitoring follicular fluid granulocyte colony-stimulating factor (G-CSF)

American Journal of Reproductive Immunology **75** (2016) 382–387

for the selection of embryos with a better implantation potential might improve the efficiency of assisted reproduction. During this trial, follicular fluid G-CSF was measured with Luminex technology in 523 individual follicular fluid samples from 78 patients undergoing intracytoplasmic sperm injection (ICSI). The results showed that follicular G-CSF concentrations were highly predictive of subsequent implantation.[47] Yet up to now neither G-CSF nor other follicular fluid markers have been routinely used for embryo selection in clinical practice.

Analysis of embryo culture media is based on the supposedly different metabolic activity of competent and impaired embryos. This would be reflected by the amount of certain molecules consumed from or released to the culture medium during development.[48] Research has focussed on amino acid[49] and glucose metabolism[48–50] as well as oxygen consumption.[51]

Recently, Monstko et al.[52] reported a 100% identification of non-viable embryos by the presence of a haptoglobin fragment in embryo culture medium. Although only 55% of successfully implanting embryos could be identified, this would still be a useful tool for embryo selection, but liquid chromatography together with mass spectrometry can hardly be expected to become available for routine use in IVF centres. The same problem, for example the need for special equipments and expertise to perform the tests, applies to the other approaches mentioned above.

Table I Methods for Assessing Embryo Quality

	Advantages	Disadvantages
Embryo morphology	Inexpensive, easy to perform, routine procedure in many laboratories	Subjective, no proven correlation with embryo quality
Time-lapse imaging	Stable culture conditions Dynamic measurements	Not enough data to prove its benefits
Polar body biopsy	Non-invasive	Reflects only the maternal side. High incidence of misdiagnosis
Biopsy of cleavage-stage embryo	Detects meiotic errors of both partners	Invasive and might affect embryo development Cannot handle embryonic mosaicism
Trophectoderm biopsy	Non-invasive	Too late for fresh transfer
Analysis of follicular fluid	Non-invasive	No randomized trials
Analysis of embryo culture media	Non-invasive	Complicated, time-consuming methods No randomized trials

Summary and conclusions

Handling the increasing rate of infertility requires adequate methods to select the embryo which is most likely to implant. The practice of transferring two or three embryos not only increases the chance of pregnancy, but also that of multiple pregnancies, the latter being one of the main causes of preterm birth. The ideal situation would be a single transfer of a well-characterized embryo with a high implantation potential. Recently, enormous efforts have been devoted to finding the appropriate method to identify the competent embryo. The methods that have been suggested so far are summarized in Table I.

Selection based on the morphological features of the embryo – still used in many laboratories – gives rather subjective results. Morphokinetic measurements provide more objective data. By time-lapse imaging, the development of the embryo can be observed in a closed system, and thus, in contrast to morphological evaluation, the culture conditions are stable. Some studies reported an elevated pregnancy rate when morphokinetic parameters were used for embryo selection, yet large randomized trials are still missing.

In spite of its invasive nature, pre-implantation genetic screening for aneuploidy looked very promising. It is very logical that excluding aneuploid embryos should increase the pregnancy rate. However, although the initial (non-randomized) studies suggested a favourable effect, recent randomized trials did not support this.

This can be partly explained by the invasiveness of the method itself, because biopsy might negatively influence further development of the embryo. Another explanation is the inherent problems in testing embryos at different stages of development. Although polar body testing is considered non-invasive as it does not directly affect the embryo, there is a high chance for misdiagnosis. Results from 3-day cleavage embryos are more informative, but because of a high rate of embryonic mosaicism at this stage, the diagnosis is often incorrect.

The ideal test should be non-invasive, simple and quick, so that it could be performed before fresh transfer. This would imply detecting changes in the spent embryo culture medium that would reflect the physiological state of the embryo. Unfortunately, tests detecting the glucose or amino acid metabolism or oxygen consumption of the embryo require sophisticated equipment and thus are not suitable for high-throughput routine screening. Determination of G-CSF in follicular fluid looks promising. The measurement is easy to perform, results can be obtained within a reasonable time, and a recent study performed on more than 500 samples revealed a good correlation between follicular fluid G-CSF concentrations and implantation.

Despite a plethora of good ideas and high number of clinical studies, there is no way at present to tell with certainty, which embryo should be selected for transfer. Several of the methods are promising, but most of them are still experimental. Randomized clinical studies are needed to confirm the usefulness of these tests before they can be put to use in everyday practice.

Acknowledgements

This work was supported by SROP-4.2.2.A-11/1/KONV-2012-0053, SROP-4.2.2.D-15/1/KONV-2015-0004 and by PTE ÁOK-KA 2015-12.

References

1 Munné S, Alikani M, Tomkin G, Grifo J, Cohen J: Embryo morphology, developmental rates and maternal age are correlated with chromosome abnormalities. *Fertil Steril* 1995; 64:382–391.
2 Magli MC, Gianaroli L, Ferrareti AP: Chromosomal abnormalities in embryos. *Mol Cell Endocrinol* 2001; 183(suppl. 1):S29–S34.
3 Márquez C, Sandalinas M, Bahçe M, Alikani M, Munné S: Chromosome abnormalities in 1255 cleavage-stage human embryos. *Reprod BioMed Online* 2000; 1:17–26.
4 Bielanska M, Tan SL, Ao A: High rate of mixoploidy among human blastocysts cultured in vitro. *Fertil Steril* 2002; 78:1248–1253.
5 Rai R, Regan L: Recurrent miscarriage. *Lancet* 2006; 368:601–611.
6 Mertzanidou A, Wilton L, Cheng J, Spits C, Vanneste E, Moreau Y, Vermeesch JR, Sermon K: Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos. *Hum Reprod* 2013; 28:256–264.
7 Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, Melotte C, Debrock S, Amyere M, Vikkula M, Schuit F, Fryns JP, Verbeke G, D'Hooghe T, Moreau Y, Vermeesch JR: Chromosome instability is common in human cleavage-stage embryos. *Nat Med* 2009; 15:577–583.
8 Quenby S, Vince G, Farquharson R, Aplin J: Recurrent miscarriage: a defect in nature's quality control? *Hum Reprod* 2002; 17:1959–1963.
9 Stephenson MD, Awartani KA, Robinson WP: Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case-control study. *Hum Reprod* 2002; 17:446–451.
10 Macklon NS, Geraedts JP, Fauser BC: Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. *Hum Reprod Update* 2002; 8:333–343.
11 Porat O, Clark DA: Analysis of immunosuppressive molecules associated with murine in vitro fertilized embryos. *Fertil Steril* 1990; 54:1154–1161.
12 Kelemen K, Paldi A, Tinneberg H, Torok A, Szekeres-Bartho J: Early recognition of pregnancy by the maternal immune system. *Am J Reprod Immunol* 1998; 39:351–355.
13 Dimitriadis E, White CA, Jones RL, Salamonsen LA: Cytokines, chemokines and growth factors in endometrium related to implantation. *Human Reprod Update* 2005; 11:613–630.
14 Gellersen B, Brosens IA, Brosens JJ: Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. *Semin Reprod Med* 2007; 25:445–453.
15 Macklon NS, Brosens JJ: The human endometrium as a sensor of embryo quality. *Biol Reprod* 2014; 91:1–8.
16 Teklenburg G, Salker M, Molokhia M, Lavery S, Trew G, Aojanepong T, Mardon HJ, Lokugamage AU, Rai R, Landles C, Roelen BA, Quenby S, Kuijk EW, Kavelaars A, Heijnen CJ, Regan L, Brosens JJ, Macklon NS: Natural selection of human embryos: decidualizing endometrial stromal cells serve as sensors of embryo quality upon implantation. *PLoS ONE* 2010; 5:e10258.
17 Brosens JJ, Salker MS, Teklenburg G, Nautiyal J, Salter S, Lucas ES, Steel JH, Christian M, Chan YW, Boomsma CM, Moore JD, Hartshorne GM, Sućurović S, Mulac-Jericevic B, Heijnen CJ, Quenby S, Koerkamp MJ, Holstege FC, Shmygol A, Macklon NS: Uterine selection of human embryos at implantation. *Sci Rep* 2014; 6:3894.
18 Das SK, Wang XN, Paria BC, Damm D, Abraham JA, Klagsbrun M, Andres GK, Dey SK: Heparin-binding EGF-like growth factor gene is induced in the mouse uterus temporally by the blastocyst solely at the site of its apposition: a possible ligand for interaction with blastocyst EGF receptor in implantation. *Development* 1994; 120:1071–1083.
19 Dey SK, Lim H, Das SK, Reese BC, Paria T, Daikoku T, Wang H: Molecular clues to implantation. *Endocr Rev* 2004; 25:341–373.
20 Singh H, Nardo L, Kimber SJ, Aplin JD: Early stages of implantation as revealed by an in vitro model. *Reproduction* 2010; 139:905–914.
21 Scott KL, Hong KH, Scott RT Jr: Selecting the optimal time to perform biopsy for preimplantation genetic testing. *Fertil Steril* 2013; 100:608–614.
22 Scott RT Jr, Ferry K, Su J, Tao X, Scott K, Treff NR: Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. *Fertil Steril* 2012; 97:870–875.
23 Steer CV, Mills CL, Tan SL, Campbell S, Edwards RG: The cumulative embryo score: a predictive embryo scoring technique to select the optimal number of embryos to transfer in an in-vitro fertilization and embryo transfer programme. *Hum Reprod* 1992; 7:117–119.
24 Gardner DK, Schoolcraft WB: In vitro culture of human blastocysts. In Toward Reproductive Certainty: Fertility and Genetics Beyond. R Jansen, D Mortimer (eds). London, Parthenon Publishing, 1999, pp 378–388.
25 Gardner DK, Sakkas D: Assessment of embryo viability: the ability to select a single embryo for transfer–a review. *Placenta* 2003; 24 (Suppl. B):S5–S12.

26 Montag M, Liebenthron J, Koster M: Which morphological scoring system is relevant in human embryo development? *Placenta* 2011; 32:S252–S256.

27 Kirkegaard K, Hindkjaer JJ, Grondahl ML, Kesmodel US, Ingerslev HJ: A randomized clinical trial comparing embryo culture in a conventional incubator with a time-lapse incubator. *J Assist Reprod Genet* 2012; 29:565–572.

28 Meseguer M, Herrero J, Tejera A, Hilligsøe KM, Ramsing NB, Remohı′ J: The use of morphokinetics as a predictor of embryo implantation. *Hum Reprod* 2011; 26:2568–2571.

29 Meseguer M, Rubio I, Cruz M, Basile N, Marcos J, Requena A: Embryo incubation and selection in a time-lapse monitoring system improves pregnancy outcome compared with a standard incubator: a retrospective cohort study. *Fertil Steril* 2012; 98:e10.

30 Campbell A, Fisher S, Bowman N, Duffy S, Sedler M, Hickman CFL: Modelling a risk classification of aneuploidy in human embryos using non-invasive morphokinetics. *Reprod Biomed Online* 2013; 26:477–485.

31 Chamayou S, Patrizio P, Storaci G, Tomaselli V, Alecci C, Ragolia C, Crescenzo C, Guglielmino A: The use of morphokinetic parameters to select all embryos with full capacity to implant. *J Assist Reprod Genet* 2013; 30:703–710.

32 Chavez SL, Loewke KE, Han J, Moussavi F, Colls P, Munne S, Behr B, Reijo Pera RA: Dynamic blastomere behaviour reflects human embryo ploidy by the four-cell stage. *Nat Commun* 2012; 3:1251.

33 Wong CC, Loewke KE, Bossert NL, Behr B, de Jonge CJ, Baer TM, Reijo Pera RA: Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. *Nat Biotechnol* 2010; 28:1115–1121.

34 Kaser DJ, Racowsky C: Clinical outcomes following selection of human preimplantation embryos with time-lapse monitoring: a systematic review. *Hum Reprod Update* 2015; 21:154.

35 Rienzi L, Capalbo A, Stoppa M, Romano S, Maggiulli R, Albricci L, Scarica C, Farcomeni A, Vajta G, Ubaldi FM: No evidence of association between blastocyst aneuploidy and morphokinetic assessment in a selected population of poor-prognosis patients: a longitudinal cohort study. *Reprod BioMed Online* 2015; 30:57–66.

36 Fragouli E, Alfarawati S, Daphnis DD, Goodall NN, Mania A, Griffiths T, Gordon A, Wells D: Cytogenetic analysis of human blastocysts with the use of FISH, CGH and aCGH: scientific data and technical evaluation. *Hum Reprod* 2011; 26:480–490.

37 Haddad G, He W, Gill J, Witz C, Wang C, Kaskar K, Wang W: Mosaic pregnancy after transfer of a "euploid" blastocyst screened by DNA microarray. *J Ovarian Res* 2013; 6:70.

38 Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR: Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. *Fertil Steril* 2013; 100:624–630.

39 Scott RT Jr, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, Tao X, Treff NR: Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. *Fertil Steril* 2013; 100:697–703.

40 Northrop LE, Treff NR, Levy B, Scott RT Jr: SNP microarray-based 24 chromosome aneuploidy screening demonstrates that cleavage-stage FISH poorly predicts aneuploidy in embryos that develop to morphologically normal blastocysts. *Mol Hum Reprod* 2010; 16:590–600.

41 Papanikolaou EG, Camus M, Kolibianakis EM, van Landuyt L, van Steirteghem A, Devroey P: In vitro fertilization with single blastocyst-stage versus single cleavage-stage embryos. *N Engl J Med* 2006; 354:1139–1146.

42 Mann MRW, Denomme MM: Maternal control of genomic imprint maintenance. *Reprod Biomed Online* 2013; 27:629–636.

43 Young LE, Sinclair KD, Wilmut I: Large offspring syndrome in cattle and sheep. *Rev Reprod* 1998; 3:155–163.

44 Andersen CY: Characteristics of human follicular fluid associated with successful conception after in vitro fertilization. *J Clin Endocrinol Metab* 1993; 77:1227–1234.

45 Ledee N, Frydman R, Osipova A, Taieb J, Gallot V, Lombardelli L, Logiodice F, Petitbarat M, Fanchin R, Chaouat G, Achour-Frydman N, Piccinni MP: Levels of follicular G-CSF and interleukin-15 appear as noninvasive biomarkers of subsequent successful birth in modified natural in vitro fertilization/intracytoplasmic sperm injection cycles. *Fertil Steril* 2011; 95:94–98.

46 Shaikly VR, Morrison IE, Taranissi M, Noble CV, Withey AD, Cherry RJ, Blois SM, Fernandez N: Analysis of HLA-G in maternal plasma, follicular fluid, and preimplantation embryos reveal an asymmetric pattern of expression. *J Immunol* 2008; 180:4330–4337.

47 Ledee N, Gridelet V, Ravet S, Jouan C, Gaspard O, Wenders F, Thonon F, Hincourt N, Dubois M, Foidart JM, Munaut C, Perrier d'Hauterive S: Impact of follicular G-CSF quantification on subsequent embryo transfer decisions: a proof of concept study. *Human Reprod* 2013; 28:406–413.

48 Gardner DK, Lane M, Stevens J, Schoolcraft WB: Noninvasive assessment of human embryo nutrient consumption as a measure of developmental potential. *Fertil Steril* 2001; 76:1175–1180.

49 Leese HJ: Metabolism of the preimplantation embryo: 40 years on. *Reproduction* 2012; 143:417–427.

50 Lane M, Gardner DK: Selection of viable mouse blastocysts prior to transfer using a metabolic criterion. *Hum Reprod* 1996; 11:1975–1978.

51 Scott L, Berntsen J, Davies D, Gundersen J, Hill J, Ramsing N: Symposium: innovative techniques in human embryo viability assessment. Human oocyte respiration-rate measurement – potential to improve oocyte and embryo selection? *Reprod Biomed Online* 2008; 17:461–469.

52 Montskó G, Zrínyi Z, Janáky T, Szabó Z, Várnagy Á, Kovács GL, Bódis J: Noninvasive embryo viability assessment by quantitation of human haptoglobin alpha-1 fragment in the in vitro fertilization culture medium: an additional tool to increase success rate. *Fertil Steril* 2015; 103:687–693.