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SYMPOSIUM: FUTURES IN REPRODUCTION
REVIEW

Selecting the 'best' embryos: prospects for improvement

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Abstract This review considers why and how embryos are selected for transfer and with what consequences. It concludes that: (i) current selection methods are inadequate or at least inadequately subjected to evidential scrutiny; (ii) decisions about number of embryos should be based not solely on input (numbers transferred) but on the likelihood of the transfer resulting in multiple pregnancies — out turn; and (iii) what is needed are better methods not just for selecting better embryos, but also for selecting responsible clinicians who collude less with their patients' demands but advise them more responsibly.

KEYWORDS: embryo quality, evidence base, IVF, morphokinetics, multiple pregnancy, PGS, responsibility
VIDEO LINK: http://sms.cam.ac.uk/media/1400963

Introduction

In the attempt to select the 'best' embryo for transfer, clinicians need to address three factors: (i) Why are embryos selected? (ii) What outcome is hoped to achieve by this selection? and (iii) Are patients being served well by embryo selection?

In the days when IVF started, especially when either mild stimulation with clomiphene was used or natural cycles advocated, and when in-vitro development of embryos was suboptimal, there were few embryos available for transfer (Edwards and Steptoe, 1980; Trounson et al., 1981). In the USA, and later in other countries, where ovarian superovulation was the norm, many more oocytes were collected and embryos derived, prompting decisions as to which embryos to select for transfer and how many (Cohen et al., 2005). The 'surplus' also led to the first steps in embryo cryopreservation (Trounson and Mohr, 1983).

It had been clear for some time, and was demonstrated recently in a statistical analysis of over 400,000 cycles in

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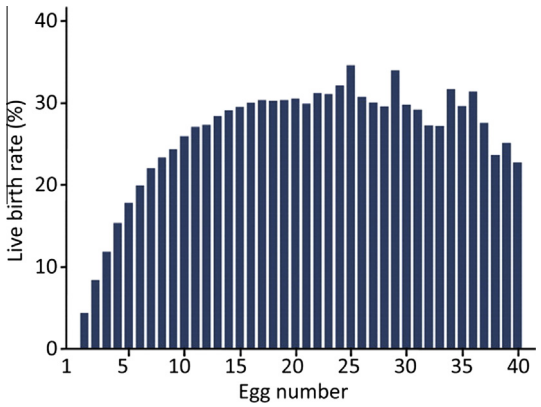

Figure 1 Association between live birth rate and number of eggs retrieved per cycle. Graph compiled from 400,135 cycles from the HFEA database for IVF cycles performed in the UK from 1991 to 2008. Reproduced from Sunkara et al. (2011).

the Human Fertilisation and Embryology Authority database (Sunkara et al., 2011) that, since the quality of eggs is variable and the development of embryos similarly so, the chances of pregnancy increase significantly with increasing numbers of eggs retrieved (**Figure 1**). However, the returns from ovarian stimulation are limited; success begins to plateau around 15 eggs (**Figure 1**), and obtaining numbers beyond that may be detrimental (**Figure 2**). Furthermore, successful outcome is age related — as the woman becomes older, so the likelihood of a successful outcome will reduce (**Figure 2**; Sunkara et al., 2011), either because increasing the dose of gonadotrophins has no further effect or because the additional eggs that are obtained are less likely to be viable. Indeed, one problem today is that women are delaying attempts at childbearing (Bewley et al., 2005). Thus, the median age of women undergoing IVF in UK is around 36 and by that time more that 90% of the ovarian pool has been lost, and the chances of a live birth following IVF are severely compromised (**Figure 3**). Not surprisingly, these women are vulnerable to the marketing ploys of those who offer any alternative that might improve their already slender chances to avoid involuntary childlessness. These desperate women fall victim to a myriad of treatment variations despite the facts that there are no data as to their proven clinical efficacy such as to justify their ubiquitous use and, even worse, available evidence has failed to demonstrate benefit in their use. Examples of such alternatives are acupuncture, aspirin, sildenafil (Viagra), steroids, heparin, growth hormone, immune therapies including intravenous immunoglobulin and endometrial biopsy (Segev et al., 2010).

One anecdote illustrating this type of problem appeared in *Hello* magazine in December 2010 in which the treatment of a famous singer was recounted — then supported in various internet blogs:

> At 42 years old and on her sixth attempt at IVF with her 68 year old husband and assisted by acupuncture, the treatment finally worked. She was originally expecting triplets, which spontaneously reduced to twins, and were delivered prematurely by Caesarean section at just over 5lbs.

This example says everything about the modern practice of assisted reproduction: the expectations of the technology, especially by older woman, how clinicians collude with alternative treatments despite absent or negative data and how they transfer multiple embryos knowing full well that multiple birth is the single biggest risk to the health and welfare of children born after IVF due to the increased hazard of significant prematurity with twin and triplet pregnancy (Braude, 2006; Grady et al., 2012).

The fact that all clinicians, all nurses, all obstetrician gynaecologists are aware of the statistics, and most will have some first hand experience of their consequences, why is it still the case, as documented by the Practice Committee of the ASRM (**Figure 4**) that 50% of all children conceived by assisted reproduction in the USA are born in a multiple pregnancy? (ASRM, 2012). Clinicians recognize the problem but for various reasons fail to act responsibly. It is our duty to try to improve IVF outcome by improving efficacy and safety: efficacy for those women not yet pregnant and desperate to be so — through better embryo selection; and safety by avoiding complications in those who do

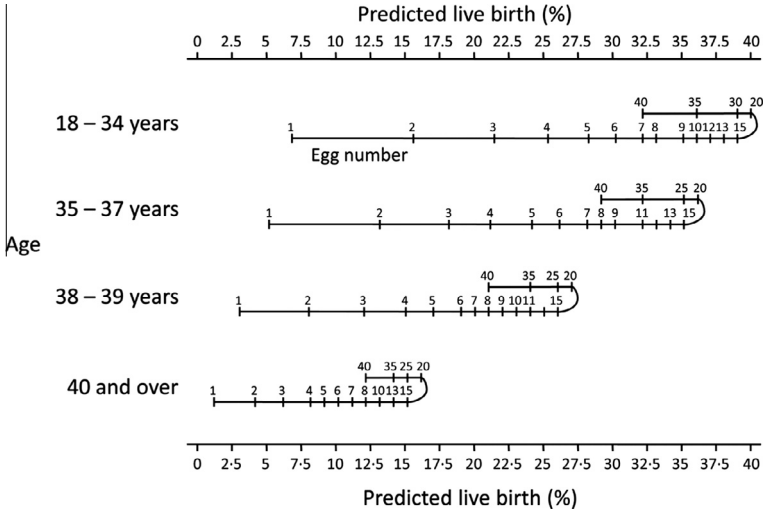

Figure 2 Nomogram to calculate predicted live birth probability given egg number and age. Reproduced from Sunkara et al. (2011).

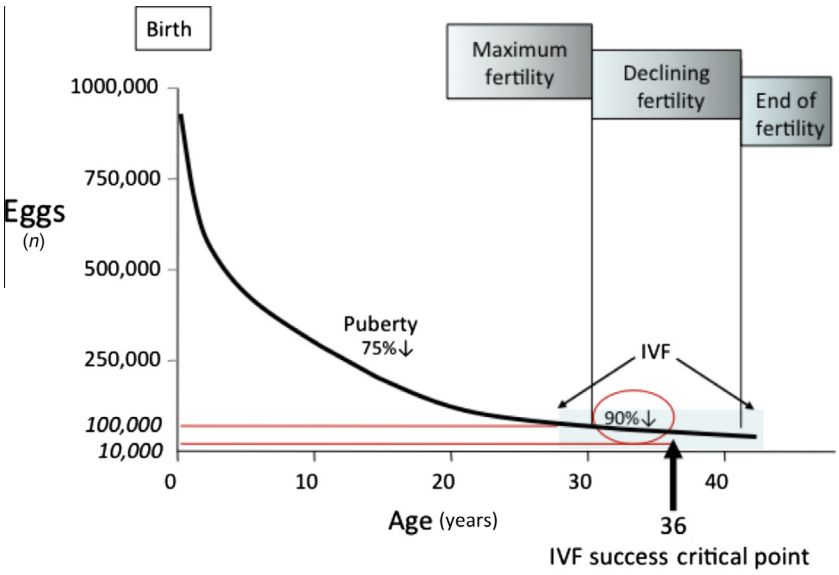

Figure 3 The decrease in number of eggs in ovaries with age, and times of likely fertility and most frequent use of IVF treatment. Reproduced with permission by Professor Scott Nelson, Glasgow.

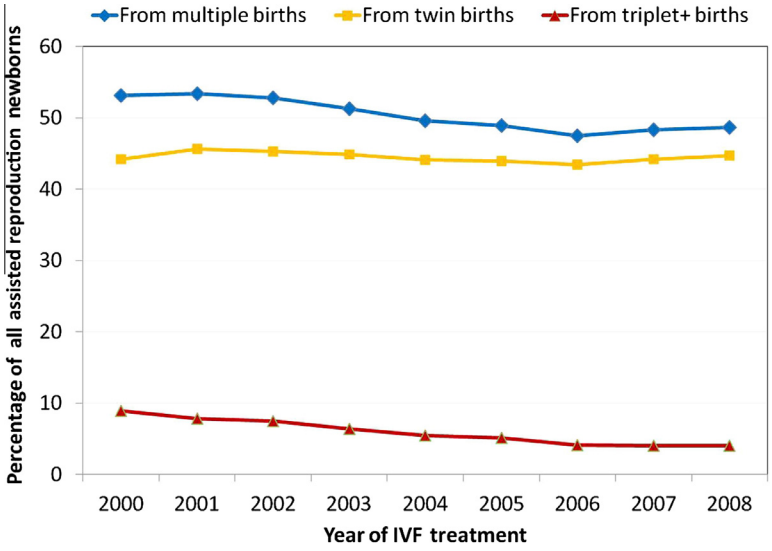

Figure 4 Proportion of all children resulting from assisted conception in the USA that were born in multiple births. Reproduced from ASRM and Practice Committee of SART and ASRM (2012).

become pregnant by encouraging use of single-embryo transfer. 'The ultimate of goal of IVF is to achieve healthy single live births following each single embryo transfer' (Cohen et al., 2012).

Factors that affect the ways in which embryos are selected

The stage at which embryos are transferred

The stage at which embryos are transferred has changed considerably over the years from pronucleate, zygote intra-Fallopian (ZIFT), the 2- or 4-cell stage (day 2), cleavage-stage (day 3), to most recently, blastocyst-stage transfer (day 5 or 6). The change in preference has been driven largely by improvements in embryology and, in the absence of any reliable endometrial data, the prevailing prejudice of clinicians about the 'best' time to transfer. While today most transfers take place on day 2 or 3, striking a balance between ease of embryo culture and perceived simplicity of staging, there appear to be some advantages to later-stage transfer, although there are some who have concerns.

The availability of video-morphokinetic data

Automated time-lapse examination of individually developing embryos is an emerging technique, which allows decision-making to straddle developmental stages (Aparicio et al., 2013). Here, added value is presumed by observing

and measuring real-time changes in the early cleavage process to predict which embryos are most likely to form well-structured blastocysts and hence to have enhanced implantation potential while maintaining transfer on day 3 (Dal Canto et al., 2012; Wong et al., 2010).

Although the derived models look promising (Campbell et al., 2013a,b), clinical efficacy is yet to be shown in prospective randomized trials. It is entirely possible that any purported advantage could be explained by the fact that development is maintained in a closed system with a tightly controlled environment, thereby avoiding the changes in light, pH and temperature that inevitably accompany intermittent microscopic examination of the embryo. Prospective studies to examine this hypothesis are lacking (Montag, 2013).

Nevertheless, this technique has provided important insights into early human development and the current arbitrary nature of embryo selection. It has identified that timing and synchrony of the first two cleavage divisions may be critical in the normal development of the human embryo and that the traditional reliance on a snapshot at a single stage of development is unreliable since cleavage is dynamic, fragments appear to come and go and the blastocyst routinely collapses and re-expands *in vitro*. This latter factor is particularly relevant since grading of blastocysts takes into account degrees of expansion (Gardner et al., 2000; Stephenson et al., 2006).

Blastocyst transfer

But why try and predict at cleavage stages which embryos are capable of making a blastocyst when this stage can be attained *in vitro* prior to transfer, thus removing all predictive guesswork, especially since it has been shown clearly that a single-blastocyst transfer is much more likely to result in a singleton live birth than does transfer of a single good-quality cleavage-stage embryo on day 3, and the pregnancy loss rate is lower (Papanikolaou et al., 2006). However, blastocyst culture and transfer is not universally accepted as safe: there may be hazards and disadvantages. Slow freezing of blastocysts may not be as effective as at the cleavage stage (Stehlik et al., 2005), and animal data have shown that prolonged in-vitro culture may lead to epigenetic effects, such as large calf syndrome (Mann and Denomme, 2013; Young et al., 1998).

Despite the improved implantation potential of the blastocyst, blastocyst transfer is still used inappropriately. Although use of blastocyst transfer is increasing, Human Fertilisation and Embryology Authority data reveal that, in the UK, so is the rate of double-blastocyst transfer (HFEA, 2011; **Figure 5**). Many have not yet chosen to accept the concept that although transfer of two blastocysts might produce a marginal improvement in the overall chances of initiating pregnancy (**Figure 6**), it undoubtedly will increase significantly the chances of a multiple pregnancy (Criniti et al., 2005; Gardner et al., 2000). Indeed, data that refer to a twin or multiple pregnancy rate belie the true clinical implication (**Figure 7**). For example, the clinical effect of a 42% twin rate is that nearly 60% of children conceived through assisted reproduction will be born in a multiple birth, a significant cohort of which will be born prematurely. Nevertheless guidance from the ASRM Practice Committee still condones the option of two blastocysts in patients over 34 years with an otherwise favourable prognosis (**Table 1**; ASRM, 2013). The problem seems not to be with selection, but in the actions that follow. What is needed are better methods not just for selecting better embryos, but also for selecting responsible clinicians.

The decision about number of embryos should be based not solely on input (numbers transferred) but on out turn — the likelihood of the actions resulting in multiple pregnancies. Replacement of multiple embryos within a poor-

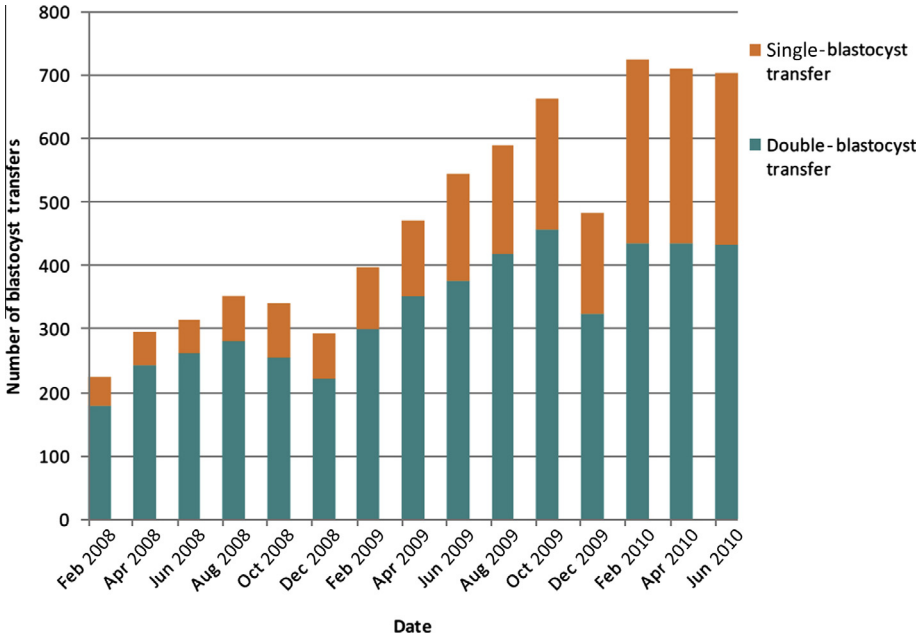

Figure 5 Proportion of fresh blastocysts which were used in single or double transfers (Jan 2008—June 2010). Data from HFEA (2011).

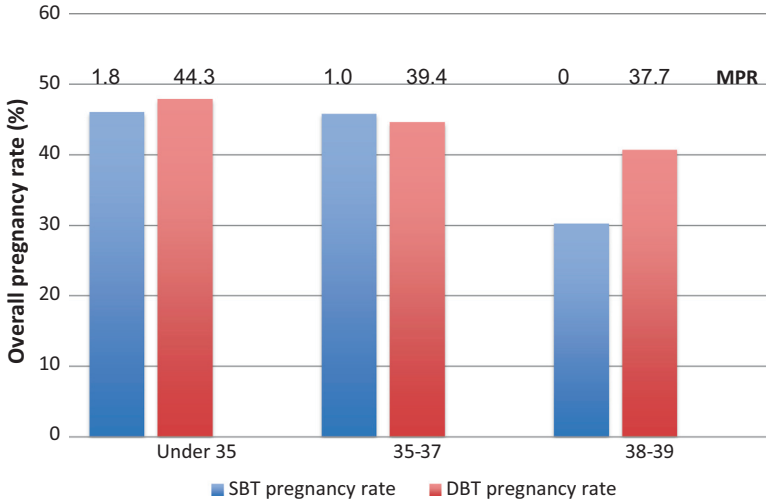

Figure 6 Multiple pregnancy rate (MPR) with double- (DBT) or single- (SBT) blastocyst transfer. Data from Table 3 in HFEA (2010).

Real meaning of a 42% twin rate

100 IVF deliveries

42 twin (84) + 58 singleton

= 142 babies

~60% (84/142) born in a multiple birth

47% (40/84) of these likely to be born premature

Figure 7 The effect of a 42% twin rate on the number of children born in multiple births.

quality ineffective IVF programme is much less likely to result in multiple births since each embryo will not have its full implantation potential realized. In an effective programme with a quality embryology laboratory and clinicians who are good at transfer, limiting the numbers of embryos to be transferred becomes crucial as each embryo has such good implantation potential. Moreover, in such programmes, cryopreservation is also likely to be effective and hence overall (cumulative) chances of pregnancy will be high. It is in these high-quality programmes that the risk of multiples is highest and therefore encouraging responsible attitudes is essential if the problem of multiple births from IVF is to be solved.

Effectiveness of cryopreservation

Poor results with slow freezing of blastocysts in some clinics is another factor that has hampered the move to single-blastocyst transfer; the preference being to adhere to cleavage-stage freezing and hence to transfer more than one cleavage-stage embryo at a time. While some clinics can achieve 85–90% survival of slow-frozen blastocysts, it appears that vitrification is more universally applicable with high success at warming (Youssry et al., 2008). In a recent Chinese study (Feng et al., 2012), live birth rates following single-thawed embryo transfer were almost as good as fresh transfer of embryos from the same cycle (37% versus 43%)

Table 1 Maximum number of embryos to transfer by age, embryo stage and prognosis. Data from ASRM and Practice Committee of SART and ASRM (2013).

Prognosis	*Age (years)*			
	<35	*35–37*	*38–40*	*>40*
Cleavage-stage embryos (day 2 or 3)				
Favourable	1–2	2	3	5
All others	2	3	4	5
Blastocyst-stage embryos (day 5 or 6)				
Favourable	1	2	2	3
All others	2	2	3	3

The ASRM identifies the following characteristics as being associated with a 'more favourable prognosis': first IVF cycle; good embryo quality by morphology-grading criteria; excess embryos available for freezing; and having a previous successful IVF cycle.

accepting that the best-quality embryos would likely be transferred fresh. Thus single-embryo transfer and cryopreservation should be used in concert to improve cumulative success and enhance the likelihood of healthy singleton births.

Preimplantation genetic screening

The logic behind preimplantation genetic screening (PGS) is incontrovertible: if one could use genetic testing to remove from transfer those embryos that are clearly aneuploid and therefore will fail to implant, or will miscarry if they do, then live birth rates could be significantly improved. For this logic be effective: (i) the sample (biopsy) to be analysed must be representative of the whole embryo; (ii) a test result must be likely on each embryo; and (iii) the result must be of sufficient robustness and certainty to allow those embryos found to be abnormal to be excluded from transfer and discarded.

I have no wish to be labelled a Luddite, a term allegedly after Ned Ludd, one of a group of English textile artisans who, beginning in 1811 protested against, and smashed, new labour-saving machinery in the early industrial revolution, and now a derogatory term for one opposed to new technology. It is not new technology about which I am suspicious, but fashionable technology shown not to work.

In order for a new embryo selection technique to be adopted ethically, it must: (i) have clear and established indications; (ii) be repeatable and reliable in any reasonably competent professional hands; (iii) exhibit a low false-negative or false-positive rate; (iv) make a significant clinical difference in properly constructed trials; and (v) be cost effective from the perspective of the patient or healthcare provider (see also Evers, 2013). Sadly, PGS using fluorescence in-situ hybridization (FISH) falls at every one of these hurdles. The indications are imprecise, whether that be in the definition of advanced maternal age, repeated miscarriage (two or three or more) or what constitutes recurrent implantation failure (does this mean at the same or another clinic which might have been of lesser quality) (Zamora et al., 2011). Criticism by proponents was levelled at the authors of the first large randomized trial (Mastenbroek et al., 2007), that the operators did not know how to biopsy or how to do the test, hence their inability to demonstrate effectiveness (Cohen and Grifo, 2007). However, there are now over 12 randomized trials, all of which show either no benefit or adverse effect of cleavage-stage biopsy and FISH with such confidence that further studies seem unethical (Mastenbroek et al., 2008, 2011).

It's not about the technology; it's about the biology

One of the reasons for the failure of PGS as a tool to improve IVF success may be due to the frequency of false-positive results due to the ubiquity of chromosomal mosaicism in cleavage-stage embryos (Mertzanidou et al., 2013; Vanneste et al., 2009). What is still unclear is whether this is a normal feature of early human embryos or whether it is an artefact created by artificial stimulation and control of oocyte development or by in-vitro conditions. What is clear is that mosaicism can impact on the reliability of test results and may have important consequences for development and testing at the blastocyst stage, depending on how cell progeny is distributed thereafter.

An examination by Northrop et al. (2010) of 50 cleavage-stage embryos found to be aneuploid on cleavage-stage biopsy and FISH, then reanalysed at the blastocyst stage using microarrays to assess ploidy, revealed that 58% were euploid on retesting at the blastocyst stage. This result suggests that the cell removed was not representative, that the FISH diagnosis was incorrect or that some form of self-correction took place. Of the 21 embryos confirmed at the blastocyst stage to be aneuploid, less than half were consistent with the earlier biopsy result, whereas 12 (57%) showed an aneuploidy different from that originally diagnosed. Furthermore, each blastocyst was biopsied in three places on the trophectoderm and one in the inner cell mass. Surprisingly, even when the same abnormality as that found at earlier biopsy was found in one of the trophectoderm biopsies, it was not necessarily found in the others or in the inner cell mass.

The finding by Northrop et al. (2010) emphasize not only the vagaries of testing using FISH on few cells, but also the possible effect of compartmentalization of the aneuploidy found in early cleavage stages. The progeny of an abnormal cell within the cleavage-stage embryos could be uniformly distributed to inner cell mass and trophectoderm or alternatively could be restricted to one or other (Robberecht et al., 2010; **Figure 8**). In the latter case, a blastocyst-stage biopsy might not produce a result indicative of the overall genetic status of the developing embryo. Thus it is the biology, not necessarily the technology, that has so far led, or at least contributed to, the failure of PGS as a selection tool, and changing the technology to comparative genomic hybridization or single-nucleotide polymorphism arrays may not necessarily solve the problem. In addition, copy number variations and interchromosomal inconsistencies (Vanneste et al., 2009, 2012) now detected by these new techniques make interpretation more difficult and declaration of euploidy less reliable.

The question still to be answered is whether the test being offered to patients purportedly to improve their chance of success is robust enough to allow the discarding of some of their embryos, especially when at advanced maternal age they may have very few anyway. Seldom are the additional costs charged for these sorts of test benchmarked against the overall likelihood of success per cycle embarked upon, rather than whenever there are embryos for transfer. Responsible practice requires responsible clinicians and embryologists who are prepared to test their results in proper clinical trials as the minimum standard before offering PGS, a fact acknowledged by some teams trying to implement these new technologies:

> Despite the growing evidence that FISH-based aneuploidy screening does not work, the concept of improving outcomes by aneuploidy screening should not be considered invalid. However, development and validation of these new technologies should be held to a higher standard than FISH ... should include demonstration of preclinical accuracy, consistency and reliability. In addition, completion of randomized trials with class-I strength of evidence for clinical benefit, and experimentally demonstrating acceptable risk of clinical implementation

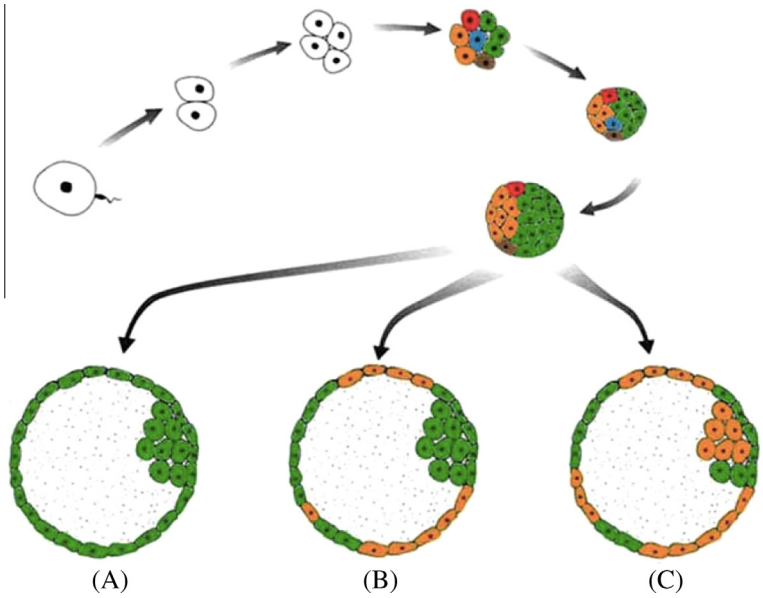

Figure 8 Origins of mosaicism within the blastocyst. The different colours at the 8-cell stage represent mosaicism of normal blastomeres (green) and blastomeres carrying mitotically derived aneuploidies and mitotic structural aberrations (orange, red, blue and brown). When the embryo reaches blastocyst stage, the aberrant cells can be lost by negative selection (A); they can segregate to the trophectoderm only, leading to confined placental mosaicism (B); or they can be found in both the inner cell mass and the trophectoderm resulting in an embryo that is affected in certain tissues (C). Reproduced from Robberecht et al. (2010).

> (i.e. a negligible impact of biopsy), should be required before any new aneuploidy screening technologies are offered as a clinical routine (Treff et al., 2010, p. 588).

Although there is not yet any clear evidence that biopsy at later stages and use of new technologies can or will improve outcome, they have helped in understanding of some of the mechanisms that might be operating throughout the PGS process. For example in the study by Scott et al. (2012b), two embryos were transferred, only one of which had been biopsied with the result unavailable at the time of transfer. Any implanting embryo could be identified at delivery by single-nucleotide polymorphism haplotyping back to the parents. The rate of miscarriage in the two groups (biopsied or not) was unaltered (similar). In one group the losses were due to, or attributed to, the diagnosed aneuploidy, whereas in the other group the cause was unknown. Thus, the expected reduction in miscarriage was not seen, accepting that failure of implantation (no pregnancy) could have biased the result. Nevertheless, in this study, the delivery rate of healthy children was much higher (133, 41%) in the group where a euploid embryo was confirmed than in those where an aneuploid embryo was known to be present (99, 4%). There was also a suggestion of a reduced implantation potential in the embryos biopsied at cleavage stage compared with those at the blastocyst stage. Whether this effect is real will have to await further properly randomized trials, but it does raise the question as to whether PGS results could have been affected by the process of biopsy itself, or whether a cell removed at the cleavage stage might be crucial for the further normal development of the embryo (Zernicka-Goetz, 2005).

As yet, there are no decent-size, properly controlled, truly randomized clinical trial results available for the newer modalities, although I believe that some are now being put in place. 'Potential benefit' in highly selected patients is not the same as demonstrated clinical efficacy — this was the problem with PGS using FISH which for 15 years stood as 'effective' in hundreds of published papers but was only recently debunked and has generally been abandoned as a technology for PGS (at least in the USA) following well-designed trials in Europe and Australia, despite vast numbers of patients having being subjected to it, especially in the USA.

Sadly, the implementation of these powerful new technologies has continued in the same vein as previously, where so-called 'trials' fail to be randomized properly, indications are muddled and even patient choice is included as an indication (Forman et al., 2012). Often only first transfers are reported, not the subsequent transfers nor the availability of cryopreserved embryos for this purpose; cumulative pregnancy rates per stimulation cycle would soon reveal the effect of discarding of some of the cohort due to the PGS, a fact seldom taken into account in presenting results to patients or in the literature. As Mastenbroek et al. argue in their rebuttal of embryo selection and PGS:

> In a scenario where all available embryos can be cryopreserved and transferred in subsequent cycles without impairment of pregnancy rates, or maybe even with improvement of pregnancy rates, no selection method will ever lead to improved live birth rates, as, by definition, the live birth rate per stimulated IVF cycle can never be improved when all embryos are serially transferred (Mastenbroek et al., 2011).

The mantra should be that now is the time for science and not for more marketing, for science is what we should do when we don't know what we are doing. Science and dis-

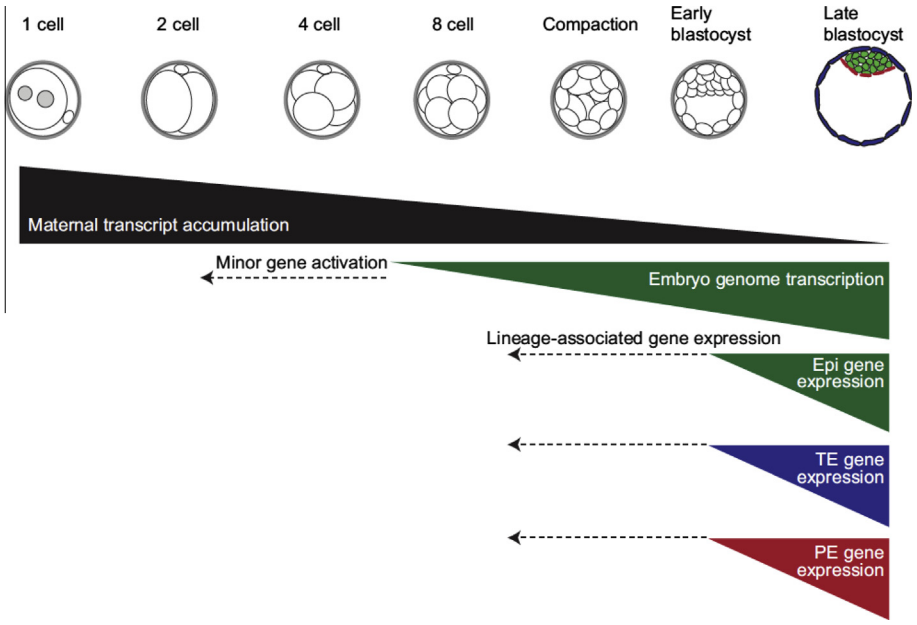

Figure 9 Genetic networks of human preimplantation development. Stages in development when genetic changes might influence outcome of preimplantation development. Maternal transcripts inherited from the oocyte are degraded through subsequent rounds of cell division. Human genome activation principally occurs between the 4- and 8-cell stages, and perhaps as early as the 2-cell stage. It is unclear when genes associated with the restriction of the TE or ICM cell lineage are expressed in human embryos, but data suggest that these lineage-associated genes are expressed in human embryos later than in mice at around the early blastocyst stage. Epi = epiblast; PE = primitive endoderm; TE = trophectoderm. Reproduced from Niakan et al. (2012).

covery are more important than earnings potential. Sadly, we have failed abysmally in applying many new fashionable modalities to reproductive technology and none so badly than with PGS. Unless we are responsible and methodical, it will take another 15 years before the full implication of offering PGS with new technologies is appreciated — we owe it to our patients to act in their interests and not play to their fears (Franklin, 2013; Daly and Bewley, 2013).

Application of science and embryo research

Indeed, the study of embryos is already revealing facts that may not have been appreciated before — that aneuploidy and mosaicism and segmental imbalances are common in human IVF embryos (Vanneste et al., 2012). The question to be asked is 'Why?' (Mantikou et al., 2012). Is it the technology that is causing a problem or is this a normal part of early human development only revealed by the close examination of the embryo *in vitro* for clinical purposes? Could it be an artefact created by what is done to eggs and embryos during ovarian stimulation and culture? (Mantikou et al., 2013). Perhaps mosaicism is the normal state of humans and is not a problem (Lucas et al., 2013). Eventually, the science will need to be performed by experiments on embryos at earlier stages of development — before and during activation of gene expression — and under varied culture conditions (Niakan et al., 2012; Zernicka-Goetz et al., 2009; **Figure 9**).

However, it is increasingly difficult to justify the use of patients' embryos at early stages. When Bob Edwards and indeed my own group were researching these early stages, blastocyst culture was awful (about 15% of embryos made it to that stage; Bolton et al., 1989) and cryopreservation was intolerably inefficient. Today, every patient should have the opportunity to have all their embryos cultured to day 5 or 6 to see if they can make it to the blastocyst stage to have a single embryo transferred and/or cryopreservation for a subsequent single-embryo transfer should the first be unsuccessful. There is no longer any excuse for sequestering patients' embryos for research and doing so is considered unethical (Scott et al., 2012a). But there are other ways that this important research can be promoted ethically. Some women who are now freezing their eggs as insurance that they may be too old to conceive when they do meet 'Mr Right' (Lockwood, 2011) may well end up forming successful relationships and having families without the need for these stored eggs. These eggs could be donated for this type of important research. Later stages may be more easily accessible ethically (Stephenson et al., 2009): with increasing success of IVF, many women will have completed their families but have surplus embryos cryopreserved. Although donation of such embryos to others is possible, many women are understandably uncomfortable with this option but may be willing to donate them for bone-fide research projects (Franklin et al., 2008).

A scientific approach to understanding development by study of early embryos without compromising patient treatment was the cornerstone of Bob's efforts and something that we should be proud to follow.

References

Aparicio, B., Cruz, M., Meseguer, M., 2013. Is morphokinetic analysis the answer?. Reprod. BioMed. Online 27, 654–663.

ASRM, Practice Committee of SART and ASRM, 2012. Elective single-embryo transfer. Fertil. Steril. 97, 835–842.
ASRM, Practice Committee of SART and ASRM, 2013. Criteria for number of embryos to transfer: a committee opinion. Fertil. Steril. 99, 44–46.
Bewley, S., Davies, M., Braude, P., 2005. Which career first? BMJ 331, 588–589.
Bolton, V.N., Hawes, S.M., Taylor, C.T., Parsons, J.H., 1989. Development of spare human preimplantation embryos in vitro: an analysis of the correlations among gross morphology, cleavage rates, and development to the blastocyst. J In Vitro Fert Embryo Transf 6, 30–35.
Braude, P., 2006. One Child at a Time: Reducing Multiple Births after IVF. HFEA.
Campbell, A., Fishel, S., Bowman, N., Duffy, S., Sedler, M., Hickman, C.F.L., 2013a. Modelling a risk classification of aneuploidy in human embryos using non-invasive morphokinetics. Reprod. Biomed. Online 26, 477–485.
Campbell, A., Fishel, S., Bowman, N., Duffy, S., Sedler, M., Thornton, S., 2013b. Retrospective analysis of outcomes after IVF using an aneuploidy risk model derived from time-lapse imaging without PGS. Reprod. Biomed. Online 27, 140–146.
Cohen, J., Grifo, J.A., 2007. Multicentre trial of preimplantation genetic screening reported in the New England Journal of Medicine: an in-depth look at the findings. Reprod. Biomed. Online 15, 365–366.
Cohen, J., Trounson, A., Dawson, K., Jones, H., Hazekamp, J., Nygren, K.-G., Hamberger, L., 2005. The early days of IVF outside the UK. Hum. Reprod. Update 11, 439–460.
Cohen, J., Alikani, M., Bisignano, A., 2012. Past performance of assisted reproduction technologies as a model to predict future progress: a proposed addendum to Moore's law. Reprod. Biomed. Online 25, 585–590.
Criniti, A., Thyer, A., Chow, G., Lin, P., Klein, N., Soules, M., 2005. Elective single blastocyst transfer reduces twin rates without compromising pregnancy rates. Fertil. Steril. 84, 1613–1619.
Dal Canto, M., Coticchio, G., Mignini Renzini, M., De Ponti, E., Novara, P.V., Brambillasca, F., Comi, R., Fadini, R., 2012. Cleavage kinetics analysis of human embryos predicts development to blastocyst and implantation. Reprod. Biomed. Online 25, 474–480.
Daly, I., Bewley, S., 2013. Reproductive ageing and conflicting clocks: King Midas' touch. Reprod. Biomed. Online 27, 722–732.
Edwards, R.G., Steptoe, P.C., 1980. A matter of Life: The Story of a Medical Breakthrough. Hutchinson, London.
Evers, H., 2013. The wobbly evidence base of reproductive medicine. Reprod. Biomed. Online 27, 742–746.
Feng, G., Zhang, B., Zhou, H., Shu, J., Gan, X., Wu, F., Deng, X., 2012. Comparable clinical outcomes and live births after single vitrified–warmed and fresh blastocyst transfer. Reprod. Biomed. Online 25, 466–473.
Forman, E.J., Tao, X., Ferry, K.M., Taylor, D., Treff, N.R., Scott Jr., R.T., 2012. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. Hum. Reprod. 27, 1217–1222.
Franklin, S., 2013. Conception through a looking glass: the paradox of IVF. Reprod. Biomed. Online 27, 747–755.
Franklin, S.B., Hunt, C., Cornwell, G., Peddie, V., Desousa, P., Livie, M., Stephenson, E.L., Braude, P.R., 2008. HESCCO: development of good practice models for hES cell derivation. Regen. Med. 3, 105–116.
Gardner, D.K., Lane, M., Stevens, J., Schlenker, T., Schoolcraft, W.B., 2000. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil. Steril. 73, 1155–1158.
Grady, R., Alavi, N., Vale, R., Khandwala, M., McDonald, S.D., 2012. Elective single embryo transfer and perinatal outcomes: a systematic review and meta-analysis. Fertil. Steril. 97, 324–331.
HFEA, 2010. Overall Pregnancy Rates (per Blastocyst Transfer) and Multiple Pregnancy Rates from Blastocyst Transfers in Year 1 of the Policy January 2009–April 2010. Human Fertilisation and Embryology Authority, London, http://www.hfea.gov.uk/6193.html.
HFEA, 2011. Improving Outcomes for Fertility Patients: Multiple Births: A Statistical Report. Human Fertilisation and Embryology Authority, London.
Lockwood, G.M., 2011. Social egg freezing: the prospect of reproductive 'immortality' or a dangerous delusion?. Reprod. Biomed. Online 23, 334–340.
Lucas, E.S., Madhuri, S., Salker, M.S., Brosens, J.J., 2013. Uterine plasticity and reproductive fitness. Reprod. Biomed. Online 27, 506–514.
Mann, M.R.W., Denomme, M.M., 2013. Maternal control of genomic imprint maintenance. Reprod. Biomed. Online 27, 629–636.
Mantikou, E., Wong, K.M., Repping, S., Mastenbroek, S., 2012. Molecular origin of mitotic aneuploidies in preimplantation embryos. Biochim. Biophys. Acta 1822, 1921–1930.
Mantikou, E., Youssef, M.A., van Wely, M., van der Veen, F., Al-Inany, H.G., Repping, S., Mastenbroek, S., 2013. Embryo culture media and IVF/ICSI success rates: a systematic review. Hum. Reprod. Update 19, 210–220.
Mastenbroek, S., Twisk, M., Van Echten-Arends, J., Sikkema-Raddatz, B., Korevaar, J.C., Verhoeve, H.R., Vogel, N.E., Arts, E.G., de Vries, J.W., Bossuyt, P.M., Buys, C.H., Heineman, M.J., Repping, S., van der Veen, F., 2007. In vitro fertilization with preimplantation genetic screening. N. Engl. J. Med. 357, 9–17.
Mastenbroek, S., Scriven, P., Twisk, M., Viville, S., van der Veen, F., Repping, S., 2008. What next for preimplantation genetic screening? More randomized controlled trials needed? Hum. Reprod. 23, 2626–2628.
Mastenbroek, S., Twisk, M., van der Veen, F., Repping, S., 2011. Preimplantation genetic screening: a systematic review and meta-analysis of RCTs. Hum. Reprod. Update 17, 454–466.
Mertzanidou, A., Wilton, L., Cheng, J., Spits, C., Vanneste, E., Moreau, Y., Vermeesch, J., Sermon, K., 2013. Microarray analysis reveals abnormal chromosomal complements in over 70% of 14 normally developing human embryos. Hum. Reprod. 28, 256–264.
Montag, M., 2013. Morphokinetics and embryo aneuploidy: has time come or not yet? Reprod. Biomed. Online 26, 528–530.
Niakan, K.K., Han, J., Pedersen, R.A., Simon, C., Pera, R.A., 2012. Human pre-implantation embryo development. Development 139, 829–841.
Northrop, L.E., Treff, N.R., Levy, B., Scott Jr., R.T., 2010. 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. 16, 590–600.
Papanikolaou, E.G., Camus, M., Kolibianakis, E.M., van Landuyt, L., van Steirteghem, A., Devroey, P., 2006. In vitro fertilization with single blastocyst-stage versus single cleavage-stage embryos. N. Engl. J. Med. 354, 1139–1146.
Robberecht, C., Vanneste, E., Pexsters, A., D'hooghe, T., Voet, T., Vermeesch, J.R., 2010. Somatic genomic variations in early human prenatal development. Curr. Genomics 11, 397.
Scott, R., Williams, C., Ehrich, K., Farsides, B., 2012a. Donation of 'spare' fresh or frozen embryos to research: who decides that an embryo is 'spare' and how can we enhance the quality and protect the validity of consent? Med. Law Rev. 20, 255–303.
Scott Jr., R.T., Ferry, K., Su, J., Tao, X., Scott, K., Treff, N.R., 2012b. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a

prospective, blinded, nonselection study. Fertil. Steril. 97, 870—875.
Segev, Y., Carp, H., Auslender, R., Dirnfeld, M., 2010. Is there a place for adjuvant therapy in IVF? Obstet. Gynecol. Surv. 65, 260—272.
Stehlik, E., Stehlik, J., Katayama, K.P., Kuwayama, M., Jambor, V., Brohammer, R., Kato, O., 2005. Vitrification demonstrates significant improvement versus slow freezing of human blastocysts. Reprod. Biomed. Online 11, 53—57.
Stephenson, E.L., Braude, P.R., Mason, C., 2006. Proposal for a universal minimum information convention for the reporting on the derivation of human embryonic stem cell lines. Regen. Med. 1, 739—750.
Stephenson, E.L., Mason, C., Braude, P.R., 2009. Preimplantation genetic diagnosis as a source of human embryonic stem cells for disease research and drug discovery. BJOG 116, 158—165.
Sunkara, S.K., Rittenberg, V., Raine-Fenning, N., Bhattacharya, S., Zamora, J., Coomarasamy, A., 2011. Association between the number of eggs and live birth in IVF treatment: an analysis of 400135 treatment cycles. Hum. Reprod. 26, 1768—1774.
Treff, N.R., Levy, B., Su, J., Northrop, L.E., Tao, X., Scott, R.T., 2010. SNP microarray-based 24 chromosome aneuploidy screening is significantly more consistent than FISH. Mol. Hum. Reprod. 16, 583—589.
Trounson, A., Mohr, L., 1983. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. Nature 305, 707—709.
Trounson, A.O., Leeton, J.F., Wood, C., Webb, J., Wood, J., 1981. Pregnancies in humans by fertilization in vitro and embryo transfer in the controlled ovulatory cycle. Science 212, 681—682.
Vanneste, E., Voet, T., le Caignec, C., Ampe, M., Konings, P., Melotte, C., Debrock, S., Amyere, M., Vikkula, M., Schuit, F., Fryns, Verbeke, G., D'hooghe, T., Moreau, Y., Vermeesch, J.R., 2009. Chromosome instability is common in human cleavage-stage embryos. Nat. Med. 15, 577—583.
Vanneste, E., van der Aa, N., Voet, T., Vermeesch, J.R., 2012. Aneuploidy and copy number variation in early human development. Semin. Reprod. Med. 30, 302—308.
Wong, C.C., Loewke, K.E., Bossert, N.L., Behr, B., de Jonge, C.J., Baer, T.M., Reijo Pera, R.A., 2010. Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage. Nat. Biotechnol. 28, 1115—1121.
Young, L.E., Sinclair, K.D., Wilmut, I., 1998. Large offspring syndrome in cattle and sheep. Rev. Reprod. 3, 155—163.
Youssry, M., Ozmen, B., Zohni, K., Diedrich, K., Al-Hasani, S., 2008. Current aspects of blastocyst cryopreservation. Reprod. Biomed. Online 16, 311—320.
Zamora, S., Clavero, A., Gonzalvo, M.C., De Dios Luna Del Castillo, J., Roldan-Nofuentes, J.A., Mozas, J., Castilla, J.A., 2011. PGS-FISH in reproductive medicine and perspective directions for improvement: a systematic review. J. Assist. Reprod. Genet. 28, 747—757.
Zernicka-Goetz, M., 2005. Cleavage pattern and emerging asymmetry of the mouse embryo. Nat. Rev. Mol. Cell Biol. 6, 919—928.
Zernicka-Goetz, M., Morris, S.A., Bruce, A.W., 2009. Making a firm decision: multifaceted regulation of cell fate in the early mouse embryo. Nat. Rev. Genet. 10, 467—477.

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