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ORIGINAL ARTICLE *ESHRE pages*

The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting†

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BACKGROUND: Many variations in oocyte and embryo grading make inter-laboratory comparisons extremely difficult. This paper reports the proceedings of an international consensus meeting on oocyte and embryo morphology assessment.

METHODS: Background presentations about current practice were given.

RESULTS: The expert panel developed a set of consensus points to define the minimum criteria for oocyte and embryo morphology assessment.

CONCLUSIONS: It is expected that the definition of common terminology and standardization of laboratory practice related to embryo morphology assessment will result in more effective comparisons of treatment outcomes. This document is intended to be referenced as a global consensus to allow standardized reporting of the minimum data set required for the accurate description of embryo development.

Key words: embryo assessment / assisted conception / consensus meeting

Introduction

Although the advent of '-omics'-based technologies may ultimately enhance the non-invasive assessment of human embryos *in vitro*, there are still no routinely applicable techniques or analytical devices available. Hence, IVF clinics worldwide continue to select embryos for transfer based on their development rate and morphological features as assessed by light microscopy. However, the many variations in embryo grading schemes applied by different clinics make inter-clinic comparisons extremely difficult, if not impossible. Although national consensus schemes exist in some countries, e.g. Spain and the UK, these are relatively few. Having an international consensus on embryo assessment would also help to validate the use of embryo morphology as an end-point in clinical trials and other studies to assess new technologies and products in IVF, if it were shown to act as at least a partial surrogate for clinical pregnancy outcome—one example might be registration of new drugs for approval by the US FDA. Therefore, it has been suggested that if common primary end-points based on embryo quality could be defined and validated, it might be possible to develop and register new fertility products and technologies more readily. This is also an extremely important element of the continual drive to improve the safety and efficacy of clinical IVF treatments.

The Alpha Executive, and ESHRE Special Interest Group of Embryology, in response to suggestions and requests from members of both international societies concerning the need for international consensus in the morphological assessment of embryos, convened a 2-day workshop to address this need. The workshop was held on 26 and 27 February 2010 in Istanbul, Turkey. In order to realize an effective consensus, the meeting had to be sufficiently small to allow consensus to be reached, while at the same time involving enough recognized experts to support the credibility of the consensus. The ultimate goal of the workshop was to establish common criteria and terminology for grading oocytes, zygotes and embryos that would be amenable to routine application in any IVF laboratory.

This report presents the proceedings of this Expert Meeting, incorporating the text of the presentations as well as the consensus points developed.

Workshop presentations

ESHRE Embryology SIG Atlas project (presented by Cristina Magli)

It is recognized that embryology is the central reference point for all of the Special Interest Groups and Taskforces of ESHRE, and therefore

†List of participants are given in Appendix.

that there is a need for consensus in the way embryos are assessed and described. To work towards this consensus, an Atlas of Embryology was published in 2000 (Gianaroli *et al.*, 2000) using images of oocyte and embryo development submitted by members of the ESHRE Special Interest Group of Embryology.

The next step in this project will be to design an embryo scoring system that can be shared among all embryologists. Once this is achieved, the Atlas will be revised to provide photographic illustrations for each of the points of the scoring system. In this way, the scoring system will be a practical reference for all embryologists.

The current state of consensus

Spain: The ASEBIR consensus scheme (presented by Gloria Calderón)

ASEBIR is the Spanish society for every professional working in the IVF laboratory. Since embryo morphology is currently the most important factor for the prediction of pregnancy, ASEBIR agreed that a dynamic system of embryo scoring was required that included all stages from gamete to blastocyst. A consensus was reached for scoring, which was then tested in a multicentre trial of IVF laboratories across Spain, with each reporting the scores throughout embryo development, and outcomes, for 15 cycles. Overall, pregnancy rates were higher when Day-3, rather than Day-2, embryos were replaced (Torelló *et al.*, 2005).

Oocyte scoring

The factors that were included in the evaluation of oocyte quality were oocyte cytoplasmic dysmorphisms, extracytoplasmic dysmorphisms and the oocyte–corona–cumulus complex. It was concluded that extracytoplasmic anomalies were phenotypic deviations.

Zygote scoring

The morphological parameters for zygote scoring were polarization, the presence of a cytoplasmic halo, the number of pronuclei and pronuclear appearance. It was agreed that since the morphological features are related to the time post-fertilization, zygote scoring must be performed within a fixed time period post-insemination. The ASEBIR consensus was that if a zygote had one polar body and two pronuclei, it should be discarded, whereas if there were two polar bodies and one pronucleus, it was the individual laboratory's decision whether to follow development *in vitro*.

Cleavage-stage embryo scoring

It was agreed that embryos would be scored in four categories:

A = top quality
B = good quality (not for elective single embryo transfer)
C = impaired embryo quality
D = do not recommend to transfer (includes all multinucleated embryos).

Because the culture medium and culture system were recognized as having a significant impact on embryo morphology, they need to be taken into account when making these comparisons. Therefore, each laboratory was encouraged to develop their own descriptions for embryos in each of these categories, based on existing observations. The ASEBIR consensus scoring for embryos is presented in Table I.

Blastocyst scoring

It was agreed that embryos should be assessed on Day 4 for evidence of compaction, as this was a good prognosis for blastocyst

Table I ASEBIR embryo assessment criteria (for confirmation by individual laboratories, based on existing observations of implantation potential).

Grade	Day	Cell number	Fragmentation (%)	Symmetry	Multi-nucleation	Vacuoles	Zona Pellucida
A	2	4	<10[a]	Even	No	No	Normal
	3	4(d2) →7–8 (d3)	<10[a]	Even	No	No	Normal
B	2	2 or 5	<26[a]	Even	No	No	Normal
		4	11–25[a]	Even	No	No	Normal
	3	4(d2) →7–8 (d3)	11–25[a]	Even	No	No	Normal
		4(d2) → ≥9 (d3)	<26[a]	Even	No	No	Normal
C	2	2–6	26–35[a]	Uneven	No	Few	Abnormal[b]
		3[c] or 6	<35[a]	Uneven	No	Few	Abnormal[b]
	3	2, 4, 6 (d2) → >7(d3)	26–35[a]	Uneven	No	Few	Abnormal[b]
		6(d2) → >8 (d3)	<35[a]	Uneven	No	Few	Abnormal[b]
		2 or 4 (d2) → 6(d3)	<35[a]	Uneven	No	Few	Abnormal[b]
		3[c](d2) → >6 (d3)	<35[a]	Uneven	No	Few	Abnormal[b]
D	2	1 or >6	>35		Yes	Many	Abnormal
	3		>35	Even	Yes	Many	Abnormal
	3	1 or >6 (d2) → any number of cells (d3)	>35		Yes	Many	Abnormal
		Any number of cells (d2) → <6 (d3)	>35		Yes	Many	Abnormal
		(d2) → (d3), only one additional cell	>35		Yes	Many	Abnormal

[a]Large fragments (i.e. not dispersed throughout the embryo).
[b]Without assisted hatching.
[c]One large and two small blastomeres.

development. In addition, delayed blastocyst development (Days 7 or 8) was considered a poor prognosis for implantation.

UK: The UK/ACE grading scheme (presented by Daniel Brison)

In the UK, the Human Fertilisation and Embryology Authority (HFEA) aims to reduce the incidence of iatrogenic multiple pregnancy, with a goal of a 10% twin rate by 2012. It was recognized that elective single embryo transfer would be the most effective strategy to achieve this goal, and therefore that there had to be a way to identify those embryos most likely to implant and lead to the establishment of a viable pregnancy. However, it was also identified that the development of such a scheme would be complicated by the available data, as most of the published embryo grading studies were small and generally from single centres, and so were potentially subjective and likely to vary between operators and laboratories. As a result, the Association of Clinical Embryologists (ACE) and the British Fertility Society (BFS) developed and published practice guidelines that included embryo morphology assessment (Cutting *et al.*, 2008; Table II). For cleavage-stage embryos, this scheme utilizes a combination of blastomere number, blastomere size [graded from 1 to 4 (best)] after Hardarson *et al.*, 2001, and degree of fragmentation [graded from 1 (most) to 4 (least)] after van Royen *et al.*, 2003. For blastocysts, a three-part grading system is used, based on the one originally reported by Gardner and Schoolcraft (1999a,b), with modifications by Stephenson *et al.* (2007) as part of an international grading scheme for the derivation of human embryonic stem cell lines.

In introducing this scheme, ACE recognized the need for an External Quality Assurance system for training, and for ongoing quality assurance, in embryo morphology scoring. A pilot study in 2003 using still images was largely unsuccessful, and so a new scheme that uses video clips of embryos being rolled, and includes embryo grading for cleavage-stage embryos and blastocysts, is due to be launched during 2010. Because this scheme is web based, it will be available to all laboratories in the UK and internationally.

USA: The USA scheme (presented by Joe Conaghan)

There is no consensus on embryo morphology assessment in the USA and there is no requirement, legal or otherwise, to report information specific to any embryo to a government or other agency. However, practitioners of IVF are required to report outcome data to the Centers for Disease Control (CDC) under a bill passed in 1992. In practice, many facilities report their data voluntarily to the Society for Assisted Reproductive Technology (SART) which then forwards it to the CDC.

Although the federal bill does not require the collection of information on individual embryos, and the CDC only collect data on the number of embryos transferred in an IVF cycle, in recent years SART has developed a standardized embryo scoring system and implemented the collection of data from individual practices for IVF cycles completed after mid-2006. Under this voluntary reporting system, data have only been collected for embryos that were transferred in cycles that used the patients' own oocytes. In 2007, the latest year from which data collection has been completed, specific embryo data were reported for 32% of all embryo transfers carried out at SART member clinics in the USA.

While various scoring systems exist for both cleavage stage (Veeck, 1999) and blastocyst stage embryos (Dokras *et al.*, 1993; Gardner and Schoolcraft, 1999a,b; Veeck and Zaninovic, 2003; Balaban *et al.*, 2006), the SART approach was to develop a simple universal system for embryo assessment that could be easily applied. Firstly, the embryo stages were defined and a concise list developed (Table III). A simple grading system (good, fair, poor) was devised that could be applied to all embryos. For cleavage-stage embryos, it was decided to record fragmentation and symmetry using simple scales, and for blastocysts the morphology of the inner cell mass (ICM) and trophectoderm (TE) are graded in the same way as whole embryos.

During 2008 and 2009 there was much discussion on the use of the embryo assessment system, and in particular about making data reporting mandatory for SART member clinics. With no clear consensus from the membership, the council took the decision to make reporting of data for embryos transferred in fresh cycles without the use of donor oocytes mandatory for SART member clinics as of March 2010. The data that have already been submitted have been used in two studies to date. In the first, Vernon *et al.* (2009) showed that the embryo assessments correlated well with live birth rate for 70 000 transferred embryos, and suggested that these assessments would therefore be a good national standard for quality assurance. In the second, Racowsky *et al.* (2009) validated the collection of stage, fragmentation and symmetry data for Day-3 embryos as they were each correlated with live birth rate. More studies are expected as the database grows, but the system is now firmly in place and it is proving to be useful.

Table II BFS and ACE cleavage stage embryo grading system (after Cutting *et al.*, 2008).

Criterion	Grade	Description
Blastomere number		Presented as *nc* (where *n* = cell no)
Blastomere size	4	Regular, even division
	3	<20% difference (cell diameter)
	2	20–50% difference
	1	>50% difference
Fragmentation	4	<10% fragmentation by volume
	3	10–20%
	2	20–50%
	1	>50%

Assessing oocytes (Day 0)

The molecular and cellular anatomy of a cytoplasmic dysmorphism in the mature human oocyte: physiological implications for normal development (Jonathan van Blerkom)

It is largely recognized in clinical IVF that the developmental competence of the human embryo is directly influenced by the normality

Table III Embryo assessment criteria as defined by SART.

Grade	Cleavage stage: cell number (1 → >8)		Morula/blastocyst: early/expanded/hatching	
	Fragmentation (%)	Symmetry	Inner cell mass	Trophectoderm
Good	0 1–10%	Perfect	Good	Good
Fair	11–25%	Moderate asymmetry	Fair	Fair
Poor	>25%	Severe asymmetry	Poor	Poor
Unknown	Unknown	Unknown	Unknown	Unknown
Not entered	Not entered	Not entered	Not entered	Not entered

Grade applies to all embryos regardless of transfer day.

of nuclear (meiotic) and cytoplasmic maturation during the pre-ovulatory period. The detection of certain cytoplasmic irregularities or defects, first termed 'cytoplasmic dysmorphisms' by Van Blerkom and Henry (1992), have since been used to select oocytes for insemination or assess the relative developmental competence of early embryos. However, while certain so-called dysmorphic oocytes fail to fertilize by conventional IVF, they do so after ICSI and many appear to develop in an apparently normal manner during the early preimplantation stages. However, high frequencies of embryo demise prior to the blastocyst stage or during the first few weeks following transfer suggest the real possibility that inherent defects exist in the oocyte that can have adverse downstream developmental consequences.

Despite the recognition of ooplasmic features that may be associated with compromised potential, little is known about (i) their origins, (ii) if, when and how they may perturb normal development processes, and (iii) whether downstream effects could involve altered expression of critical molecular, regulatory or signaling pathways. An understanding of which defects are more apparent than real, and which could have important consequences for an individual conceived by IVF from a 'dysmorphic' oocyte, are fundamental issues both for purposes of oocyte and embryo selection, and for understanding the normal developmental biology of the latter stages of human oogenesis and early embryogenesis.

This presentation focused on a single dysmorphism, the aggregation of smooth-surfaced endoplasmic reticulum (sER) as a disc-like aggregate(s), and (i) how normal peri-fertilization activities that involve calcium signaling and mitochondrial bioenergetics are perturbed in these oocytes and (ii) why such perturbations can have both immediate and downstream developmental consequences. Although its occurrence is relatively rare in cohorts of oocytes produced by different regimens of controlled ovarian hyperstimulation, this particular dysmorphism has been a subject of experimental analysis because both published studies and anecdotal findings suggest that among all the actual (i.e. developmentally significant) dysmorphic phenotypes, sER aggregation may be associated with early fetal demise and in newborns, with certain imprinting disorders (e.g. Beckwith-Wiedemann Syndrome; Otsuki *et al.*, 2004).

The possible molecular 'connection' between fetal demise and imprinting disorders for this dysmorphism seems to be related to the level of intracellular calcium released upon activation, the so-called first calcium transient, which is significantly higher and of longer duration than in morphological normal siblings or counterparts. Abnormally elevated levels of intracellular calcium have been detected in every MII oocyte with this dysmorphic phenotype that we have examined to date ($n = 49$), and shortly after this initial calcium surge, levels of mitochondrial ATP synthesis are at least two to three times higher than normal, but slowly return to normal levels over a 20-h period. Time-lapse imaging during the post-activation period show unusually robust cytoplasmic activity that abruptly ceases, including the rapid movement of the sER disc within the ooplasm. These findings were discussed in the context of the developmental abnormalities that occur during organogenesis but not during preimplantation embryogenesis that in other mammals are associated with experimentally elevating intracellular calcium levels at oocyte activation.

Assessing and grading oocytes (Thomas Ebner)

Controlled ovarian hyperstimulation recruits both good quality oocytes and gametes that would never become mature without the use of external hormones. Consequently, embryologists have to deal with oocytes of different qualities. This is mostly due to a desynchronization of nuclear and cytoplasmic maturation (Ebner *et al.*, 2006). In some cases, however, oocytes are 'over-mature' since they are aged *in vivo* or *in vitro* (Miao *et al.*, 2009). In case of 'immaturity' any impact of nuclear maturation could theoretically result in formation of a giant egg (diploid; Rosenbusch *et al.*, 2002) or in failure of meiotic spindle development (which is not visible at the light-microscope level). Changes in cytoplasmic maturation would consequently impair cytoplasm function. Therefore, any impact on further preimplantation development is closely correlated to the size and the number of anomalies. The only exception is the so-called clustering of the smooth endoplasmic reticulum (sER), which is the worst dysmorphism observable considering the reported consequences (Otsuki *et al.*, 2004; Ebner *et al.*, 2008; Akarsu *et al.*, 2009). To facilitate discussion amongst scientists, all anomalies should be subdivided into intracytoplasmic (incorporations, refractile bodies, dense central granulation, vacuoles, aggregation of sER) and extracytoplasmic dysmorphisms (*first polar body morphology, perivitelline space size and granularity*, discoloration, zona pellucida defects, shape anomalies). Some of these latter dysmorphisms (in italics) are indicators of oocyte aging. To conclude, two anomalies remain that should be handled with caution: (i) 'giant' oocytes because of their likely abnormal

genetic constitution and (ii) sER clusters because of their potentially lethal outcomes.

Assessing fertilization and zygotes (Day 1)

Assessing fertilization (James Catt)

Assessment of fertilization should be straightforward, as a fertilized oocyte should have two pronuclei and two polar bodies. However, this definition of fertilization is a snapshot from a continuum of events, as has been illustrated through time-lapse photography (Payne *et al.*, 1997). In the time course leading to the initiation of pronuclear formation, zygotes arising from IVF are observed to be ~1 h behind those arising from ICSI, provided that the spermatozoa used for IVF have been preincubated under conditions that support capacitation. Therefore, since fertilization is usually assessed 16–18 h post-insemination, this may not be the most appropriate time for assessment. In a clinical study, of 22 308 fertilized oocytes assessed at 17 ± 1 h post-insemination, 8% were already in syngamy—suggesting that it may be more appropriate to assess for fertilization sooner. Another confounding aspect of the definition of fertilization is the requirement for two polar bodies to be identified, as polar bodies can fragment and disintegrate before the fertilization check.

Assessing early cleavage (James Catt and Thorir Hardarson)

At present, the use of early cleavage/early syngamy in scoring regimens varies greatly between laboratories. As for all embryo assessments, the assessment of syngamy or time of first cleavage provides a snapshot of development within a continuum of events. Because of this, the morphology is subject to change over relatively short time periods, and so the time of assessment post-insemination must be standardized. An important aspect to consider is the difference between zygotes originating from ICSI or standard IVF, as ICSI bypasses several time-consuming processes in oocyte fertilization (Nagy *et al.*, 1998).

The assessment of syngamy is of potential value in laboratory quality control, as the proportion of zygotes in syngamy 24 h post-insemination is a very sensitive key performance indicator and a *post hoc* indicator for oocyte maturity (Lawler *et al.*, 2007).

The time of the first cell cleavage of the zygote has been shown to predict both embryo quality and implantation (Shoukir *et al.*, 1997; Sakkas *et al.*, 1998; Lundin *et al.*, 2001; Salumets *et al.*, 2003; Hammoud *et al.*, 2008). In addition, early-cleaving embryos have been reported to cleave more evenly which in turn has been strongly correlated with a lower incidence of chromosomal errors (Hardarson *et al.*, 2001). However, it should be cautioned that embryos with precocious development (cleavage earlier than 20 h post-insemination) have a poorer prognosis. The assessment of early cleavage can also be used to select against zygotes that cleave directly into three or more cells, which has been shown to be associated with chromosomal abnormality (Hardarson *et al.*, 2006).

In the future the more widespread use of time-lapse recordings of early human embryonic development may alter the way we consider many of the morphological parameters currently in use. In any case, time-lapse assessment would certainly provide a powerful tool with which to ascertain both cleavage rates and subtle morphological changes (Lemmen *et al.*, 2008).

Pronuclear morphology (Lynette Scott)

The positive predictive value of pronuclear scoring has been the subject of some debate, with some papers showing a prognostic effect (e.g. Scott and Smith, 1998; Tesarik and Greco, 1999; Scott *et al.*, 2000; Tesarik *et al.*, 2000; Balaban *et al.*, 2001; Nagy *et al.*, 2003; Scott 2003), while others identified a correlation with aneuploidy (e.g. Sadowy *et al.*, 1998; Gianaroli *et al.*, 2003; Edirisinghe *et al.*, 2005), and still others found no positive predictive value (Salumets *et al.*, 2001; James *et al.*, 2006; Weitzman *et al.*, 2010). However, in some countries legislation requires embryos for culture to be selected at the zygote stage, and pronuclear scoring allows the identification (and hence, elimination from culture) of embryos with limited implantation potential. In addition, abnormal gametes generally do not produce normal embryos, and the assessment of early embryo parameters (Days 1 and 2) can provide a *post hoc* indication of gamete quality. Later embryo development (Day 3 to Day 5) reflects gene expression, differentiation and developmental controls.

It is usual for the pronuclei to be of similar size, closely apposed, and centrally located in the fertilized oocyte. Pronuclear scoring takes into account the symmetry and alignment of the pronuclei, and involves the assessment of the number and relative position of the nucleolar precursor bodies (NPBs) which are established in the pronuclei. Ideally, there should be five to seven NPBs in each pronucleus, with similar distributions in each. Any inequality in number or distribution of the NPBs within the pronuclei is considered to be abnormal. For this reason, zygotes should be rolled as part of the scoring procedure, to ensure an optimal plane of observation.

Animal studies have indicated the importance of NPBs for normal embryo development. A lack of NPBs has been associated with imprinting errors in the mouse, and the delayed embryonic genome activation observed in nuclear transfer embryos has been attributed to the late onset of functional NPB and nucleoli formation (Svarcova *et al.*, 2009)

Assessing cleavage-stage embryos (Days 2 and 3)

Fragmentation (Kersti Lundin)

A fragment can be defined as an anuclear, membrane-bound extracellular cytoplasmic structure. The incidence of fragmentation is difficult to evaluate, as it is first necessary to differentiate fragments from cells, and then estimate the relative proportion of the embryo that is fragmented. Johansson *et al.* (2003) defined fragments as cells that were <45 μm in diameter for Day-2 embryos, and <40 μm in diameter for Day-3 embryos.

The impact of <10% fragmentation in Day-3 embryos on implantation rate has been found to be negligible (Van Royen *et al.*, 2001), and a trend was found between the level of fragmentation and the incidence of aneuploidy (Ziebe *et al.*, 2003; Munné, 2006). In another study, a review of 1273 single embryo transfers of 4-cell embryos at Sahlgrenska Hospital identified no difference in live birth rates up to 20% fragmentation (K. Lundin, unpublished data). However, the

same study found that embryos with 10–20% fragmentation and uneven cell sizes had the same live birth rate as embryos with >20% fragmentation—indicating that fragmentation should not be the only morphological criterion assessed.

Multinucleation (Thorir Hardarson)

A blastomere containing more than a single interphase nucleus is defined as being multinucleated. The presence of multinucleation is considered abnormal and has been reported in both *in vivo* (Hertig *et al.*, 1954) and, in particular, *in vitro* embryos (Tesarik *et al.*, 1987; Winston *et al.*, 1991; Pickering *et al.*, 1995). The reported multinucleation rates per treatment vary greatly. For example, Balakier and Cadesky (1997) reported that at least 44% patients had one or more embryo with multinucleation while both Jackson *et al.* (1998) and Van Royen *et al.* (2003) reported its occurrence in up to 87% of cycles with 31–33% of the embryos affected.

Factors that have been suggested to affect the rate of multinucleation include culture media (Winston *et al.*, 1991), and improper temperature control especially in relation to oocyte retrieval (Pickering *et al.*, 1990). Different mechanisms leading to multinucleated blastomeres have been suggested: (i) karyokinesis without cytokinesis; (ii) partial fragmentation of nuclei or (iii) defective migration of chromosomes at mitotic anaphase (Staessen and Van Steirteghem, 1998). Munné and Cohen (1993), using fluorescence *in situ* hybridization (FISH) demonstrated that all these mechanisms may be involved.

Multinucleation has been well documented to correlate with a high degree of chromosomal aberration (Kligman *et al.*, 1996; Hardarson *et al.*, 2001) as well as a higher degree of fragmentation and number of blastomeres on Days 2 and 3 (Van Royen *et al.*, 2003). Multinucleation has also been associated with uneven cell size (Hardarson *et al.*, 2001). Replacement of embryos with multinucleated blastomeres has been shown to lead to lower implantation, pregnancy and birth rates (Jackson *et al.*, 1998; Pelinck *et al.*, 1998; Hardarson *et al.*, 2001; Van Royen *et al.*, 2003).

The use of multinucleated blastomere scoring is widespread, although there may be differences in the evaluation criteria between different laboratories, depending on a number of factors, including the availability of extended culture to blastocyst.

Cleavage (Thorir Hardarson)

Uneven cleavage

Uneven cellular cleavage is commonly found in human embryos *in vitro*. Several studies have identified the phenomenon of uneven cleavage leading to unequal cell size, the first being Puissant *et al.*, 1987 who defined an uneven embryo as one in which the blastomeres had >1/3 difference in size (Puissant *et al.*, 1987). Later, the existence of uneven cleavage and its negative impact on pregnancy outcome was confirmed by several authors (Giorgetti *et al.*, 1995; Ziebe *et al.*, 1997; Hardarson *et al.*, 2001).

Genetic analysis of the blastomeres resulting from uneven cleavage has been correlated with multinucleation and a higher degree of chromosomal aberration (Hardarson *et al.*, 2001). This impairment may also be due to uneven distribution of proteins, mRNA, mitochondria and furthermore may possibly disturb the polarized allocation of certain proteins and genes in both oocytes and embryos (Antczak and van Blerkom, 1999).

Cleavage rate

The single most important indicator of embryo viability is the occurrence of cellular division. Numerous authors have reported that too slow or too fast embryo cleavage rate has a negative impact on implantation rate (Edwards *et al.*, 1980; Giorgetti *et al.*, 1995; Ziebe *et al.*, 1997; Van Royen *et al.*, 1999).

A correlation between 'normal cell number' and chromosomal constitution has also been reported (Almeida and Bolton, 1996; Magli *et al.*, 2007).

Morphology and chromosome abnormalities (Santiago Munné)

Cleavage stage

A number of studies have assessed the correlation between embryo morphology and chromosome abnormalities (reviewed by Munné *et al.*, 2007). Most dysmorphisms (fragmentation, multinucleation, asymmetry, etc.) tend to occur in the same embryos (Hardarson *et al.*, 2001; Van Royen *et al.*, 2003), and are associated with increased risk of post-meiotic abnormalities, such as mosaicism, monospermic polyploidy and haploidy.

The incidence of chromosome abnormalities has been reported to increase from 50 to 60% in non-fragmented embryos to 70–90% in embryos with >35% fragmentation. However, while fragmentation was strongly correlated with mosaicism and other post-zygotic abnormalities, it was not correlated with aneuploidy (Munné and Cohen, 1998; Magli *et al.*, 2001; Munné *et al.*, 2007).

Several studies have analyzed multinucleated embryos, preferentially observing them at the 2-cell stage, and all reported high rates of chromosome abnormalities, ranging from 55 to 100%, depending on the number of chromosomes analyzed (reviewed by Munné, 2007).

Giant embryos develop from giant oocytes (>200 μm in diameter), and have invariably been found to be triploid or triploid mosaics (Munné *et al.*, 1994; Balakier *et al.*, 2002; Rosenbusch *et al.*, 2002). In contrast, elongated embryos have been found to have similar rates of chromosomal abnormalities as spherical embryos (Magli *et al.*, 2001).

Cleavage patterns of embryos from Days 1 to 3 are at least as important as morphological patterns in selecting embryos of high potential. 'Arrested' embryos are those that have not cleaved during a 24-h period. 'Slow' embryos have 6 or fewer cells on Day 3 (68 ± 1 h post-insemination), but have cleaved during the preceding 24-h period. 'Normal' embryos reach 7–9 cells by Day 3, with <15% fragmentation and no multinucleation, and have cleaved during the preceding 24 h. 'Accelerated' embryos have >9 cells by Day 3.

Several studies have each reported the chromosomal analysis of more than 500 embryos, and the pooled results from a total of 1255 embryos from two of these studies (Munné *et al.*, 1995; Márquez *et al.*, 2000) demonstrated a highly significant relationship between maternal age and aneuploidy ($P < 0.001$), and between decreasing developmental competence (from normal to arrested) and an increase in post-meiotic abnormalities ($P < 0.001$). Two larger studies, each including over 4000 embryos, confirmed that the incidence of chromosomal abnormalities was significantly higher

in arrested, slow-cleaving and accelerated embryos compared with normally developing embryos (Magli *et al*., 2007; Munné *et al*., 2007).

All of these studies found that aneuploidy did not lead to developmental arrest during the cleavage stage, probably because the embryonic genome is not yet fully active (Braude *et al*., 1988; Tesarik *et al*., 1988). Thus, aneuploidy does not increase with decreasing developmental potential and cannot be selected against through cleavage-stage morphology selection. In contrast, post-meiotic abnormalities increase with decreasing embryonic competence, probably as a synchronous effect of the same mechanism producing the zygotic dysmorphism (Silber *et al*., 2003). These post-meiotic chromosomal abnormalities are not affected by maternal age.

Blastocyst stage

Many studies have assessed the chromosome composition of surplus blastocysts (reviewed by Munné, 2007). The frequency of mosaicism detected by FISH is high, but the proportion of abnormal cells is no more than 30% on average, with the majority of abnormal cells being tetraploid (23–86% of all blastocysts), in addition to other abnormalities. No differences in the rate of mosaicism between the ICM and TE were reported by Evsikov and Verlinsky (1998).

While higher rates of chromosome abnormalities have been found in blastocysts developing from embryos that had poor Day 3 morphology (Bielanska *et al*., 2002; Hardarson *et al*., 2003), another study found that 65% of mosaic blastocysts had good morphology (Bielanska *et al*., 2005). Thus, extended culture is generally not an appropriate tool to screen against chromosomal abnormalities. Although early studies reported that fewer chromosomally abnormal embryos reached blastocyst stage (Magli *et al*., 2000; Sandalinas *et al*., 2001), later studies using different culture media have found little correlation between blastocyst morphology and chromosomal abnormalities, with all types of aneuploidies being detected at the blastocyst stage (Fragouli *et al*., 2010; Schoolcraft *et al*., 2010).

In summary, careful evaluation of embryo morphology will detect dysmorphic and arrested embryos, at least 50% of which are chromosomally abnormal, which should not be replaced if morphologically better embryos are available. However, this evaluation does not allow selection against aneuploidy, the incidence of which in normal embryos increases from 30% in women 35–39 years of age to >60% in women >40 (Munné *et al*., 2007). Culture to blastocyst stage eliminates more post-meiotic abnormalities, but not aneuploidy. The remainder of chromosomal abnormalities can only be identified through preimplantation genetic diagnosis.

Hierarchy of embryo morphology assessment (Dominique Royère)

While many parameters have been reported to correlate with embryo implantation or blastocyst development, few studies have focused on the interdependence of these parameters, and even fewer have aimed at determining a relative weight for these parameters to predict implantation or developmental potential. However, using this strategy, Sjöblom *et al*. (2006) identified five parameters (cytoplasmic appearance, pronuclei and nucleoli, cytoplasmic deficit and cell number) as well as the presence of multinucleated blastomeres at Day 2, which gave a strong correlation with implantation. Similarly, Scott *et al*. (2007) identified that a lack of pronuclear symmetry, unevenly sized blastomeres and multinucleation at Day 2 were consistently correlated with failure of implantation and fetal development, and that early parameters such as pronuclear morphology, number and ratio of NPBs per nucleus, and Day 2 morphology of cleaving embryos were strong positive predictors of implantation.

An alternative approach for the derivation of weighted parameters is the use of logistic regression to evaluate their interdependency. In a prospective study, Holte *et al*. (2007) assessed the outcome of 2266 double-embryo Day 2 transfers, and determined that an integrated morphology cleavage score that included cell number, equal blastomere size and the number of mononucleated blastomeres on Day 2 had a significant predictive value for implantation. In a study of the development of 4042 individually cultured embryos, Guerif *et al*. (2007) observed that cell number at Day 2 and the incidence of early cleavage were the most predictive parameters for good blastocyst quality, while combining all parameters (pronuclear morphology, early cleavage, cell number, and incidence of fragmentation) gave a relatively poor prediction of embryo viability. However, in the same study, Guerif *et al*. (2007) also observed that the Day 2 morphology was not correlated with implantation potential once an embryo had reached the blastocyst stage and had good morphology. Using a model that included cell number and embryo development scores, Rehman *et al*. (2007) also found that later stages of embryo development had higher sensitivity and specificity in the prediction of implantation. These observations suggest that there is an additional value in assessing blastocyst development for the prediction of embryo potential.

Thus, all of the parameters of embryo development *in vitro* need to be considered when developing an embryo classification and scoring system.

Assessing morulae and blastocysts (Days 4–6)

Historical overview of blastocyst assessment (David Gardner)

The significance of examining the embryo post-compaction is the ability to examine it after embryonic genome activation. Furthermore, the obvious benefit of looking at the blastocyst is the ability to examine both of the cell types. The extent to which the TE develops will reflect the embryo's ability to attach and implant in the endometrium, while development of the ICM is obviously crucial for the development of the fetus itself (Kovacic *et al*., 2004). There are numerous papers discussing the merits of blastocyst transfer and which patients will benefit from such a laboratory procedure (e.g. review by Gardner and Balaban, 2006).

It has been shown that there is a strong relationship between embryo cell number on Day 3 and blastocyst development (Langley *et al*., 2001). Clearly, this is of value in establishing algorithms for patient selection in specific cases, and establishes the need to quantify the stage of development at any given time.

The grading system proposed by Gardner and Schoolcraft (1999a,b) was an initial attempt by the team in Colorado to classify the extent of blastocoel development. The aim was to grade the size of the blastocysts quickly on a stereo microscope. It was felt that grading expansion

was important as production of the cavity requires both extensive energy utilization through the sodium/potassium ATPases on the basolateral membrane of the TE and formation of effective tight junctions between TE cells to form a barrier, and so expansion is therefore a reflection of embryo competence.

It was also clear that when an embryo had started to expand [i.e. for blastocysts graded as 3–6 (full blastocysts onwards)], it was then possible to assign independent scores to the ICM and the TE. This next step of the grading was designed to be performed on an inverted microscope. The use of the grades A, B and C was an attempt to make the system user-friendly in the first case. For ICM: grade A indicated a tightly packed ICM with many cells; grade B, a loosely grouped ICM with many cells and grade C, an ICM with very few cells. For TE: grade A indicated a TE with many cells forming a cohesive epithelium; grade B, a TE with few cells forming a loose epithelium and grade C, a TE with very few cells.

It was anticipated that the scoring system would then be modified and refined once the significance of the scores was understood. For example, one later study added a further letter, D, to imply the presence of Degenerative tissue (Veeck and Zaninovic, 2003), while another included ICM grades of D and E (Stephenson *et al*., 2007).

A retrospective analysis of 301 cycles in which two blastocysts were transferred showed a significant linear trend in implantation rate related to the number of top-scoring blastocysts transferred (Gardner *et al*., 2000). These data have since been confirmed in almost 1000 non-donor cases, and so it is recommended that AA blastocyst be transferred individually. However, it is also of note that even blastocysts with a low score implant at a relatively high rate compared with cleavage-stage embryos.

Consensus points

Following discussions related to each of the presentations, the following consensus points were developed. It should be noted that these are the first set of consensus recommendations for oocyte and embryo scoring, and will need to be reviewed at regular intervals. In addition, it should be understood that these consensus points represent the 'minimum standards' for oocyte and embryo morphology scoring and, as such, do not restrict laboratories from performing additional observations or including additional details per observation. In other words, while some laboratories will likely choose to perform additional evaluations of oocyte and embryo morphology, all laboratories performing ART should be able to provide the following information. It was noted that more frequent or prolonged observations of oocytes and embryos carries the risk (albeit small) of an impact on their developmental potential. Thus, practitioners must consider the cost versus benefit to making additional observations while ensuring that all observations be performed in a way that imposes minimal risk to embryo development.

Timing and reporting of observation of fertilized oocytes and embryos

It was agreed that standardized timing of observations is critical to the ability to compare results between different laboratories, and that this should be relative to the time of insemination (Table IV), and uniformly presented in assessment reports as hours post-insemination. Furthermore, it was noted that there is an inherent variability in timing of all biological processes and the times given reflect the times at which these events occur in the majority of patients/cases.

For embryos, it was noted that each observation has two parts, cell number/stage and grading. The consensus was that these must be reported separately, in association with the time post-insemination.

Oocyte Scoring

It was the consensus opinion that the optimal oocyte morphology is that of a spherical structure enclosed by a uniform zona pellucida, with a uniform translucent cytoplasm free of inclusions and a size-appropriate polar body. Furthermore, it was noted that oocytes undergo both nuclear and cytoplasmic maturation, and that these processes are neither the same nor necessarily even synchronous.

Cumulus-oocyte complex scoring

It was the consensus that, although at present there is little corroborated evidence to support a correlation with embryo developmental competence, cumulus–oocyte complex (COC) scoring provides an important tool for troubleshooting. This should be a binary score (0 or 1), with a 'good' COC (score of 1) defined as having expanded cumulus and a radiating corona.

Zona pellucida scoring

The panel could find no specific benefit to measuring zona thickness, as it was agreed that there is insufficient evidence for any effect on outcome. However, it was noted that there could be patient-specific effects, and so a note should be made of exceptional observations regarding the colour or thickness of the zona pellucida.

Perivitelline space

It was agreed that the presence of inclusions in the perivitelline space is anomalous. However, there was insufficient evidence to support any specific prognosis associated with this observation. Therefore, it was

Table IV Timing of observation of fertilized oocytes and embryos, and expected stage of development at each time point.

Type of observation	Timing (hours post-insemination)	Expected stage of development
Fertilization check	17 ± 1	Pronuclear stage
Syngamy check	23 ± 1	Expect 50% to be in syngamy (up to 20% may be at the 2-cell stage)
Early cleavage check	26 ± 1 h post-ICSI 28 ± 1 h post-IVF	2-cell stage
Day-2 embryo assessment	44 ± 1	4-cell stage
Day-3 embryo assessment	68 ± 1	8-cell stage
Day-4 embryo assessment	92 ± 2	Morula
Day-5 embryo assessment	116 ± 2	Blastocyst

ICSI, intracytoplasmic sperm injection.

the consensus that while the observation of inclusions should be noted, there is no requirement to count or measure them.

It was further agreed that a note of the perivitelline space should only be made if it is exceptionally large.

Polar body scoring

The presence or absence of the first polar body should be noted in the uninseminated oocyte, where possible (this may not be possible for oocytes that are inseminated via IVF, rather than ICSI).

The size of the polar body should only be noted if it is exceptionally large. It was the consensus that oocytes with an abnormally large polar body should not be inseminated, due to the risk of oocyte aneuploidy.

Cytoplasm scoring

The consensus was that homogeneous cytoplasm is expected, and that non-homogeneous cytoplasm is of unknown biological significance, and based on current evidence, may represent variability between oocytes rather than a 'dysmorphism' of developmental significance.

Further to this, it was agreed that 'granularity' of the cytoplasm is ill-defined, and distinctly different from clustering of organelles. Clustering is detectable by any form of microscopy, whereas 'granularity' is often only seen by modulation of the optical path in phase contrast microscopy. It was agreed that clustering is associated with lower implantation potential.

It was also agreed that sER disks are associated with the risk of a serious, significantly abnormal outcome (Otsuki *et al*., 2004). It is the strong recommendation of the Expert Panel that oocytes with this feature should not be inseminated. In addition, it was noted that the sibling oocytes should also be examined for the presence of sER disks, presenting either as a single disk or as a series of smaller plaques.

Vacuolization

It was agreed that a few small vacuoles (5–10 μm in diameter) that are fluid filled but transparent are unlikely to be of biological consequence. In contrast, large vacuoles (>14 μm in diameter) are associated with fertilization failure. In oocytes that are fertilized, those vacuoles that persist past syngamy can interfere with cleavage planes, resulting in a lower blastocyst rate.

Hence, the observation of large vacuoles in the oocyte should be noted.

Fertilization check

The optimal fertilized oocyte should be spherical, and have two polar bodies, with two centrally located, juxtaposed pronuclei that are even sized, with distinct membranes. The pronuclei should have equivalent numbers and size of NPBs that are ideally equatorially aligned at the region of membrane juxtaposition.

It was agreed that both pronuclear size and location should be assessed at fertilization check (Table IV). The consensus was that the following features of pronuclei are severely atypical: widely separated pronuclei; pronuclei of grossly different sizes; micronuclei. The presence of sER disks should be assessed as part of the fertilization check (if IVF, rather than ICSI was performed). Normally fertilized oocytes in which sER disks are observed should not be transferred.

The consensus was that at present, there is insufficient evidence to support a prognostic value for the observation of a peripheral cytoplasmic translucency in the fertilized oocyte (a 'halo').

Table V Consensus scoring system for pronuclei.

Category	Rating	Description
1	Symmetrical	Equivalent to Z1 and Z2
2	Non-symmetrical	Other arrangements, including peripherally sited pronuclei
3	Abnormal	Pronuclei with 0 or 1 NPB

NPB, nucleolar precursor body; Z, Z-score (Scott, 2003).

The decision to perform a second Day 1 assessment is at the discretion of the laboratory, and may be either a syngamy or an early cleavage assessment (Table IV). The purpose of the second assessment can be for either quality control (syngamy) or prognostic (early cleavage) reasons, which will define the assessment time selected.

Pronuclear scoring

It was agreed that pronuclear scoring is of value, as it can provide additional information to the fertilization check, and that both should be performed at the same time.

The consensus on pronuclear scoring was that there should be three categories: symmetrical; non-symmetrical; and abnormal (Table V). The abnormal category includes pronuclei with no NPBs (so-called 'ghost pronuclei'), and those with a single nucleolar precursor body ('bulls-eye pronuclei"), which have been associated with abnormal outcomes in animal models.

Cleavage-stage embryos

Assessment of cell number

The expected stages of development at each of the nominated time points post-insemination were agreed (Table IV).

The consensus was that, on average, embryos that have cleaved more slowly than the expected rate have a reduced implantation potential, and that embryos that have cleaved faster than the expected rate are likely to be abnormal and have a reduced implantation potential.

Therefore, the consensus was that the current expected observation for embryo development is 4 cells on Day 2 and 8 cells on Day 3, depending on the time elapsed post-insemination. It was noted, however, that this may change in the future, depending upon the culture media being used.

Fragmentation

A fragment was defined as an extracellular membrane-bound cytoplasmic structure that is <45 μm diameter in a Day-2 embryo and <40 μm diameter in a Day-3 embryo. The relative degrees of fragmentation were defined as: mild (<10%); moderate (10–25%) and severe (>25%). The percent values are based on the cell equivalents, so for a 4-cell embryo, 25% fragmentation would equate to one blastomere in volume.

The consensus was that a definition of the impact of fragment localization could not be included, as this can be a dynamic phenomenon, i.e. the fragments can move within the embryo.

Multinucleation

Multinucleation was defined as the presence of more than one nucleus in a blastomere, and includes micronuclei. The consensus was that multinucleation is associated with a decreased implantation potential, and that multinucleated embryos are associated with an increased level of chromosome abnormality and, as a consequence, increased risk of spontaneous abortion.

It was agreed that multinucleation assessment should be performed on Day 2 (i.e. 44 ± 1 h post-insemination), and that the observation of multinucleation in one cell is sufficient for the embryo to be considered to be multinucleated. Laboratories should record the incidence of multinucleation in each embryo, and ideally, the nucleation status of each blastomere in each Day-2 embryo. It was further agreed that multinucleation assessment on Day 3 would be complicated by the much smaller cell size, and therefore would be less reliable.

The grading scheme for multinucleation should be binary, noting its presence or absence.

Cell size

It was agreed that for embryos at the 2-, 4- and 8-cell stages, blastomeres should be even sized. For all other cell stages, one would expect a size difference in the cells, as the cleavage phase has not been completed.

The grading scheme for cell size should be binary, noting whether all cell sizes are stage appropriate.

Other morphological features of Day-2 and -3 embryos

Other morphological features, such as cytoplasmic granularity, membrane appearance and the presence of vacuoles, can also be scored as part of the morphological assessment of Day-2 and Day-3 embryos. It is important to understand that these features can vary between a patient's embryos and between patients.

It was the consensus that at this stage, there is no significant body of evidence to support a clear biological effect of these features on implantation potential. Therefore, more research is required to identify which, if any, of these features are correlated with (or indicative of) implantation potential.

It was also the consensus that for embryos with apparent spatial disorganization, i.e. those that do not have the expected three-dimensional arrangement of blastomeres, there is no conclusive evidence that they are abnormal. In addition, it was noted that while early compaction on Day 3 is atypical, this observation is of unknown biological significance.

Cleavage-stage embryo scoring system

It was the consensus opinion that an optimal Day-2 embryo (44 ± 1 h post-insemination) would have 4 equally sized mononucleated blastomeres in a three-dimensional tetrahedral arrangement, with <10% fragmentation. It was the consensus opinion that an optimal Day-3 embryo (68 ± 1 h post-insemination) would have 8 equally sized mononucleated blastomeres, with <10% fragmentation. The consensus scoring system for cleavage-stage embryos is presented in Table VI. The scoring format would be cell number, grade and reason for the grade (e.g. 4-cell, grade 2, fragmentation).

Table VI Consensus scoring system for cleavage-stage embryos (in addition to cell number).

Grade	Rating	Description
1	Good	• <10% fragmentation • Stage-specific cell size • No multinucleation
2	Fair	• 10–25% fragmentation • Stage-specific cell size for majority of cells • No evidence of multinucleation
3	Poor	• Severe fragmentation (>25%) • Cell size not stage specific • Evidence of multinucleation

Table VII Consensus scoring system for Day-4 embryos.

Grade	Rating	Description
1	Good	• Entered into a fourth round of cleavage. • Evidence of compaction that involves virtually all the embryo volume.
2	Fair	• Entered into a fourth round of cleavage. • Compaction involves the majority of the volume of the embryo
3	Poor	• Disproportionate compaction involving less than half of the embryo, with two or three cells remaining as discrete blastomeres

Day 4 assessment (Morula stage)

It was the consensus that an optimal embryo at this stage (92 ± 2 h; Table IV) would be compacted or compacting, and have entered into a fourth round of cleavage. Compaction should include virtually all the embryo volume.

It was noted that variations in Day-4 embryo morphology will include apparently excluded cells, the effect of which is unclear. The exception is that if more than half of the embryo is excluded, it was agreed that this is likely to be associated with a poor prognosis (Tao *et al.*, 2002).

The consensus scoring system for Day-4 embryos is presented in Table VII. This system shares some similarities with that proposed by Feil *et al.* (2008), although the consensus system uses three grades, rather than four. As for the cleavage-stage embryo scoring system, the reason for a fair or poor grade should also be included, to ensure that relevant information is not lost.

Day 5 assessment (Blastocyst stage)

It was the consensus that an optimal embryo at this developmental stage (116 ± 2 h; Table IV) will be a fully expanded through to hatched blastocyst with an ICM that is prominent, easily discernible and consisting of many cells, with the cells compacted and tightly adhered together, and with a TE that comprises many cells forming a cohesive epithelium. It was agreed that while the ICM has a high prognostic value for implantation and fetal development, a functional TE is also essential.

Table VIII Consensus scoring system for blastocysts.

	Grade	Rating	Description
Stage of development	1		Early
	2		Blastocyst
	3		Expanded
	4		Hatched/hatching
ICM	1	Good	Prominent, easily discernible, with many cells that are compacted and tightly adhered together
	2	Fair	Easily discernible, with many cells that are loosely grouped together
	3	Poor	Difficult to discern, with few cells
TE	1	Good	Many cells forming a cohesive epithelium
	2	Fair	Few cells forming a loose epithelium
	3	Poor	Very few cells

The scoring system for blastocysts is a combination of the stage of development, and of the grade of the ICM and of the TE (e.g. an expanded blastocyst with a good ICM and a fair TE would be scored as 312). It is a numerical interpretation of the Gardner scale (Gardner and Schoolcraft, 1999a,b).

Common variants with unknown significance include the presence of cytoplasmic 'strings' linking different cells and cell types, and the presence of cellular or acellular structures within the perivitelline space or the blastocoel cavity.

The consensus for a blastocyst scoring system was that there should be a combination of stage and score (Table VIII). It was agreed that 'hatching' is defined as the obvious emergence of the TE with enclosed blastocoel through a thinning zona pellucida. It was also agreed that hatching cannot be reliably assessed in embryos with an artificially breached zona pellucida (with the exception of the breach made during ICSI). For each of the developmental stages, it was agreed that the ICM and TE should be graded relative to the Gardner A–C scale, but that a grade of 1–3 (rather than A–C) should be given—with Grade 1 equivalent to Gardner A. The rationale for this change is to support the entry of scores into numeric databases and facilitate statistical analysis.

It was noted that if a blastocyst is collapsed at the time of assessment, it cannot be graded reliably. These blastocysts should be re-evaluated 1–2 h later, as regular cycles of collapse and re-expansion of blastocysts is normal.

Definition of a non-viable embryo

It was the consensus opinion that a non-viable embryo is an embryo in which development has been arrested for at least 24 h, or in which all the cells have degenerated or lysed.

Conclusion

It is hoped that these consensus points will form the common language for embryologists worldwide to describe oocyte and embryo morphology. It is understood that some laboratories will continue to score other facets of embryo morphology, and provided that this scoring does not alter the developmental trajectory, these enhancements may provide future prognostic indicators and should be encouraged. However, in the meantime, the use of a common minimum data set for descriptive scoring system in publications, along with reference to the new edition of the Atlas of Embryology, will enhance our understanding of the applicability of the findings to our day-to-day practice, and may lead to improved patient outcomes.

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Appendix

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