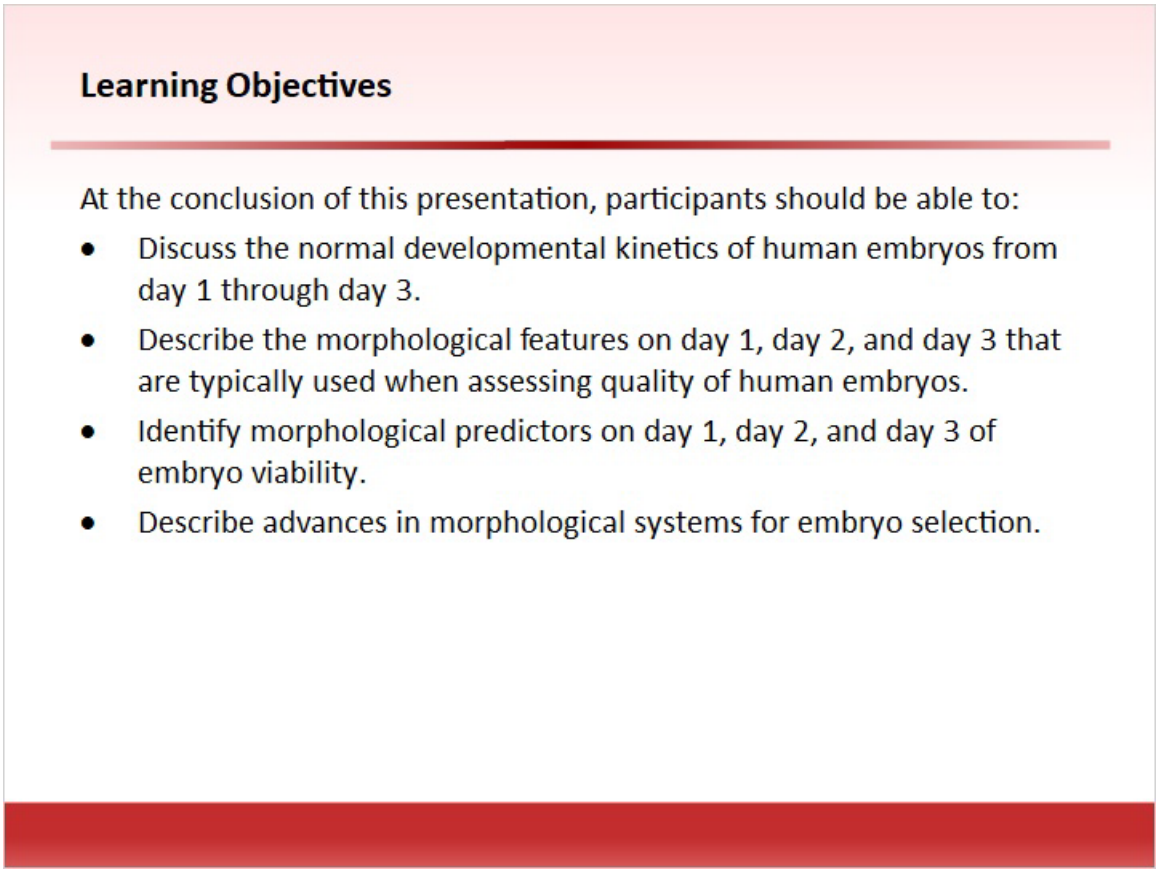
LABCC100 Lesson 20

1.1 Cleavage-Stage Embryo Grading

Notes:

Welcome to the American Society for Reproductive Medicine's eLearning modules. This module addresses the topic of cleavage-stage embryo grading.

1.2 Learning Objectives

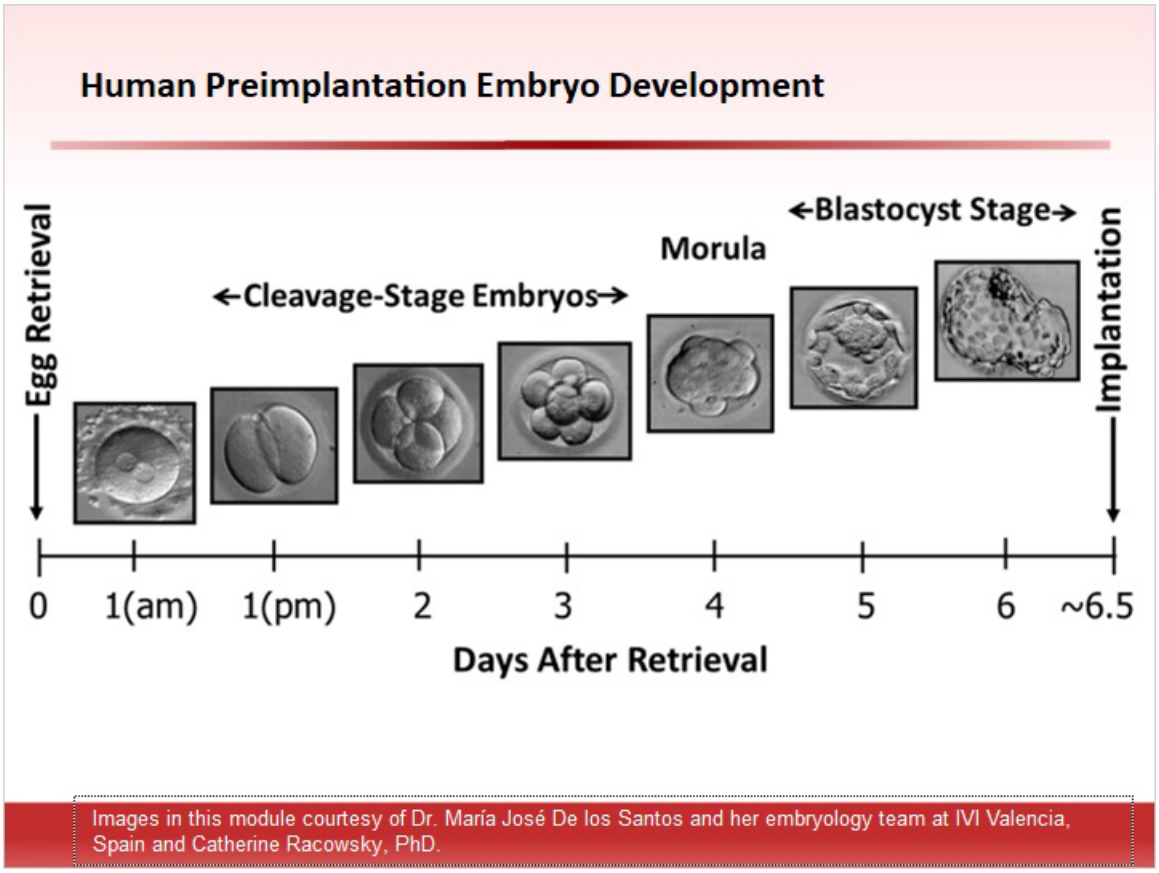


Notes:

At the conclusion of this presentation, participants should be able to:

- Discuss the normal developmental kinetics of human embryos from day 1 through day 3.
- Describe the morphological features on day 1, day 2, and day 3 that are typically used when assessing quality of human embryos.
- Identify morphological predictors on day 1, day 2, and day 3 of embryo viability.
- Describe advances in morphological systems for embryo selection.

1.3 Human Preimplantation Embryo Development

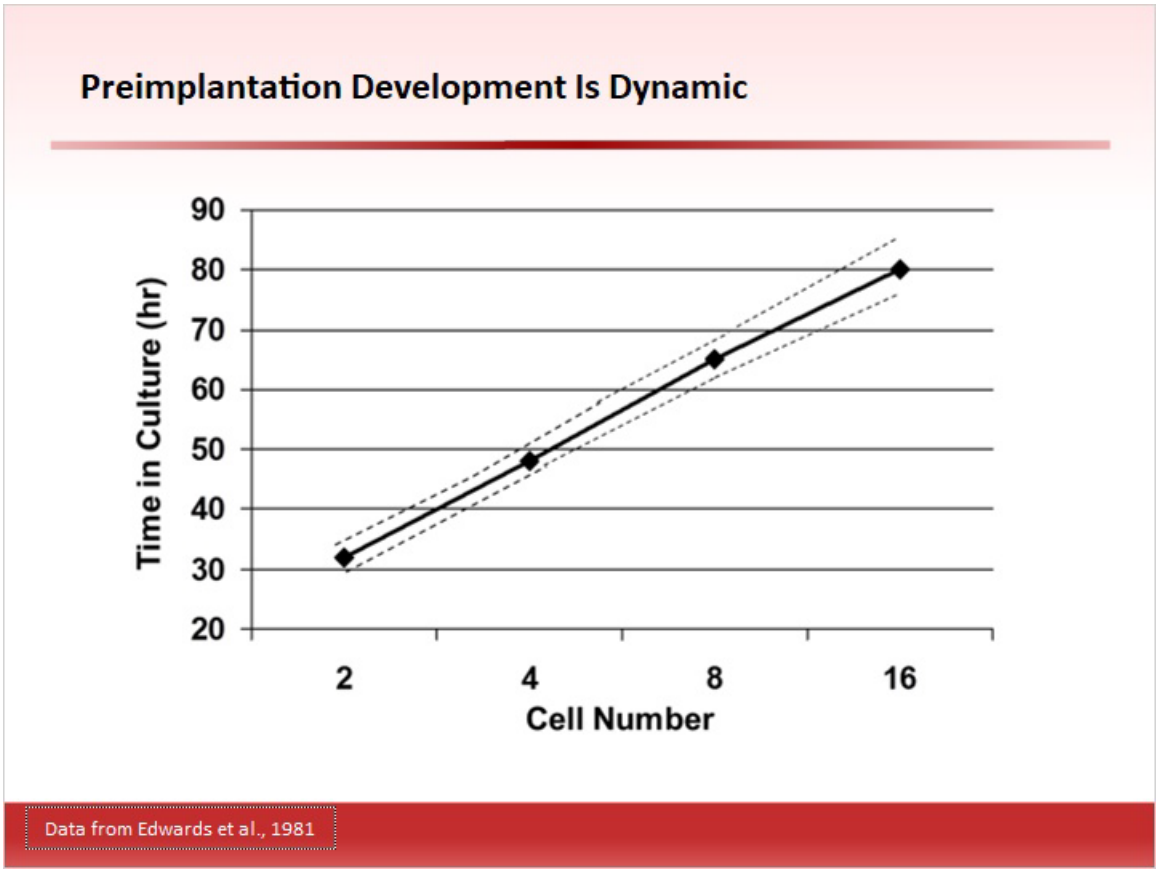


Notes:

Preimplantation development in the human begins after fertilization (day 1), when the fusion of the sperm and oocyte occurs leading to the transition of an oocyte to a zygote. A series of mitotic cell divisions on days 1, 2, and 3 lead to an embryo composed of 2, 4, and 8 cells, respectively. Following embryonic genome activation on day 3, the embryo continues to divide to 16 to 32 cells. This is followed by tight junction formation between the blastomere membranes, compaction, and formation of a morula on day 4. Small pockets of fluid then begin to accumulate between the blastomeres, which ultimately coalesce to form the blastocelic cavity and blastocyst formation on day 5. Subsequent divisions lead to distinct differentiation of the trophectoderm and inner cell mass lineages and ultimately a fully expanded blastocyst. The blastocyst then hatches from its zona pellucida, and the exposed trophoblast cells invade the endometrial epithelial cells to initiate implantation, which occurs on approximately day 6.5.

This module will summarize current knowledge regarding the earliest stages of human preimplantation development, occurring from days 1 through 3, and highlight how studies have provided key insights into morphological evaluations to improve embryo selection and enhance implantation rates.

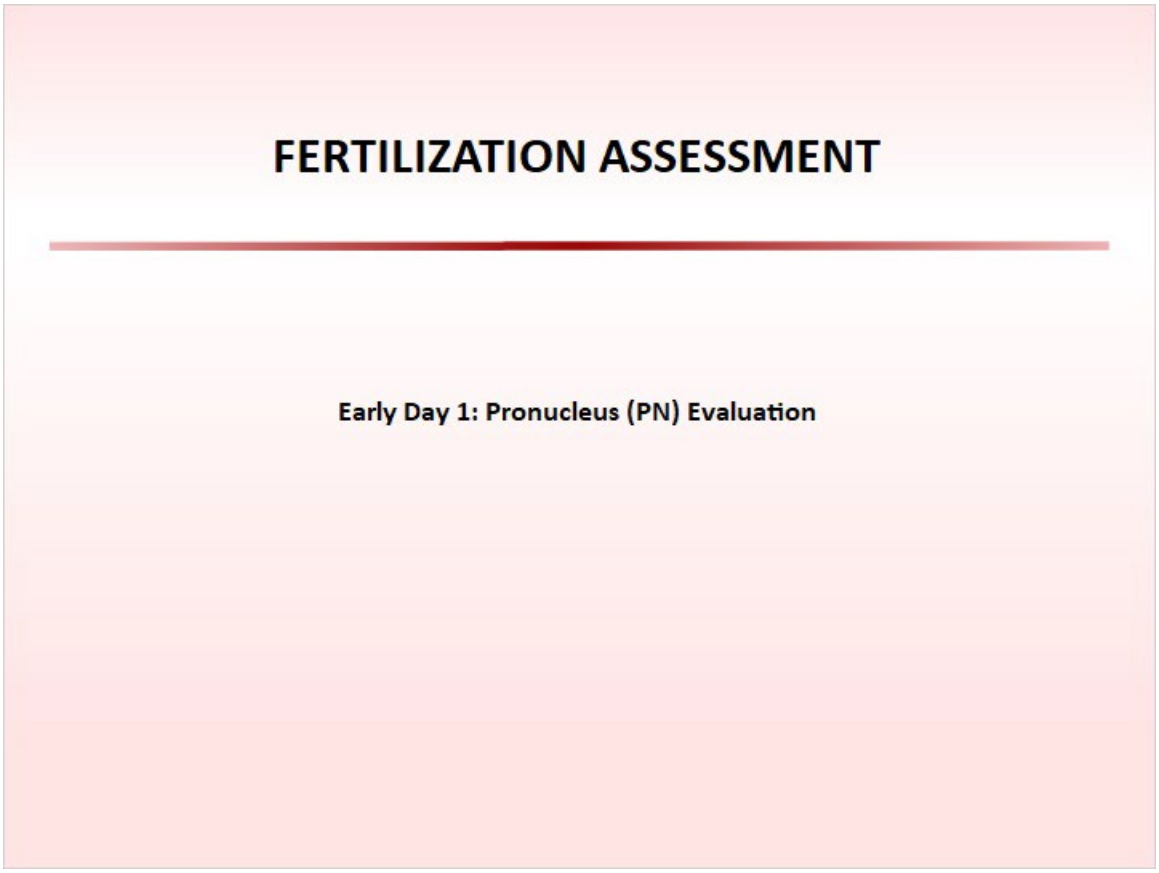
1.4 Preimplantation Development Is Dynamic



Notes:

Developmental kinetics through the first 3 mitotic divisions of the human embryo were first described by Sir Robert Edwards and his colleagues in 1981. This slide shows this timeline, which has stood the test of time.

1.5 FERTILIZATION ASSESSMENT

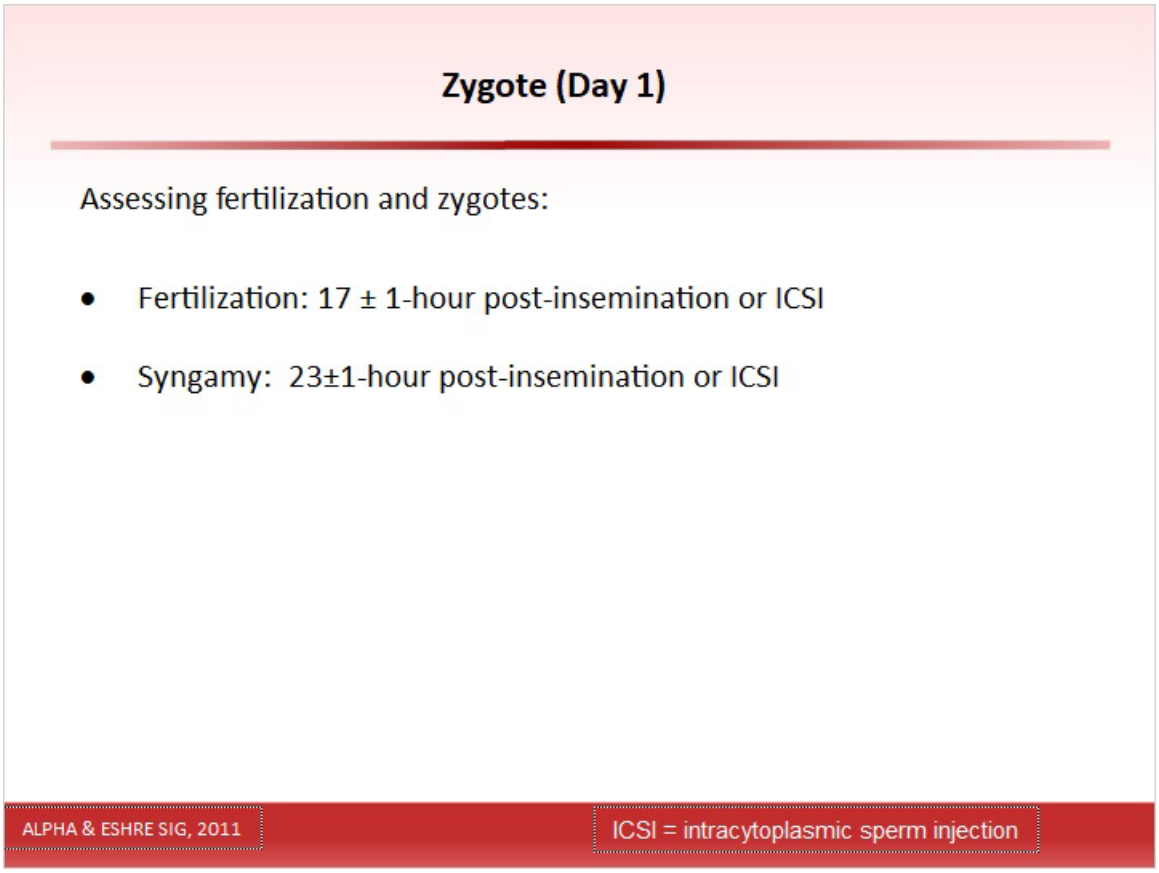


Notes:

Fertilization assessment consists of evaluating inseminated or injected oocytes for the presence of 2 pronuclei (2PNs). In normally fertilized oocytes, one of these pronuclei will contain the oocyte chromosomes, while the other (which is typically larger) will contain the sperm chromosomes. Evaluation of the pronuclear characteristics of human zygotes has been proposed as an indicator of developmental competence and chromosomal normality.

This section will discuss pronuclear assessment for detection of abnormal fertilizations, and consider the prognostic value of pronuclear disposition and 1-cell morphology when appraising embryo development.

1.6 Zygote (Day 1)



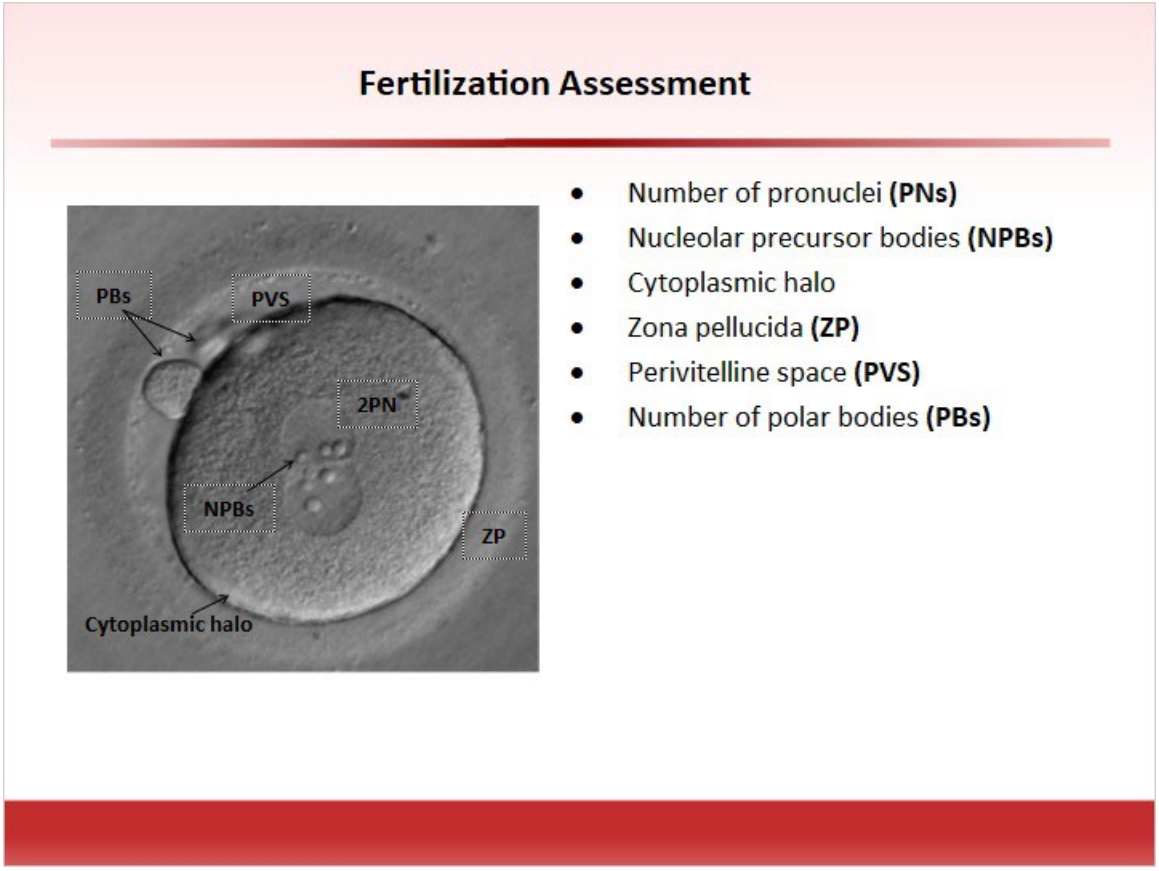
Notes:

Consideration of the timing of appearance of the 2 pronuclei is critical as their formation and subsequent disappearance occurs within a relatively tight time window; once the zygote disappears but before its entry into the first mitotic division, it is impossible to determine definitively whether fertilization has occurred and, if so, whether it was by only a single sperm. A large body of data shows that the optimum time for observing the pronuclei is 16-18 hours post-insemination or after intracytoplasmic sperm injection (ICSI). Performing the fertilization check by 18 hours reduces the risk that the pronuclei have become invisible as a result of syngamy (i.e., mingling of the sperm and oocyte DNA) and dissolution of their nuclear membranes. The typical timing for syngamy is 22-24 hours post-insemination or ICSI, but may occur earlier in some zygotes.

The initial steps for fertilization assessment are quite different for in vitro fertilization (IVF) versus ICSI oocytes. During standard IVF insemination, the cumulus cells are left surrounding the oocyte. While the sperm will normally have dispersed the expanded cumulus mass (by release of hyaluronidase, among other enzymes) during co-incubation, any residual corona/cumulus must be removed to facilitate accurate assessment of the number of pronuclei present. In contrast, fertilization assessment of ICSI oocytes does

not require removal of these follicular cells as this has been done in preparation for injection.

1.7 Fertilization Assessment

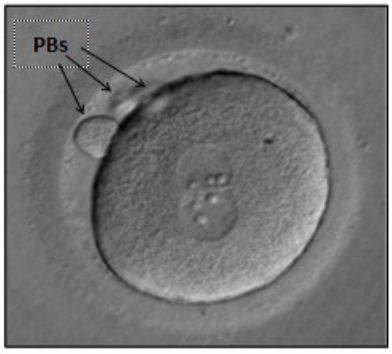


Notes:

Zygotes having 2PNs are considered "normally" fertilized. However, the presence of 2PNs does not tell us about the chromosomal complement (i.e., the ploidy) of the zygote, which can only be determined by genetic analysis. Besides counting the number of pronuclei, other gross morphological characteristics of the zygote that may be scored include the presence of a cytoplasm halo and the appearance of the zona pellucida and perivitelline space. However, there is not strong evidence that these additional features have value in predicting embryo quality.

1.8 Polar Bodies

Polar Bodies



3 PBs, 2 of which are out of the focal plane

- **Number**
  - 2 polar bodies (2PB)

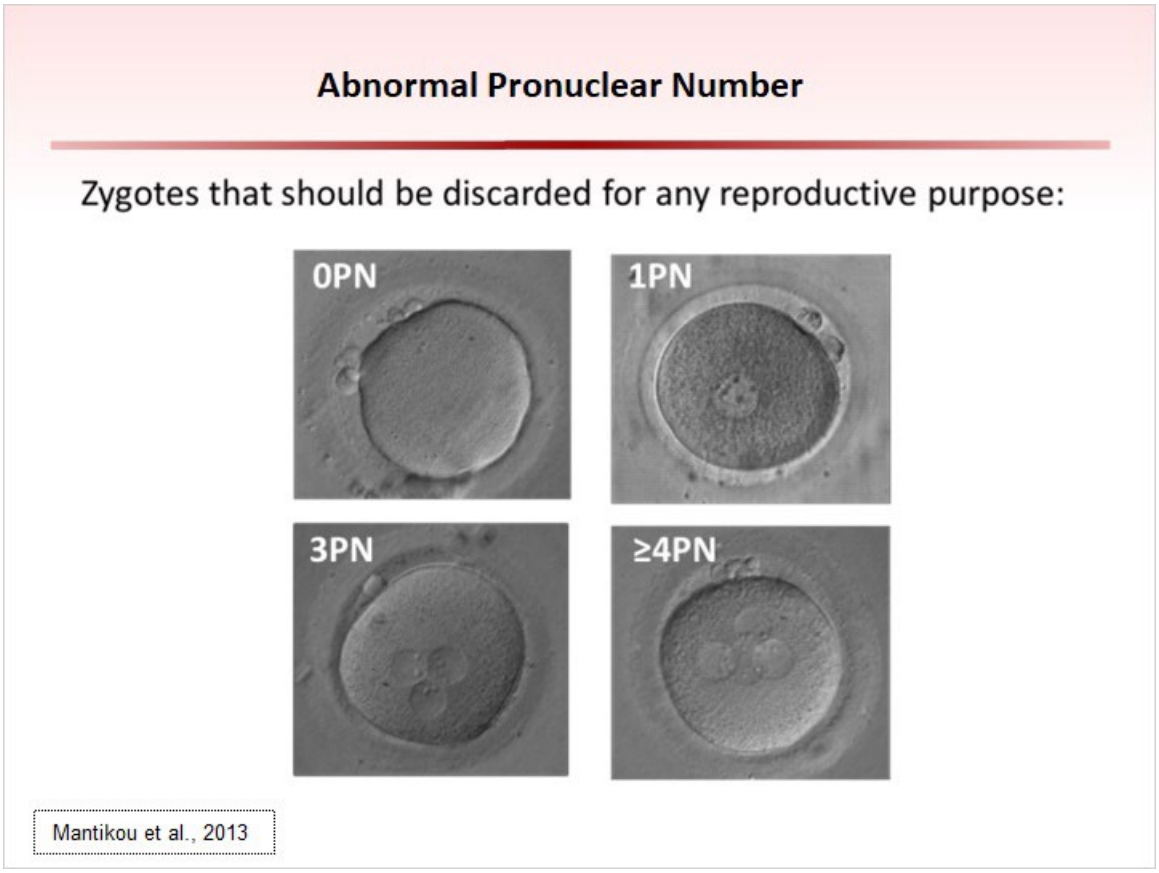
BUT

- PBs frequently undergo division or fragmentation, so the number of PBs is not a useful marker of normality when 2PNs are present.

Notes:

Some embryologists also count the number of polar bodies because a normally fertilized oocyte should have 2 that are closely apposed. However, as the polar bodies frequently either divide or undergo fragmentation, polar body number is not generally considered a useful marker of fertilization.

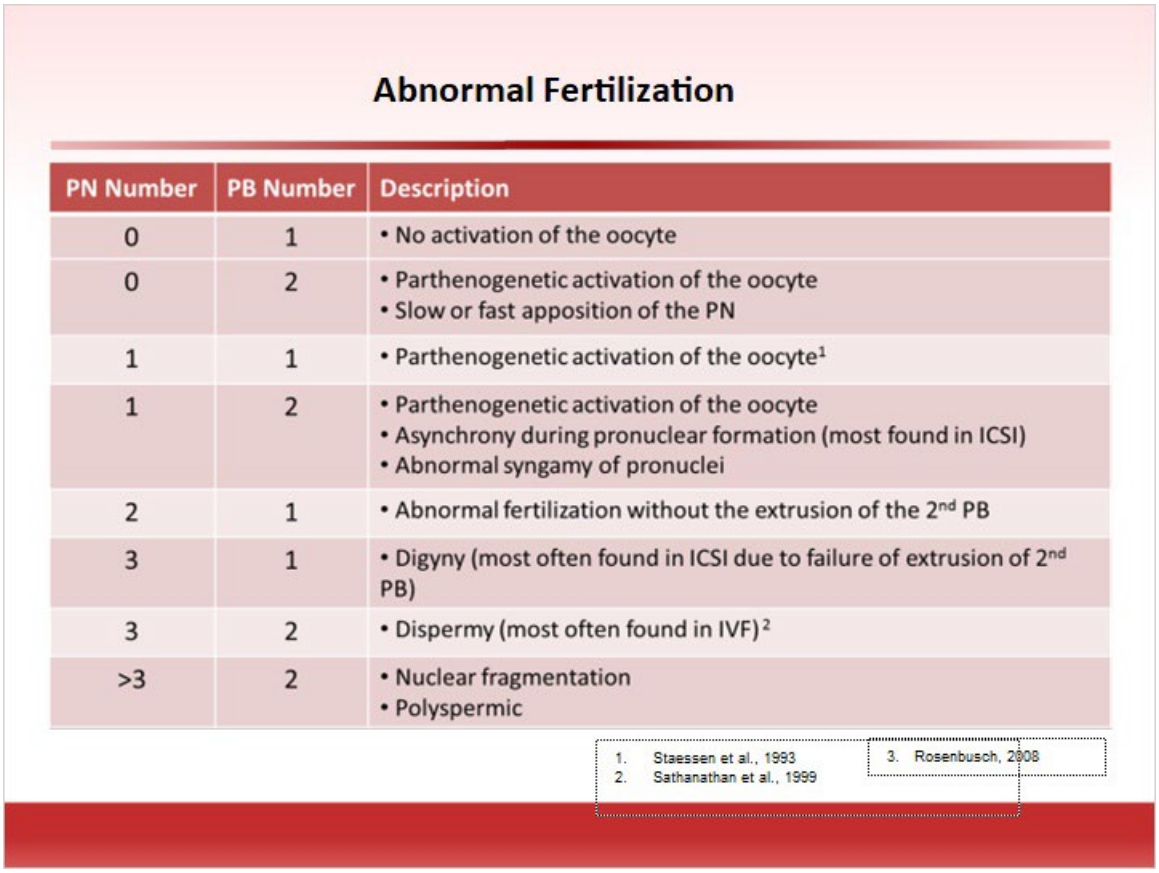
1.9 Abnormal Pronuclear Number



Notes:

Assuming that the fertilization assessment is performed within the optimum time window, oocytes with 3 or more than 3 pronuclei should be discarded for any reproductive purpose. Those with 0PN or 1PN have a very low reproductive potential, but if cultured to day 5 will allow identification of those capable of developing to the blastocyst stage. While most dispermic zygotes are mosaic, digynic embryos present lower rates of mosaicism as they undergo regular chromosome segregation, albeit with failure of emission of the second polar body (at telophase II). Even though pronuclear removal in digynic embryos may restore the chromosomal state, there is no report of a human pregnancy after microsurgical correction of 3PN zygotes.

1.10 Abnormal Fertilization



Notes:

Various mechanisms have been proposed to explain the origin of 0PN, 1PN, or ≥3 pronuclei with or without the extrusion of the second polar body.

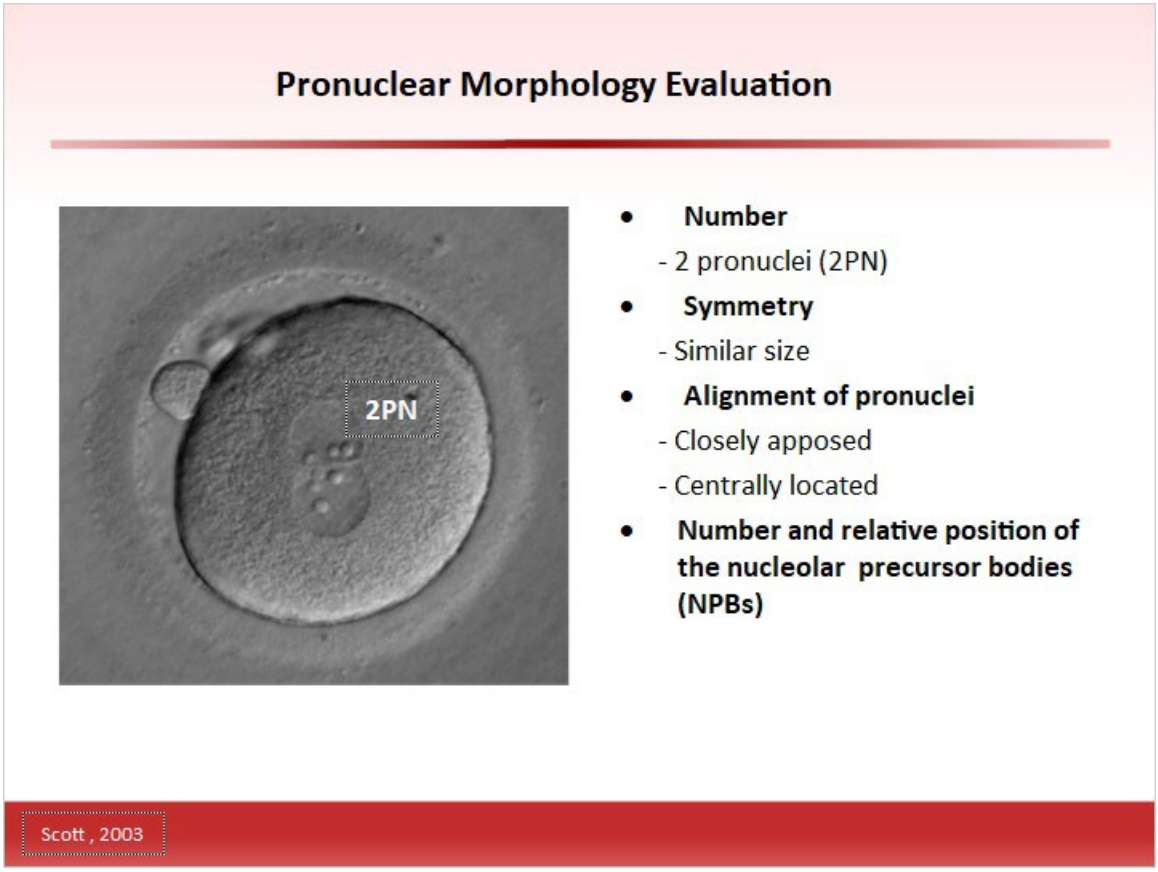
The absence of any pronuclei along with the presence of only a single polar body indicates that the oocyte has not been activated. However, when 2 polar bodies are observed, this may be due either to parthenogenetic activation or rapid apposition and subsequent disappearance of pronuclei after fertilization.

1PN zygotes may result from parthenogenetic activation of the oocyte, in which case the oocyte will be haploid. Alternatively, mononucleation may be due to asynchrony during pronuclear formation or abnormal syngamy of pronuclei, with or without the extrusion of the second polar body which, in some cases, can lead to diploid zygotes.

3PN zygotes after standard IVF insemination typically originate when oocytes are fertilized by 2 spermatozoa, and from ICSI, after fertilization by a single sperm but failure to extrude the second polar body.

The presence of more than 3 PNs with the extrusion of the 2 polar bodies could be due to polyspermic fertilization or nuclear fragmentation.

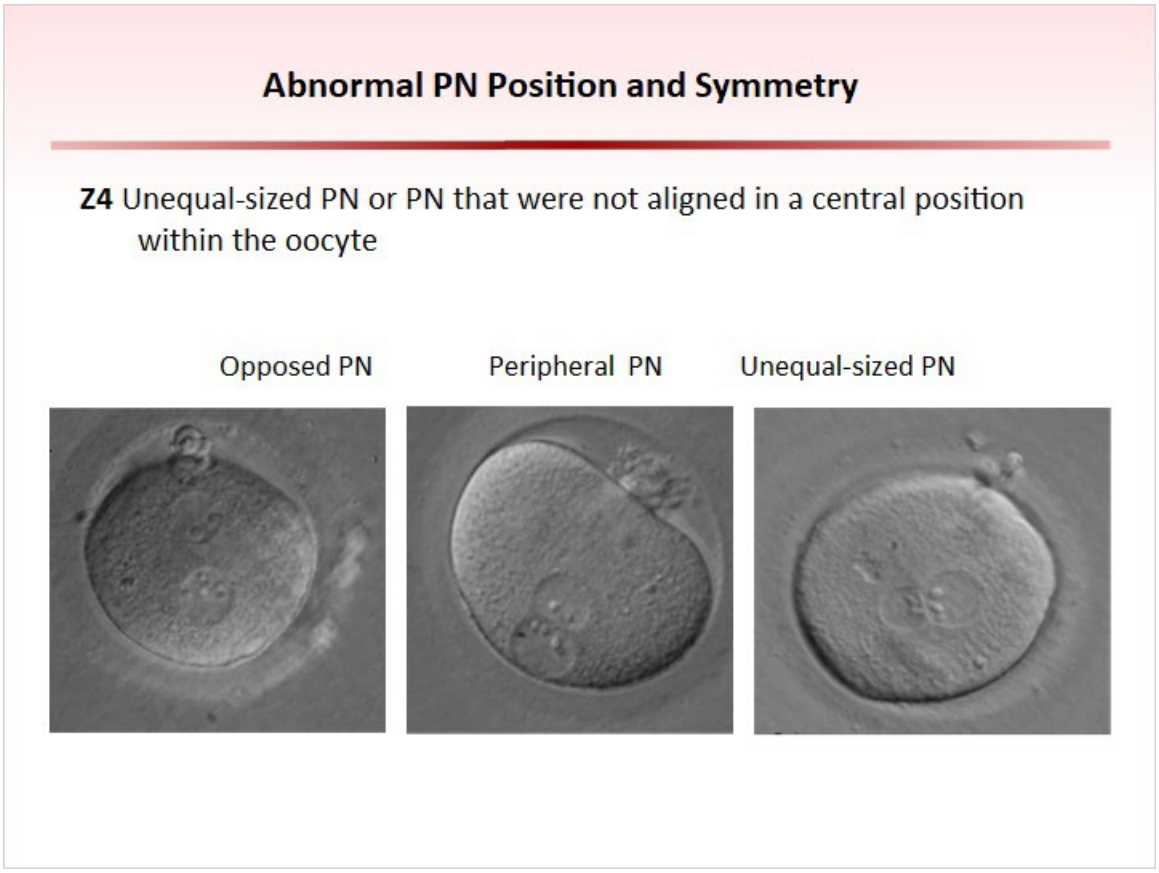
1.11 Pronuclear Morphology Evaluation



Notes:

The two PNs should be similar in size, closely apposed, and centrally located in the zygote. Examples of zygotes exhibiting abnormal PN position and symmetry are shown next.

1.12 Abnormal PN Position and Symmetry

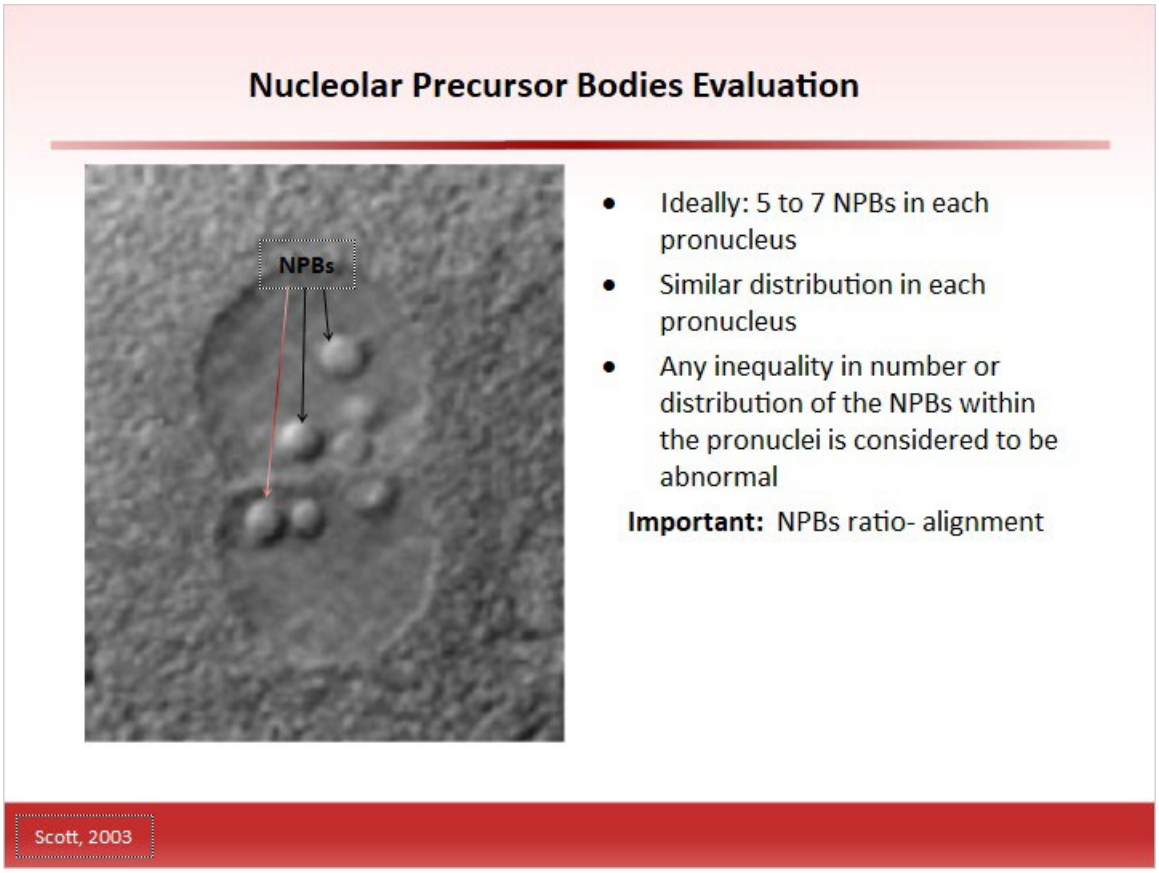


Notes:

Zygotes that are scored as abnormal and should be discarded are those in which the pronuclei have not come together by 16-18 hours after insemination, those in which the pronuclei are displaced to the periphery of the cell, and those with unequal size.

While pronuclei that are not centrally located could reflect failure of microtubule or aster formation, those presenting with unequal size have been found to have low developmental potential and a high incidence of aneuploidy.

1.13 Nucleolar Precursor Bodies Evaluation

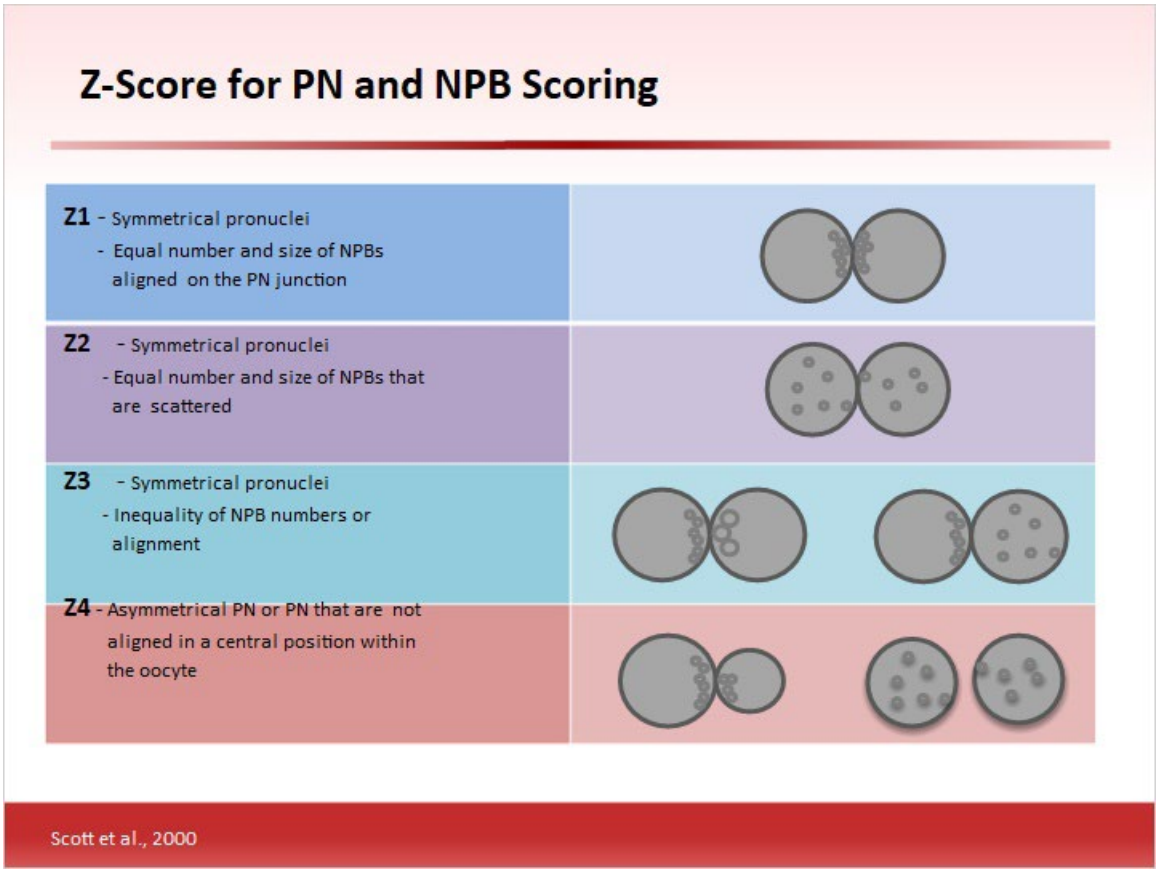


Notes:

There is some evidence that the number and disposition of the nucleolar precursor bodies (NPBs) are predictive of zygote developmental potential (see Scott et al., 2000), although other studies have not shown NPB scoring to be helpful.

Ideally, each of the 2 pronuclei should have 5 to 7 NPBs that are similarly distributed between the 2 pronuclei. Some studies have shown that zygotes exhibiting any inequality in number or distribution of the NPBs within the pronuclei have reduced developmental competence compared with those that are uniform and polarized.

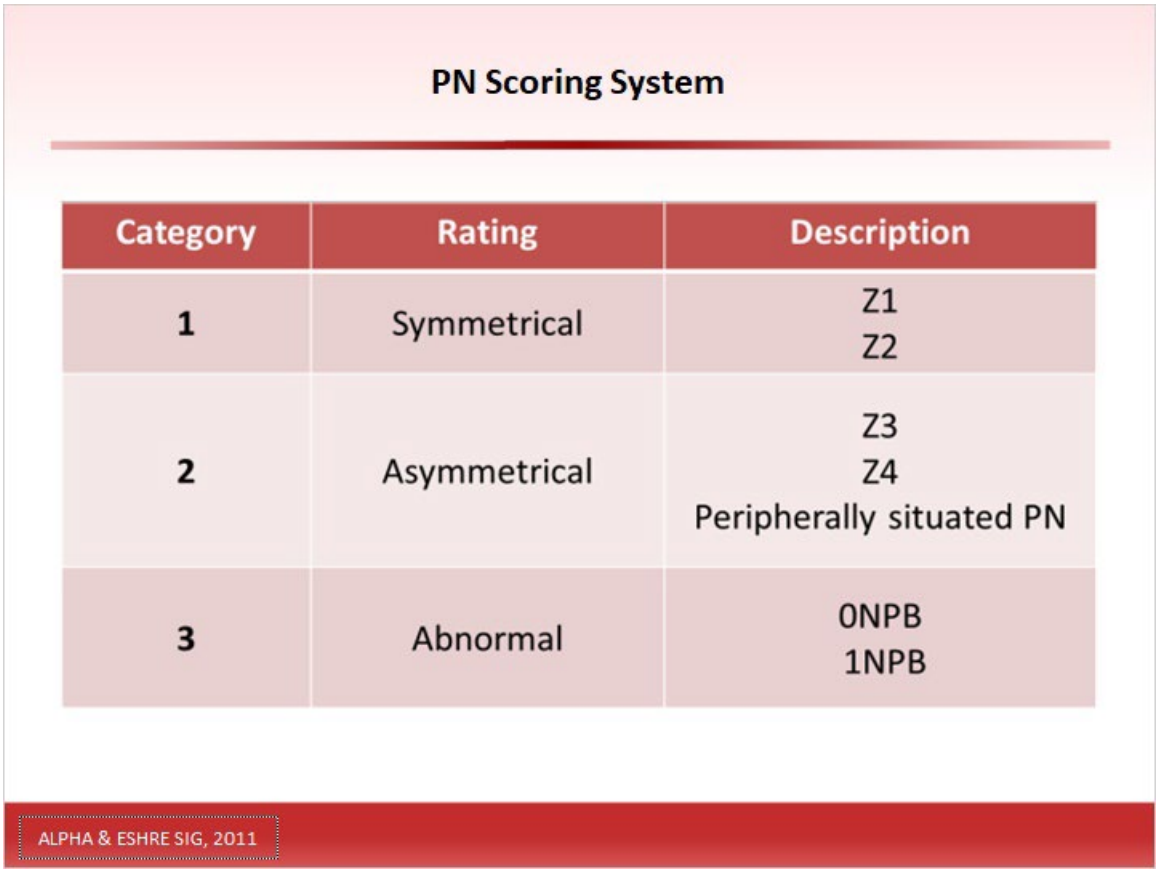
1.14 Z-Score for PN and NPB Scoring



Notes:

Although different PN grading systems have been proposed, the most widely accepted is Z-scoring by Scott et al. 2000, with several studies confirming its prognostic value for embryo selection.
As shown in this slide, this scoring system involves classifying the zygotes into 4 groups according to pronuclear size and location as well as the size, number, and distribution of the NPBs. While embryos with Z1 and Z2 scoring are considered of highest quality, those with a Z3 score are considered to be of inferior quality, while Z4 embryos have been reported to have a very low implantation potential. Due to the complexity of this scoring system, an attempt has been made to simplify the classifications.

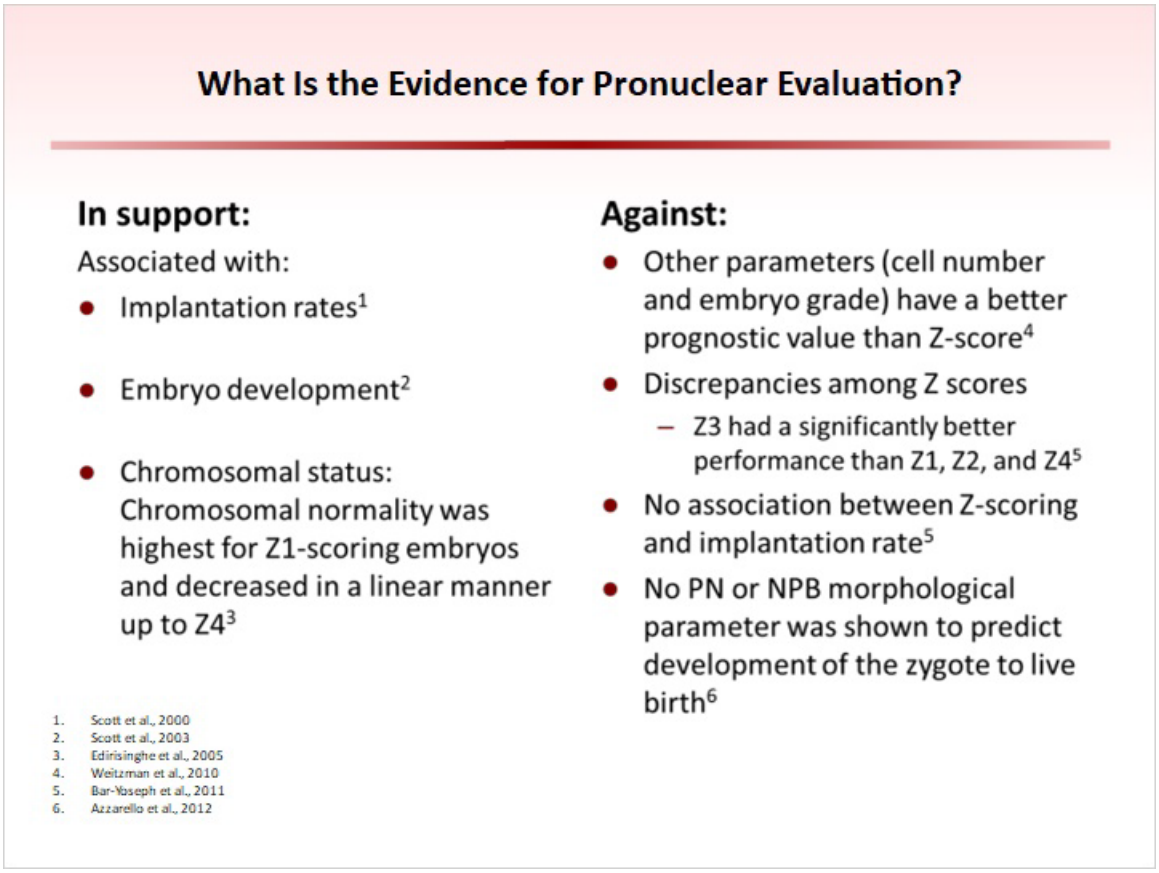
1.15 PN Scoring System



Notes:

During the so-called “Consensus of Istanbul” of the Alpha and ESHRE Special Interest Group in 2011, Scott's Z-scoring system was standardized into 3 categories. The symmetrical category corresponds to Z1 and Z2. The nonsymmetrical category covers Z3 and Z4, including peripherally positioned pronuclei, and the third one is the abnormal category which corresponds to pronuclei with none or only 1 NPB.

1.16 What Is the Evidence for Pronuclear Evaluation?



Notes:

There are disagreements among studies dealing with pronuclear evaluation. Some studies correlate pronuclear scoring as a predictor of embryo development resulting in higher implantation rates per transfer. Likewise, some studies have correlated the pronuclear scores with chromosomal status, with euploidy being higher in Z1-scoring embryos, with a decrease in incidence of euploid zygotes in a linear manner up to Z4.

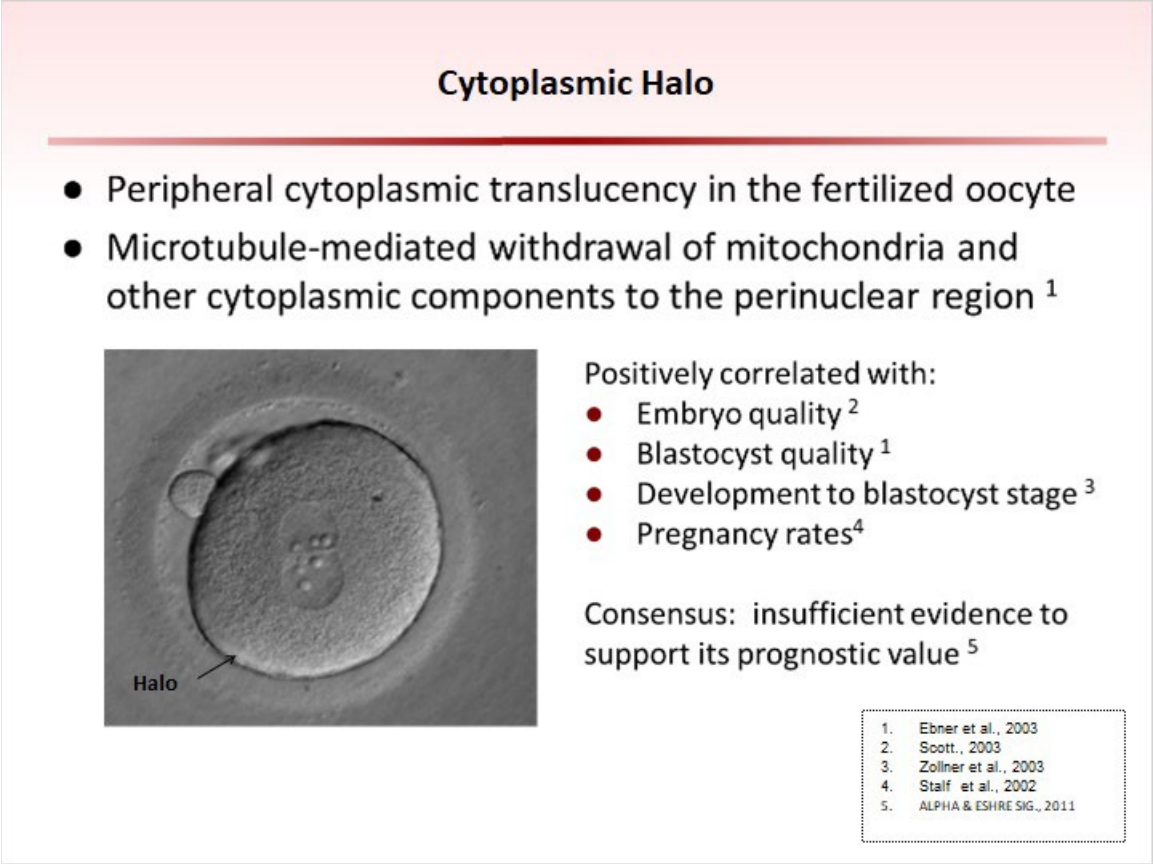
However, other studies highlight that other parameters such as the cell number and embryo grade have a better prognostic value than Z-score. Also some discrepancies were found among the Z scores showing that Z3 had a significantly better performance than Z1, Z2, and Z4. Moreover, other studies did not find any association between Z-scoring and implantation rate, and no morphological parameter was shown to predict development of the zygote to live birth.

Overall, Z-scoring provides limited additional value in predicting embryo quality above that obtained from other parameters in cleavage- and/or blastocyst-stage embryos. However, if a lab is going to use Z-scoring as part of their grading system, it is

recommended that there is very tight control over the timing of the fertilization check as the disposition of the pronuclei and the size and number of the NPBs are exquisitely dynamic.

The next section will consider whether there is value in grading the cytoplasmic halo and perivitelline space.

1.17 Cytoplasmic Halo



Notes:

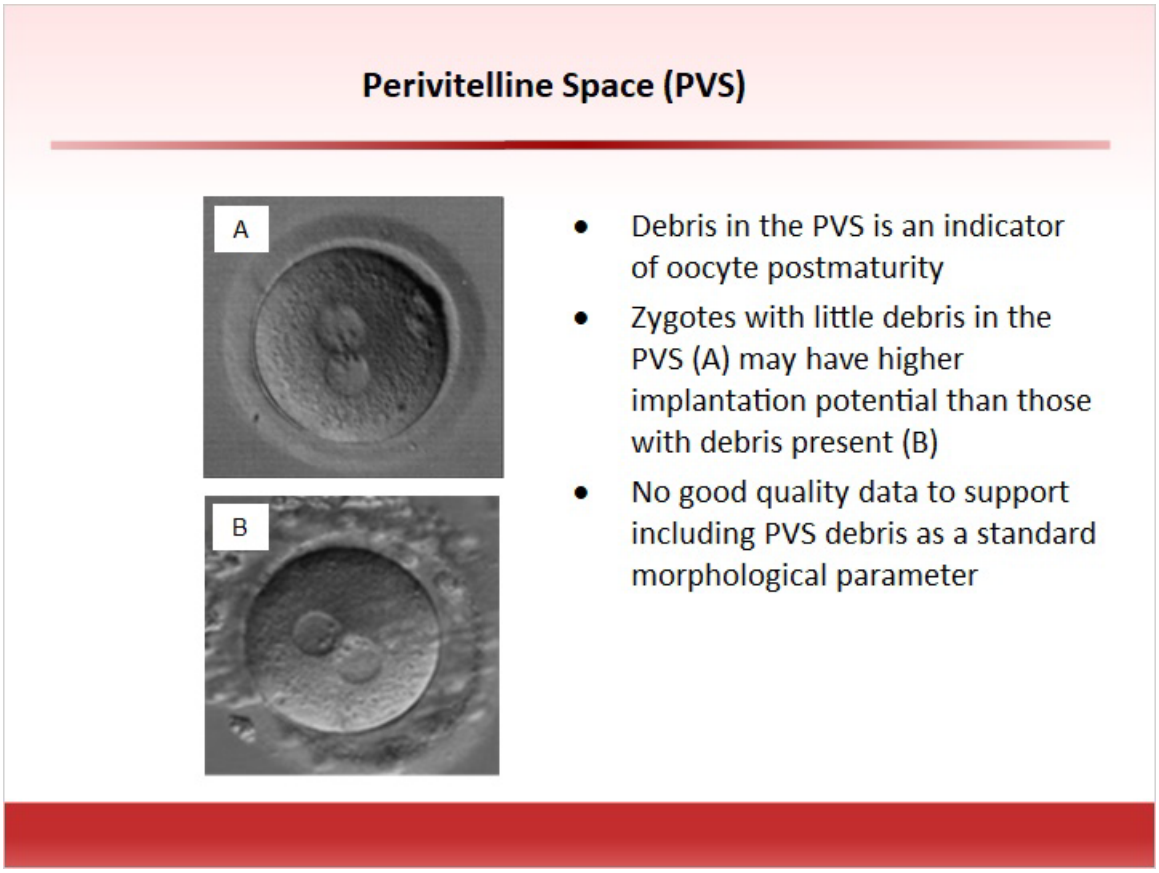
The cytoplasmic halo is defined as a peripheral cytoplasmic translucency in the fertilized oocyte that reflects microtubule-mediated withdrawal of mitochondria and other cytoplasmic components to the perinuclear region.

The presence of a cytoplasmic halo at the zygote stage has been shown to be associated with increased embryo quality as evidenced by enhanced development of higher-quality blastocysts. Moreover, embryos from halo-positive zygotes have been reported to

implant at an increased rate compared with those from zygotes that do not reveal halos.

However, there is still disagreement among studies and, overall, there is insufficient evidence to support its prognostic value as a routine marker of developmental competence.

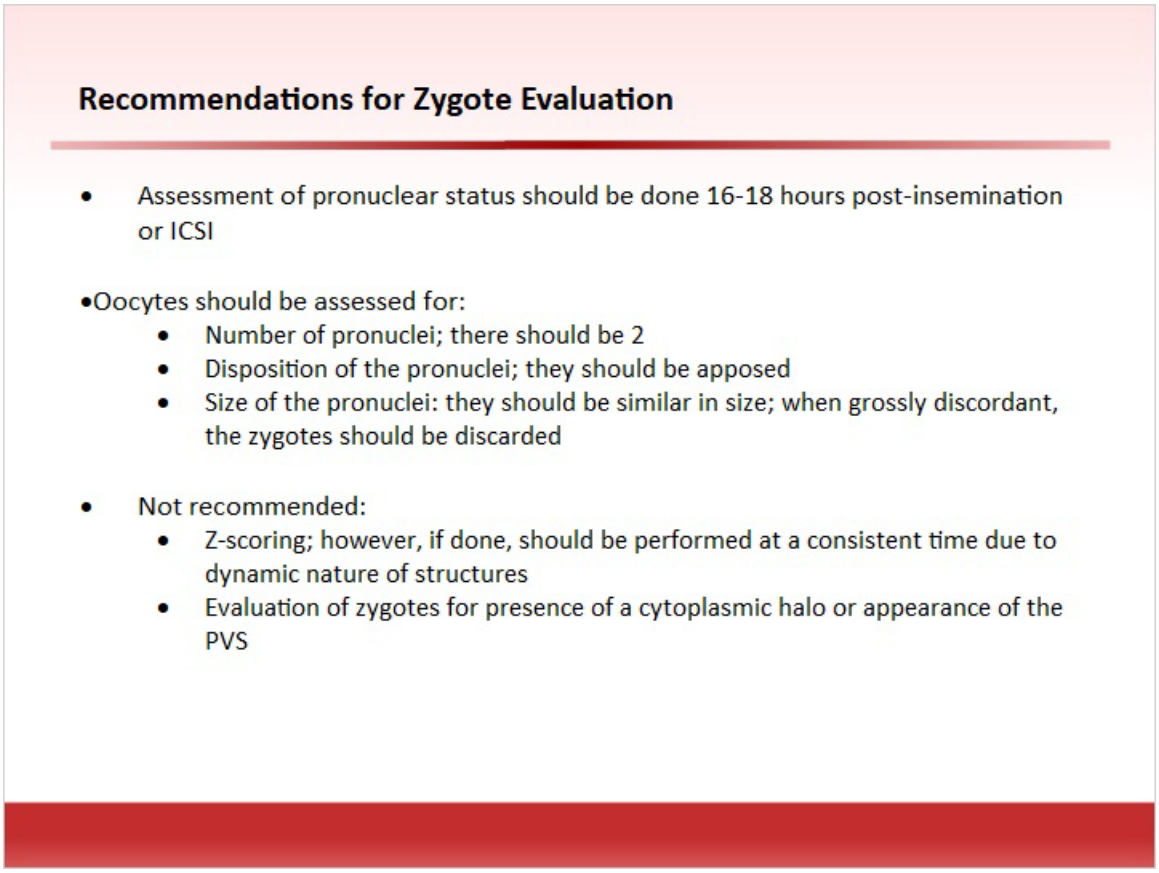
1.18 Perivitelline Space (PVS)



Notes:

The perivitelline space (PVS) is the space between the vitelline membrane and the zona pellucida. Debris in the PVS may be an indicator of oocyte postmaturity. Zygotes with little debris in the PVS may have higher implantation potential than those with debris present. However, there is currently insufficient good quality data to support including PVS debris as a standard morphological parameter.

1.19 Recommendations for Zygote Evaluation

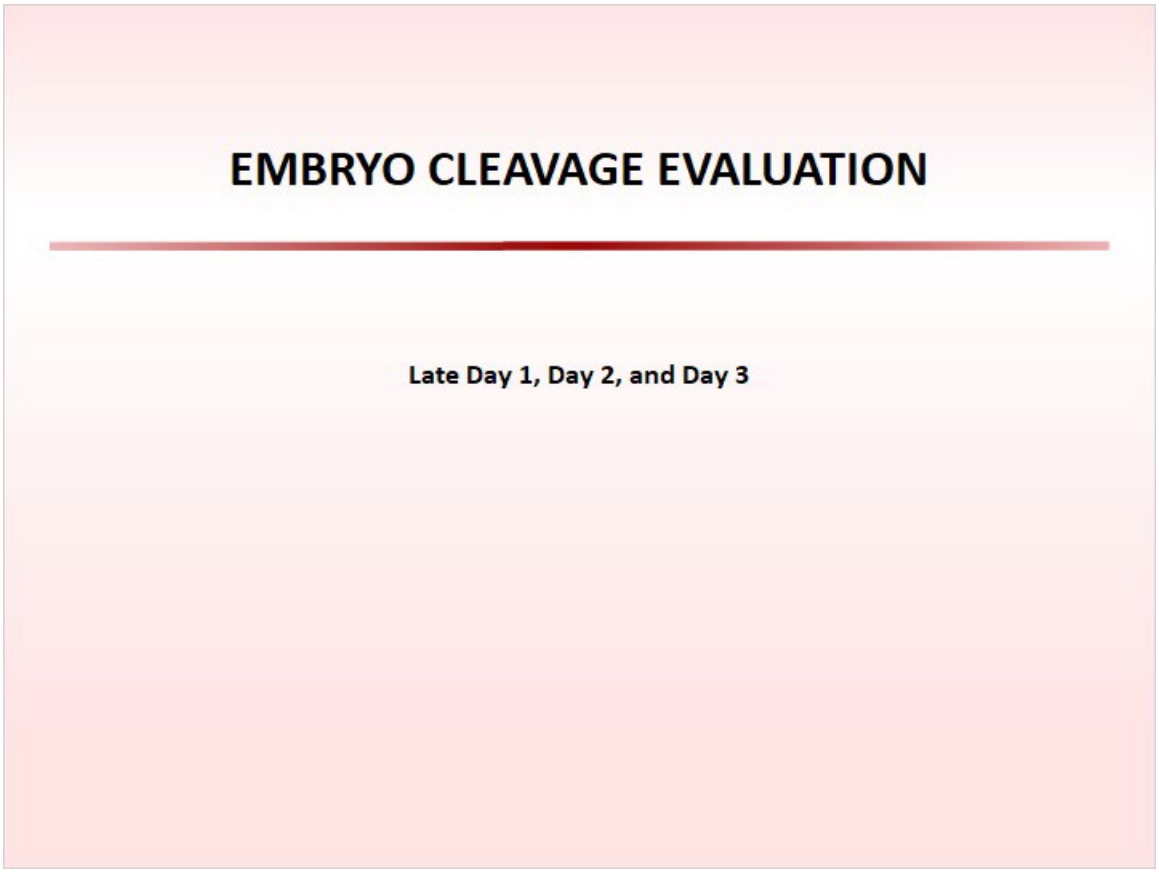


Notes:

Assessment of pronuclear status should be done 16-18 hours post-insemination or -ICSI. Oocytes should be assessed for the number of pronuclei (there should be 2), disposition of the pronuclei (they should be apposed), and size of the pronuclei (they should be similar in size). When the size is grossly discordant, the zygotes should be discarded.

Z-scoring is not recommended. However, if done, it should be performed at a consistent time due to the dynamic nature of the structures. Evaluation of zygotes for presence of a cytoplasmic halo or appearance of the PVS is also not recommended.

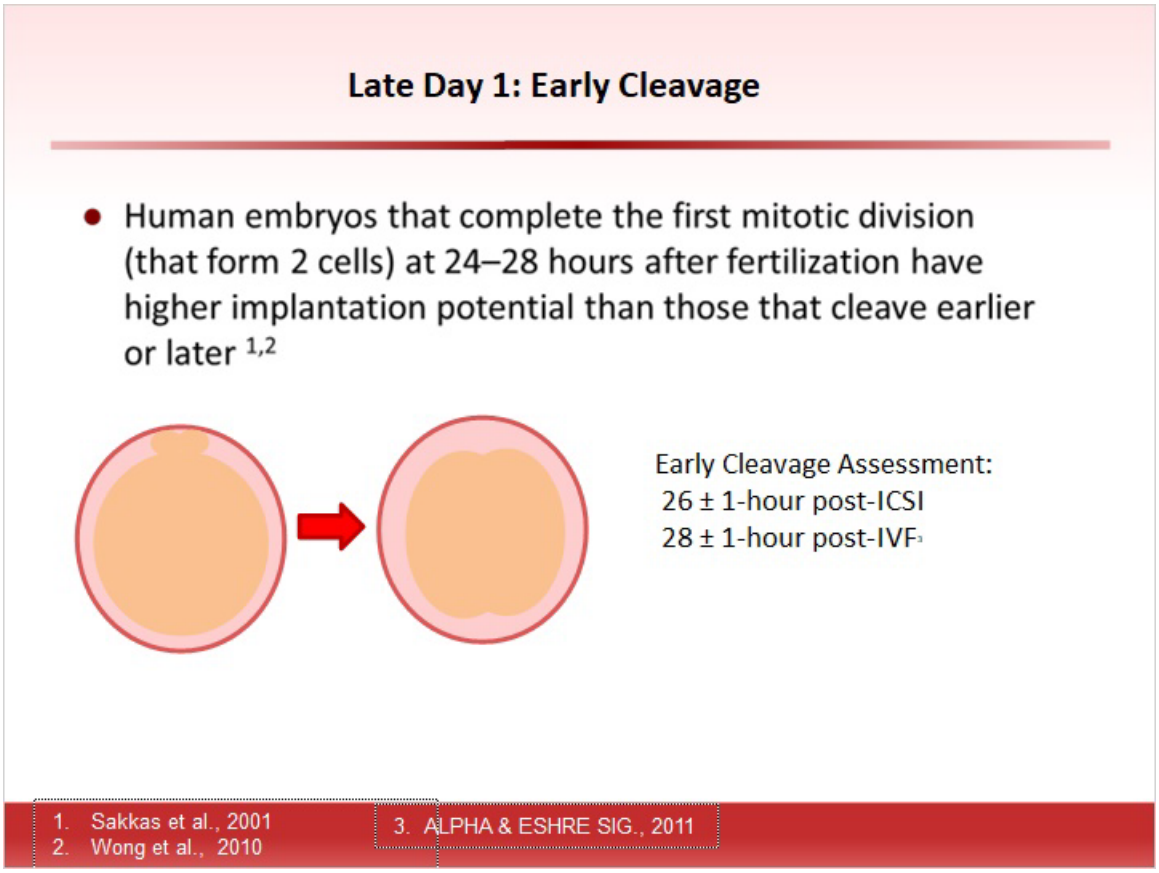
1.20 EMBRYO CLEAVAGE EVALUATION



Notes:

Cleavage evaluation of embryos from late day 1, day 2, and day 3 is an important parameter that has to be considered for embryo selection.

1.21 Late Day 1: Early Cleavage

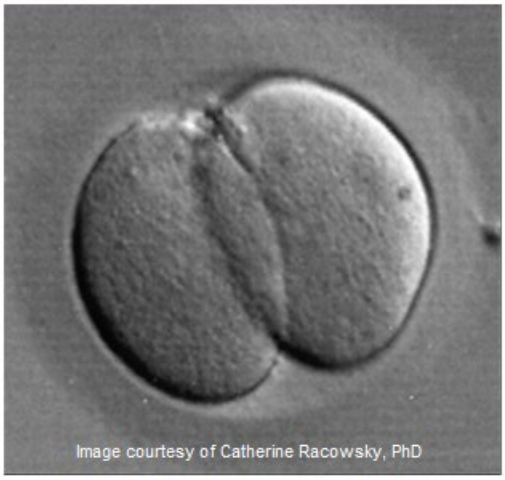


Notes:

Many studies have investigated whether so-called “early-cleavage” assessment is useful for determining embryo developmental competence. In this context, “early cleavage” refers to those zygotes that complete the first mitotic division within a defined time range after insemination or ICSI compared with those outside of the range. The overall weight of the data shows that those having 2 cells at approximately 24-28 hours after fertilization have higher implantation potential than those that cleave earlier or later. According to these criteria, “early-cleavage” assessment should be done at 26 ± 1 hour after ICSI or 28 ± 1 hour after standard IVF insemination.

1.22 Early Cleavage

Early Cleavage



Associated with increased:

- Embryo quality[1]
- Embryo viability[2]
- Blastocyst development[1,3]
- Pregnancy outcomes[2,3,4,5]
- Likelihood of embryo selection at the time of transfer on day 2 and day 3[2]
- Obstetric outcomes[6]

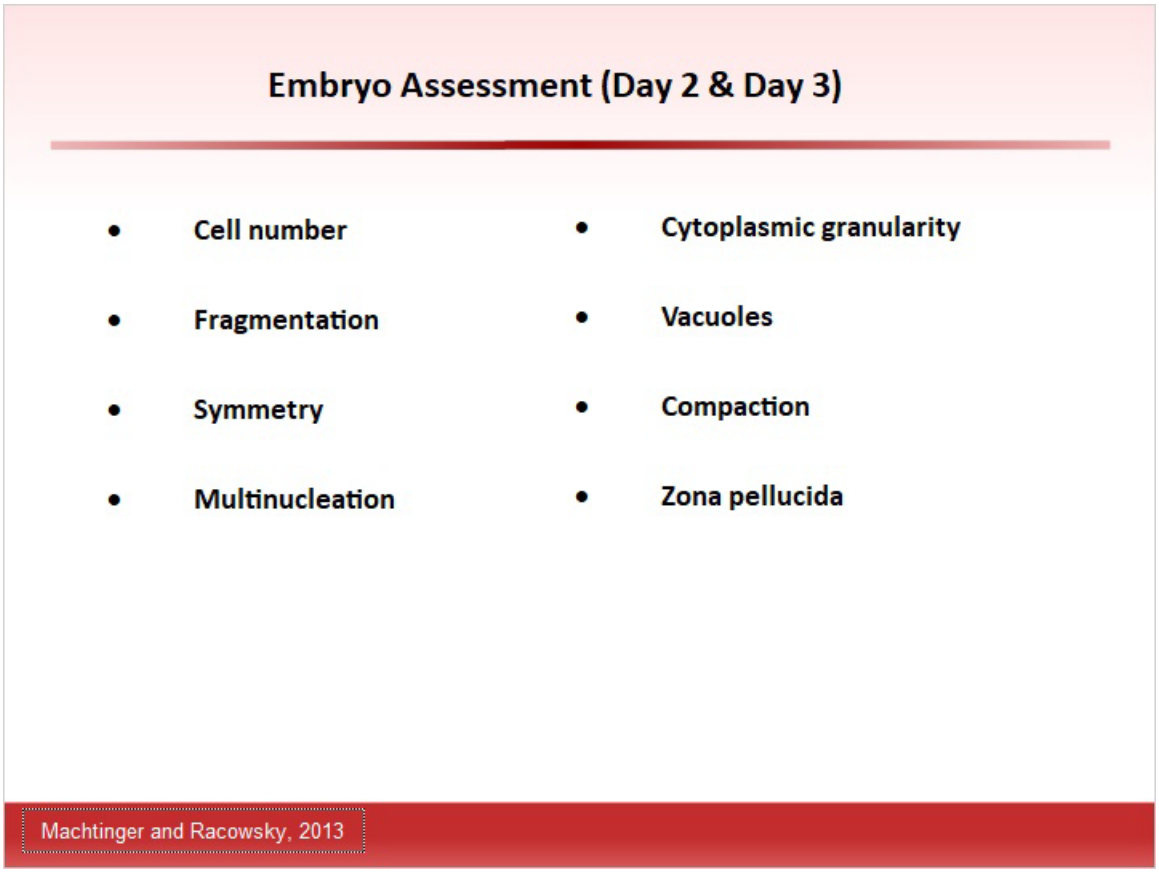
1. Neuber et al., 2003
2. Sakkas et al., 2001
3. Salumets et al., 2003
4. Lundin et al., 2001
5. Van Montfoort et al., 2004
6. Wennerholm, et al., 2009

Notes:

Compared with non-early cleavers, “early-cleaved” embryos are associated with superior morphological quality on days 2 and 3. These embryos also have an increased potential to develop to the blastocyst stage, which is also reflected in pregnancy outcomes. Therefore, knowing which embryos were “early cleavers” late on day 1 may enhance selection when choosing between embryos of otherwise identical morphological appearance.

Despite the recent success with blastocyst vitrification, cryopreservation of cleavage embryos remains an area of high interest, particularly in countries in which extended culture is not popular. Wennerholm et al. (2009) concluded, by reviewing several studies, that “early-cleavage” embryos had better or at least as good obstetric outcome, measured as preterm birth and low birth weight for children born after cryopreservation, when compared with children born after a fresh cycle.

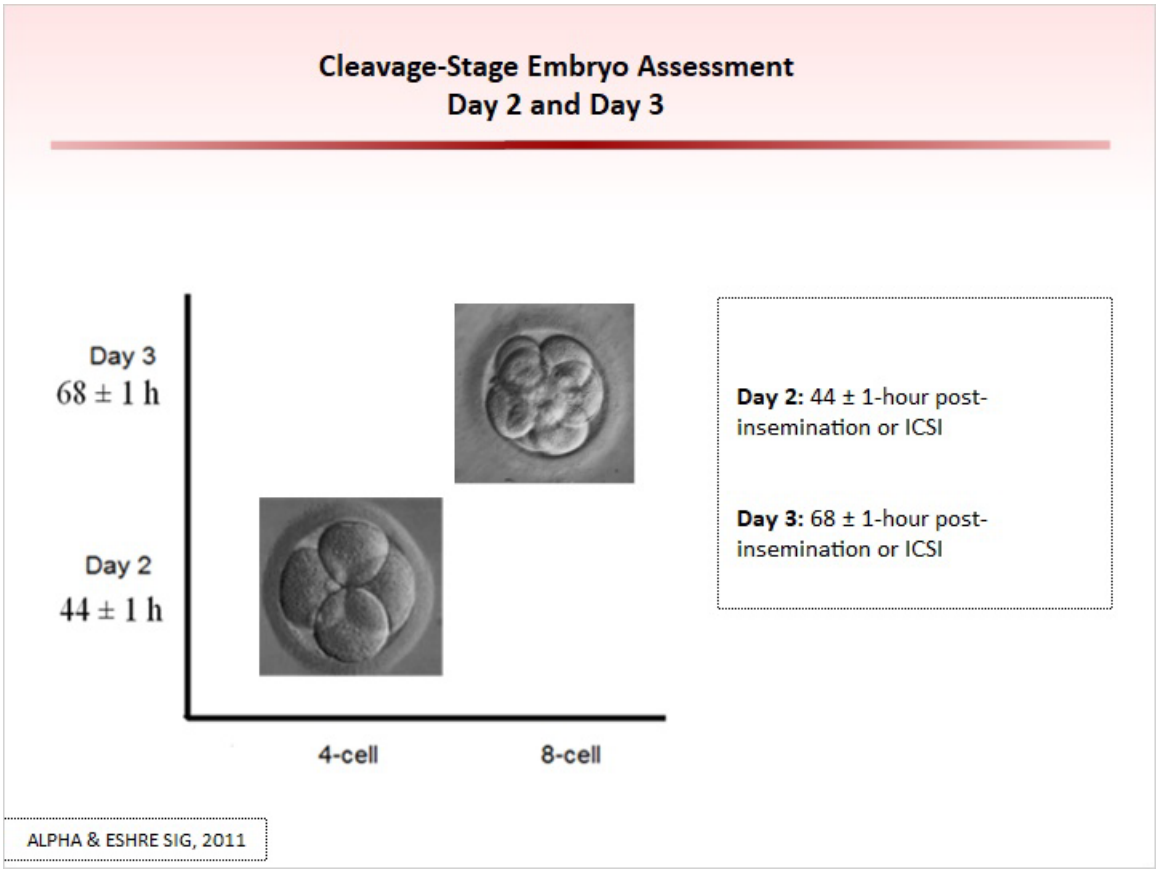
1.23 Embryo Assessment (Day 2 & Day 3)



Notes:

Morphological assessment of embryos on days 2 and 3 should include evaluation of several characteristics, the most important being cell number, degree of fragmentation, extent of symmetry, and presence or absence of multinucleation. Some embryologists also grade embryos for the presence of cytoplasmic granularity and vacuoles, as well as early signs of compaction and appearance of the zona pellucida.

1.24 Cleavage-Stage Embryo Assessment



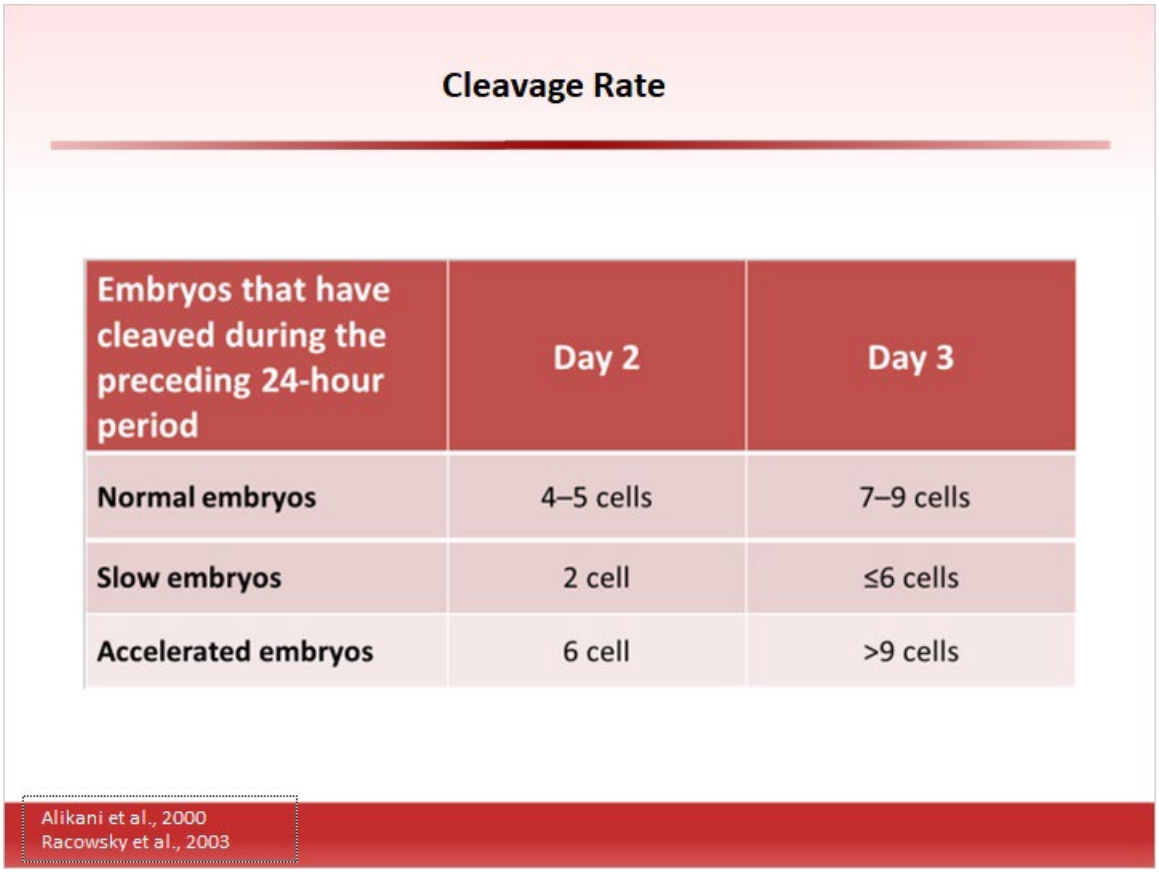
Notes:

As discussed earlier, normal developing human preimplantation embryos progress along a predictable timeline, forming 4 cells on day 2 and 8 cells on day 3. Numerous studies indicate that when divisions to these stages occur within specific time windows, the embryos are more likely to be viable. Therefore, the number of cells in an embryo at a specific time post-insemination or ICSI is considered a key characteristic when scoring the morphology of cleavage-stage embryos.

Following insemination or ICSI:

The evaluation time for identification of 4-cell embryos on day 2 has been standardized to 44 ± 1 hour and the evaluation time for identification of 8-cell embryos on day 3 has been standardized to 68 ± 1 hour.

1.25 Cleavage Rate

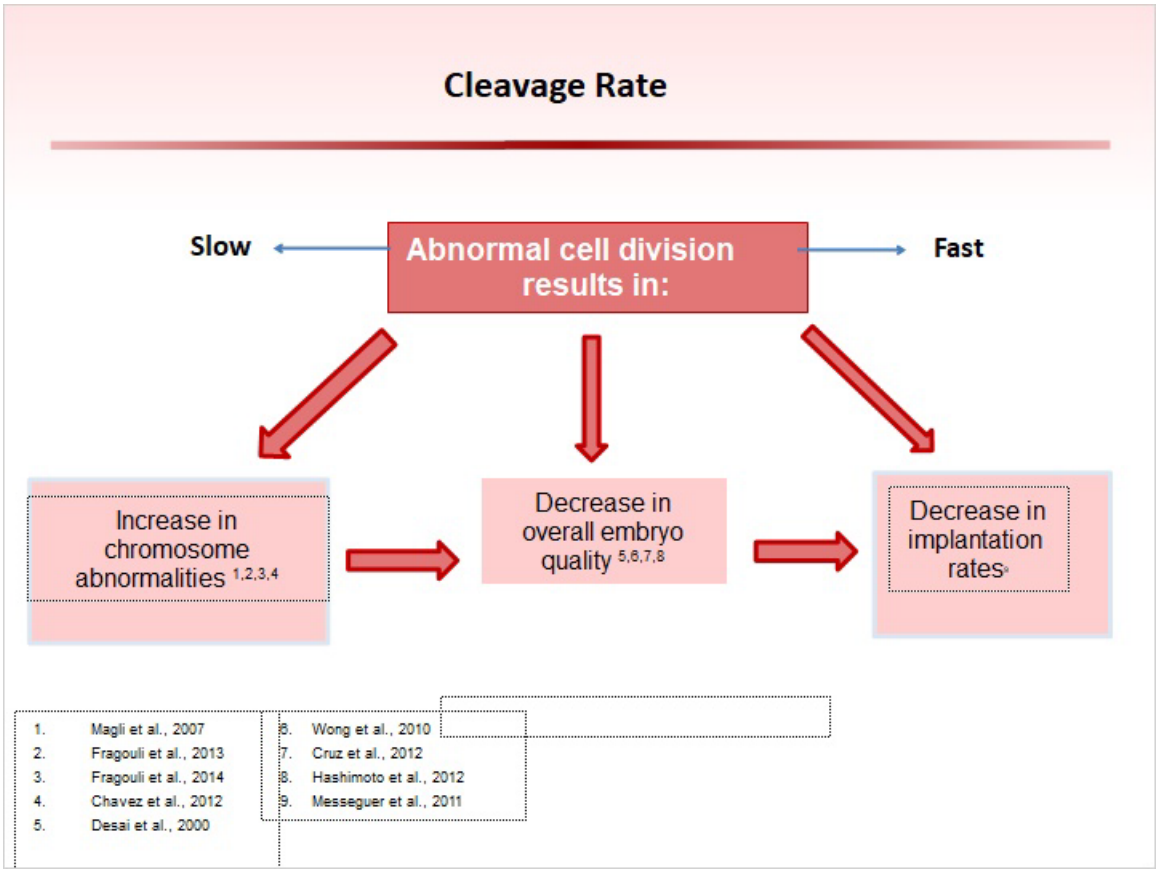


Notes:

Embryos are classified according to the numbers of cells that are present on day 2 and day 3, which together reflect the cleavage rate.

Assuming that evaluations are done at the optimum times:
Embryos with 4-5 cells on day 2 and 7-9 cells on day 3 are considered to be developing at the "normal" cleavage rate. Embryos with only 2 cells on day 2 and ≤6 cells on day 3 are considered "slow." Embryos with 6 cells on day 2 and >9 cells on day 3 are considered "accelerated."
"Slow" or "accelerated" embryos are considered suboptimal as these embryos are less likely to reach a normal blastocyst stage.

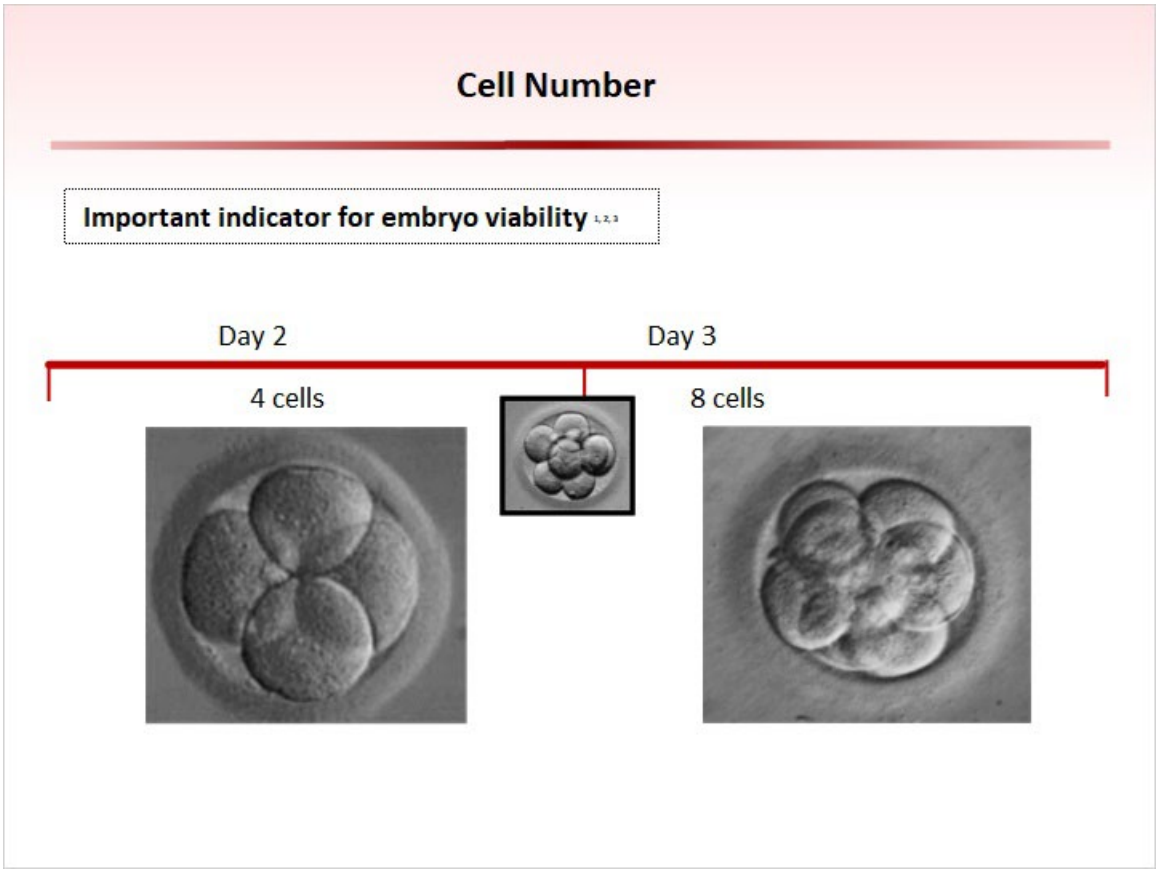
1.26 Cleavage Rate



Notes:

Embryo cleavage outside of the optimum time ranges is associated with an increased incidence in chromosomal abnormalities, and a decrease in embryo quality and implantation rates.

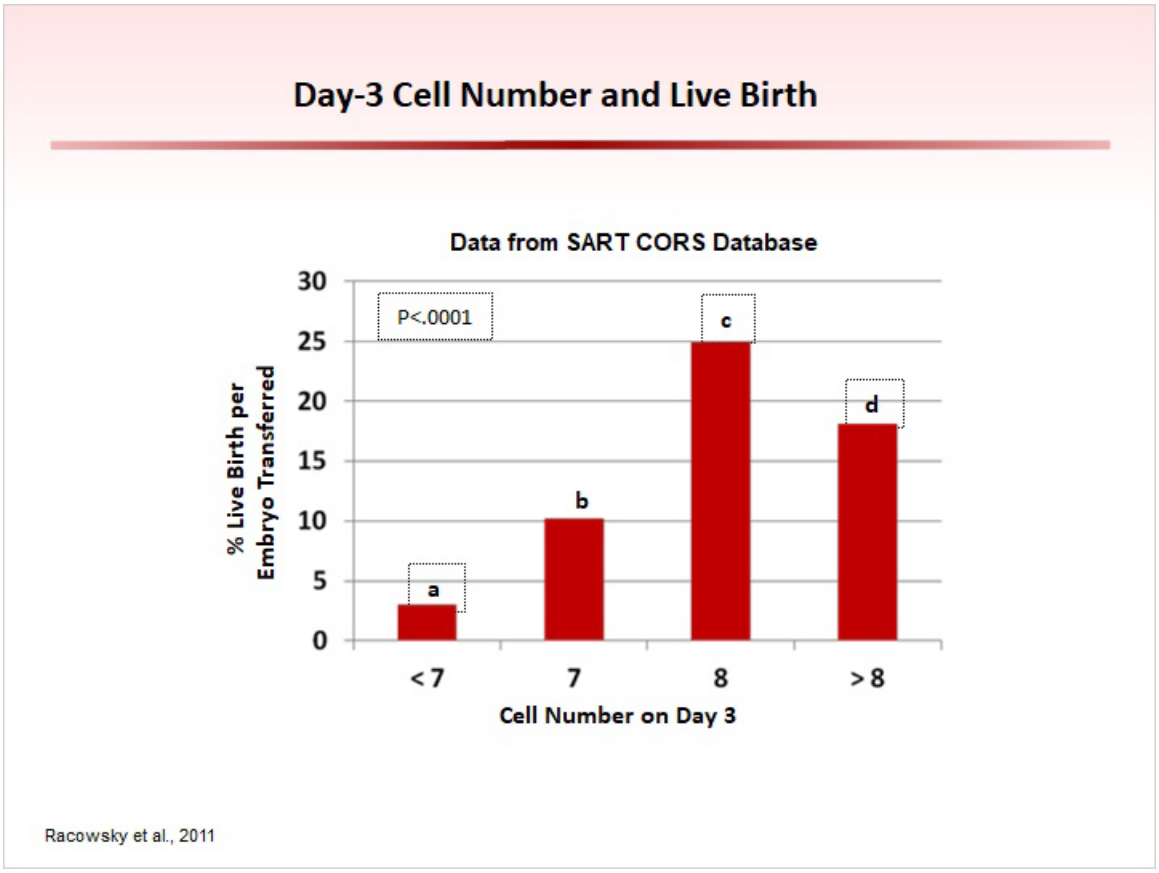
1.27 Cell Number



Notes:

One of the most commonly used predictors of embryo viability in cleavage-stage embryos is the number of cells. According to this criterion, top-quality embryos undergo the first cleavage division early on day 2 at around 26-28 hours after fertilization, and it is subsequently composed of 4 cells on day 2 and 8 cells on day 3, before compacting.

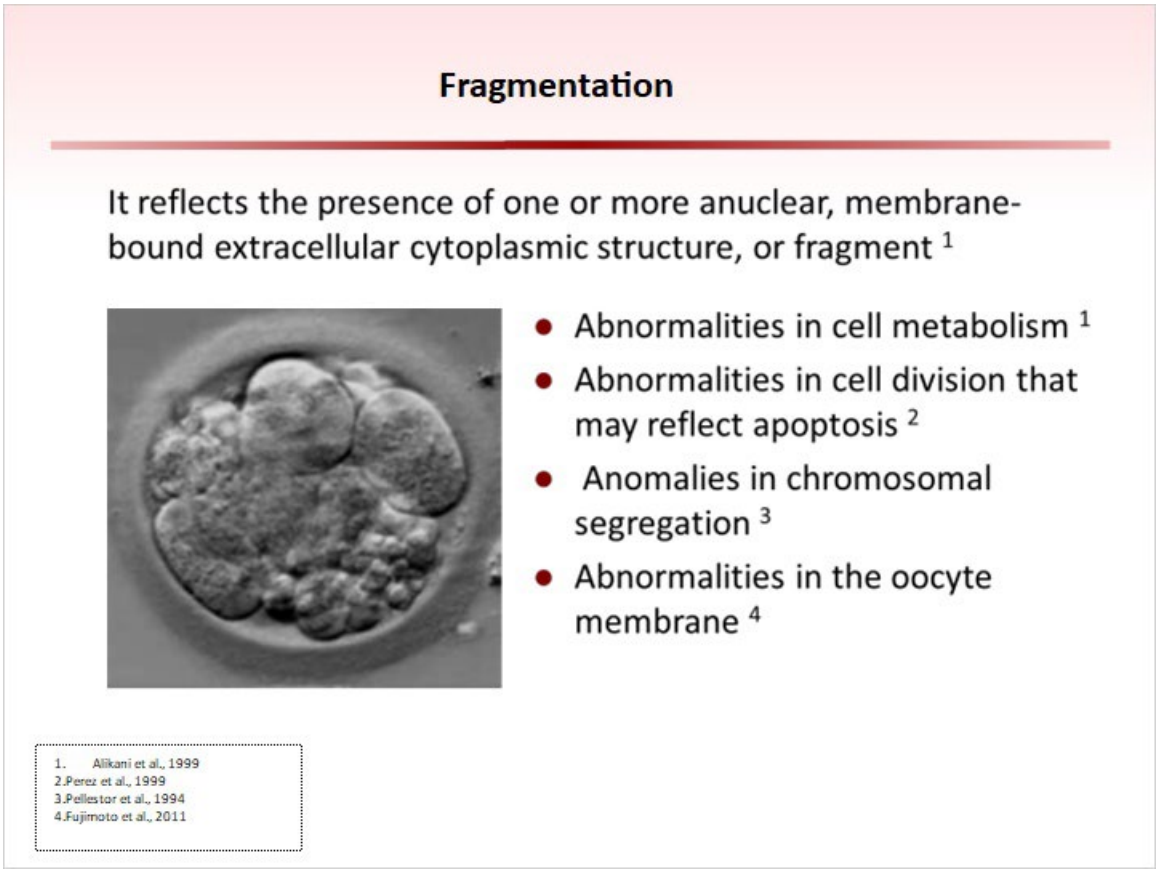
1.28 Day-3 Cell Number and Live Birth



Notes:

Many studies have investigated the relationship between the number of cells on day 3 and embryo viability. The consistent finding is that 8-cell embryos are associated with a significantly higher live-birth rate than embryos having any other number of cells. Of importance, embryos with more than 8 cells have a lower live-birth rate than those with 8 cells. Moreover, embryos with less than 7 cells have a <10% likelihood of resulting in a live birth. The data shown in this slide were derived from national data collected in the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) database.

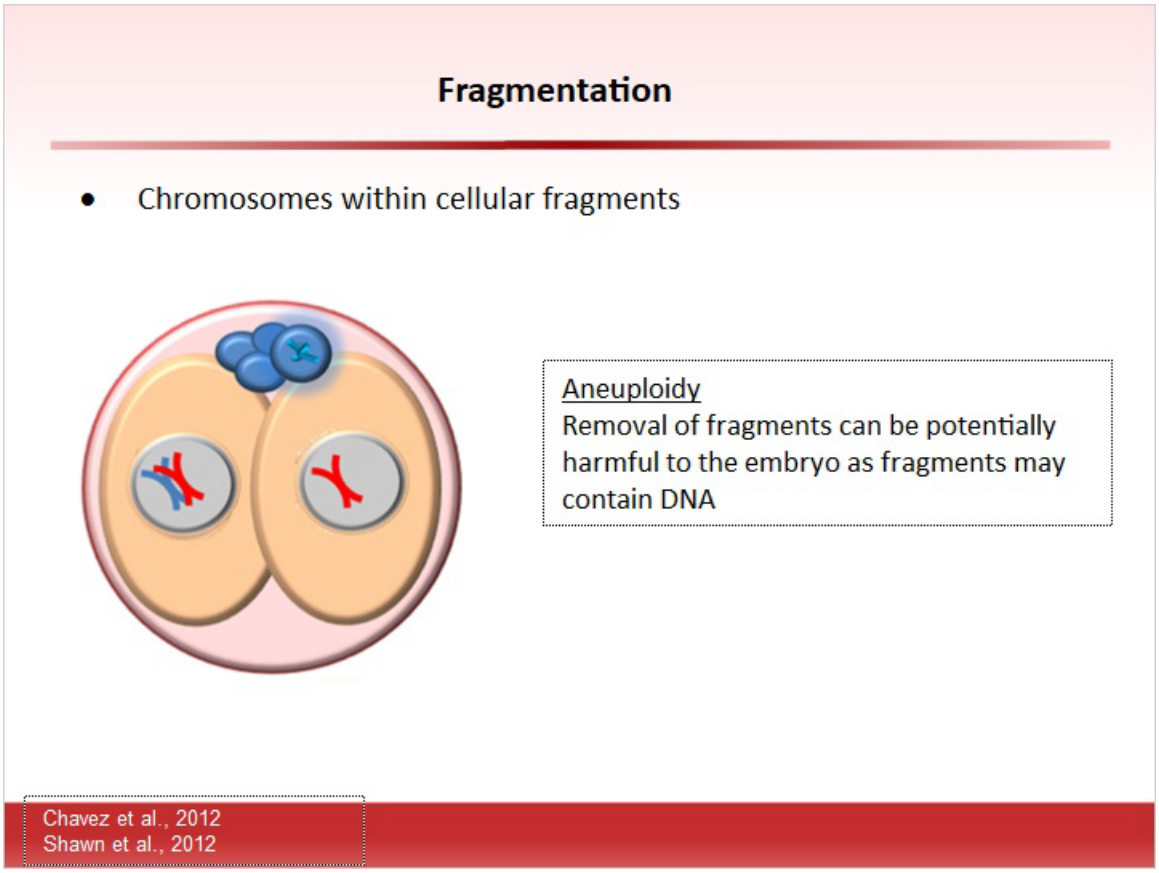
1.29 Fragmentation



Notes:

Fragmentation is a common characteristic of human cleavage-stage embryos. It reflects the presence of one or more anuclear, membrane-bound extracellular cytoplasmic structure, or fragment, that reduces the cell volume. The appearance of fragmentation has been suggested to be due to abnormalities in cell metabolism, abnormalities in cell division that may reflect apoptosis, abnormalities in chromosomal segregation, or abnormalities in the oocyte membrane.

1.30 Fragmentation

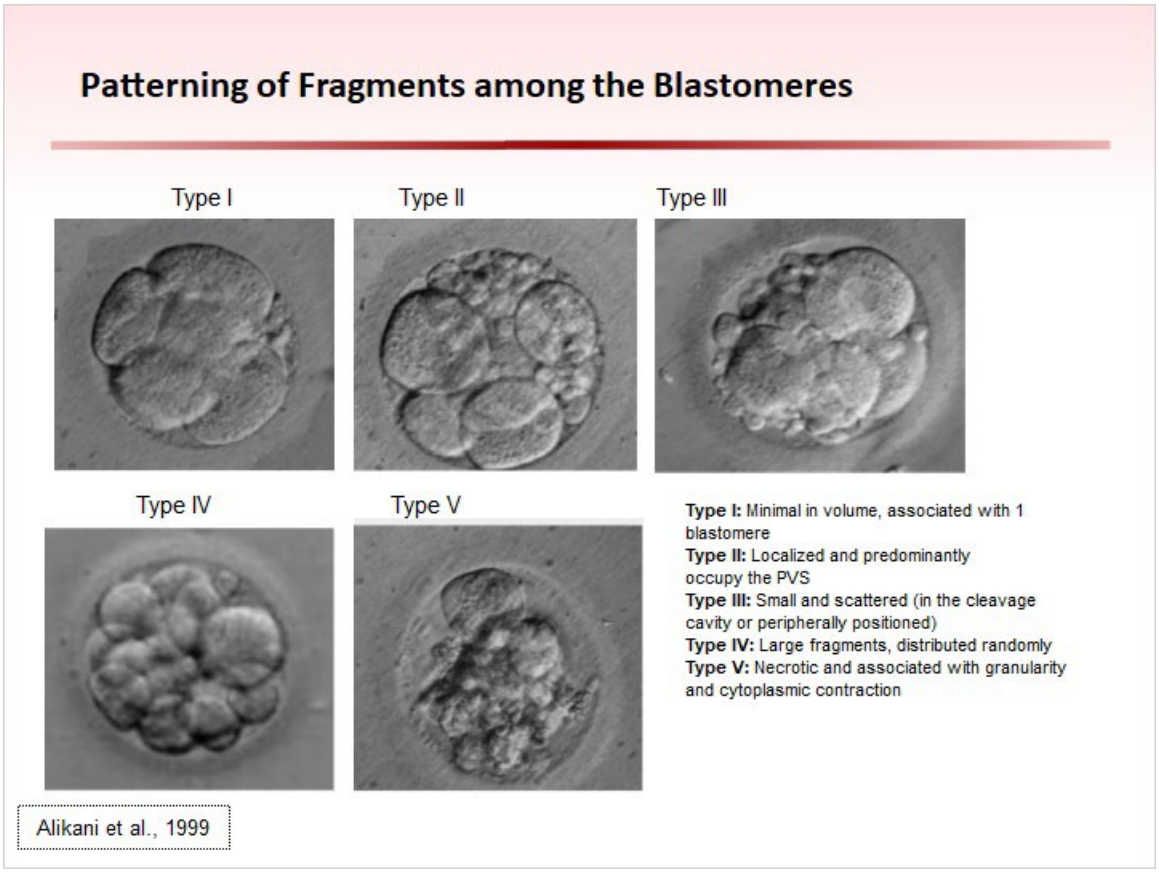


Notes:

Using fluorescence in situ hybridization (FISH), chromosomes have been shown to be present within fragments. This suggests that fragmentation of blastomeres may contribute to blastomere aneuploidy. Nonetheless, the removal of fragments could be potentially harmful to the embryo as it could also lead to removal of DNA.

Fragments may result from the demise of an entire blastomere(s), in which case the fragments are likely to be localized, or they may form after cytoplasmic extrusions from multiple blastomeres. The extent of fragmentation varies considerably and various patterns of fragmentation have been described. Fragmentation patterning has led to the development of a scoring system.

1.31 Patterning of Fragments among the Blastomeres



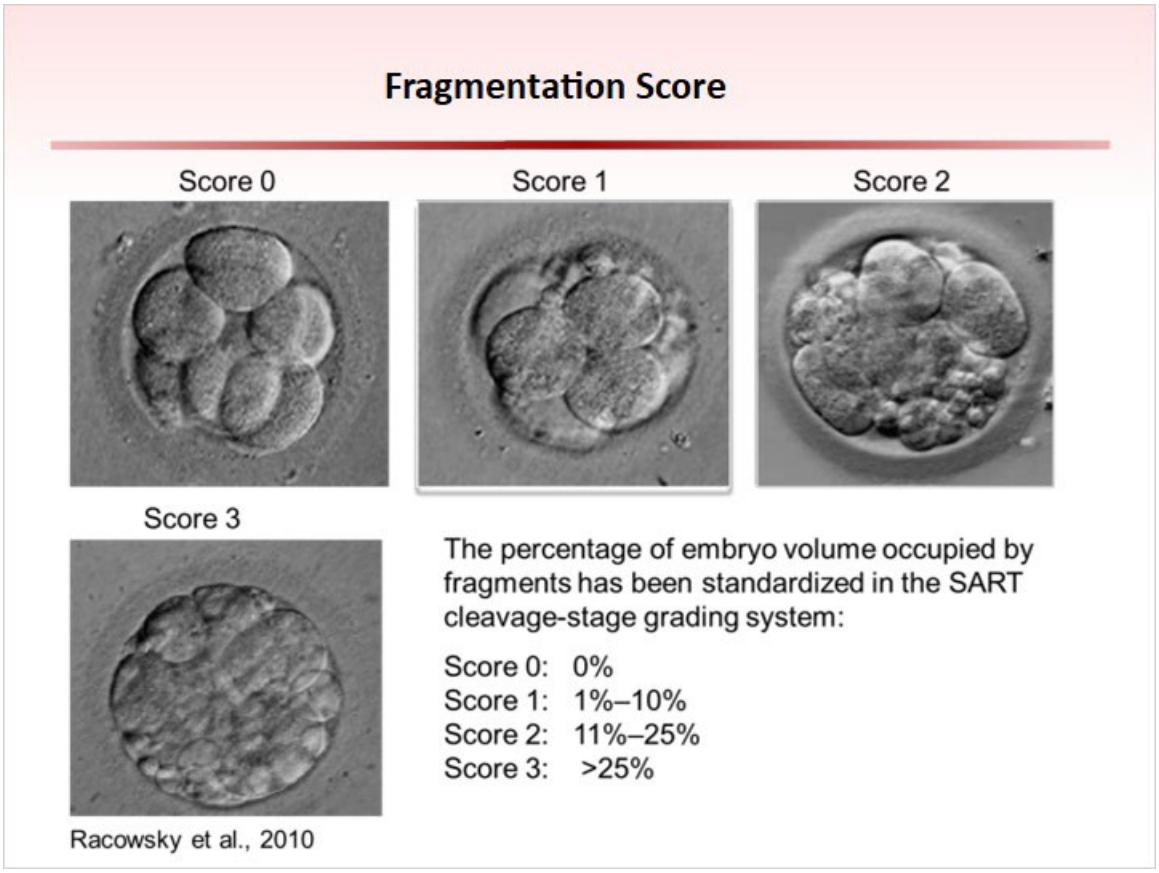
Notes:

The patterning (or disposition) of fragments among the blastomeres is classified in 5 types:

Type I:	Minimal in volume, associated with 1 blastomere
Type II:	Localized and predominantly occupying the PVS
Type III:	Small and scattered (in the cleavage cavity or peripherally positioned)
Type IV:	Large fragments, distributed randomly
Type V:	Necrotic and associated with granularity and cytoplasmic contraction

However, most systems describe the percentage of the overall volume of the embryo that is fragmented, rather than different patterns of fragmentation.

1.32 Fragmentation Score

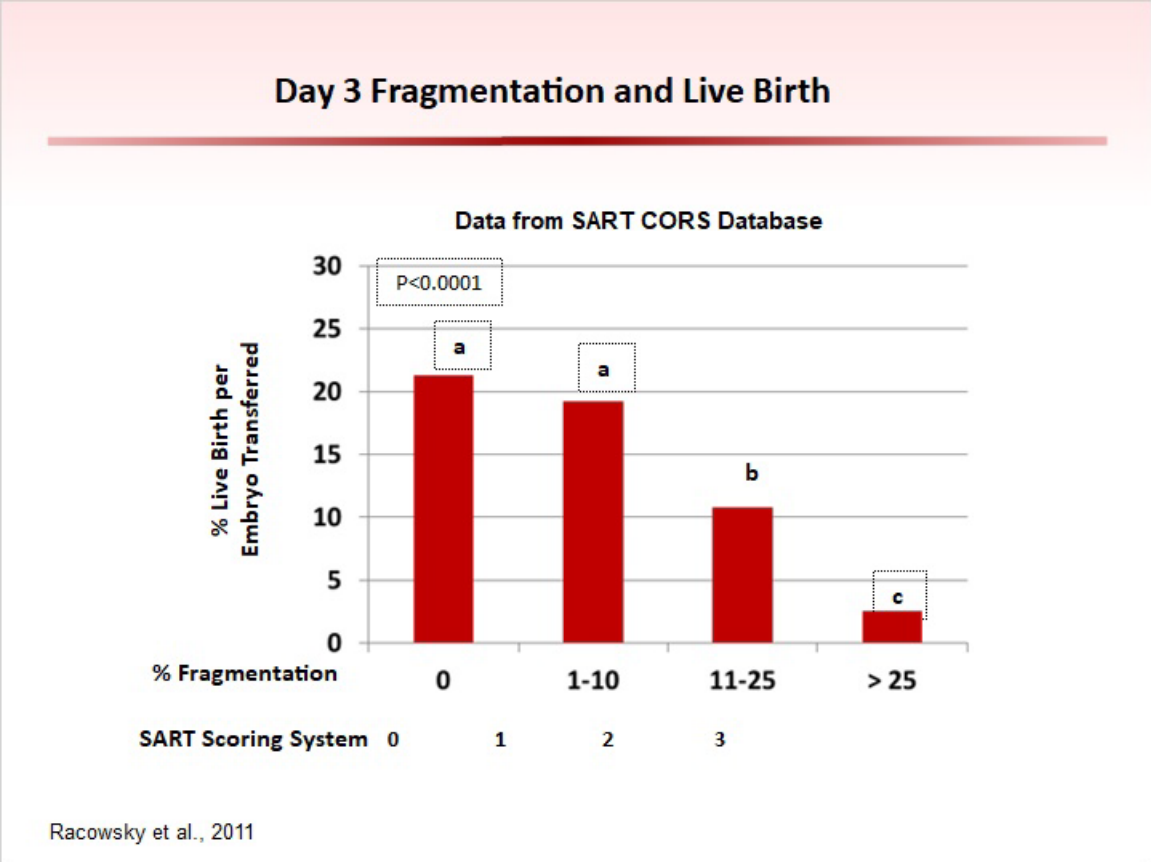


Notes:

Several different fragmentation scoring systems have been proposed that describe the percentage of the volume of the embryo that is fragmented; such a system for the evaluation of fragmentation on day 3 was standardized by SART in 2010.

The SART scoring is as follows:
Score 0: 0%
Score 1: 1%-10%
Score 2: 11%-25%
Score 3: >25%

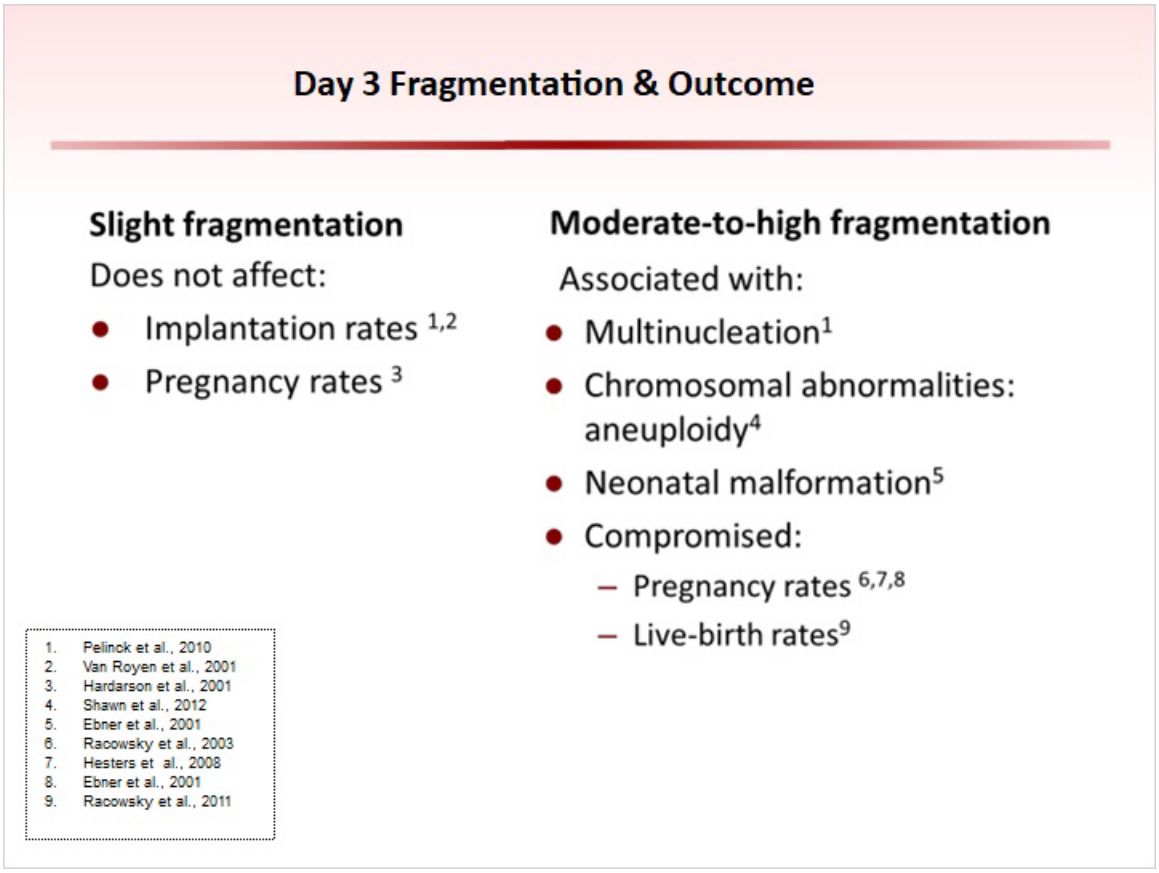
1.33 Day 3 Fragmentation and Live Birth



Notes:

While slight fragmentation (≤10%) does not affect the live-birth rate, there is a progressive decrease in live-birth rate with increasing percentage of fragmentation beyond 10%. These results were obtained from a study using national data reported into the SART CORS database.

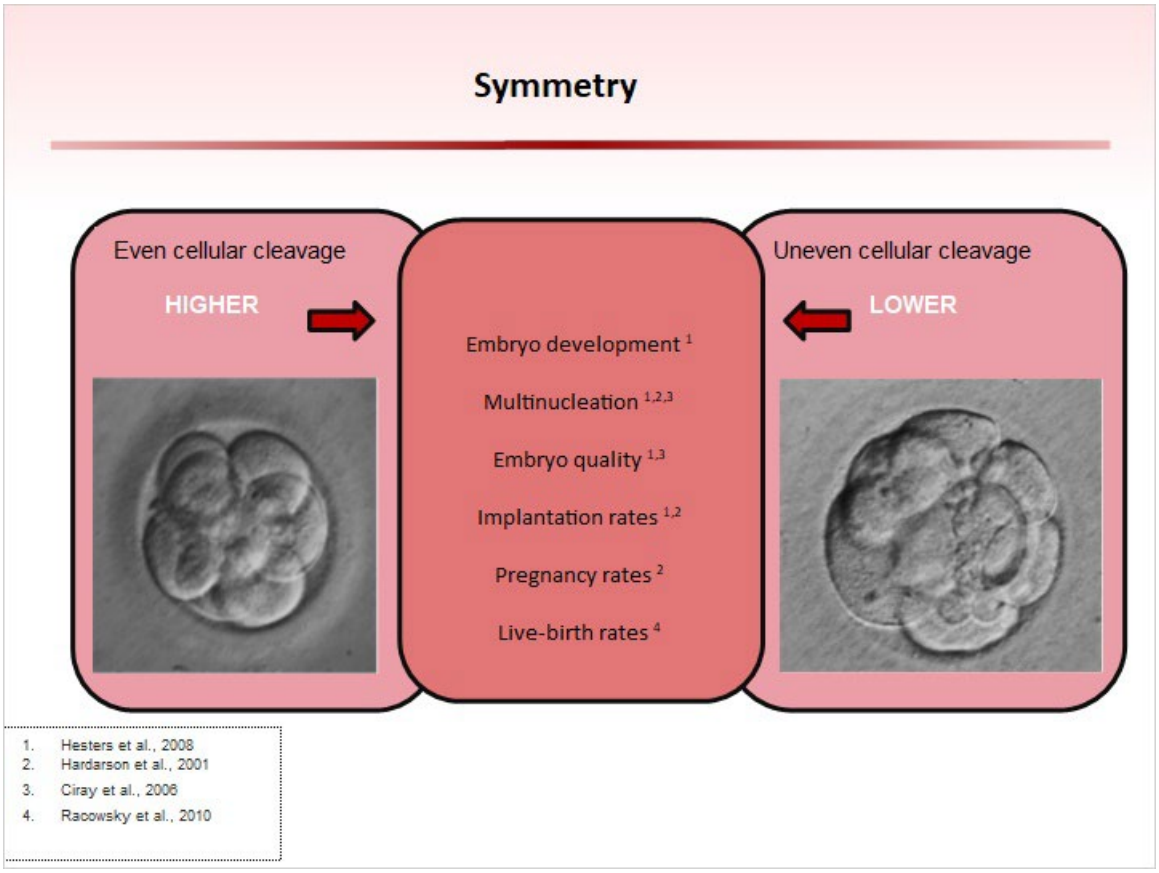
1.34 Day 3 Fragmentation & Outcome



Notes:

However, moderate-to-high fragmentation is associated with blastomere multinucleation and chromosomal abnormalities. An increased incidence of neonatal malformations as well as reduced pregnancy and live-birth rates have been reported when >50% fragmentation is present.

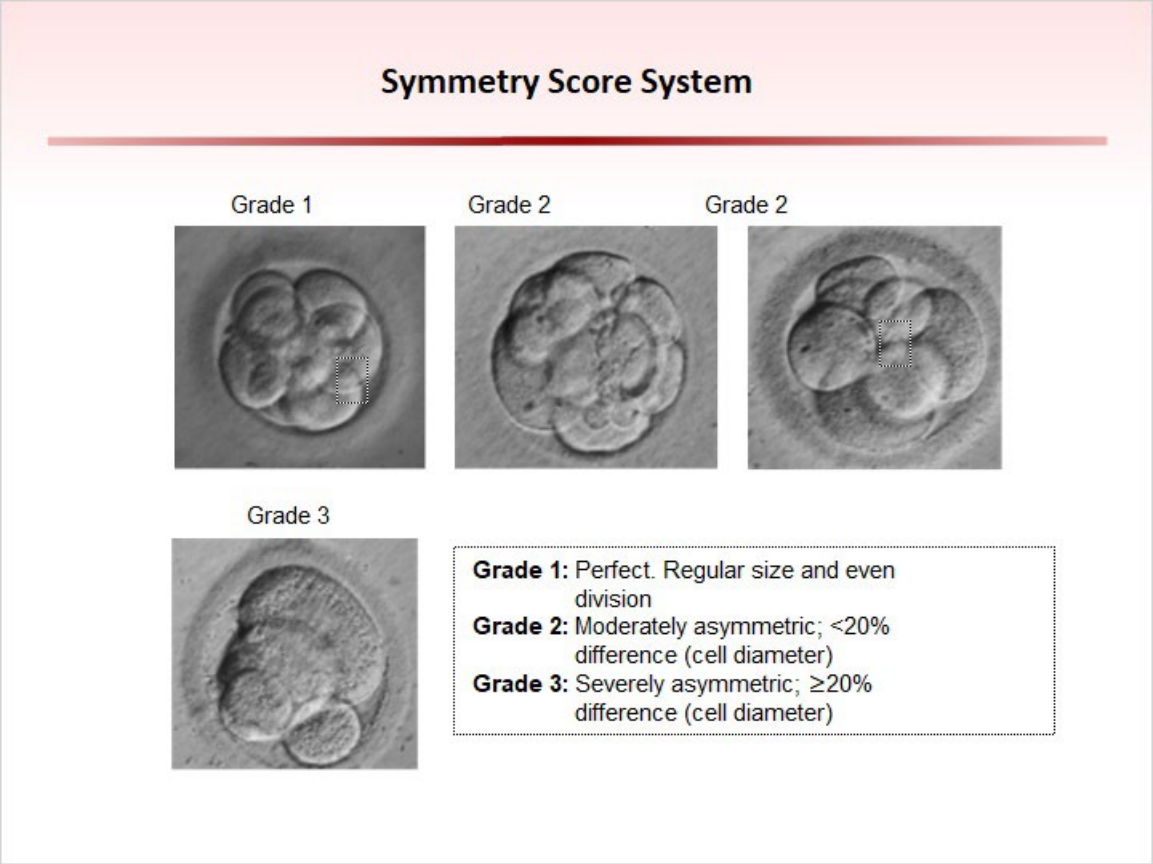
1.35 Symmetry



Notes:

Symmetry scoring typically describes assessment of the size and shape of the blastomeres relative to one another, rather than the overall shape of the embryo. Mitosis typically results in even cytokinesis, resulting in the formation of daughter blastomeres of similar size. However, when the blastomeres divide unequally, the distribution of proteins, mRNA, and organelles will not be homogeneous to the daughter cells. This likely induces a loss of essential molecules to blastomeres, with a negative effect on subsequent cell divisions, increased probability of multinucleation, and a compromise to development and implantation.

1.36 Symmetry Score System

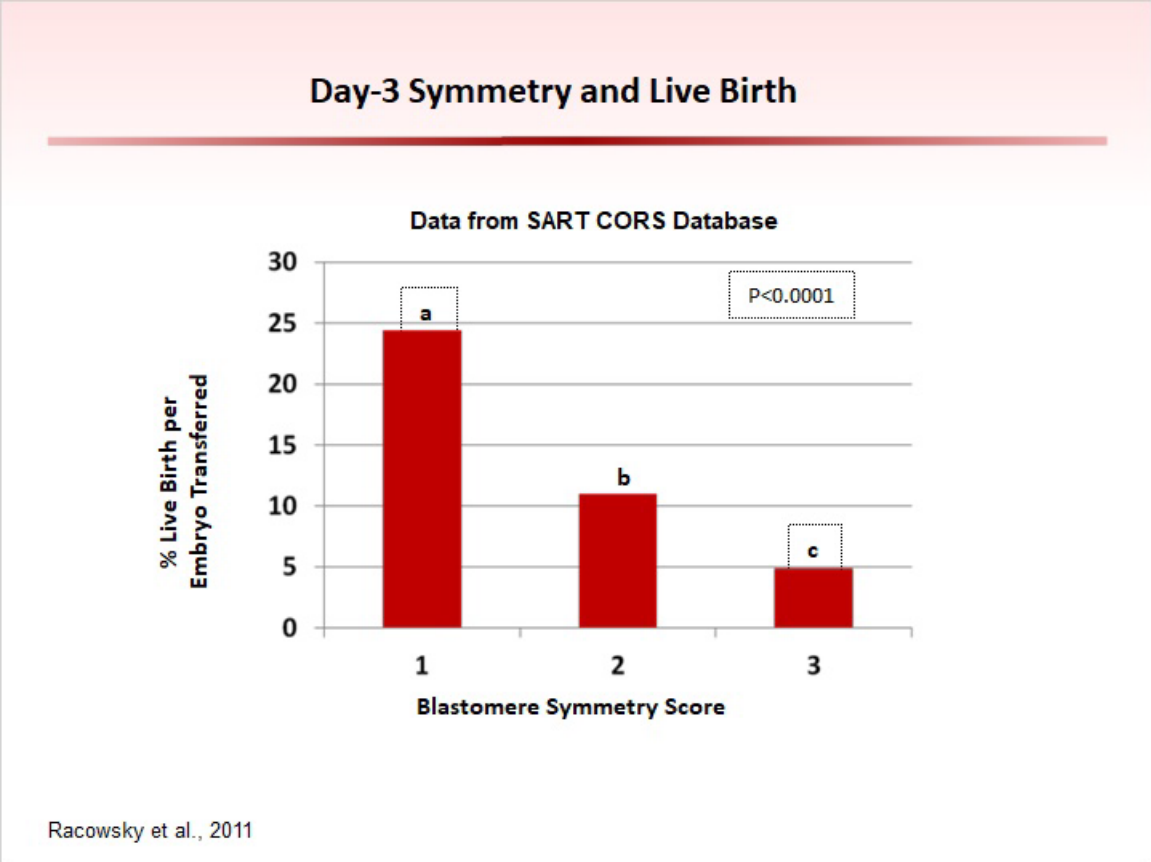


Notes:

A typical scoring system for blastomere symmetry is as follows:

Grade 1: Perfect. Regular size and even division
Grade 2: Moderately Asymmetric; <20% difference in blastomere cell diameters
Grade 3: Severely Asymmetric; ≥20% difference in blastomere cell diameters

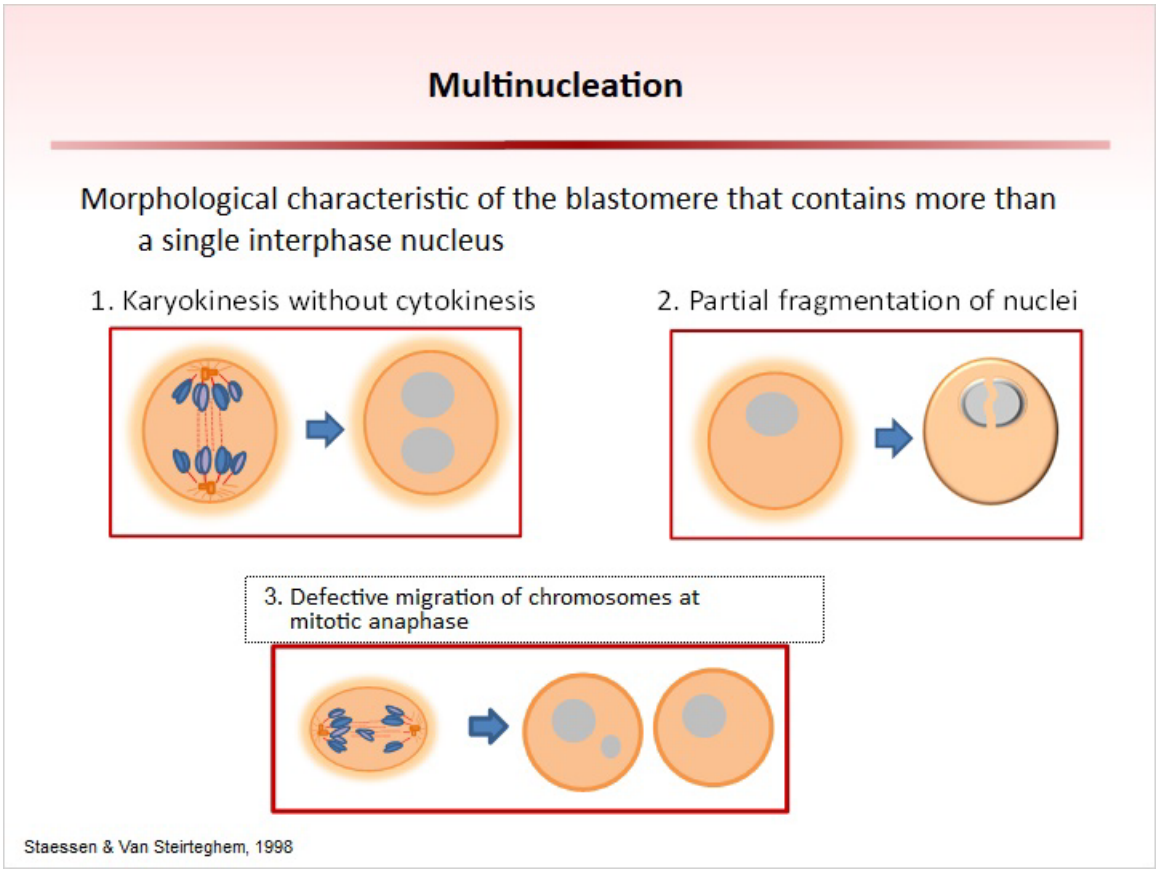
1.37 Day-3 Symmetry and Live Birth



Notes:

As shown in this slide, analysis of data obtained from the SART CORS database shows that live-birth rate is significantly associated with the extent of blastomere symmetry in day-3 embryos when this standardized system for the evaluation of symmetry on day 3 was applied.

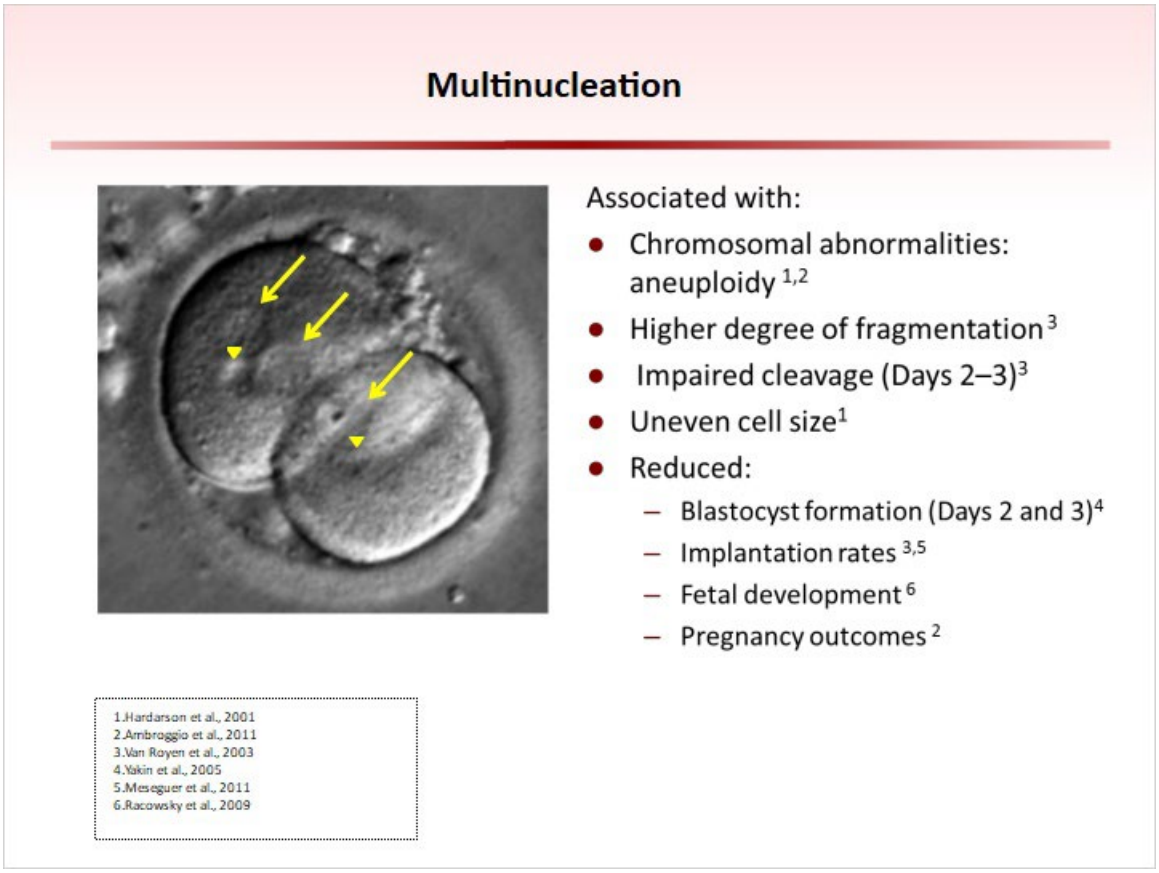
1.38 Multinucleation



Notes:

Multinucleation is defined as the presence of more than one interphase nucleus in a blastomere. Three mechanisms have been proposed for the formation of multinucleated blastomeres, all of which have been associated with chromosome aberrations: karyokinesis without cytokinesis, partial fragmentation of nuclei, and defective migration of chromosomes at mitotic anaphase.

1.39 Multinucleation



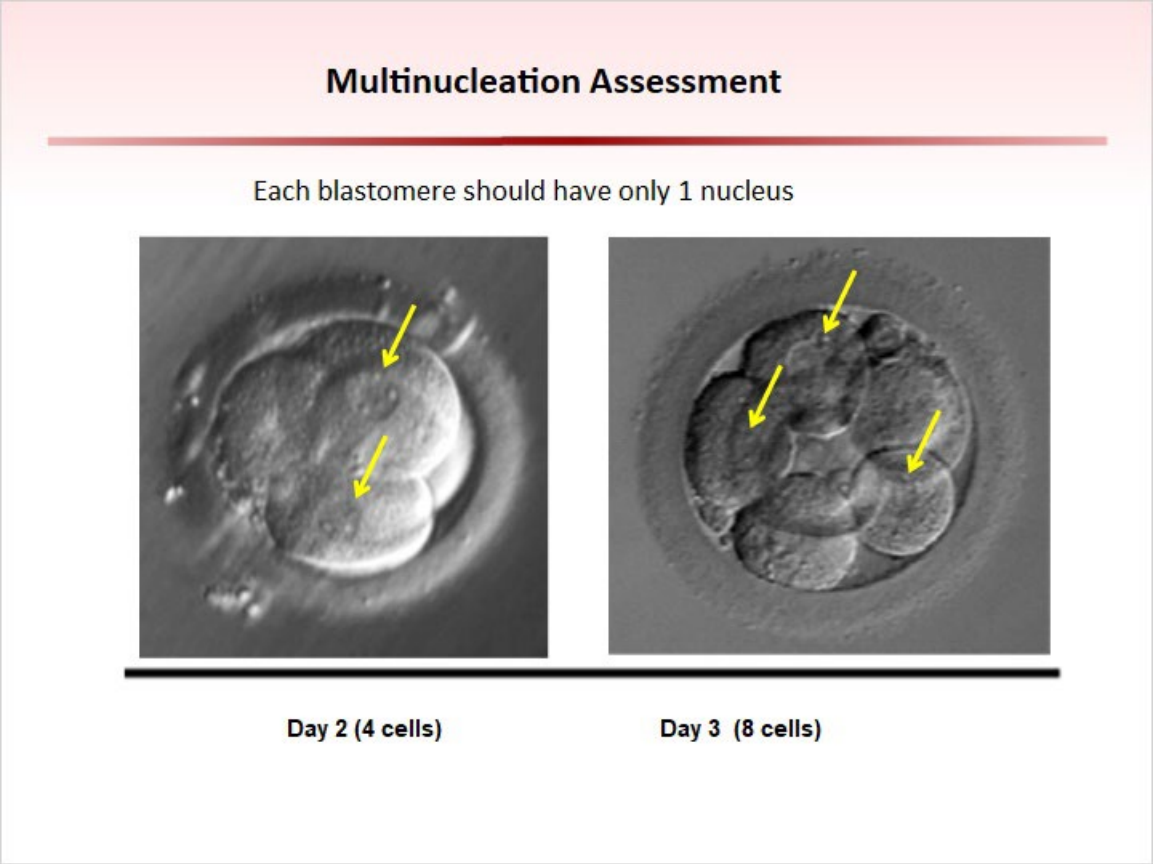
Notes:

Multinucleation is associated with higher rates of aneuploidy, as confirmed by preimplantation genetic screening. Moreover, there is a relationship between high multinucleation and high fragmentation. The same is true is for cleavage rate and asymmetry, as multinucleation is associated with less or more blastomeres than the optimal number, as well as an increased likelihood of asymmetric blastomeres.

All cleavage embryos should be evaluated for multinucleation as this abnormality is associated with compromised embryo quality, implantation rates, fetal development, and pregnancy rates.

In the embryo shown, the blastomere on the left has two nuclei (arrows) and a micronucleus (arrowhead), while that on the right has only one nucleus (arrow) and a micronucleus (arrowhead).

1.40 Multinucleation Assessment

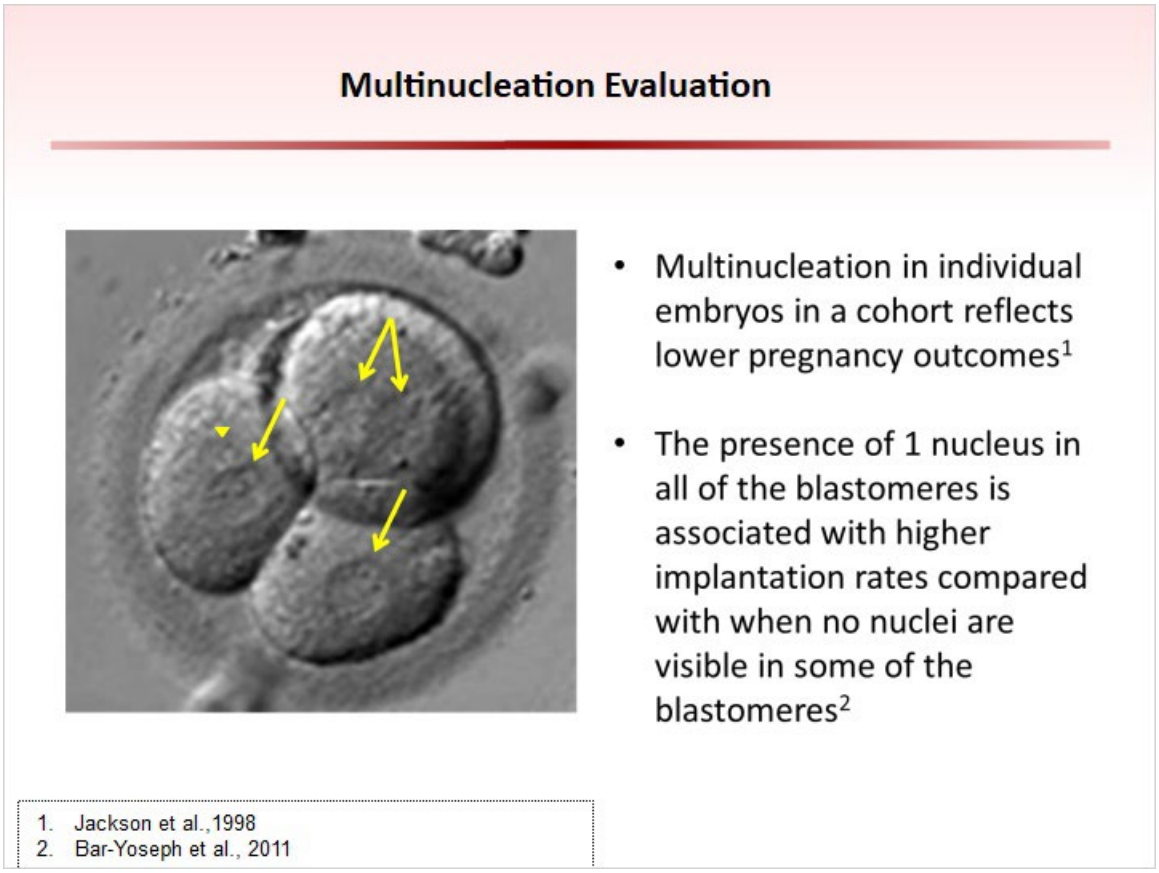


Notes:

The multinucleation assessment should be performed on day 2 (44±1 hour post-insemination) rather than on day 3, as it is frequently difficult to visualize nuclei in embryos with higher numbers of cells. Even multinucleation in only 1 blastomere is sufficient for the embryo to be considered to be multinucleated.

Both embryos shown here each have blastomeres with only a single visible nucleus (arrows).

1.41 Multinucleation Evaluation



Notes:

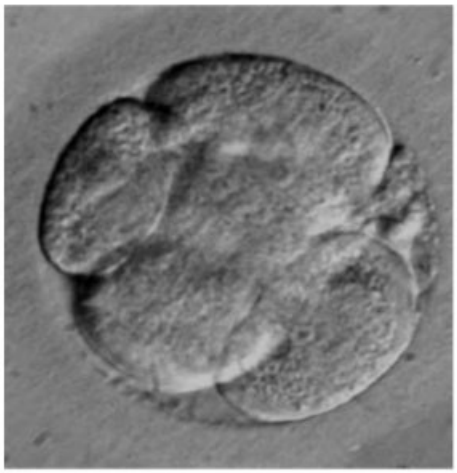
Multinucleation in any embryo in a cohort is associated with reduced implantation rates, even if the multinucleated embryo(s) is not transferred. Moreover, the presence of 1 nucleus in all of the blastomeres also reflects higher implantation rates compared with when no nuclei are visible in some of the blastomeres.

In the embryo shown here, the top right blastomere has 2 nuclei (arrows), the bottom blastomere has only 1 nucleus, and the blastomere on the left has a micronucleus (arrowhead) in addition to a more normal-sized nucleus.

1.42 Cytoplasmic Granularity

Cytoplasmic Granularity

The presence of cytoplasmic granularity on day 3 is considered a sign of activation of the embryo genome.



However, it does not appear to be correlated with:

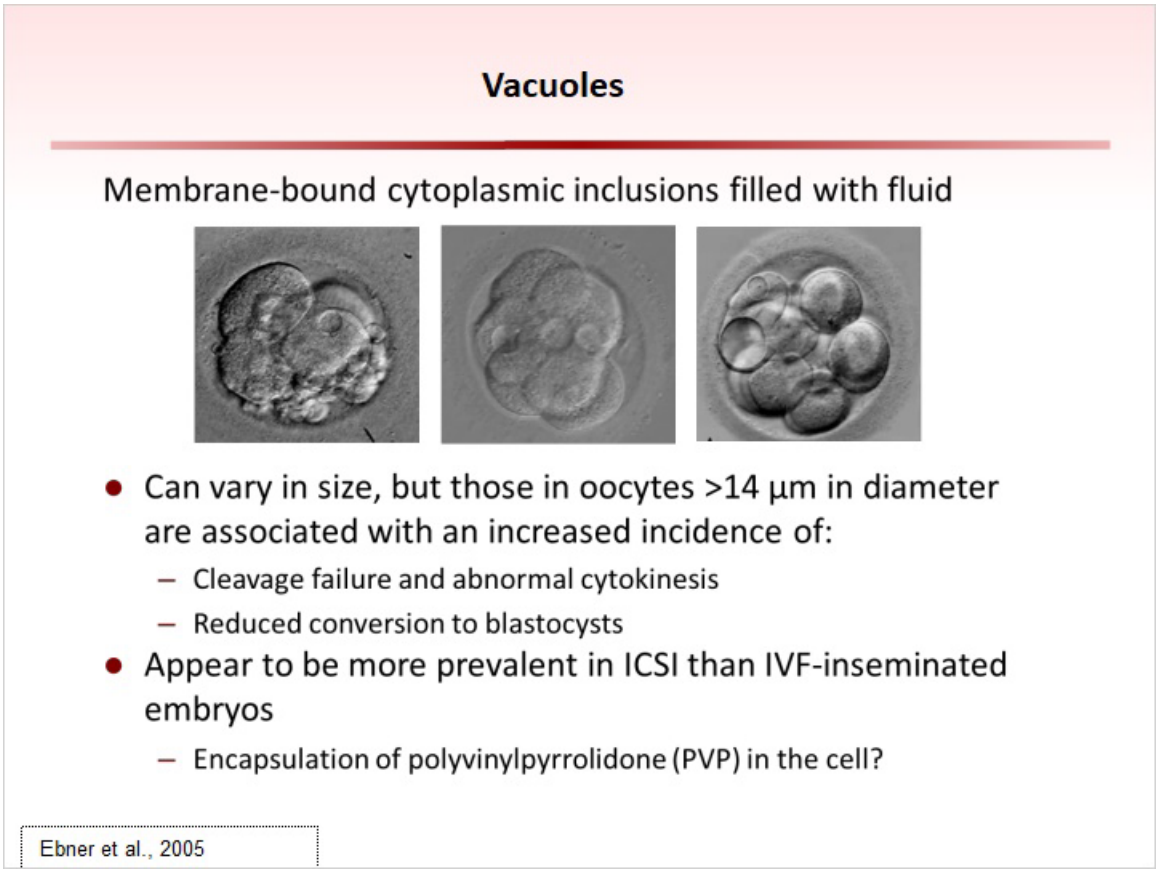
- Embryo quality[1]
- Pregnancy outcomes[2]

1. Rienzi et al., 2003
2. Desai et al., 2000

Notes:

The presence of cytoplasmic granularity on day 3 is considered as a sign of activation of the embryo genome as transcriptional activity is increased. This phenomenon has been associated with embryos most likely to continue to develop. However, the few studies investigating this characteristic have not shown any association between granularity and either overall embryo quality or pregnancy outcomes.

1.43 Vacuoles

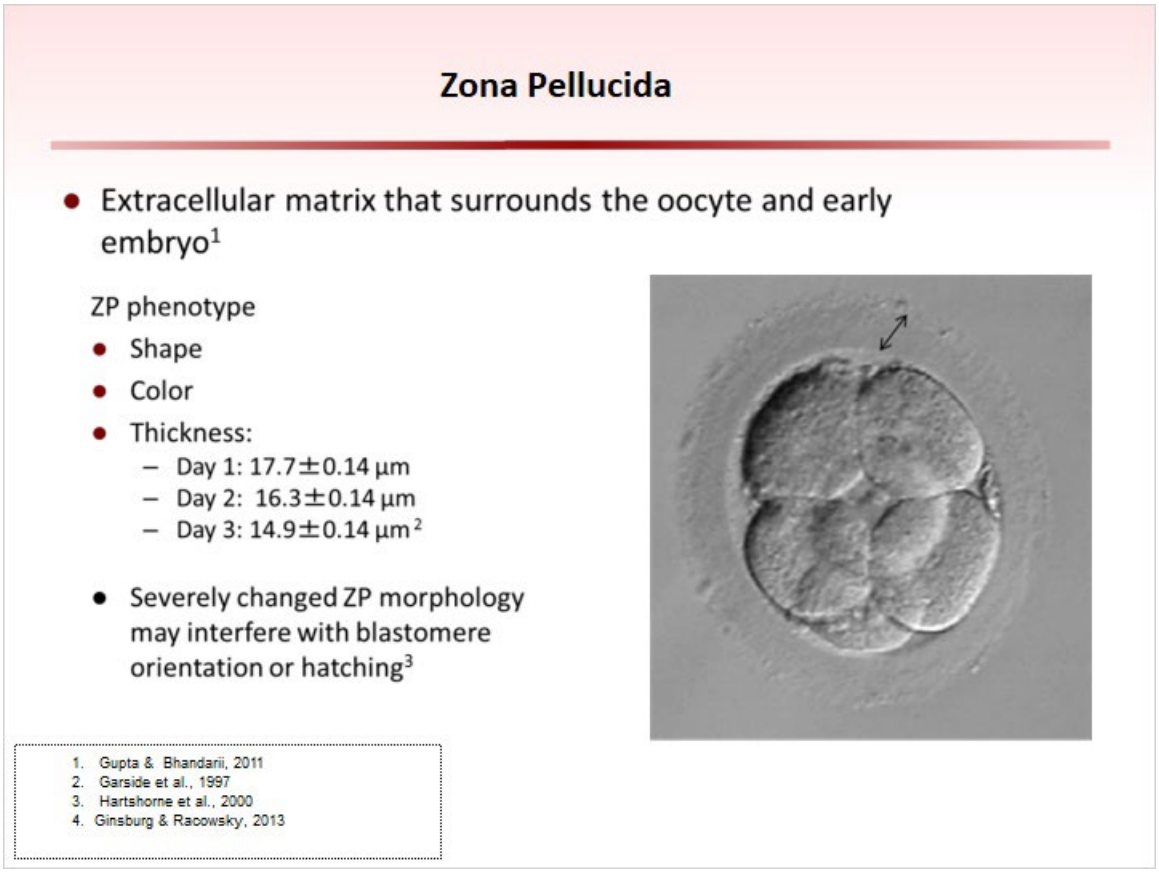


Notes:

Vacuoles are membrane-bound cytoplasmic inclusions filled with fluid that is virtually identical to perivitelline fluid. Vacuoles can vary both in size and number. Vacuolization is probably the most apparent and dynamic cytoplasmic dysmorphism in human oocytes. The presence of vacuoles in oocytes that are >14 µm of diameter is associated with cleavage failure or abnormal cytokinesis during early preimplantation development.

Limited data suggest that the later the vacuoles emerge during early development, the more negative the effect on blastocyst formation. Moreover, significantly fewer IVF-inseminated zygotes have vacuoles compared with those from ICSI. This result suggests a direct influence of the ICSI procedure (when injecting the sperm) that could lead to an encapsulation of PVP in the cell.

1.44 Zona Pellucida

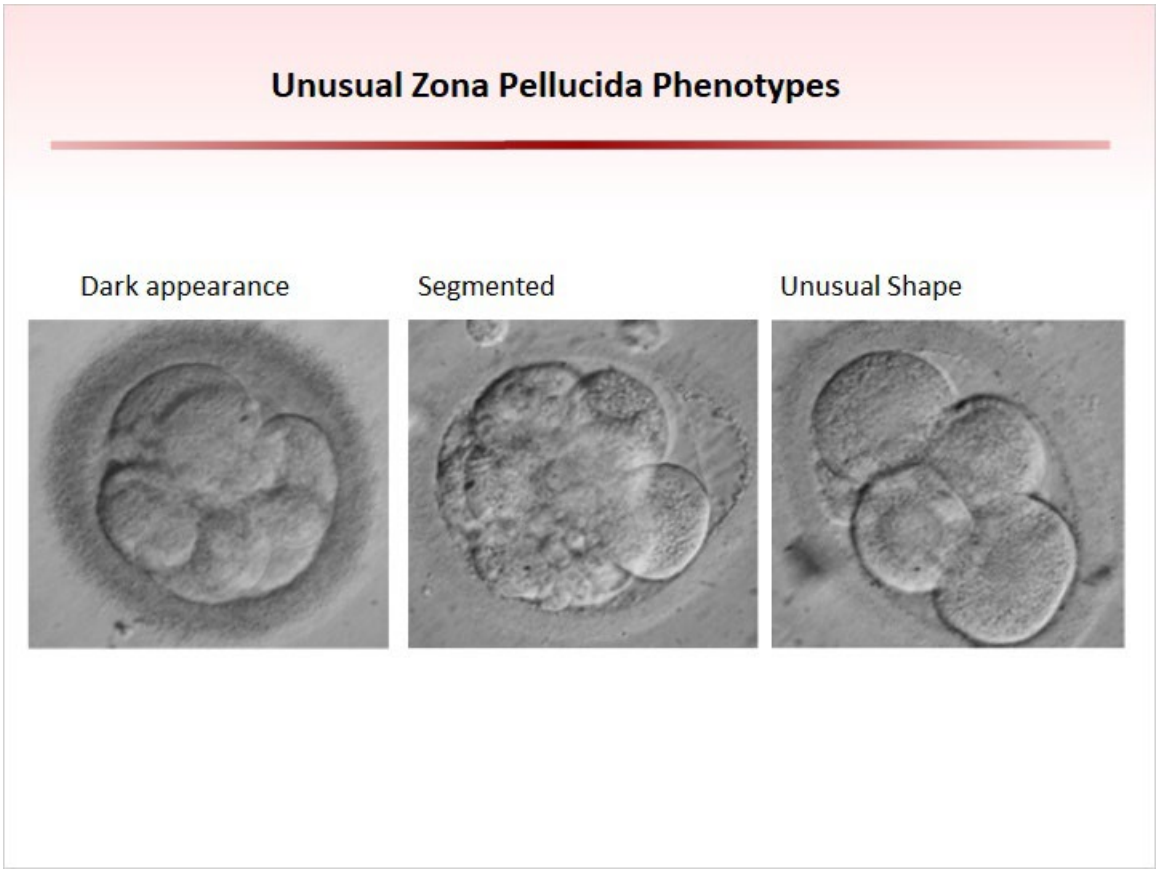


Notes:

The zona pellucida (ZP) is an extracellular matrix surrounding the oocyte and early embryo that is composed of 4 glycoproteins (ZP1, ZP2, ZP3, and ZP4). Unusual forms of the zona pellucida can include different shapes, colors, and thicknesses.

The average zona thickness on day 1 is 17.7±0.14 µm, 16.3±0.14 µm on day 2, and 14.9±0.14 µm on day 3.

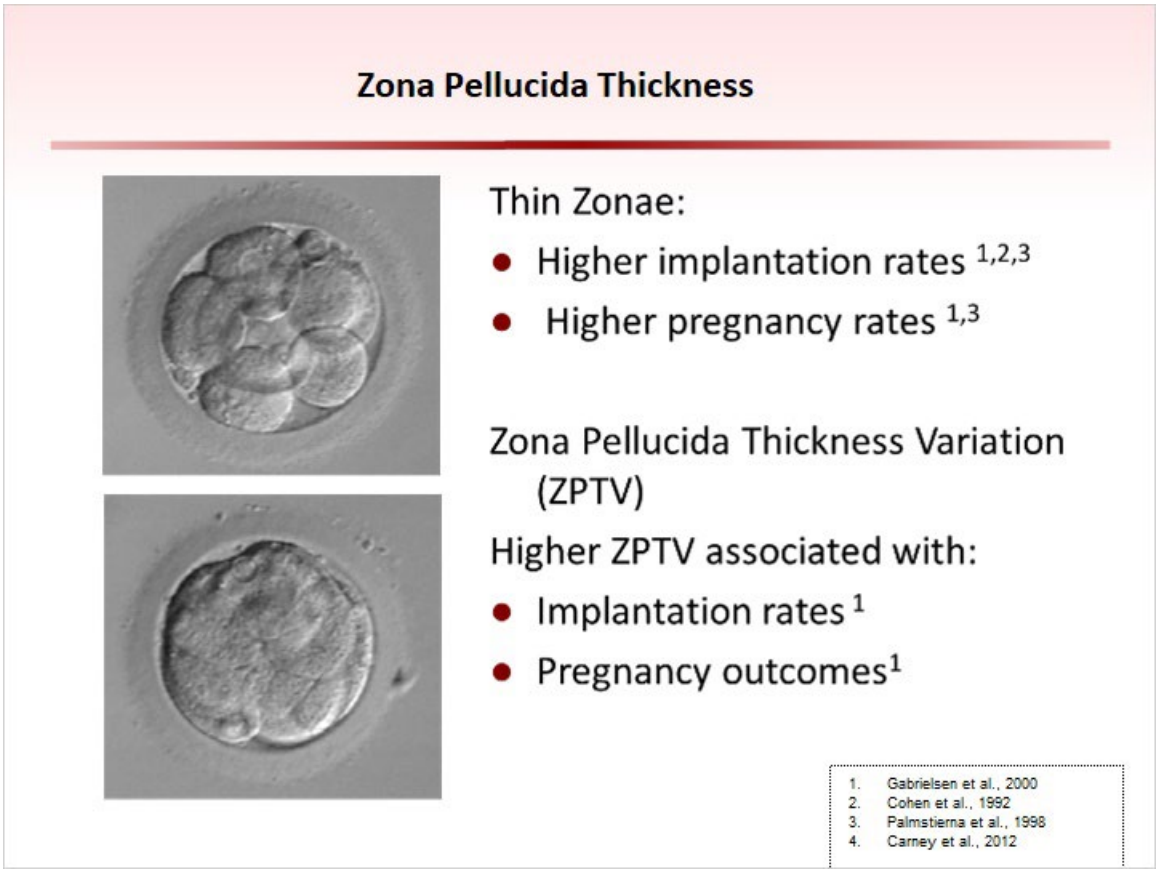
1.45 Unusual Zona Pellucida Phenotypes



Notes:

Unusual forms of zona pellucidae are shown here.

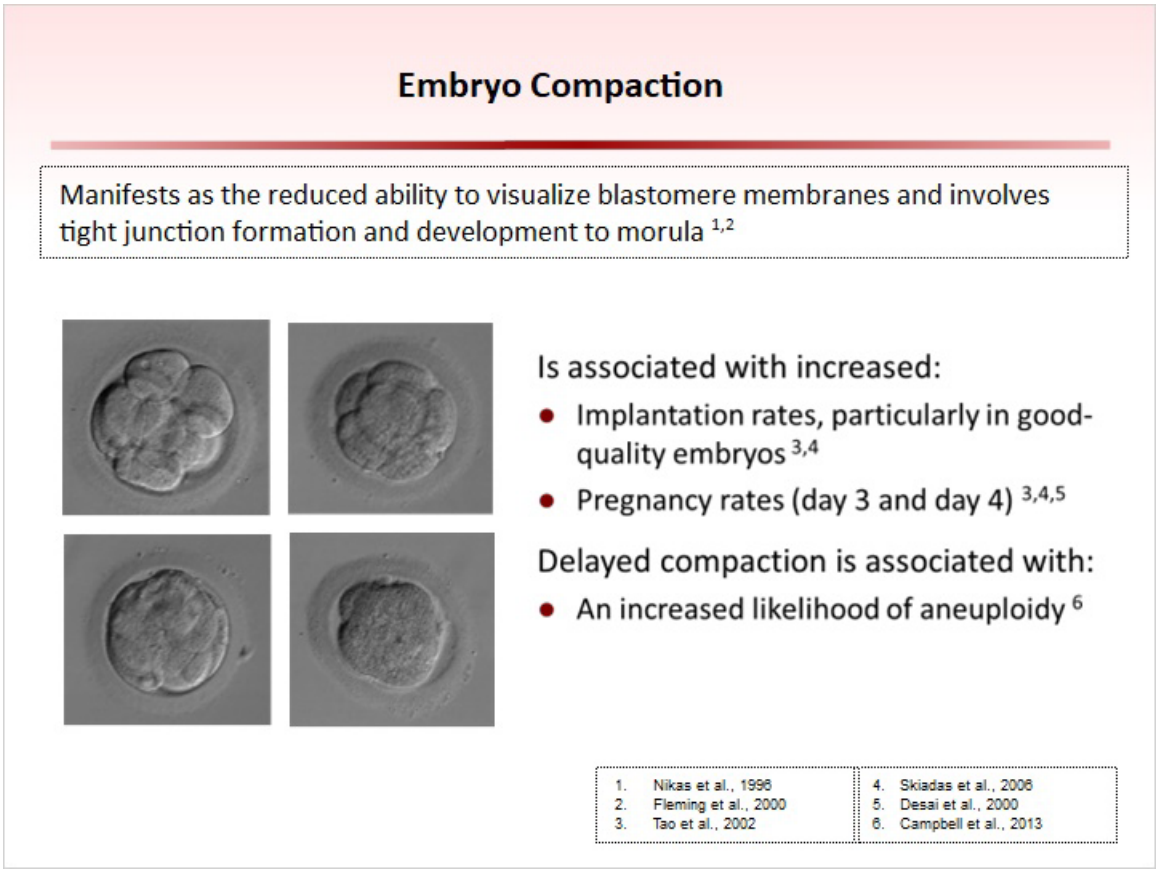
1.46 Zona Pellucida Thickness



Notes:

The zona pellucida thickness has been suggested to be a strong predictor of implantation and pregnancy, and so should be a useful tool to determine when assisted hatching (AH) should be performed. In fact, embryos with a thinner zona and increased variation in the thickness have higher implantation and pregnancy rates. However, a 2012 Cochrane Review (Carney et al., 2012) concluded that there is only moderate-quality evidence that AH increases the chance of clinical pregnancy (odds ratio 1.13, 95% confidence interval 1.01 to 1.27), and that the take-home baby rate was not shown to be increased by AH. Of note, most of the trials included in this review did not report on live-birth rates. Overall, it can be concluded that AH of cleavage-stage embryos may improve clinical outcome in select patient populations, but that each program should establish its own criteria for use of AH.

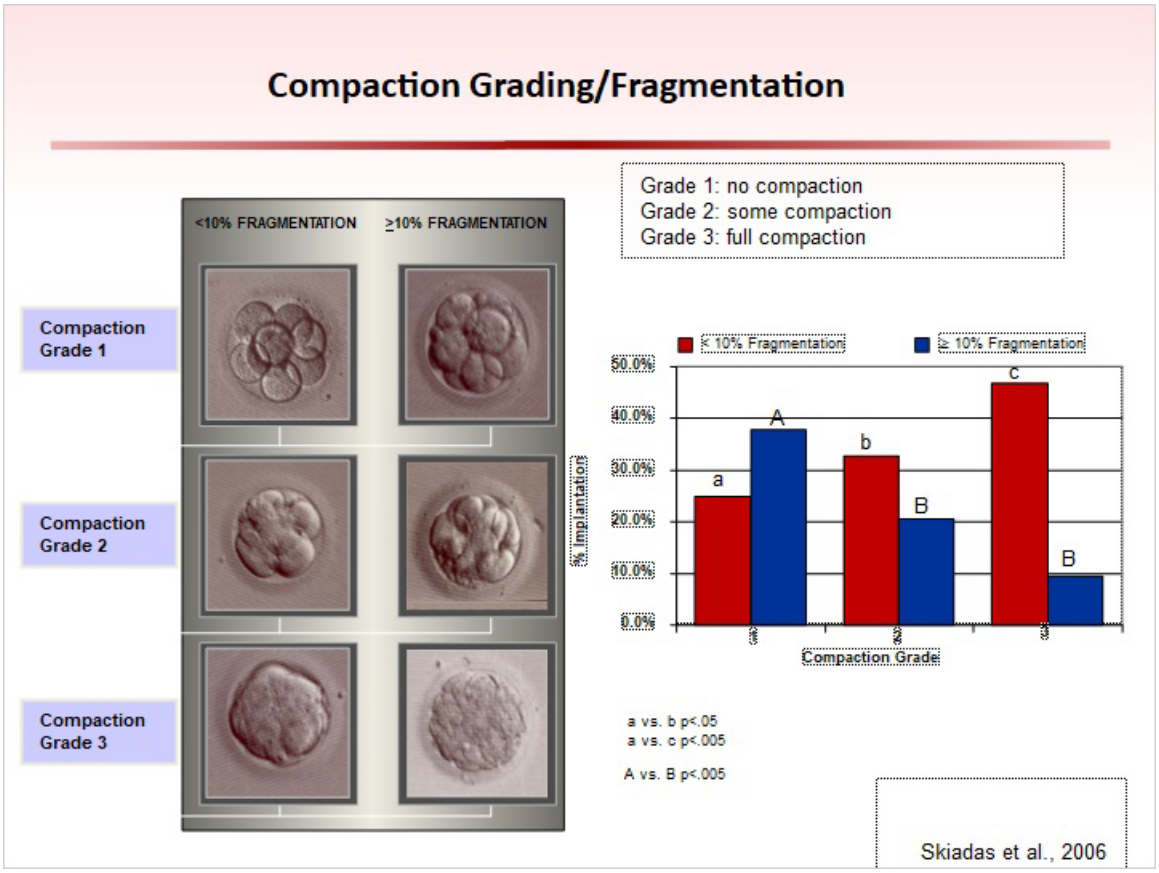
1.47 Embryo Compaction



Notes:

Compaction is the process during which tight junctions form between the blastomeres, and manifests by reduced visualization of blastomere membranes. This process usually occurs late on day 3/early on day 4, resulting in decreased resolution of the blastomeres, which ultimately leads to formation of the morula. Its presence on day 4 is generally considered a good prognostic indicator of embryo development and, when delayed, is associated with aneuploid embryos.

1.48 Compaction Grading/Fragmentation



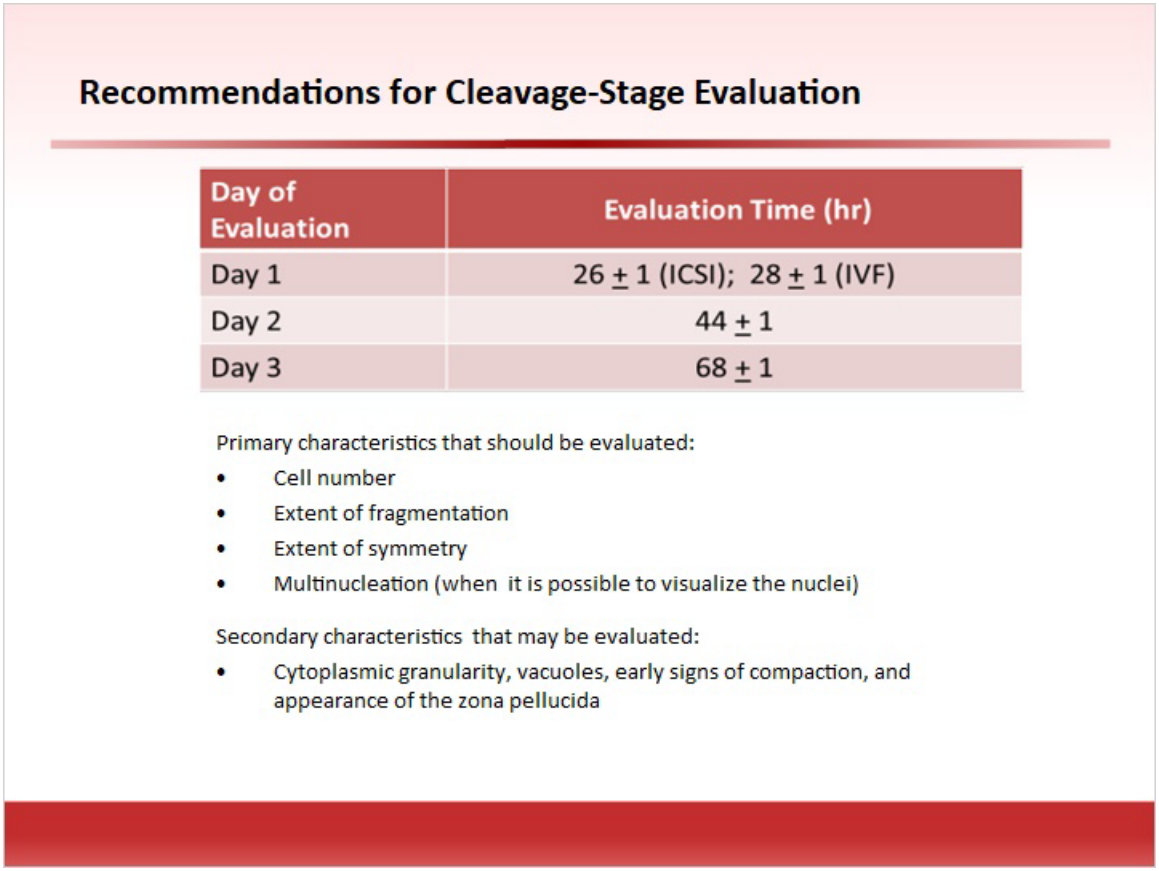
Notes:

Skiadas et al. (2006) established a system for grading compaction on day 3:
Grade 1: no compaction, i.e., no evidence of membrane fusion
Grade 2: some compaction, i.e., some blastomeres showing evidence of membrane fusion but the number of blastomeres are easily countable
Grade 3: full compaction, i.e., all blastomeres compacted with no ability to count the number of blastomeres present

Based on a retrospective study, these investigators showed a positive association between compaction grade and implantation rate when embryos had <10% fragmentation (red bars in figure), while the converse was observed for embryos with higher fragmentation (≥10% fragmentation; blue bars).

These results suggest that the relationship between degree of compaction and implantation potential is influenced by the extent of fragmentation and that compaction grading on day 3 may represent an additional tool in embryo assessment for purposes of embryo selection on day 3. However, further studies are required to confirm this possibility.

1.49 Recommendations for Cleavage-Stage Evaluation



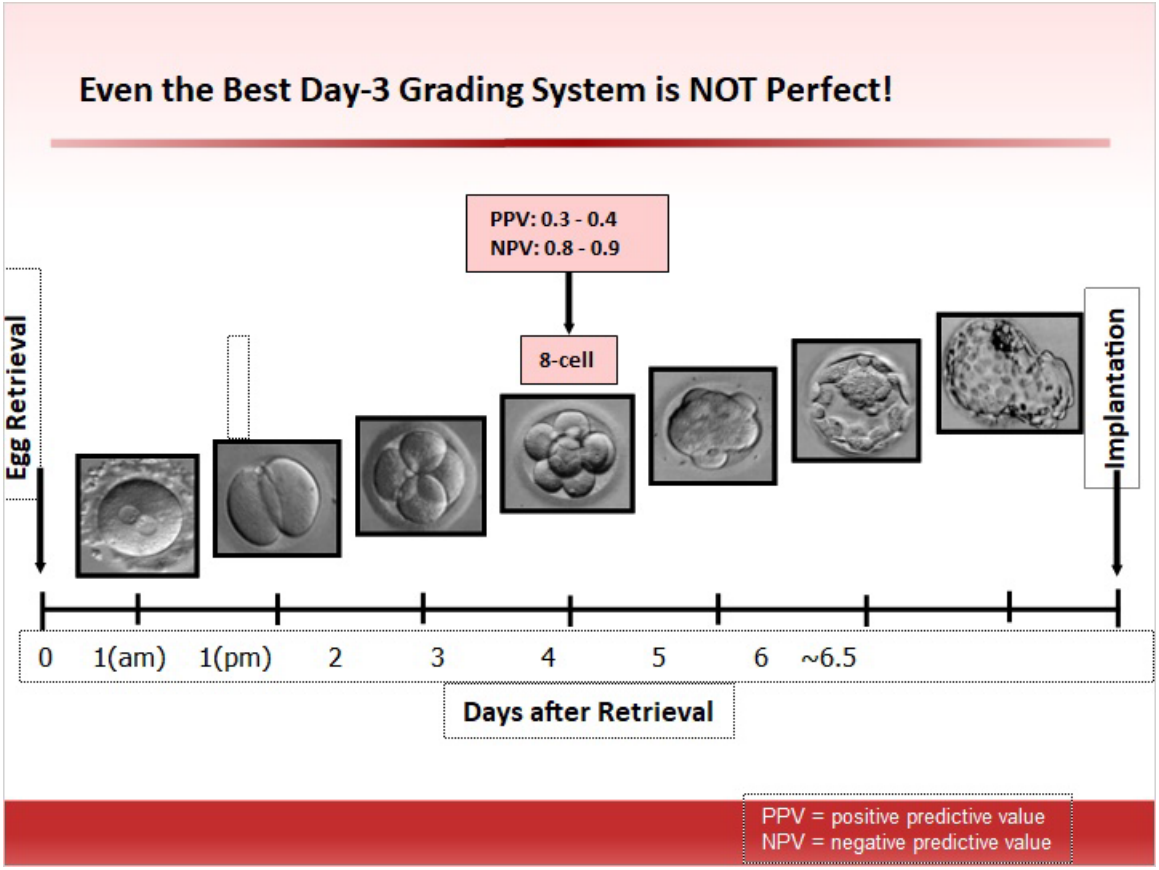
Notes:

Due to the kinetics of early development, evaluations should be performed within tight time windows after insemination/ICSI.

The ideal evaluation times shown here are those at which a normally developing human embryo is most likely to be at: the 2-cell stage on day 1 (“early cleavage”); the 4-cell stage on day 2; and the 8-cell stage on day 3.

In addition to the number of cells present, fragmentation, blastomere symmetry, and multinucleation, all have been associated with implantation potential. If choosing between 2 embryos with identical grades for these features, secondary characteristics, such as the presence or absence of cytoplasmic granularity, vacuoles and early signs of compaction, and appearance of the zona pellucida may also be considered.

1.50 Even the Best Day-3 Grading System is NOT Perfect!



Notes:

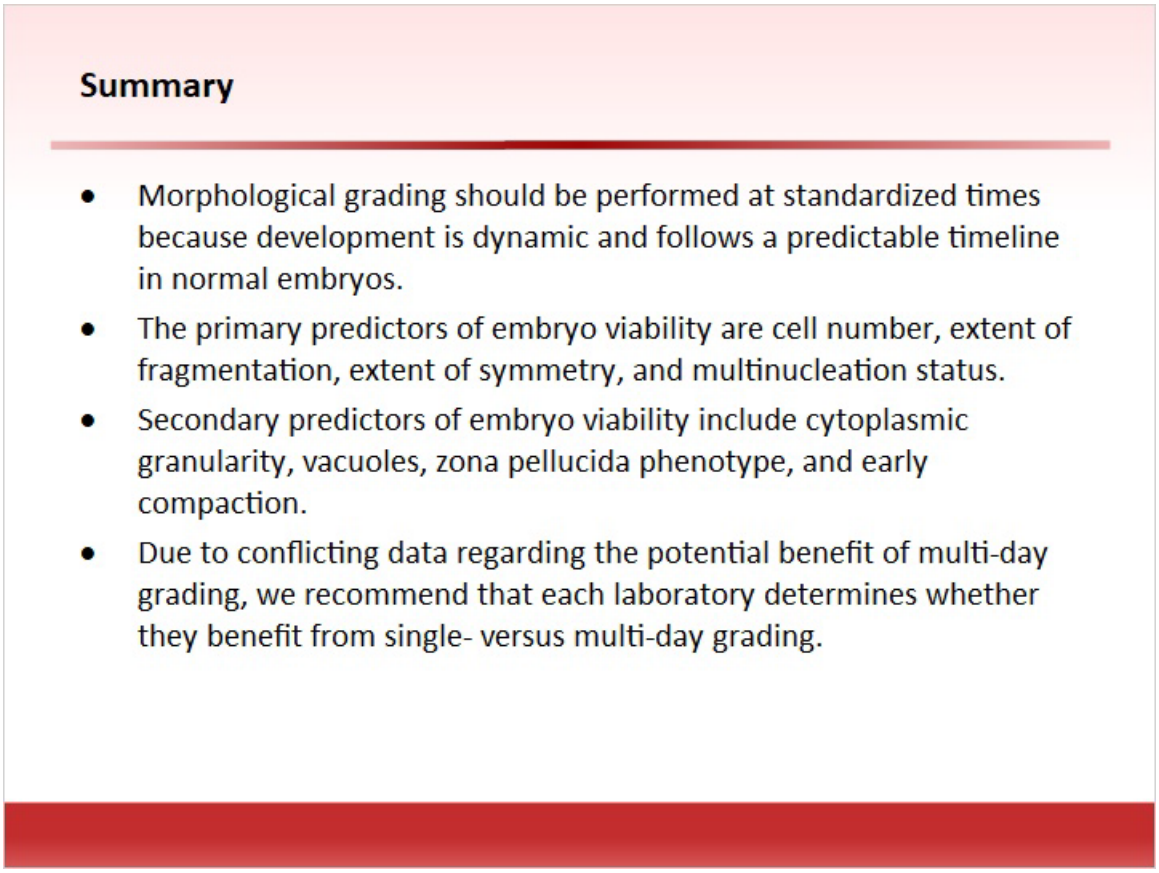
When applying these recommendations, numerous studies have shown that even the most sophisticated cleavage-stage grading systems result in only modest positive predictive values (PPV) for implantation (in the range of 0.3-0.4), in contrast to very reasonable negative predictive values (NPVs; in the range of 0.8-0.9). Remember that the PPV and NPV describe the diagnostic performance of a test. In this case, the modest PPV indicates that standard morphological methods for day-3 grading are only modestly good at predicting which embryos will implant; however, the high NPV indicates that these standard methods are good at predicting which embryos will not implant.

Because of the low PPV of most standard methods for grading cleavage-stage embryos, studies have been undertaken to determine whether grading embryos on consecutive days (i.e., multi-day grading) enables improved selection of the “best” embryo(s) compared with embryo assessment on just a single day, immediately before transfer.

Conflicting data exist in support of multi-day grading, which may be due to many factors

including variations in patient populations, inconsistencies as to how embryos are graded among embryologists on a team, how strictly the times for embryo evaluations are standardized, and how rapidly different incubator models re-equilibrate after being opened. Due to these conflicting data, it is not possible to make any recommendation regarding single- versus multi-day grading for cleavage-stage embryos. However, ongoing prospective studies assessing the efficacy of new time-lapse imaging algorithms may shed light on the utility of multiple-image analyses for embryo evaluation and selection.

1.51 Summary

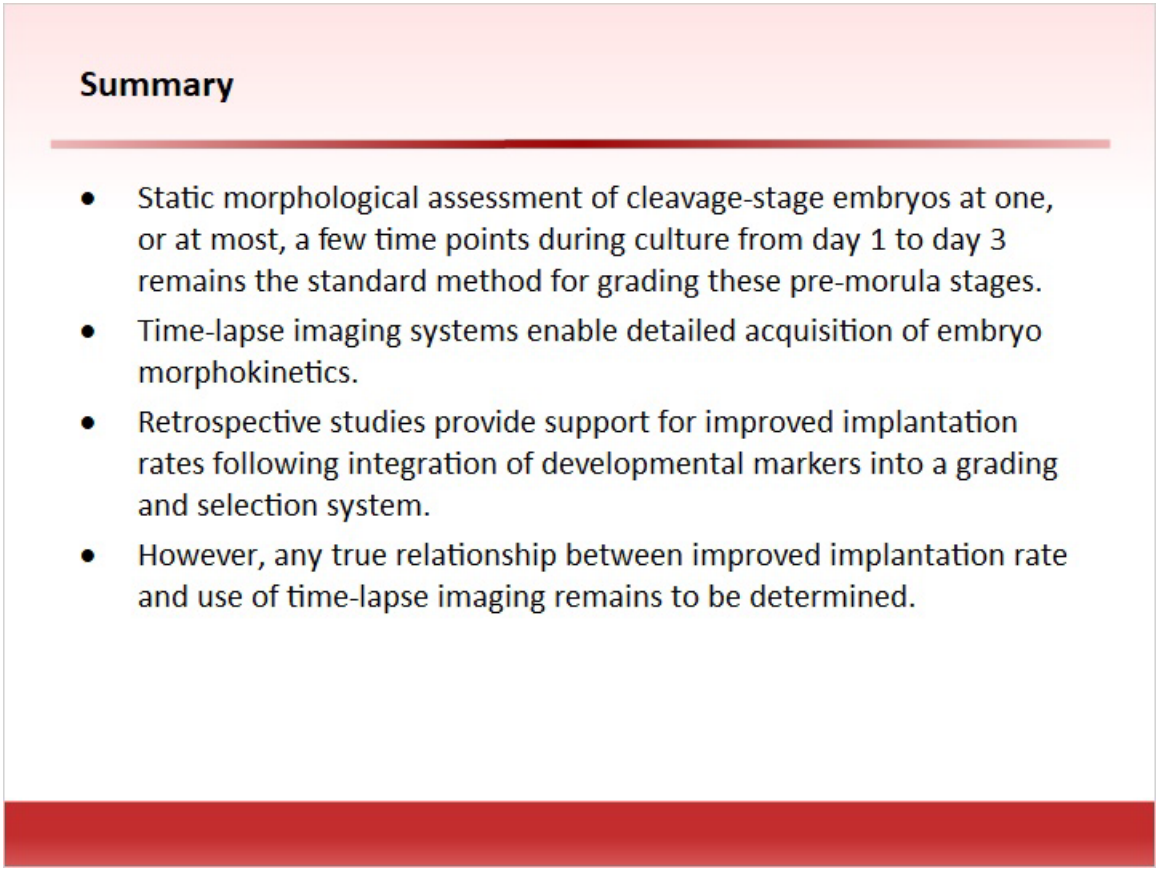


Notes:

In summary, morphological grading should be performed at standardized times because development is dynamic and follows a predictable timeline in normal embryos. The primary predictors of embryo viability are cell number, extent of fragmentation, extent of symmetry, and multinucleation status. Secondary predictors of embryo viability include cytoplasmic granularity, vacuoles, zona pellucida phenotype, and early compaction. Due to conflicting data regarding the potential benefit of multi-day grading,

each laboratory should determine whether there is benefit from single- versus multi-day grading.

1.52 Summary



Notes:

Static morphological assessment of cleavage-stage embryos at one, or at most, a few time points during culture from day 1 to day 3 remains the standard method for grading these pre-morula stages. Time-lapse imaging systems enable detailed acquisition of embryo morphokinetics. Retrospective studies provide support for improved implantation rates following integration of developmental markers into a grading and selection system. However, any true relationship between improved implantation rate and use of time-lapse imaging remains to be determined.

1.53 Thank you!

Notes:

Thank you for participating in this educational activity.