Reproductive BioMedicine Online (2012) **25**, 108–117

www.sciencedirect.com
www.rbmonline.com

REVIEW

The clinical benefit and safety of current and future assisted reproductive technology

Rachel Brown [a,*], Joyce Harper [b]

[a] *Division of Medicine, University College London, Gower Street, UK;* [b] *UCL Centre for PG and D, Institute for Women's Health, University College London, UK and The Centre for Reproductive and Genetic Health, UCLH, London, UK*
[*] Corresponding author. *E-mail address:* rmjbreb@ucl.ac.uk (R Brown).

Rachel Brown is a graduate of the University of Edinburgh, where she obtained a first-class degree in medical biology. She recently completed a Master's degree with distinction in prenatal genetics and fetal medicine at University College London. She is currently a first-year PhD student funded by the Medical Research Council and maintains an interest in genetics and reproductive biology.

Abstract Since the first birth by IVF was achieved in 1978, the techniques involved in assisted reproductive technology have grown at an enormous rate. However, new technology has rarely been robustly validated before clinical use and developing scientific understanding of the available techniques has done little to alter their use. Furthermore, there are inconsistencies in the available clinical studies and endpoints. The benefits of some technologies already established for routine use are currently dubious and there are clear ethical concerns with providing them to patients when their scientific basis is not clear. As the uptake of assisted reproductive technology increases and newer technologies continue to push the boundaries of science, it is important to consider the clinical benefits and safety of all assisted reproductive technologies. This review will discuss aspects of some of the more recent techniques, including sperm DNA-damage tests, intracytoplasmic morphologically selected sperm injection, amino acid and metabolomics profiling, preimplantation genetic screening and time-lapse imaging, and those that may have substantial impacts on the field of reproductive medicine in the future including artificial gametes, ovarian transplantation and gene therapy.

KEYWORDS: assisted reproductive technology, embryo selection, IVF, preimplantation genetic screening, sperm DNA damage, time-lapse imaging

Introduction

Since the first IVF birth in 1978 (Steptoe and Edwards, 1978), the field of assisted reproductive technology has evolved at a rapid rate. Now, in 2012, clinics can achieve pregnancies in couples where there are no spermatozoa in the ejaculate, efficiently freeze human gametes and embryos, and perform whole chromosome scanning of the preimplantation embryo. Despite being impressive achievements, historically, new technology has rarely been robustly validated before clinical use in humans (Harper et al., 2012). Ideally, new technology should be first evaluated in a suitable animal model, and only when safety has been

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2012.04.009

confirmed should it be applied to humans. Even then, newly introduced techniques should only be used routinely after randomized controlled trials (RCT) have confirmed that the technique is of benefit and that all safety issues have been addressed. As new technologies continue to push the boundaries of scientific knowledge, an appreciation of their clinical benefit and safety will become even more paramount.

This review begins by critically discussing some of the techniques already being used in clinical practice and to what end their use is supported by a strong scientific basis. Whether improvements in scientific understanding since their introduction have altered their clinical benefit and safety, and whether this information has been incorporated into clinical practice, is also considered. Some technologies that are beginning to be introduced or could have substantial impacts on reproductive medicine in the future will also be discussed. In an attempt to learn from previous mistakes, a particular focus will be placed on whether these developing technologies will provide a clear clinical benefit in addition to adhering to safety and ethical concerns.

Ongoing developments: gamete and embryo selection

Sperm DNA-damage tests

The main aim of assisted reproductive technology is to artificially achieve a pregnancy when natural conception has failed. The number of viable embryos may intuitively be improved if the most suitable gametes are involved in fertilization. Male fertility has long been assessed on conventional parameters including motility, morphology and concentration. While these parameters are important considerations, it is now understood that these alone provide only a limited diagnostic and prognostic value and an improved marker of sperm quality is desirable (Lefievre et al., 2007; Chen et al., 2009). It is intuitive that the genomic integrity of the spermatozoa is important for its cellular functions, including the ability to promote embryonic development and health after fertilization, and therefore methods to assess genomic integrity could have important clinical applications.

As early as 1980, it was reported that an appreciation of sperm DNA integrity could be a useful indicator of male fertility (Evenson et al., 1980), and an ever-increasing interest in DNA damage has followed. However, to what degree such DNA fragmentation tests are currently beneficial has now been called into question (Zini and Sigman, 2009; Barratt et al., 2010). Fundamentally, the precise nature and location of the damage detected by DNA-damage assays are, in many cases unclear, as is indeed the case for the most utilized clinical assay, the sperm chromatin structure assay (SCSA) (Aitken et al., 2009; Bungum et al., 2011). Further still, the unique packaging of sperm chromatin raises the question as to whether assay reagents are capable of accessing all areas of the genome, further complicating the correct interpretation of assay results. Several DNA-damage assays are now available for research and clinical purposes, including the SCSA (Evenson et al., 1999), sperm chromatin dispersion test (SCD) (Fernandez et al., 2003), terminal deoxynucleotidyl transferase-mediated dUDP nick-end labelling (TUNEL) (Gorczyca et al., 1993), the comet assay (Singh et al., 1989) and the sperm comet assay (Simon et al., 2010). However, the lack of validation and standardization of these numerous assays is becoming clear. While some have stated that the results from the SCSA and SCD display a high concordance, discrepancies in the absolute values of DNA fragmentation generated by these assays may lead to confusion when comparing reports using different techniques (Fernandez et al., 2005; Balasuriya et al., 2011). Others have stated that the SCSA and TUNEL assay measure different aspects of DNA damage, and therefore each may provide information of different clinical relevance (Henkel et al., 2010). It is therefore not acceptable to regard these various assays as providing comparable information. Lastly, clearly defined thresholds to distinguish fertile from infertile men on the basis of several assays have not been developed (Simon et al., 2010), and concerns over the high intra-individual variation of results, at least with the SCSA, have been raised (Erenpreiss et al., 2006). Since the SCSA has been exposed to more scrutiny than other assays (Sakkas and Alvarez, 2010), it is possible that results from these other assays will also be complicated by individuals displaying varying levels of damage in a temporal manner. It is therefore currently inappropriate to place a large emphasis on the results of single DNA-damage assays, despite such tests often being used in such a manner.

In addition to these concerns, the clinical effect that DNA damage has on outcomes and post-natal health is currently conflicting. As some studies have reported that DNA-damage assays, in particular the SCSA, can predict the success of natural conception and intrauterine conception (Evenson and Wixon, 2006; Bungum et al., 2007), it has been suggested that DNA-damage assays could be offered to all patients prior to treatment in an effort to better decide whether more invasive techniques (IVF, intracytoplasmic sperm injection (ICSI)) would be more appropriate. While this certainly would be beneficial, more evidence is required before such a routine use could be supported. Recent evidence has suggested that the association between sperm DNA damage and failure to achieve pregnancy during IVF or ICSI cycles is not strong enough to suggest DNA-damage assays have a broad clinical indication (Collins et al., 2008; Zini and Sigman, 2009; Barratt et al., 2010; Zini, 2011). While more robustly designed clinical studies will be needed to verify this statement, a recent paper noting that sperm DNA fragmentation had no clinical effect when good-quality oocytes were used raises the likelihood that an assessment of sperm DNA damage is not relevant in all cases (Meseguer et al., 2011b). Concern surrounding DNA damage has led to the suggestion that sperm DNA damage could be promutagenic, with the potential to cause molecular mutations leading to post-natal disease (Aitken et al., 2001). While animal models have raised concerns about the long-term health and behaviour of offspring conceived with spermatozoa containing damaged DNA (Fernández-Gonzalez et al., 2008), there is no conclusive evidence that DNA damage in spermatozoa is a significant risk to the post-natal health of humans, It should however be noted that it has been suggested that current studies have been underpowered to detect

overt phenotypic changes (Aitken et al., 2011) and the importance of continued follow up of assisted-conception children is not disputed.

Notwithstanding this point, it should be clear that the clinical utility of sperm DNA-damage tests should be limited until a better understanding of what they reveal and the importance of such information is consolidated. Rigorous work to understand the physiological parameters measured by each assay and how the results from different techniques relate to one another, in addition to standardizing and validating these assays, will be important to identify which is the most reliable and relevant to be used clinically.

Intracytoplasmic morphologically selected sperm injection

The ability to artificially select spermatozoa is most relevant to ICSI, where all natural barriers to fertilization are bypassed. Spermatozoa are conventionally selected for ICSI on motility and morphology parameters while other characteristics, such as genomic integrity, cannot be inferred from such a crude selection method. Consequently, a superior method is deemed desirable. In recent years, developments have been made in the form of intracytoplasmic morphologically selected sperm injection (IMSI), a method which uses high-powered magnification (x6600) to allow the visualization of intracellular structures, offering a chance to better select a single spermatozoon deemed most suitable for ICSI (Bartoov et al., 2002).

In initial studies using IMSI, the mean incidence of 'normal' nuclei (defined by shape and vacuolar area) in leftover sperm fractions was retrospectively significantly associated with fertilization and pregnancy rates (Bartoov et al., 2002). Prospective studies have since been performed, comparing IMSI patients to conventional ICSI controls (Bartoov et al., 2003) (Berkovitz et al., 2006b; Antinori et al., 2008) or analysing sequential treatments (ICSI then IMSI) where patients act as their own controls (Hazout et al., 2006). While the positive effect on fertilization is lacking support, all studies have confirmed improved implantation and pregnancy rates and decreased miscarriage rates. IMSI has also been reported to increase the number of blastocysts that develop (Vanderzwalmen et al., 2008), which has far-reaching effects on multiple-birth rates and potentially preimplantation genetic screening. Similarly, IMSI has been associated with lower rates of embryonic aneuploidy (Figueira et al., 2010). The only meta-analysis of IMSI to date verifies that implantation and pregnancy rates are 3-fold higher than conventional ICSI, and that miscarriage rates are 40% lower (Setti et al., 2010). Matched IMSI groups (differing only on nucleus 'normalcy') suggest these results occur due to morphological variations, not the sperm preparations used for IMSI (Berkovitz et al., 2005); (Berkovitz et al., 2006a). Importantly, IMSI may limit the overuse of ICSI by providing a method to assess whether IVF may be achievable before resorting to ICSI (Wittemer et al., 2006).

It is postulated that poor nucleus morphology, in particular large or numerous vacuoles (as detected by IMSI), reflects abnormalities causing poor chromatin integrity. In turn, such spermatozoa are more susceptible to DNA damage and may adversely affect ICSI outcomes (Hazout et al., 2006) (Franco et al., 2008). Consequently, selection based on fine-scale morphology improves ICSI outcomes by virtue of superior DNA integrity. Worryingly, a recent paper reported 65% spermatozoa deemed suitable for ICSI by conventional methods were subsequently deselected after high-magnification analysis (Wilding et al., 2010). How often suboptimal spermatozoa are currently used in treatment cycles, and the ramifications this may have, is a source of apprehension.

Although IMSI has thus far shown promise, the preliminary studies investigating IMSI are hindered by small sample numbers and differing study designs. These flaws complicate direct comparisons. Efficacy data are still needed to establish whether IMSI will become a routine technique or be restricted to patients with severe infertility and/or repeated ICSI failure. The expense and time-consuming nature of IMSI may well limit its clinical applicability, so concomitant research into alternative sperm selection methods is still warranted.

Amino acid and metabolomic profiling

While methods to improve gamete selection are ongoing, embryo selection is always likely to form an integral part of assisted reproductive technology and non-invasive methods are highly sought after. Houghton et al. (2002) showed that by non-invasively analysing spent culture medium, the profiles of amino acid turnover had the potential to distinguish between embryos with the competence to develop to the blastocyst and those that would later arrest. Further confidence for the benefits of amino acid profiling was found when the turnover of asparagine, glycine and leucine was found to be significantly correlated with clinical pregnancy and live birth rates, independently of other known predictors of success (Brison et al., 2004). More recently, a positive correlation between amino acid turnover and DNA damage has been reported (Sturmey et al., 2009), as has an association between amino acid metabolism and aneuploidy (Picton et al., 2010). Together, these studies highlight the potential of non-invasive metabolomic methods to identify vast amounts of information about the health and viability of embryos in vitro. Of interest, it has been noted that both human and bovine embryos of different genders metabolize amino acids differently (Picton et al., 2010; Sturmey et al., 2010). Considering sex selection for social reasons is illegal in many countries, this raises the very real possibility for a need of tight regulations governing the use of non-invasive biomarkers of gender.

As an extension of amino acid profiling, 'metabolomic profiling' involves analysing all small molecules used and metabolized by the embryo (Botros et al., 2008; Seli et al., 2010a). As for amino acid profiling, it therefore represents the functional phenotype of the embryo, is entirely non-invasive and is therefore safe with regards to the embryo. Methods to create complete metabolomic profiles as opposed to analysing specific culture metabolites have been attempted since 2007, using spectroscopic techniques to identify quantitative changes in —SH, —CH, —NH and —OH groups (Seli et al., 2007). This initial study analysed the culture of embryos for which the clinical outcome was already known, and the spectral regions most associated with positive pregnancies were identified using a genetic algo-

rithm and developed into a 'viability index'; implanted embryos had higher indices than those who did not. Depending on the precise spectroscopic method used, sensitivity reached 86% (Seli et al., 2007). Blinded, large-scale studies have since been performed which confirm these results (Scott et al., 2008); (Vergouw et al., 2008); (Bromer et al., 2008; Seli et al., 2010b) and it would therefore seem that metabolomics could represent an exciting future for assisted reproductive technology, allowing the clinical introduction of a non-invasive selection method based on a growing scientific understanding of the nutritional and metabolic requirements of early embryos. However, all of the studies aforementioned were retrospective and unfortunately the results from the first prospective randomized trial showed that this technique has failed to produce an increase in delivery rates (Hardarson et al., 2011). This finding not only exemplifies the need for optimally designed trials to prevent the premature introduction of new techniques (Harper et al., 2012) but also highlights the importance of assessing what is deemed the most important endpoint of any assisted cycle; that is a healthy delivery. The current state of metabolomic profiling is arguably in its infancy, and it is possible that it may only prove truly useful when enough scientific data has been generated and analysed to fully understand the embryonic metabolism and how this relates to developmental and implantation potential. Only then will it be possible to interpret clinical data in a manner to substantially improve embryo selection and provide patients a clear benefit.

Preimplantation genetic screening

The development of IVF in turn also permitted the establishment of preimplantation genetic diagnosis (PGD), a method to identify chromosomal and/or monogenic diseases in preimplantation embryos. The most common indication for PGD is aneuploidy screening, forming 62% of all cycles during 2008 (Harper et al., 2010b). Now more specifically termed preimplantation genetic screening (PGS) (Thornhill et al., 2005), the scientific rationale behind the technique is logical. Aneuploidies are rarely compatible with embryonic development, are the most common cause of spontaneous miscarriage, and oocyte aneuploidies increase with age, contributing to a reduction in natural fertility (Simpson, 2010). Embryo morphology alone is not an adequate predictor of euploidy and, accordingly, PGS was developed to cytogenetically analyse embryos with indications being advanced maternal age, repeated IVF failure, repeated implantation failure and severe male-factor infertility. It was assumed that by transferring only euploid embryos, implantation rates in these patients would increase.

To date, 11 RCT have been performed to assess the effectiveness of PGS (Staessen et al., 2004; Stevens et al., 2004; Mastenbroek et al., 2007; Blockeel et al., 2008; Hardarson et al., 2008; Jansen et al., 2008; Mersereau et al., 2008; Staessen et al., 2008; Meyer et al., 2009; Schoolcraft et al., 2009; Debrock et al., 2010). All have performed the cytogenetic analysis using fluorescent in situ hybridization (FISH) and, while one has used blastocyst biopsy (Jansen et al., 2008), all others have investigated embryos biopsied at the cleavage stage. The extensive use of PGS for many years prior to the first RCT exemplifies the lack of sound scientific investigation often present in this field. Unfortunately, on the evidence of these RCT, it is now widely reported that PGS at the cleavage stage using FISH is an unsuccessful and unjustified technique (Harper et al., 2010a). Additionally, several papers have now been published that have offered explanations as to why PGS, using FISH at the cleavage stage, does not improve pregnancy rates as expected. These include inherent technical problems with FISH but also improved scientific knowledge in the understanding that mosaicism is relatively common in cleavage-stage embryos, even from young and fertile women (Baart et al., 2005; Vanneste et al., 2009; Mastenbroek et al., 2011).

This improved scientific knowledge has done little to slow the use of PGS, and despite the evidence from multiple RCT to suggest it is incapable of improving clinical outcomes, PGS continues to be routinely used in a clinical setting. Nonetheless, such work has generated discussion about PGS and encouraged further developments and possible refinements to the technique. On the basis of improved understanding in the past few years, blastocyst biopsies and the introduction of array platforms to more comprehensively analyse the chromosomal complement have gained popularity for PGS (Harper and Harton, 2010). It is hoped that these newer methods will improve the efficiency of PGS, and certainly they do appear to overcome some of the difficulties associated with FISH and cleavage-stage biopsy. However, as should now be clear, even if scientific theory is sound, it may not translate into a clinical benefit. Regardless, these newer methods of PGS have been used clinically (Hellani et al., 2008) and therefore it is important to quickly and adequately assess these methods by further RCT to determine whether they are a suitable replacement for previously inadequate techniques.

Time-lapse embryo morphology scoring

In cases where no genetic analyses are performed, embryos are selected for transfer largely based on morphological criteria. A number of morphological scoring systems have been proposed with the aim of identifying embryos with the highest developmental and implantation potential, thereby improving success rates and enabling a means to reduce multiple pregnancies (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). Historically, these systems have been based on the visualization of embryos at static time points. Such a technique is hindered by the need for interruptions of the controlled incubation conditions to permit visualization by microscopy and the risk of completely missing important developmental events. Conversely, time-lapse image acquisition can be performed by integrating a monitoring system with the incubation wells, thereby minimizing incubation disruptions and providing a means to capture substantially more information. The possible benefits of time-lapse imaging as a method to strengthen current morphological selection methods have recently been reported in an encouraging study by Wong et al. (2010) who state that progression to the blastocyst stage can be predicted by day 2 by measuring just three parameters; the duration of the first cytokinesis, the time interval between the end of the first mitosis and the start

of the second and the time interval between the second and third mitoses. Importantly, the technique is non-invasive and was reported to have a sensitivity and specificity of >93% (Wong et al., 2010) and therefore holds clear clinical promise. Indeed, the first pregnancy successfully delivered after selection of a blastocyst from the information provided by time-lapse images was reported in 2010 (Pribenszky et al., 2010). Yet, although this suggests the technique is safe for the embryo, such a conclusion is far from being guaranteed and a single case report is certainly insufficient to suggest a clinical benefit of the technique. Although technically a non-invasive method, exposure to light (as needed to obtain periodical digital images) is an unnatural stressor for the embryo and is known to adversely affect animal embryo development (Oh et al., 2007; Ottosen et al., 2007). The first paper to assess the safety of time-lapse scoring has reported that the level and duration of light exposure needed for time-lapse image capturing does not adversely affect fertilization, embryo quality or cleavage rate (Nakahara et al., 2010). Another paper published this year has also reported no adverse effects on embryo development and viability, but also found no significant improvements in ongoing pregnancy rates (Cruz et al., 2011). A recent retrospective study (Meseguer et al., 2011a) has again highlighted the possible benefits of this technique, but the results of the randomized prospective study are awaited to better understand its full potential. Although the potential benefits of time-lapse imaging are clear and current data promising, on balance there is still a paucity of efficiency and safety data into this technique. Large-scale, multicentre trials are needed to ensure the accuracy and benefit of this method, and such studies will need to be extensively designed to overcome several confounding factors including uterine receptivity that can affect implantation rates. Despite this, one great use of this technology that is being realized is its ability to offer novel embryological data that enables the timing of developmental stages to be reassessed.

Areas of active research

So far this review has focused on some techniques that have recently been brought into the IVF laboratory with a variety of success. While these have predominately been concerned with the ability to improve pregnancy rates by better selecting gametes and/or embryos, there is also active research into altogether more ambitious techniques. These techniques push the boundaries of scientific knowledge, and it is therefore important to learn from mistakes with other techniques and to question not only if they are safe, but what one can hope to achieve if they become clinically available.

Overcoming a complete lack of gametes without donation

There will always be patients for whom standard techniques do not work. Cancer survival rates are increasing, and social acceptance of older parents and same-sex couples is growing. It is not unreasonable to consider an increasing cohort of patients in the near future who are infertile because they completely lack the appropriate gametes. For these patients, the ability to generate gametes from alternative cell populations would remove the need for gamete donation or adoption.

Germ cell differentiation from embryonic stem cells (ESC) has been established in murine models (Toyooka et al., 2003) (Geijsen et al., 2003) (Hubner et al., 2003) (Nayernia et al., 2006). As patients desire gametes containing their own genome, if neo-gametogenesis from ESC is to be used clinically, somatic cell nuclear transplantation (SCNT) into an enucleated oocyte would also be necessary in order to develop the necessary ESC. These could then be differentiated into gametes (of either sex if male somatic cells are used (Kerkis et al., 2007) and used for IVF. These so called 'artificial gametes' could be created for women past the menopause and to allow same-sex couples to reproduce. There is evidence that human ESC can also be differentiated into gametes (Clark et al., 2004) (Aflatoonian et al., 2005).

Gamete development from ESC is a prime example of a technique for which not only the outcome but also the means to achieve it must be considered. Methods to maintain ESC and induce differentiation remain poorly understood, while effectiveness, safety and tolerance would need to be rigorously tested before clinical use. Nonetheless, it is likely that ethical (and religious) restraints will limit this avenue of research.

Induced pluripotent stem cells (iPSC) are adult-somatic cells which have been reprogrammed to behave like ESC, therefore overcoming the concern of creating embryos purely for therapeutic reasons. Human iPSC have been created (Takahashi et al., 2007; Yu et al., 2007) and subsequently used to develop germ-line precursors (Park et al., 2009). They may therefore seem more desirable than ESC but currently iPSC display abnormal regulatory pathways and aberrant imprinting patterns. It should be remembered that the ultimate end goal from any intervention, including the use of artificial gametes, is not simply a pregnancy but the delivery of a child whose short- and long-term health is normal. Ongoing research is indisputably needed and this absolutely warrants rigorous animal trials involving long-term follow ups. Yet ethical concerns remain. Ironically iPSC differentiate most readily if co-cultured with fetal cells (Park et al., 2009), and the ability to permit reproduction to same-sex couples or older patients is unsettling for some. No forms of in vitro derived gametes are currently allowed for treatment in the UK.

Stem cell research in general will give unprecedented insight in developmental regulation and disease pathology and provide a platform on which to test drugs. Yet it is vital not to vastly overestimate the benefits of these cells. While patients undergoing infertility treatment may provide the embryos to allow such scientific research, a reciprocal benefit from stem cells is unlikely to have clinical implications in the near future. Scientific limitations, ethical considerations, safety fears and political/funding barriers will undoubtedly hinder this line of research.

Restoring fertility

Current techniques do not 'cure' infertility, they just overcome it. Artificial gametes would similarly require some degree of assisted fertilization. The most appropriate focus of future research may be to restore fertility.

Gonadal transplantation after cryopreservation is being investigated for cancer survivors (Ginsberg et al., 2009; Moffat et al., 2009). In order to restore fertility, orthotopic autografts would be necessary. Live births have been reported following orthotopic ovarian transplantation, from both spontaneous (Donnez et al., 2004; Demeestere et al., 2007) and IVF-assisted pregnancies (Meirow et al., 2005) (Andersen et al., 2008). However, there is confusion over whether these fertilized oocytes were a result of the transplant or survived chemotherapy/radiation. The ischaemic insult suffered by ovarian tissue after transplantation (during revascularization) dramatically reduces the follicle reserve, making fertility restoration transient. Whole ovarian transplantation, including the vascular supply, may become an option to overcome this but concerns over reintroducing cancer cells and the need for numerous operations are obvious. Heterotopic transplantation or in vitro maturation of gametes may be more sensible options, but again require assisted reproductive technology so do not restore fertility.

Gene therapy may also be valuable for fertility restoration. The genes identified to be involved in fertility are increasing quickly (Matzuk and Lamb, 2008) and may be propagated by ICSI. Gene therapy directed to Sertoli cells has improved fertility in mice (Kanatsu-Shinohara et al., 2002) (Kojima et al., 2003) as has spermatogonial stem cell transgenesis, albeit to a lesser extent (Takehashi et al., 2007). Gene therapy directed at embryos would allow infertility prevention. However, this avenue has yielded less success in animal models as mortality rates are unacceptably high at 80—90% (Navarro et al., 2008).

Currently, gene therapy is technically difficult, time-consuming, expensive and inefficient. However, if these caveats can be overcome, gene therapy may offer a suitable treatment for many infertile patients. When used to correct somatic cell abnormalities, gene therapy offers only a localized treatment, but the transgenesis of gametes or embryos may be less acceptable. The in vitro development of 'transgenic' humans is likely to put many people at unease, especially if viral vectors are used. Gene therapy may also lead to a 'slippery-slope' situation whereby desirable genes are modified, leading to truly designer babies. All scientific research should be conducted responsibly, offering clearly defined clinical benefits. Just because scientific knowledge allows a procedure does not imply it is necessary or reasonable to do so, and appropriate regulation will be necessary to limit the ethical dilemmas arising from developing scientific abilities.

Conclusion

This review has attempted to address the current state of several assisted reproductive technology interventions, with a particular focus on the clinical benefit provided to patients in addition to their safety. Not all established or developing techniques have been discussed as they are beyond the scope of this review, but the considerations raised will none the less be relevant for other technologies such as in vitro maturation, artificial oocyte activation, sperm selection by hyaluronic acid-binding assays and pronuclear transfer for overcoming mitochondrial disease.

As basic scientific knowledge increases, it should be translated to clinical care. While this will undoubtedly be reflected in additional technologies being introduced, such knowledge should also be used to reassess the interventions already established. It is now clear that PGS using cleavage-stage biopsy and FISH fails to offer patients a true clinical advantage. Similarly, it should be realized where basic science has been lacking from the start and how this could impact on the clinical utility of a technique. This is evident in techniques such as DNA-damage assays, which although may hold promise for improving practices, are currently surrounded with uncertainties.

Although the same could be said for all areas of medicine, assisted reproductive technology in particular has developed a very strong commercial backing. It is therefore particularly important to ensure that all new technologies are adequately and rigorously tested for both safety and efficiency, ideally before being used clinically. While not all of the techniques discussed here are routinely offered, many are reserved for use in patients with repeated IVF failure. The use of largely experimental techniques particularly in a vulnerable cohort of patients further highlights the need for the risks and benefits to be fully investigated.

In cases where pre-clinical studies may not be possible, RCT need to have proved efficiency and safety before the technique is routinely offered to patients. A clear hindrance to many areas of assisted reproducton research is an inability to compare and contrast papers professing to discuss the same topic. The recently published Istanbul consensus document on embryo assessment has called for the development of consistent and defined embryo morphology endpoints to enable both inter-clinic comparisons and to provide a clinical endpoint to which new techniques can be directly compared, much like how new pharmaceuticals must be compared with a current gold standard before approval (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). If other areas of assisted reproduction or study design could be standardized in a similar way, this might make it possible to assess new technologies more coherently and efficiently. If future studies fail to show that a technique has a benefit (either at all or relative to an already accepted alternative)m then it is in the name of best practice to ensure that the technology does not become established as an integral part of practice. Safety research is also an absolute necessity, especially as more people are likely to use assisted reproduction in the future (Harper et al., 2012) and safety studies should be conducted in a similar manner.

There is a strong and necessary relationship between basic science and clinical practice and it is important that both develop at a similar rate. In this respect, it is important to continuously ask what one is trying to achieve with the introduction of new technologies. The developing techniques discussed here, including artificial gametes, reflect just how advanced scientific understanding and ability has become since the introduction of IVF just over 30 years ago. However, such research needs to be sensibly performed with a clinical application in mind, including whether it will provide patients with a benefit beyond what is already available and whether it is safe. A final consideration is that safety in the context of assisted reproductive technology includes not only the short- and long-term physiological

aspects (to embryos and patients) but also the psychological and ethical concerns that are so evident in this field.

References

Aflatoonian, B., Fazeli, A., Ruban, L., Jones, M., Andrews, P.W. and Moore, H.D.M. (2005) Human embryonic stem cells differentiate to primordial germ cells as determined by gene expression profiles and antibody markers.

Aitken, R.J., Curry, B.J., 2011. Redox regulation of human sperm function: from the physiological control of sperm capacitation to the etiology of infertility and DNA damage in the germ line. Antioxidants and Redox Signaling 14 (3), 367–381.

Aitken, R.J., de Iuliis, G.N., McLachlan, R.I., 2009. Biological and clinical significance of DNA damage in the male germ line. International Journal of Andrology 32, 46–56.

Aitken, R.J., Krausz, C., 2001. Oxidative stress, DNA damage and the Y chromosome. Reproduction 122 (4), 497–506.

Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Human Reproduction 26, 1270–1283.

Andersen, C.Y., Rosendahl, M., Byskov, A.G., Loft, A., Ottosen, C., Dueholm, M., Schmidt, K.L.T., Andersen, A.N., Ernst, E., 2008. Two successful pregnancies following autotransplantation of frozen/thawed ovarian tissue. Human Reproduction 23, 2266.

Antinori, M., Licata, E., Dani, G., Cerusico, F., Versaci, C., D'Angelo, D., Antinori, S., 2008. Intracytoplasmic morphologically selected sperm injection: a prospective randomized trial. Reproductive Biomedicine Online 16, 835–841.

Baart, E.B., Martini, E., van den Berg, I., Macklon, N.S., Galjaard, R.J., Fauser, B., Opstal, D.V., 2005. Preimplantation genetic screening reveals a high incidence of aneuploidy and mosaicism in embryos from young women undergoing IVF. Human Reproduction.

Balasuriya, A., Speyer, B., Serhal, P., Doshi, A., Harper, J.C., 2011. Sperm chromatin dispersion test in the assessment of DNA fragmentation and aneuploidy in human spermatozoa. Reproductive Biomedicine Online.

Barratt, C.L.R., Aitken, R.J., Bjarndahl, L., Carrell, D.T., de Boer, P., Kvist, U., Lewis, S.E.M., Perreault, S.D., Perry, M.J., Ramos, L., 2010. Sperm DNA: organization, protection and vulnerability: from basic science to clinical applications: a position report. Human Reproduction 25, 824.

Bartoov, B., Berkovitz, A., Eltes, F., Kogosowski, A., Menezo, Y., Barak, Y., 2002. Real-time fine morphology of motile human sperm cells is associated with IVF-ICSI outcome. Journal of Andrology 23, 1.

Bartoov, B., Berkovitz, A., Eltes, F., Kogosovsky, A., Yagoda, A., Lederman, H., Artzi, S., Gross, M., Barak, Y., 2003. Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection. Fertility and Sterility 80, 1413–1419.

Berkovitz, A., Eltes, F., Yaari, S., Katz, N., Barr, I., Fishman, A., Bartoov, B., 2005. The morphological normalcy of the sperm nucleus and pregnancy rate of intracytoplasmic injection with morphologically selected sperm. Human Reproduction 20, 185.

Berkovitz, A., Eltes, F., Ellenbogen, A., Peer, S., Feldberg, D., Bartoov, B., 2006a. Does the presence of nuclear vacuoles in human sperm selected for ICSI affect pregnancy outcome? Human Reproduction 21, 1787.

Berkovitz, A., Eltes, F., Lederman, H., Peer, S., Ellenbogen, A., Feldberg, B., Bartoov, B., 2006b. How to improve IVF-ICSI outcome by sperm selection. Reproductive Biomedicine Online 12, 634–638.

Blockeel, C., Schutyser, V., de Vos, A., Verpoest, W., de Vos, M., Staessen, C., Haentjens, P., van der Elst, J., Devroey, P., 2008. Prospectively randomized controlled trial of PGS in IVF/ICSI patients with poor implantation. Reproductive Biomedicine Online 17, 848–854.

Botros, L., Sakkas, D., Seli, E., 2008. Metabolomics and its application for non-invasive embryo assessment in IVF. Molecular Human Reproduction 14, 679.

Brison, D.R., Houghton, F.D., Falconer, D., Roberts, S.A., Hawkhead, J., Humpherson, P.G., Lieberman, B.A., Leese, H.J., 2004. Identification of viable embryos in IVF by non-invasive measurement of amino acid turnover. Human Reproduction 19, 2319.

Bromer, J.G., Sakkas, D., Seli, E., 2008. Metabolomic profiling of embryo culture media to predict IVF outcome. Expert Review of Obstetrics and Gynecology 3, 441–447.

Bungum, M., Humaidan, P., Axmon, A., Spanò, M., Bungum, L., Erenpreiss, J., Giwercman, A., 2007. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Human Reproduction 22, 174–179.

Bungum, M., Bungum, L., Giwercman, A., 2011. Sperm chromatin structure assay (SCSA): a tool in diagnosis and treatment of infertility. Asian Journal of Andrology 13, 69–75.

Chen, X., Zhang, W., Luo, Y., Long, X., Sun, X., 2009. Predictive value of semen parameters in in vitro fertilisation pregnancy outcome. Andrologia 41, 111–117.

Clark, A.T., Bodnar, M.S., Fox, M., Rodriquez, R.T., Abeyta, M.J., Firpo, M.T., Pera, R.A.R., 2004. Spontaneous differentiation of germ cells from human embryonic stem cells in vitro. Human Molecular Genetics 13, 727.

Collins, J.A., Barnhart, K.T., Schlegel, P.N., 2008. Do sperm DNA integrity tests predict pregnancy with in vitro fertilization? Fertility and Sterility 89, 823–831.

Cruz, M., Gadea, B., Garrido, N., Pedersen, K.S., Martínez, M., Pérez-Cano, I., Muñoz, M., Meseguer, M., 2011. Embryo quality, blastocyst and ongoing pregnancy rates in oocyte donation patients whose embryos were monitored by time-lapse imaging. Journal of Assisted Reproduction and Genetics 28, 569–573.

Debrock, S., Melotte, C., Spiessens, C., Peeraer, K., Vanneste, E., Meeuwis, L., Meuleman, C., Frijns, J.P., Vermeesch, J.R., D'Hooghe, T.M., 2010. Preimplantation genetic screening for aneuploidy of embryos after in vitro fertilization in women aged at least 35 years: a prospective randomized trial. Fertility and Sterility 93, 364–373.

Demeestere, I., Simon, P., Emiliani, S., Delbaere, A., Englert, Y., 2007. Fertility preservation: successful transplantation of cryopreserved ovarian tissue in a young patient previously treated for Hodgkin's disease. The Oncologist 12, 1437.

Donnez, J., Dolmans, M.M., Demylle, D., Jadoul, P., Pirard, C., Squifflet, J., Martinez-Madrid, B., van Langendonckt, A., 2004. Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. The Lancet 364, 1405–1410.

Erenpreiss, J., Bungum, M., Spano, M., Elzanaty, S., Orbidans, J., Giwercman, A., 2006. Intra-individual variation in sperm chromatin structure assay parameters in men from infertile couples: clinical implications. Human Reproduction 21, 2061.

Evenson, D., Wixon, R., 2006. Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. Reproductive Biomedicine Online 12, 466–472.

Evenson, D.P., Darzynkiewicz, Z., Melamed, M.R., 1980. Relation of mammalian sperm chromatin heterogeneity to fertility. Science 210, 1131.

Evenson, D.P., Jost, L.K., Marshall, D., Zinaman, M.J., Clegg, E., Purvis, K., de Angelis, P., Claussen, O.P., 1999. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Human Reproduction 14, 1039.

Fernandez, J.L., Muriel, L., Rivero, M.T., Goyanes, V., Vazquez, R., Alvarez, J.G., 2003. The sperm chromatin dispersion test: a simple method for the determination of sperm DNA fragmentation. Journal of Andrology 24, 59.

Fernandez, J.L., Muriel, L., Goyanes, V., Segrelles, E., Gosãlvez, J., Enciso, M., Lafromboise, M., de Jonge, C., 2005. Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. Fertility and Sterility 84, 833–842.

Fernández-Gonzalez, R., Moreira, P.N., Pérez-Crespo, M., Sánchez-Martín, M., Ramirez, M.A., Pericuesta, E., Bilbao, A., Bermejo-Alvarez, P., De Dios Hourcade, J., de Fonseca, F.R., Gutiérrez-Adán, A., 2008. Long-term effects of mouse intracytoplasmic sperm injection with DNA-fragmented sperm on health and behavior of adult offspring. Biology of Reproduction 78 (4), 761–772.

Figueira, R.C.S., Braga, D., Setti, A.S., Iaconelli Jr., A., Borges Jr., E., 2010. Morphological nuclear integrity of sperm cells is associated with preimplantation genetic aneuploidy screening cycle outcomes. Fertility and Sterility.

Franco Jr., J.G., Baruffi, R.L.R., Mauri, A.L., Petersen, C.G., Oliveira, J.B.A., Vagnini, L., 2008. Significance of large nuclear vacuoles in human spermatozoa: implications for ICSI. Reproductive Biomedicine Online 17, 42–45.

Geijsen, N., Horoschak, M., Kim, K., Gribnau, J., Eggan, K., Daley, G.Q., 2003. Derivation of embryonic germ cells and male gametes from embryonic stem cells. Nature 427, 148–154.

Ginsberg, J.P., Carlson, C.A., Lin, K., Hobbie, W.L., Wigo, E., Wu, X., Brinster, R.L., Kolon, T.F., 2009. An experimental protocol for fertility preservation in prepubertal boys recently diagnosed with cancer: a report of acceptability and safety. Human Reproduction.

Gorczyca, W., Traganos, F., Jesionowska, H., Darzynkiewicz, Z., 1993. Presence of DNA strand breaks and increased sensitivity of DNA in situ to denaturation in abnormal human sperm cells: analogy to apoptosis of somatic cells. Experimental Cell Research 207, 202–205.

Hardarson, T., Hanson, C., Lundin, K., Hillensjã, T., Nilsson, L., Stevic, J., Reismer, E., Borg, K., Wikland, M., Bergh, C., 2008. Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial. Human Reproduction 23, 2806.

Hardarson, T., Ahlstrom, A., Rogberg, L., Botros, L., Hillensjo, T., Westlander, G., Sakkas, D., Wikland, M., 2011. Non-invasive metabolomic profiling of Day 2 and 5 embryo culture medium: a prospective randomized trial. Human Reproduction, 1–8.

Harper, J.C., Harton, G., 2010. The use of arrays in preimplantation genetic diagnosis and screening. Fertility and Sterility 94, 1173–1177.

Harper, J., Coonen, E., de Rycke, M., Fiorentino, F., Geraedts, J., Goossens, V., Harton, G., Moutou, C., Pehlivan Budak, T., Renwick, P., 2010a. What next for preimplantation genetic screening (PGS)? A position statement from the ESHRE PGD Consortium steering committee. Human Reproduction 25, 821.

Harper, J., Goossens, V., Harton, G., 2010b. Session 09: PGD and PGS Session. Human Reproduction 25, i17.

Harper, J., Magli, M.C., Lundin, K., Barratt, C.L.R., Brison, D.R., 2012. When and how should new technology be introduced into the IVF laboratory? Human Reproduction 27 (2), 303–313.

Hazout, A., Dumont-Hassan, M., Junca, A.M., Bacrie, P.C., Tesarik, J., 2006. High-magnification ICSI overcomes paternal effect resistant to conventional ICSI. Reproductive Biomedicine Online 12, 19–25.

Hellani, A., Abu-Amero, K., Azouri, J., El-Akoum, S., 2008. Successful pregnancies after application of array-comparative genomic hybridization in PGS-aneuploidy screening. Reproductive Biomedicine Online 17, 841–847.

Henkel, R., Hoogendijk, C.F., Bouic, P.J.D., Kruger, T.F., 2010. TUNEL assay and SCSA determine different aspects of sperm DNA damage. Andrologia 42, 305–313.

Houghton, F.D., Hawkhead, J.A., Humpherson, P.G., Hogg, J.E., Balen, A.H., Rutherford, A.J., Leese, H.J., 2002. Non-invasive amino acid turnover predicts human embryo developmental capacity. Human Reproduction 17, 999.

Hubner, K., Fuhrmann, G., Christenson, L.K., Kehler, J., Reinbold, R., de la Fuente, R., Wood, J., Strauss III, J.F., Boiani, M., Scholer, H.R., 2003. Derivation of oocytes from mouse embryonic stem cells. Science 300, 1251.

Jansen, R.P.S., Bowman, M.C., de Boer, K.A., Leigh, D.A., Lieberman, D.B., McArthur, S.J., 2008. What next for preimplantation genetic screening (PGS)? Experience with blastocyst biopsy and testing for aneuploidy. Human Reproduction 23, 1476–1478.

Kanatsu-Shinohara, M., Ogura, A., Ikegawa, M., Inoue, K., Ogonuki, N., Tashiro, K., Toyokuni, S., Honjo, T., Shinohara, T., 2002. Adenovirus-mediated gene delivery and in vitro microinsemination produce offspring from infertile male mice. Proceedings of the National Academy of Sciences of the United States of America 99, 1383.

Kerkis, A., Fonseca, S.A.S., Serafim, R.C., Lavagnolli, T.M.C., Abdelmassih, S., Abdelmassih, R., Kerkis, I., 2007. In vitro differentiation of male mouse embryonic stem cells into both presumptive sperm cells and oocytes. Cloning and Stem Cells 9, 535–548.

Kojima, Y., Sasaki, S., Umemoto, Y., Hashimoto, Y., Hayashi, Y., Kohri, K., 2003. Effects of adenovirus mediated gene transfer to mouse testis in vivo on spermatogenesis and next generation. The Journal of Urology 170, 2109–2114.

Lefievre, L., Bedu-Addo, K., Conner, S.J., Machado-Oliveira, G.S.M., Chen, Y., Kirkman-Brown, J.C., Afnan, M.A., Publicover, S.J., Ford, W.C.L., Barratt, C.L.R., 2007. Counting sperm does not add up any more: time for a new equation? Reproduction 133, 675.

Mastenbroek, S., Twisk, M., Van Echten-Arends, J., Sikkema-Raddatz, B., Korevaar, J.C., Verhoeve, H.R., Vogel, N.E.A., Arts, E.G.J.M., de Vries, J.W.A., Bossuyt, P.M., 2007. In vitro fertilization with preimplantation genetic screening. New England Journal of Medicine 357, 9–17.

Mastenbroek, S., Twisk, M., van der Veen, F., Repping, S., 2011. Preimplantation genetic screening: a systematic review and meta-analysis of RCTs. Human Reproduction Update 17, 454.

Matzuk, M.M., Lamb, D.J., 2008. The biology of infertility: research advances and clinical challenges. Nature Medicine 14, 1197–1213.

Meirow, D., Levron, J., Eldar-Geva, T., Hardan, I., Fridman, E., Zalel, Y., Schiff, E., Dor, J., 2005. Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. New England Journal of Medicine 353, 318.

Mersereau, J.E., Pergament, E., Zhang, X., Milad, M.P., 2008. Preimplantation genetic screening to improve in vitro fertilization pregnancy rates: a prospective randomized controlled trial. Fertility and Sterility 90, 1287–1289.

Meseguer, M., Herrero, J., Tejera, A., Hilligsoe, K.M., Ramsing, N.B., Remohi, J., 2011a. The use of morphokinetics as a predictor of embryo implantation. Human Reproduction 26 (10), 2658–2671.

Meseguer, M., Santiso, R., Garrido, N., Garcia-Herrero, S., Remohi, J., Fernandez, J.L., 2011b. Effect of sperm DNA fragmentation on pregnancy outcome depends on oocyte quality. Fertility and Sterility 95, 124–128.

Meyer, L.R., Klipstein, S., Hazlett, W.D., Nasta, T., Mangan, P., Karande, V.C., 2009. A prospective randomized controlled trial of preimplantation genetic screening in the. Fertility and Sterility 91, 1731–1738.

Moffat, R., de Geyter, C., von Wolff, M., 2009. New strategies for fertility preservation in young women with cancer. Therapeutische Umschau. Revue thérapeutique 66, 831.

Nakahara, T., Iwase, A., Goto, M., Harata, T., Suzuki, M., Ienaga, M., Kobayashi, H., Takikawa, S., Manabe, S., Kikkawa, F., 2010.

Evaluation of the safety of time-lapse observations for human embryos. Journal of Assisted Reproduction and Genetics 27, 93–96.

Navarro, J., Risco, R., Toschi, M., Schattman, G., 2008. Gene therapy and intracytoplasmatic sperm injection (ICSI) – A review. Placenta 29, 193–199.

Nayernia, K., Nolte, J., Michelmann, H.W., Lee, J.H., Rathsack, K., Drusenheimer, N., Dev, A., Wulf, G., Ehrmann, I.E., Elliott, D.J., 2006. In vitro-differentiated embryonic stem cells give rise to male gametes that can generate offspring mice. Developmental Cell 11, 125–132.

Oh, S.J., Gong, S.P., Lee, S.T., Lee, E.J., Lim, J.M., 2007. Light intensity and wavelength during embryo manipulation are important factors for maintaining viability of preimplantation embryos in vitro. Fertility and Sterility 88, 1150–1157.

Ottosen, L.D.M., Hindkjær, J., Ingerslev, J., 2007. Light exposure of the ovum and preimplantation embryo during ART procedures. Journal of Assisted Reproduction and Genetics 24, 99–103.

Park, T.S., Galic, Z., Conway, A.E., Lindgren, A., van Handel, B.J., Magnusson, M., Richter, L., Teitell, M.A., Mikkola, H.K.A., Lowry, W.E., 2009. Derivation of primordial germ cells from human embryonic and induced pluripotent stem cells is significantly improved by coculture with human fetal gonadal cells. Stem Cells 27, 783–795.

Picton, H.M., Elder, K., Houghton, F.D., Hawkhead, J.A., Rutherford, A.J., Hogg, J.E., Leese, H.J., Harris, S.E., 2010. Association between amino acid turnover and chromosome aneuploidy during human preimplantation embryo development in vitro. Molecular Human Reproduction 16, 557.

Pribenszky, C., Mátyás, S., Kovács, P., Losonczi, E., Zádori, J., Vajta, G., 2010. Pregnancy achieved by transfer of a single blastocyst selected by time-lapse monitoring. Reproductive Biomedicine Online 21, 533–536.

Sakkas, D., Alvarez, J.G., 2010. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertility and Sterility 93, 1027–1036.

Schoolcraft, W.B., Katz-Jaffe, M.G., Stevens, J., Rawlins, M., Munne, S., 2009. Preimplantation aneuploidy testing for infertile patients of advanced maternal age: a randomized prospective trial. Fertility and Sterility 92, 157–162.

Scott, R., Seli, E., Miller, K., Sakkas, D., Scott, K., Burns, D.H., 2008. Noninvasive metabolomic profiling of human embryo culture media using Raman spectroscopy predicts embryonic reproductive potential: a prospective blinded pilot study. Fertility and Sterility 90, 77–83.

Seli, E., Sakkas, D., Scott, R., Kwok, S.C., Rosendahl, S.M., Burns, D.H., 2007. Noninvasive metabolomic profiling of embryo culture media using Raman and near-infrared spectroscopy correlates with reproductive potential of embryos in women undergoing in vitro fertilization. Fertility and Sterility 88, 1350–1357.

Seli, E., Robert, C., Sirard, M.A., 2010a. OMICS in assisted reproduction: possibilities and pitfalls. Molecular Human Reproduction 16, 513.

Seli, E., Vergouw, C.G., Morita, H., Botros, L., Roos, P., Lambalk, C.B., Yamashita, N., Kato, O., Sakkas, D., 2010b. Noninvasive metabolomic profiling as an adjunct to morphology for noninvasive embryo assessment in women undergoing single embryo transfer. Fertility and Sterility 94, 535–542.

Setti, A.S., Ferreira, R.C., de Almeida Ferreira Braga, D.P., Figueira, R.C.S., Iaconelli Jr., A., Borges Jr., E., Borges, E., 2010. Intracytoplasmic sperm injection outcome versus intracytoplasmic morphologically selected sperm injection outcome: a meta-analysis. Reproductive Biomedicine Online.

Simon, L., Brunborg, G., Stevenson, M., Lutton, D., McManus, J., Lewis, S.E.M., 2010. Clinical significance of sperm DNA damage in assisted reproduction outcome. Human Reproduction 25, 1594.

Simpson, J.L., 2010. Preimplantation genetic diagnosis at 20 years. Prenatal Diagnosis 30, 682–695.

Singh, N.P., Danner, D.B., Tice, R.R., McCoy, M.T., Collins, G.D., Schneider, E.L., 1989. Abundant alkali-sensitive sites in DNA of human and mouse sperm. Experimental Cell Research 184, 461–470.

Staessen, C., Platteau, P., van Assche, E., Michiels, A., Tournaye, H., Camus, M., Devroey, P., Liebaers, I., van Steirteghem, A., 2004. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. Human Reproduction 19, 2849–2858.

Staessen, C., Verpoest, W., Donoso, P., Haentjens, P., van der Elst, J., Liebaers, I., Devroey, P., 2008. Preimplantation genetic screening does not improve delivery rate in women under the age of 36 following single-embryo transfer. Human Reproduction 23, 2818–2825.

Steptoe, P.C., Edwards, R.G., 1978. Birth after the reimplantation of a human embryo. Lancet 2, 366.

Stevens, J., Wale, P., Surrey, E.S., Schoolcraft, W.B., Gardner, D.K., 2004. Is aneuploidy screening for patients aged 35 or over beneficial? A prospective randomized trial. Fertility and Sterility 82, S249.

Sturmey, R.G., Hawkhead, J.A., Barker, E., Leese, H.J., 2009. DNA damage and metabolic activity in the preimplantation embryo. Human Reproduction 24, 81.

Sturmey, R.G., Bermejoâ Alvarez, P., Gutierrezâ Adan, A., Rizos, D., Leese, H.J., Lonergan, P., 2010. Amino acid metabolism of bovine blastocysts: a biomarker of sex and viability. Molecular Reproduction and Development 77, 285–296.

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., Yamanaka, S., 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872.

Takehashi, M., Kanatsu-Shinohara, M., Inoue, K., Ogonuki, N., Miki, H., Toyokuni, S., Ogura, A., Shinohara, T., 2007. Adenovirus-mediated gene delivery into mouse spermatogonial stem cells. Proceedings of the National Academy of Sciences 104, 2596.

Thornhill, A.R., Dedie-Smulders, C.E., Geraedts, J.P., Harper, J.C., Harton, G.L., Lavery, S.A., Moutou, C., Robinson, M.D., Schmutzler, A.G., Scriven, P.N., 2005. ESHRE PGD Consortium' Best practice guidelines for clinical preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS)'. Human Reproduction 20, 35.

Toyooka, Y., Tsunekawa, N., Akasu, R., Noce, T., 2003. Embryonic stem cells can form germ cells in vitro. Proceedings of the National Academy of Sciences of the United States of America 100, 11457.

Vanderzwalmen, P., Hiemer, A., Rubner, P., Bach, M., Neyer, A., Stecher, A., Uher, P., Zintz, M., Lejeune, B., Vanderzwalmen, S., 2008. Blastocyst development after sperm selection at high magnification is associated with size and number of nuclear vacuoles. Reproductive Biomedicine Online 17, 617–627.

Vanneste, E., Voet, T., Melotte, C., Debrock, S., Sermon, K., Staessen, C., Liebaers, I., Fryns, J.P., D'Hooghe, T., Vermeesch, J.R., 2009. What next for preimplantation genetic screening? High mitotic chromosome instability rate provides the biological basis for the low success rate. Human Reproduction.

Vergouw, C.G., Botros, L.L., Roos, P., Lens, J.W., Schats, R., Hompes, P.G.A., Burns, D.H., Lambalk, C.B., 2008. Metabolomic profiling by near-infrared spectroscopy as a tool to assess embryo viability: a novel, non-invasive method for embryo selection. Human Reproduction 23, 1499.

Wilding, M., Coppola, G., di Matteo, L., Palagiano, A., Fusco, E. and Dale, B. (2010) Intracytoplasmic injection of morphologi-

cally selected spermatozoa (IMSI) improves outcome after assisted reproduction by deselecting physiologically poor quality spermatozoa. Journal of Assisted Reproduction and Genetics, Epub ahead of print.
Wittemer, C., Pujol, A., Boughali, H., Viville, S., 2006. The impact of high-magnification evaluation of sperm on ART outcome. Human Reproduction 21, I59—I60.
Wong, C.C., Loewke, K.E., Bossert, N.L., Behr, B., de Jonge, C.J., Baer, T.M., Pera, R.A.R., 2010. Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. Nature Biotechnology 28, 1115—1121.
Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, 2007. Induced pluripotent stem cell lines derived from human somatic cells. Science 318, 1917.
Zini, A., 2011. Are sperm chromatin and DNA defects relevant in the clinic? Systems Biology in Reproductive Medicine, 78—85.
Zini, A., Sigman, M., 2009. Are tests of sperm DNA damage clinically useful? Pros and Cons. Journal of Andrology 30, 219—229.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 15 January 2012; refereed 11 March 2012; accepted 18 April 2012.