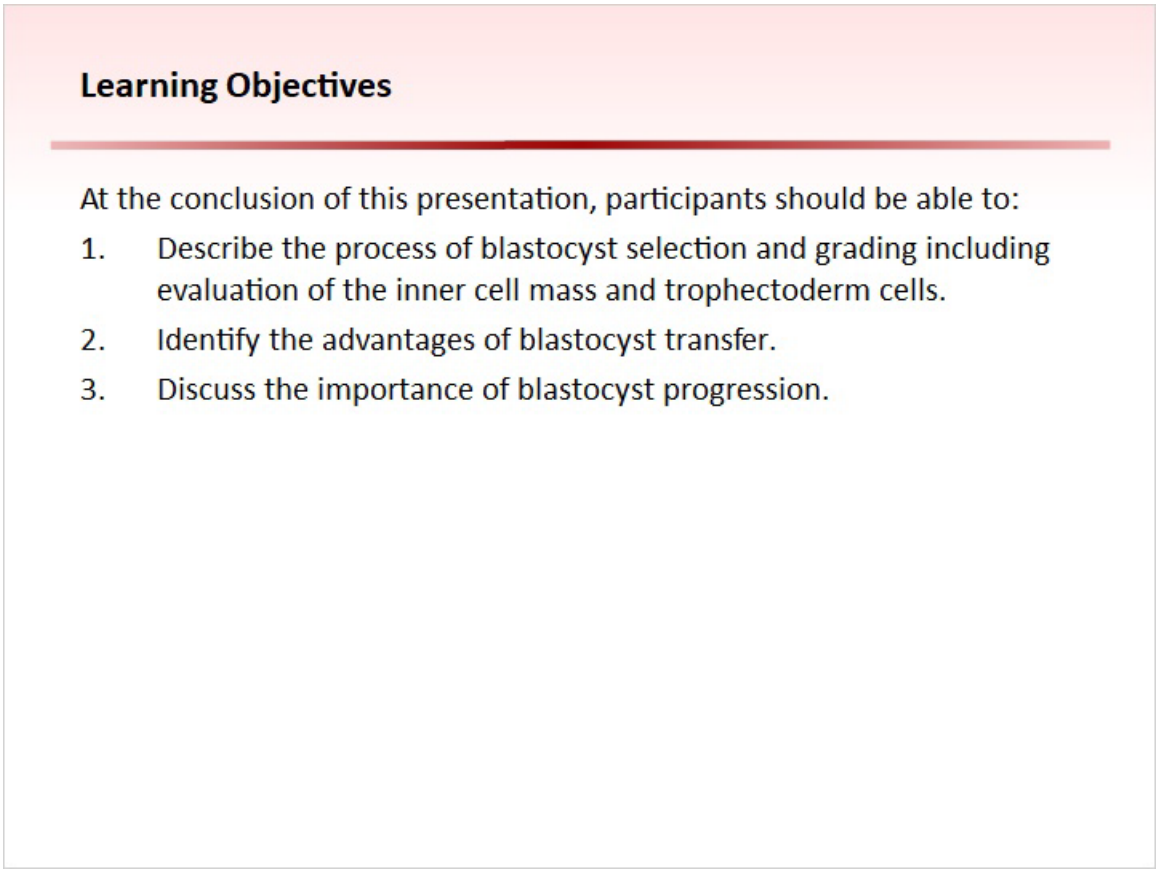
LABCC100 Lesson 21

1.1 Blastocyst Development and Grading

Notes:

Welcome to the American Society for Reproductive Medicine's eLearning modules. The subject of this presentation is Blastocyst Development and Grading.

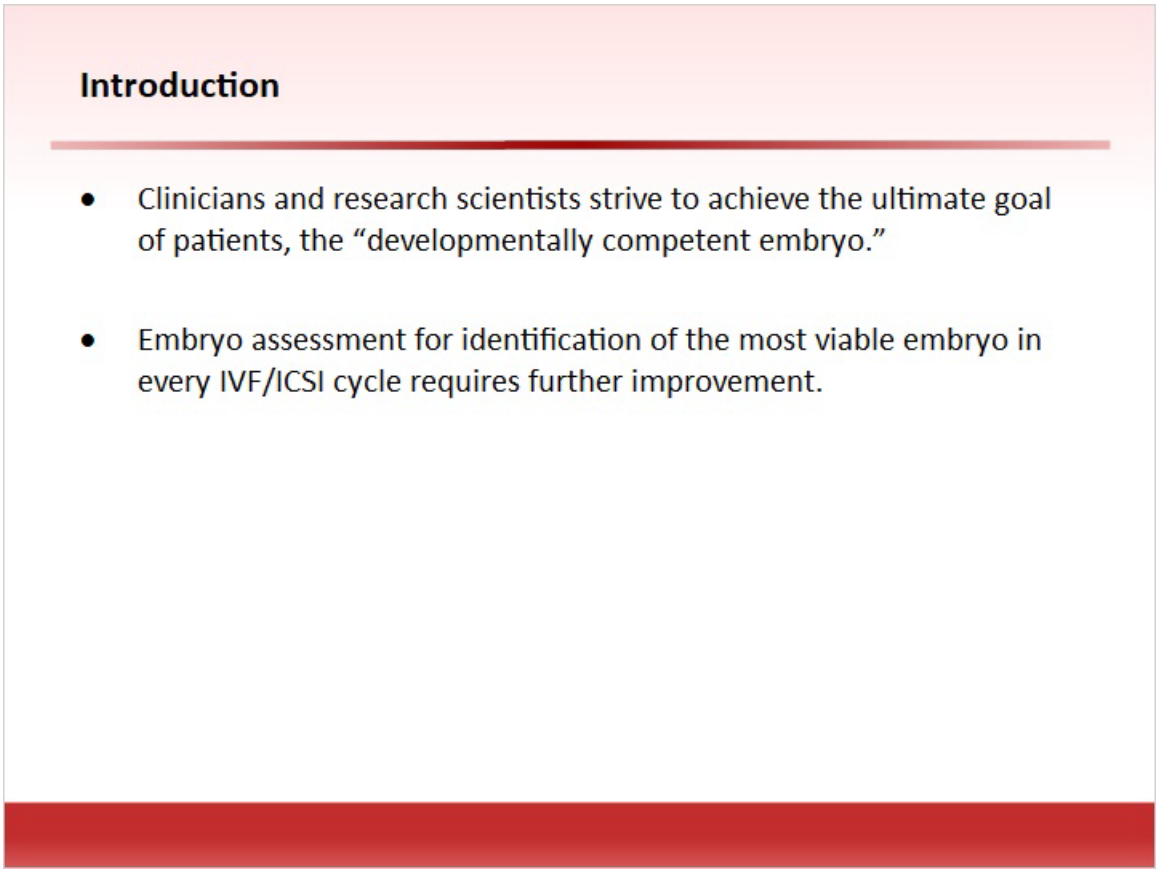
1.2 Learning Objectives



Notes:

At the conclusion of this presentation, participants should be able to:
Describe the process of blastocyst selection and grading, including evaluation of the inner cell mass and trophectoderm cells.
Identify the advantages of blastocyst transfer.
Discuss the importance of blastocyst progression.

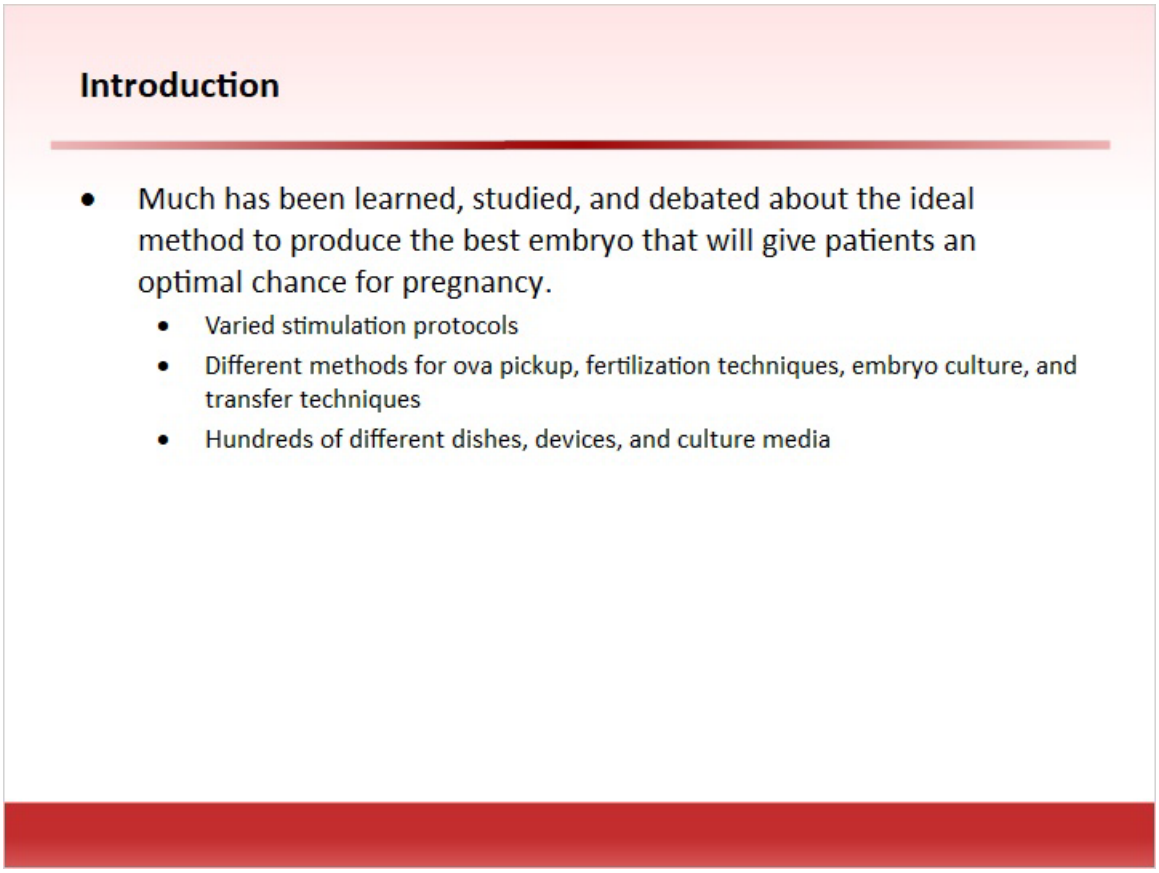
1.3 Introduction



Notes:

Clinicians and research scientists strive to achieve the ultimate goal of patients, the "developmentally competent embryo". Embryo assessment for identification of the most viable embryo in every IVF/ICSI cycle requires further improvement.

1.4 Introduction

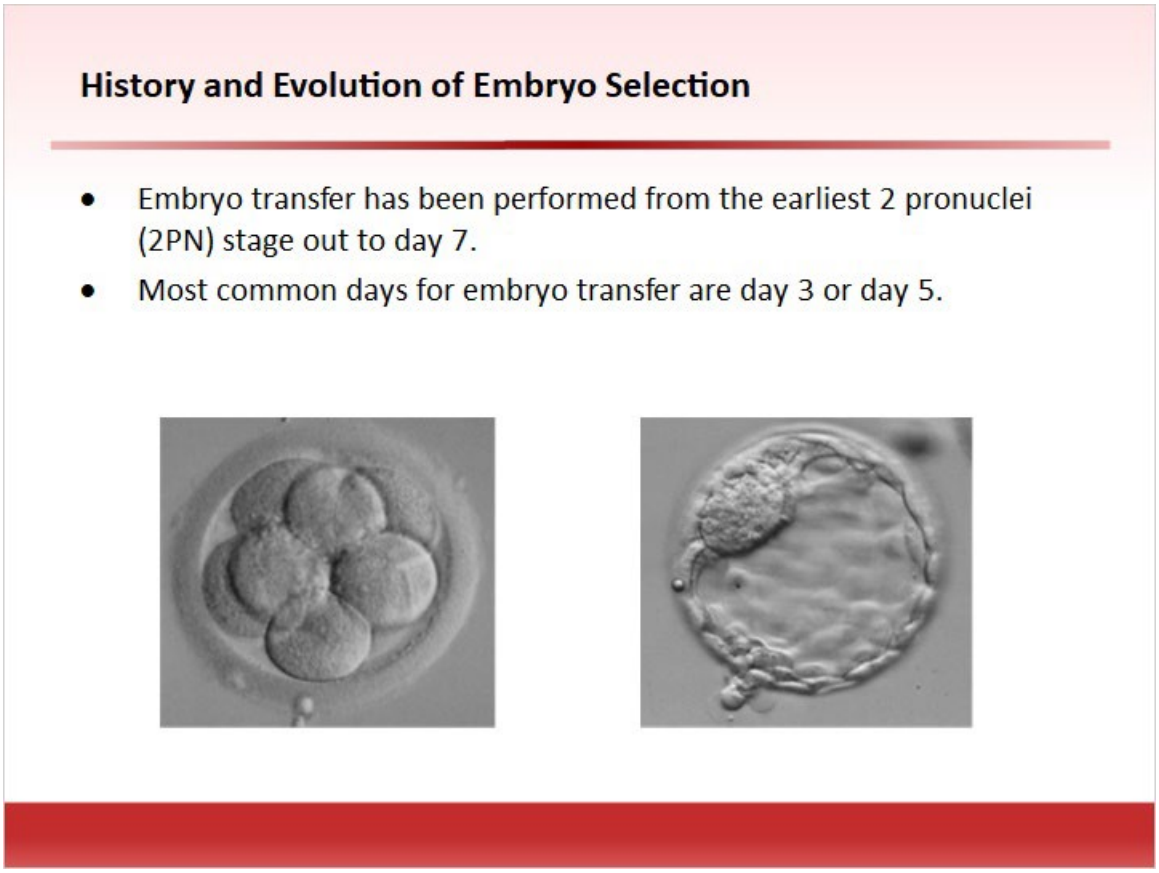


Notes:

Much has been learned, studied, and debated about the ideal method to produce the best embryo that will give patients an optimal chance for pregnancy.

Varied stimulation protocols, different methods for ova pickup, fertilization techniques, embryo culture, and transfer techniques exist that encompass hundreds of different dishes, devices, and culture media, all created to produce a better outcome.

1.5 History and Evolution of Embryo Selection

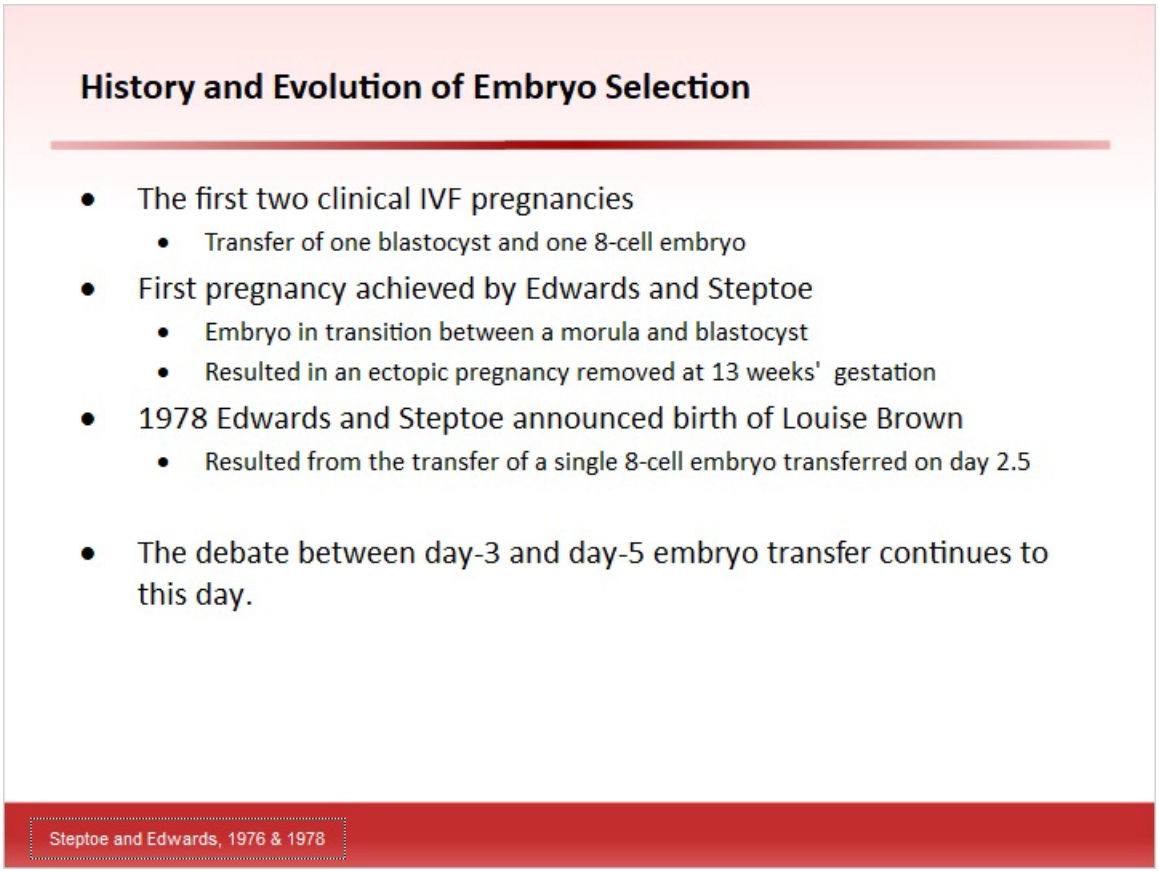


Notes:

The embryologist and laboratory personnel play critical roles in embryo selection for transfer.

Although embryo transfer has been performed from the earliest 2 pronuclei (2PN) stage out to day 7, the most common days for embryo transfer are day 3 or day 5.

1.6 History and Evolution of Embryo Selection

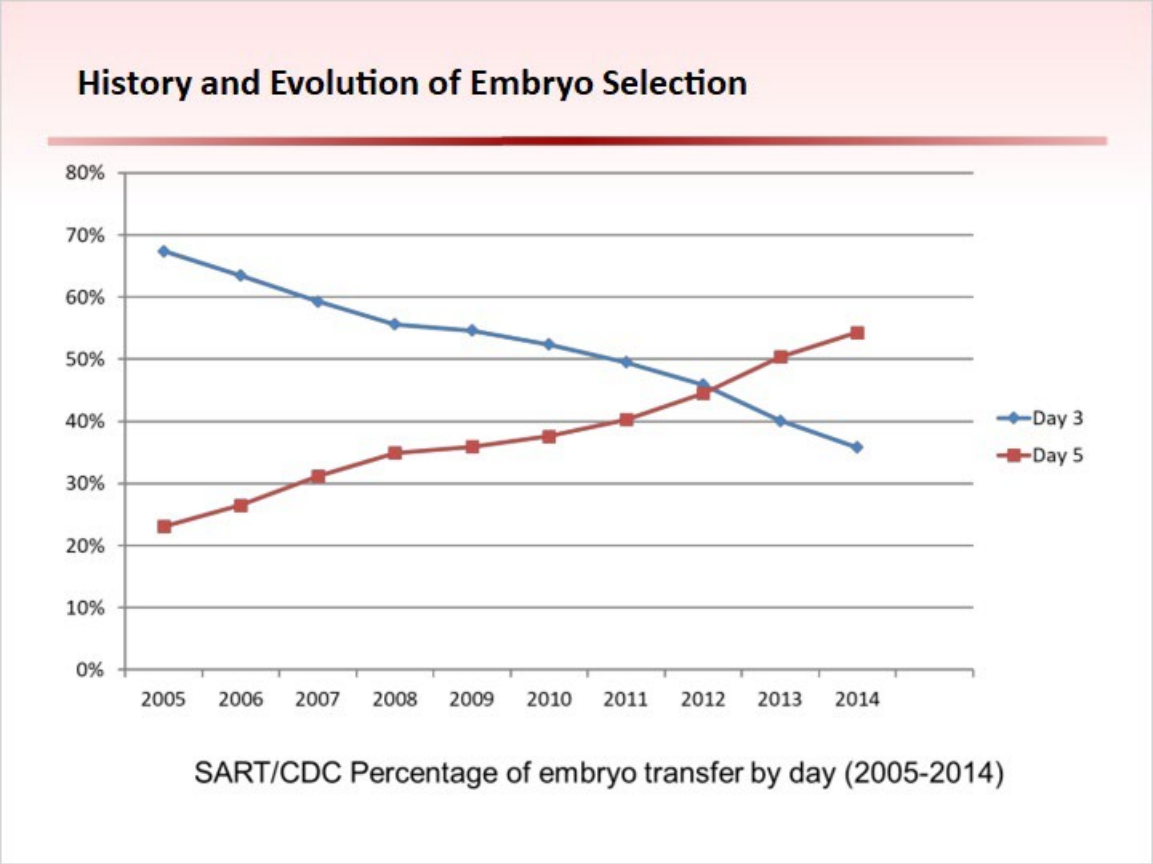


Notes:

The first two reported clinical human IVF pregnancies resulted from transferring one blastocyst and one 8-cell embryo. The first pregnancy achieved by Edwards and Steptoe was from an embryo in transition between a morula and blastocyst. However, this resulted in an ectopic pregnancy removed at 13 weeks of gestation. In 1978, Edwards and Steptoe announced the successful birth of Louise Brown, resulting from the transfer of a single 8-cell embryo transferred on day 2.5.

The debate between day-3 and day-5 embryo transfer continues to this day.

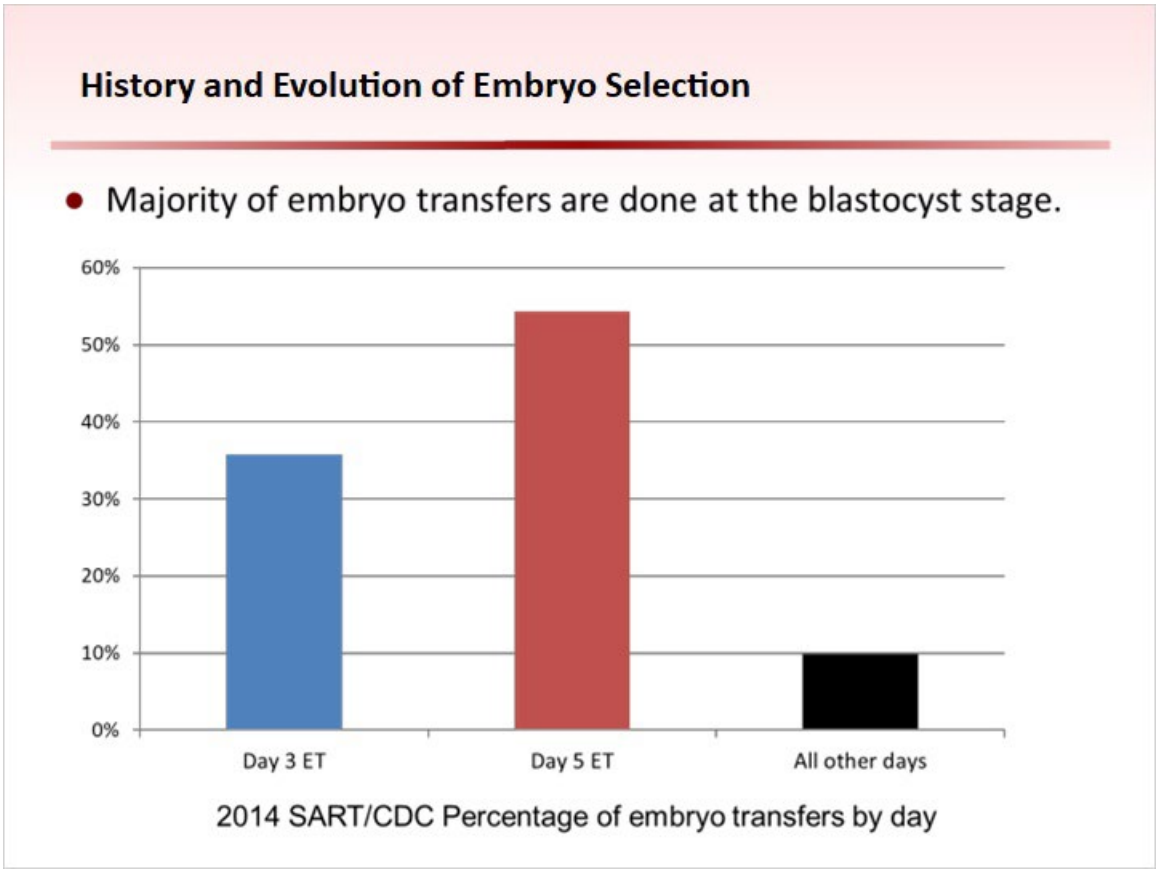
1.7 History and Evolution of Embryo Selection



Notes:

There has been an increasing trend toward blastocyst transfer since SART and the CDC began to compile transfer day statistics in 2005. The number of blastocyst transfers exceeded the number of day 3 embryo transfers beginning in 2013.

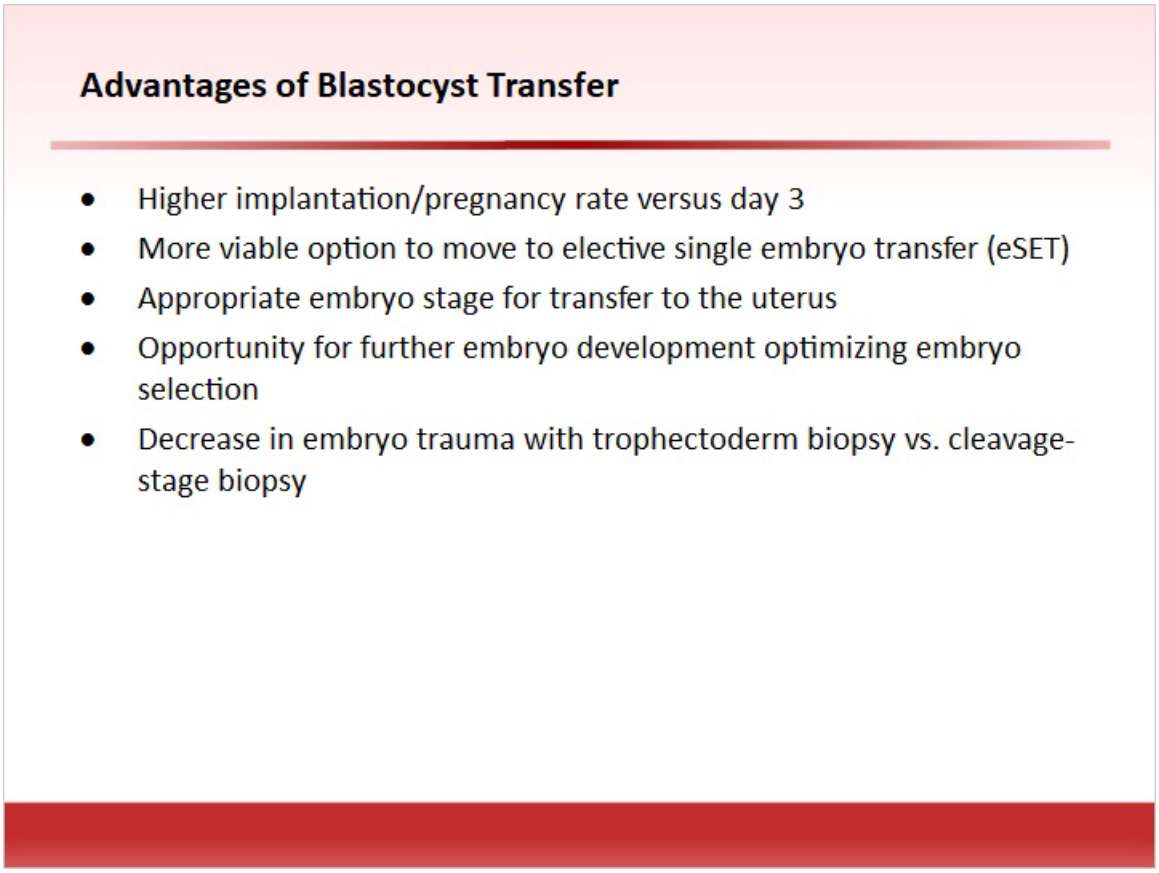
1.8 History and evolution of embryo selection



Notes:

The most current data from the Society for Assisted Reproductive Technology and the Centers for Disease Control and Prevention demonstrate an almost 20% preference toward day 5 blastocyst transfer over day 3 embryo transfer.

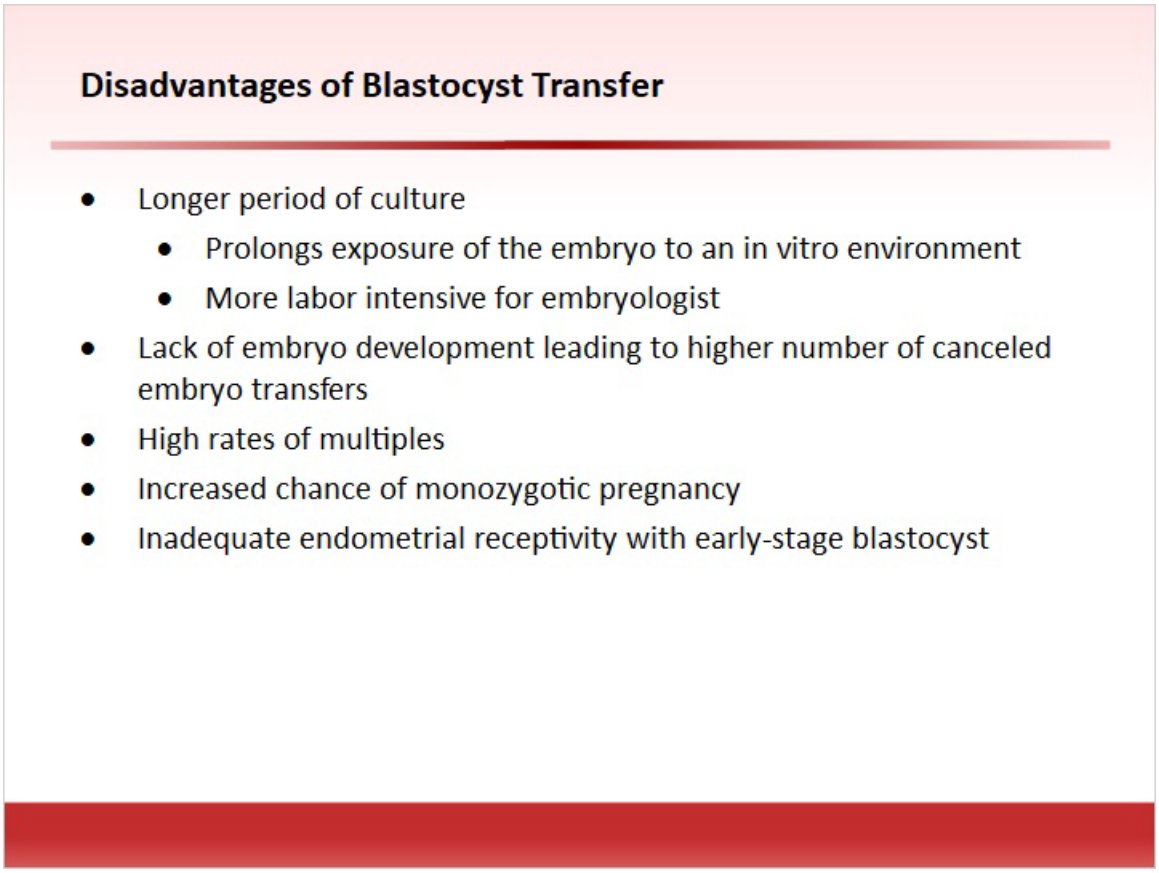
1.9 Advantages of Blastocyst Transfer



Notes:

Advantages of blastocyst transfer include:
A higher implantation/pregnancy rate with blastocyst transfer versus day 3 embryo transfer.
A more viable option to move to elective single embryo transfer.
A more appropriate embryo stage for transfer to the uterus.
An opportunity for further embryo development optimizing embryo selection.
Decreased potential for embryo trauma with trophectoderm biopsy compared with cleavage-stage biopsy.

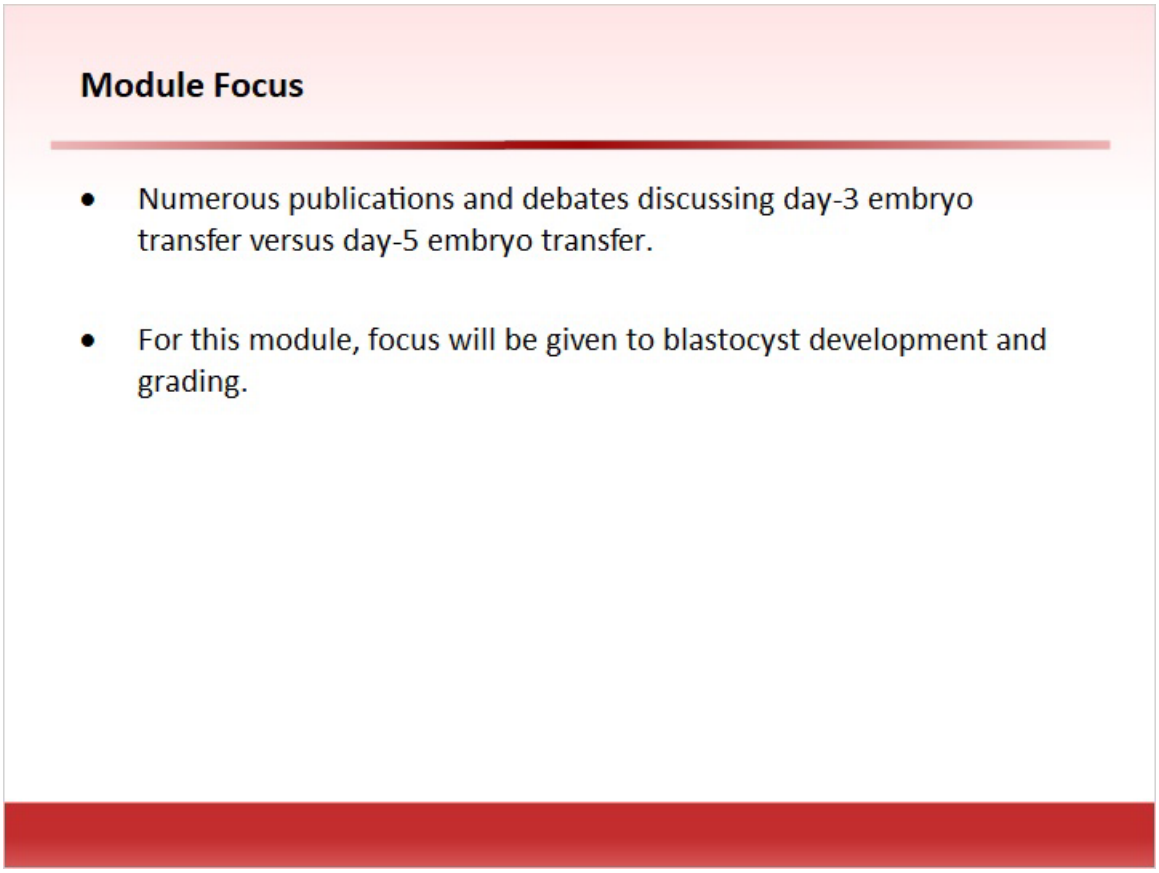
1.10 Disadvantages of Blastocyst Transfer



Notes:

Disadvantages of blastocyst transfer include:
A longer period of culture occurs with blastocyst transfer, which prolongs exposure of the embryo to an in vitro environment and is more labor intensive for the embryologist.
A chance of lack of embryo development to day 5 leading to a higher number of canceled embryo transfers.
A potential for high rates of multiples and an increase in the number of monozygotic pregnancies.
Possible inadequate endometrial receptivity with an early-stage blastocyst.

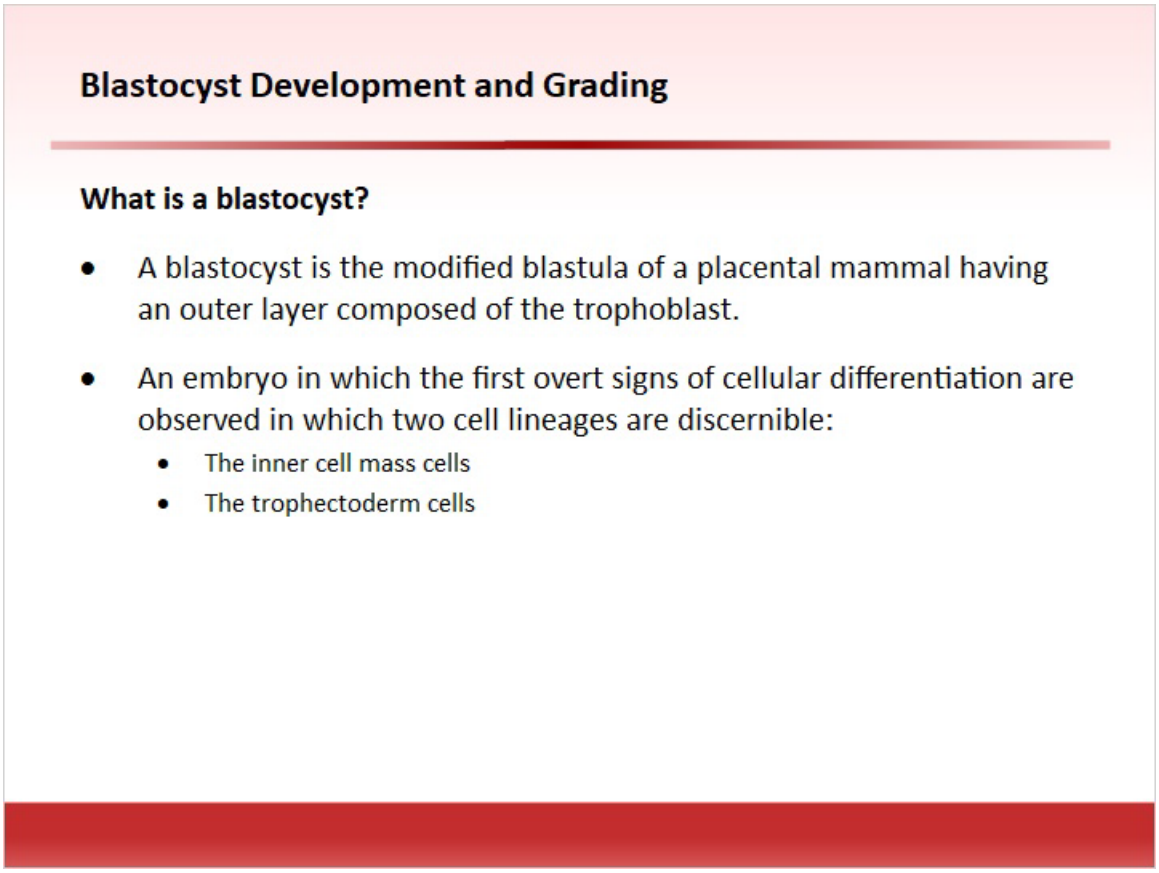
1.11 Module Focus



Notes:

Numerous publications discuss day-3 embryo transfer versus day-5 embryo transfer. However, the focus of this module will be on blastocyst development and grading.

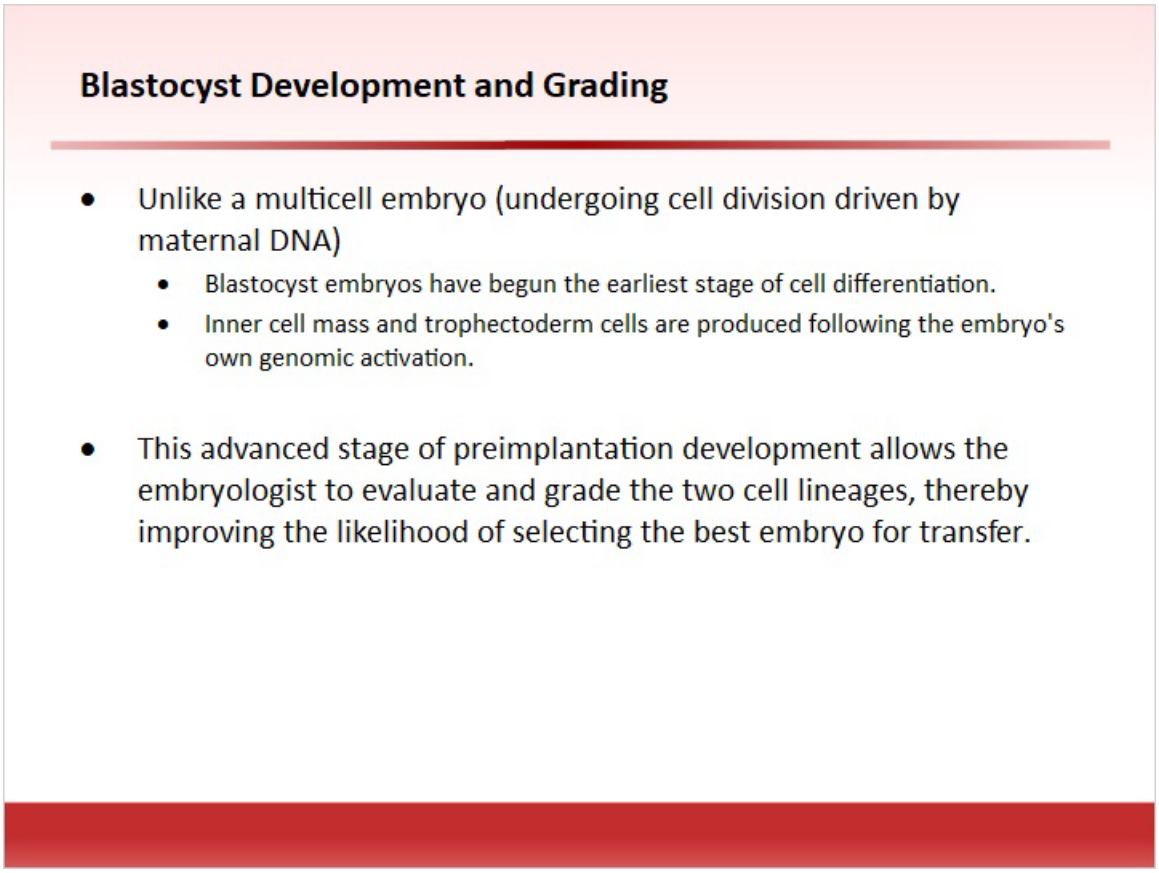
1.12 Blastocyst Development and Grading



Notes:

A blastocyst is the modified blastula of a placental mammal having an outer layer composed of the trophoblast. It is an embryo in which the first overt signs of cellular differentiation are observed in which two cell lineages are discernible:
The inner cell mass cells
The trophectoderm cells

1.13 Blastocyst Development and Grading

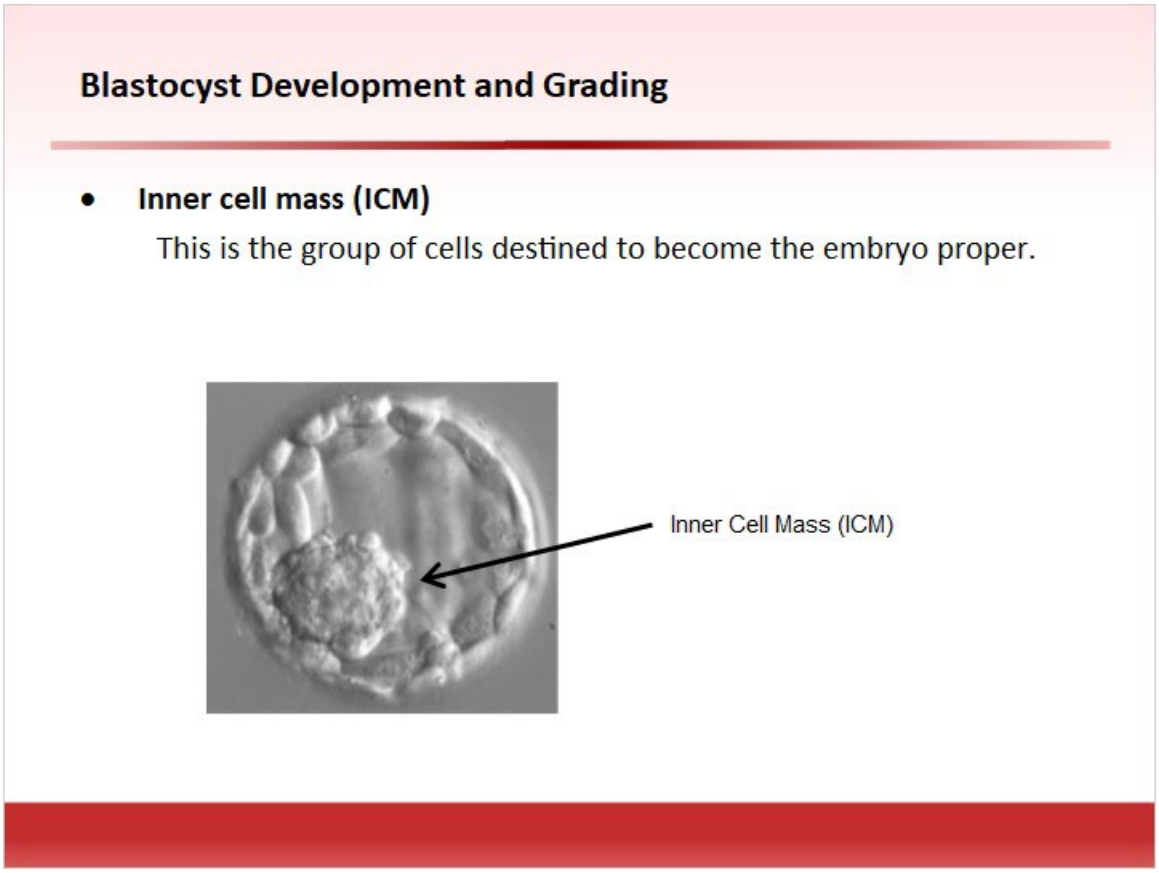


Notes:

Unlike a multicell embryo (undergoing cell division driven by maternal DNA) blastocyst embryos have begun the earliest stage of cell differentiation. Inner cell mass and trophectoderm cells develop following the embryo's own genomic activation.

This advanced stage of preimplantation development allows the embryologist to evaluate and grade the two cell lineages, thereby improving the likelihood of selecting the best embryo for transfer.

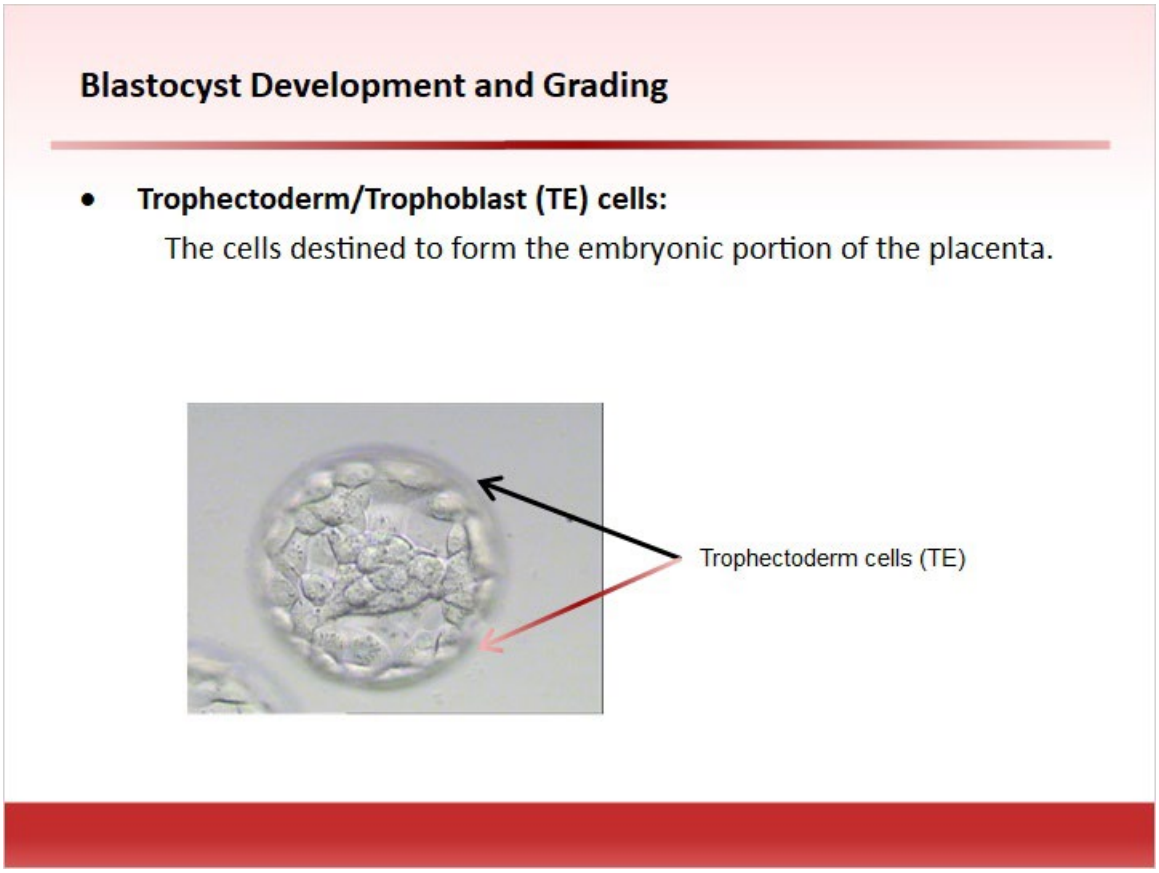
1.14 Blastocyst Development and Grading



Notes:

The inner cell mass is the group of cells destined to become the embryo proper. A well-defined ICM is shown here.

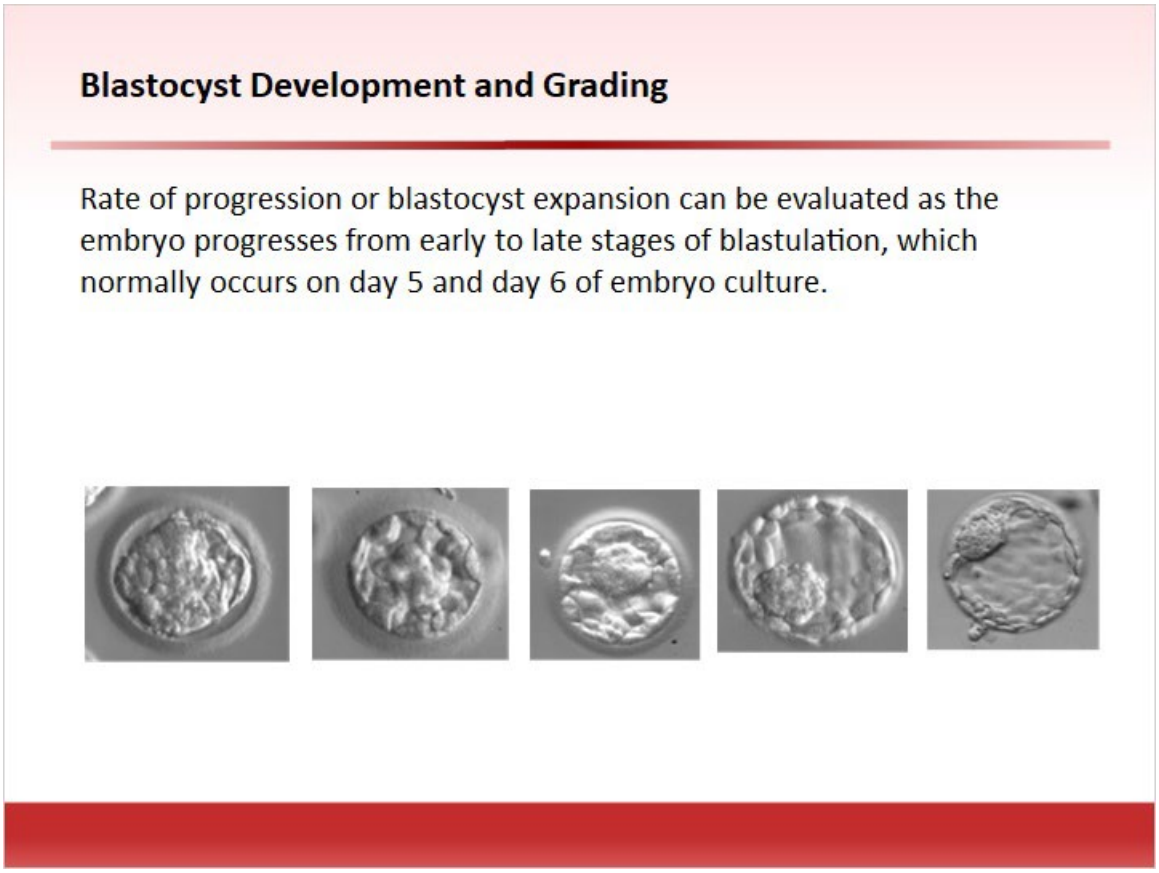
1.15 Blastocyst Development and Grading



Notes:

The trophectoderm or trophoblast cells are destined to form the embryonic portion of the placenta.

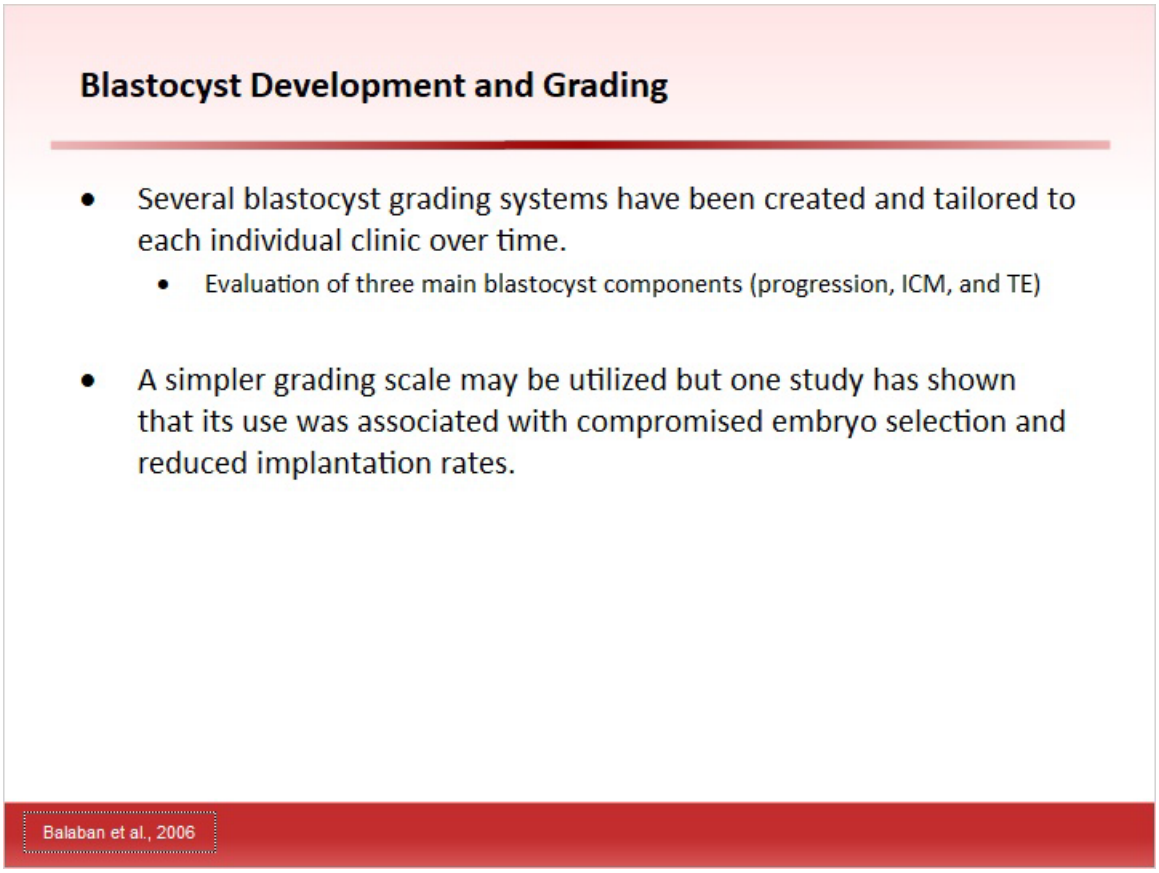
1.16 Blastocyst Development and Grading



Notes:

Along with development of the inner cell mass and trophectoderm, equally important is the rate of progression or blastocyst expansion, which can be evaluated as the embryo progresses from early to late stages of blastulation. This normally occurs on day 5 or day 6 of embryo culture.

1.17 Blastocyst Development and Grading

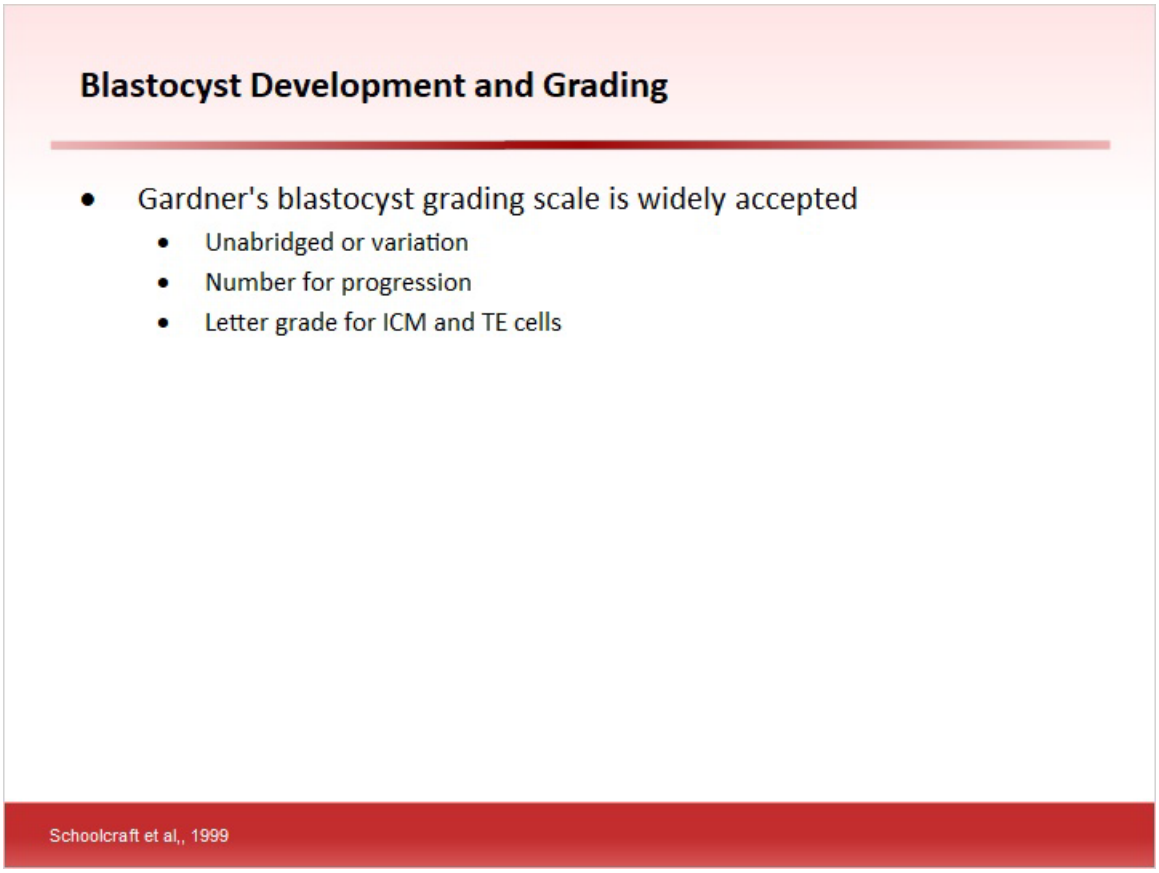


Notes:

Several blastocyst grading systems have been created and tailored to each individual clinic over time. Evaluation of the three main blastocyst components (progression, ICM and TE) comprise the core of the majority of them.

Simpler grading scales may be utilized but have been demonstrated to potentially affect outcomes. Balaban showed this using the Dokras grading system where blastocysts were simply graded as grade 1, 2, and 3.

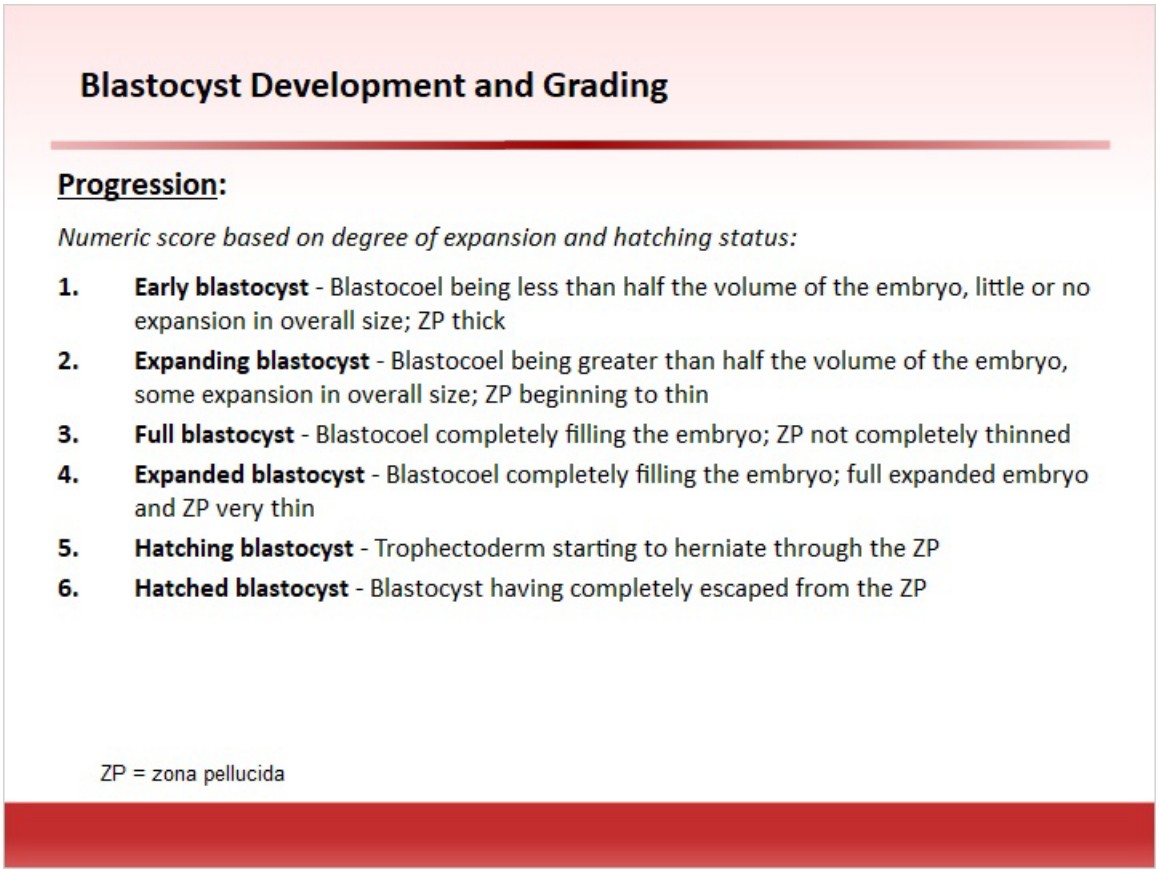
1.18 Blastocyst Development and Grading



Notes:

A widely accepted blastocyst grading scale is the “Gardner's blastocyst grading scale.” This scale assigns a number for progression and a letter grade for ICM and TE cells.

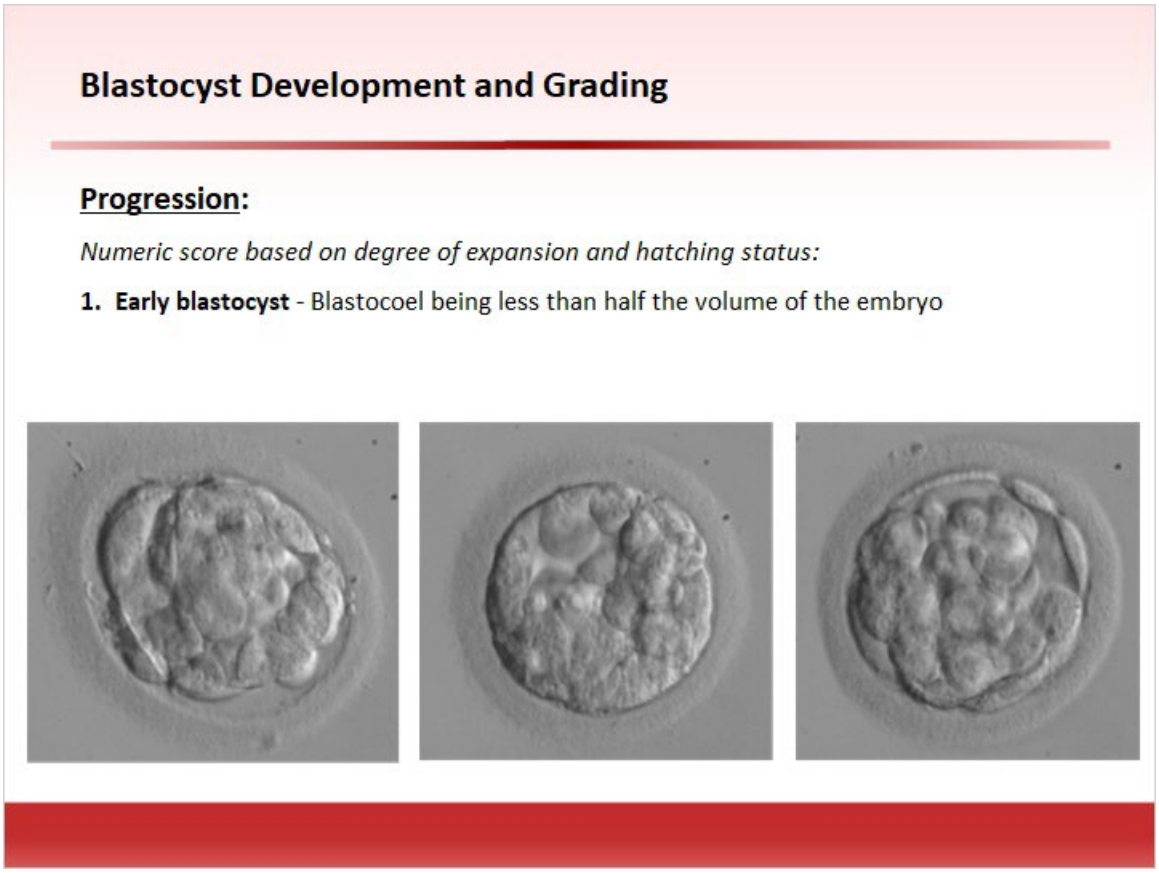
1.19 Blastocyst Development and Grading



Notes:

Progression is scored based on degree of expansion and hatching status.
Each of these stages will be discussed.

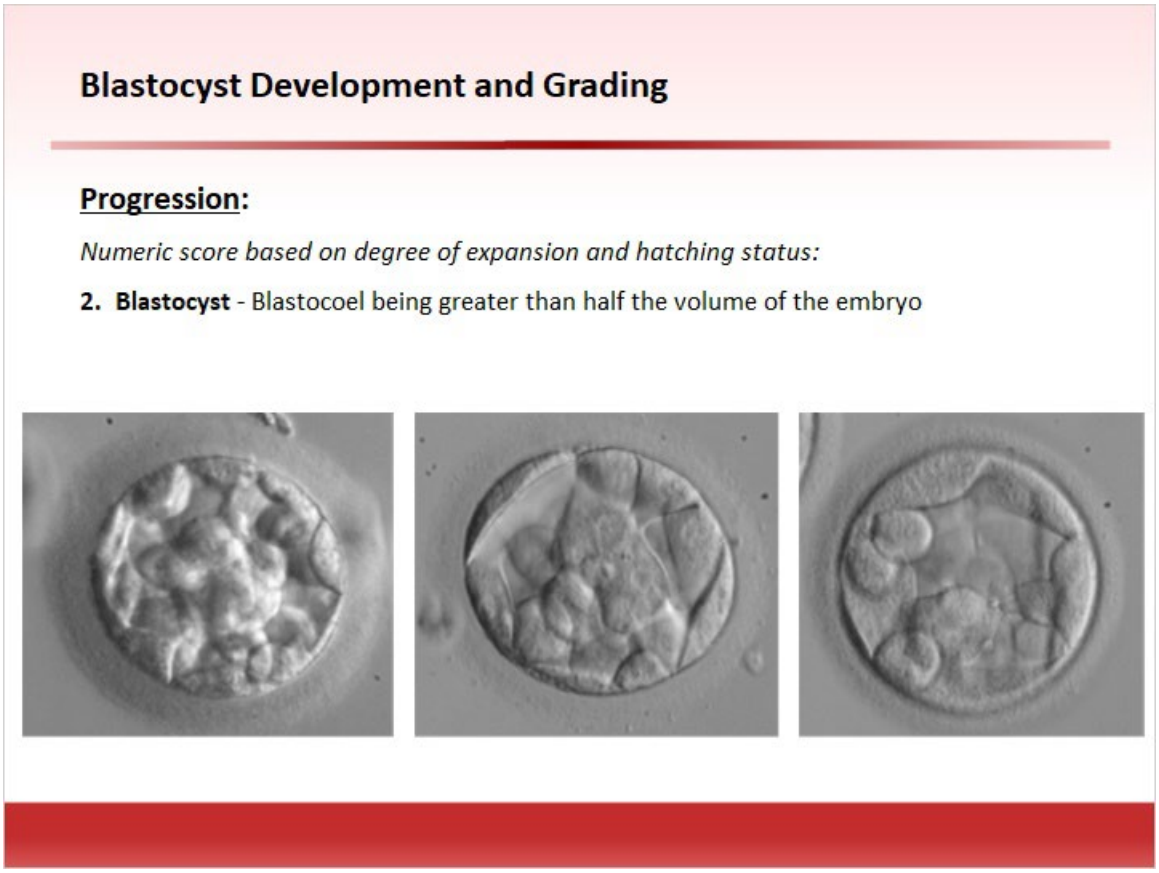
1.20 Blastocyst Development and Grading



Notes:

A blastocoel less than half the volume of the embryo is an early blastocyst.

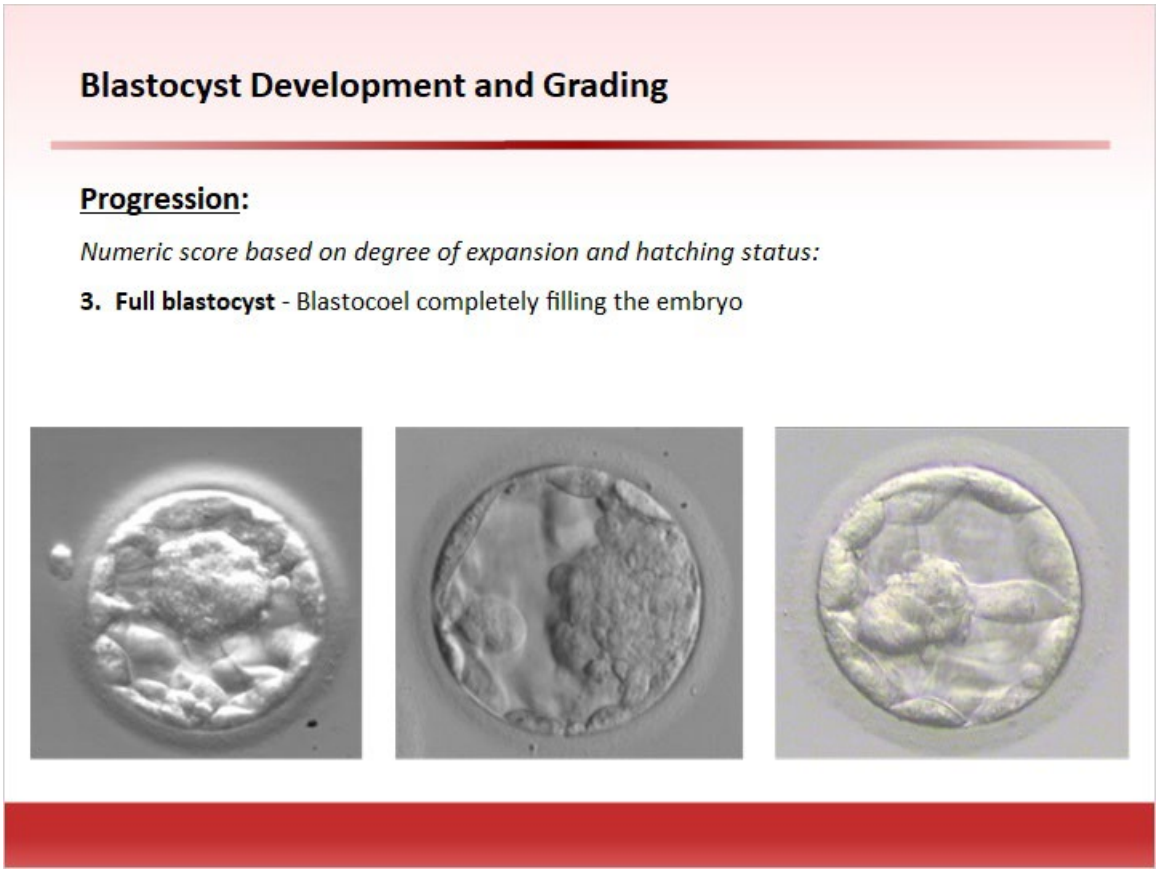
1.21 Blastocyst Development and Grading



Notes:

At blastocyst stage, the blastocoel is greater than half the volume of the embryo.

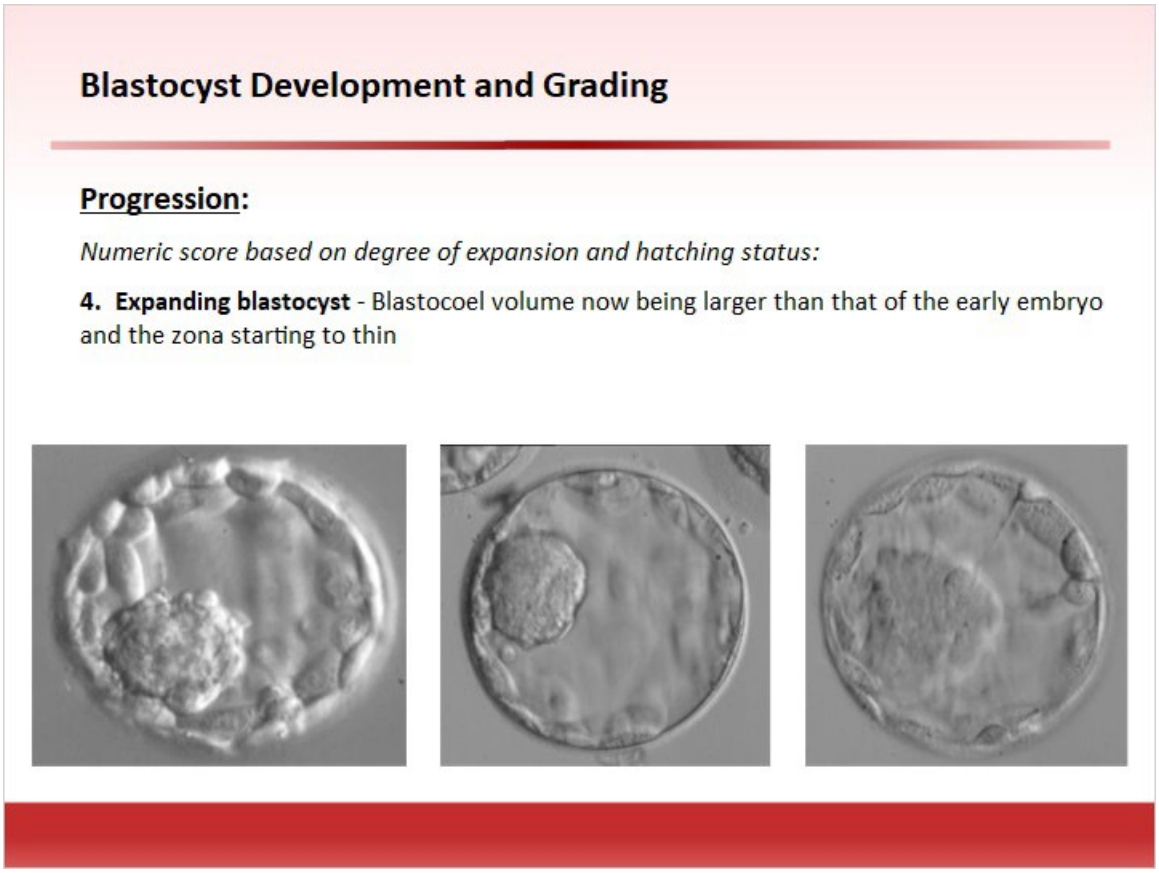
1.22 Blastocyst Development and Grading



Notes:

In a full blastocyst, the blastocoel completely fills the embryo.

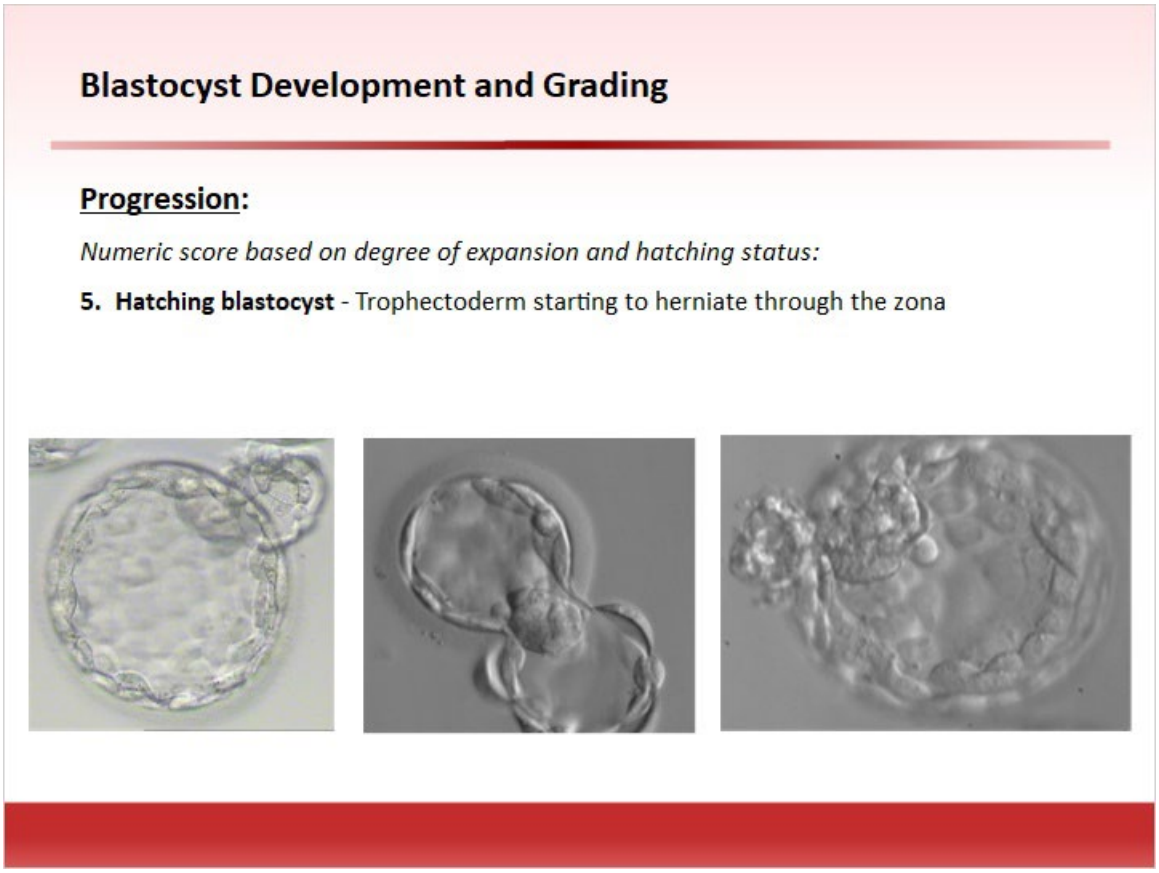
1.23 Blastocyst Development and Grading



Notes:

Expanding blastocyst stage has a blastocoel volume larger than that of the early embryo, and the zona starts to thin.

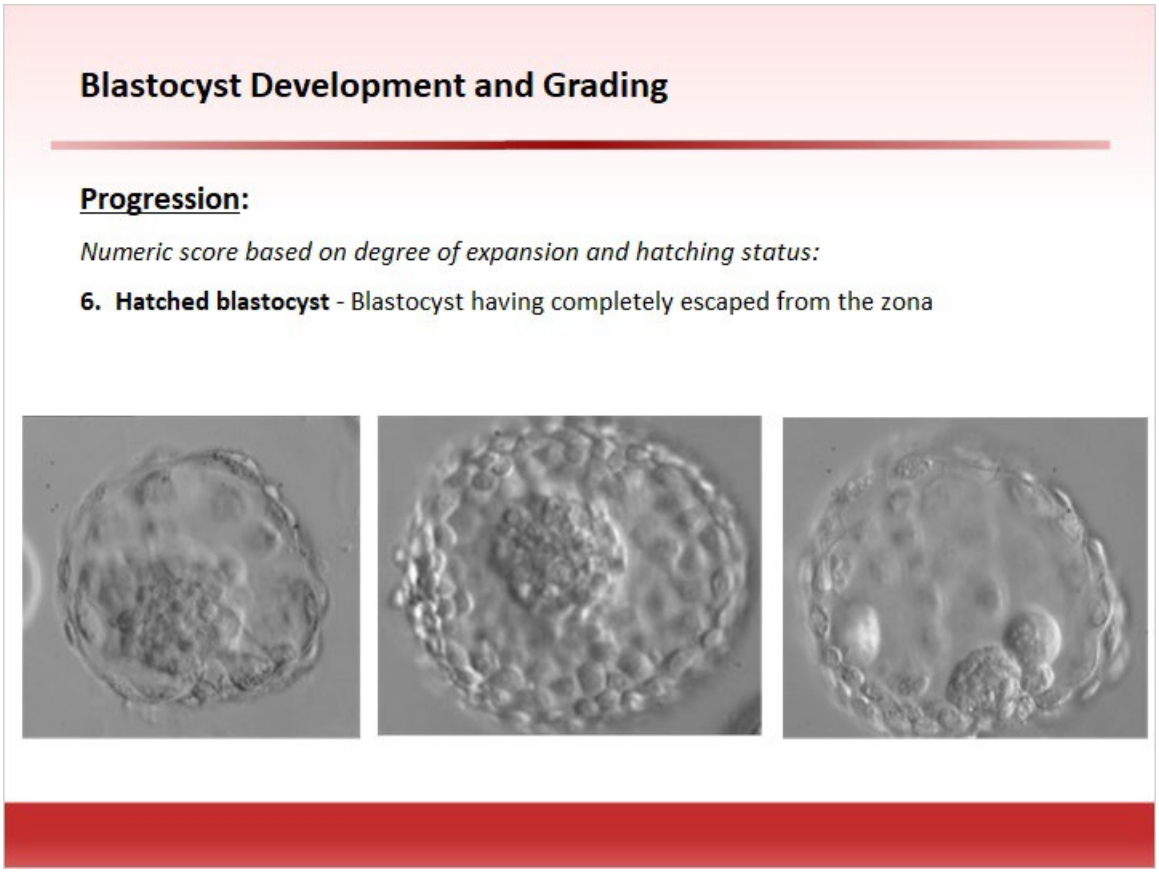
1.24 Blastocyst Development and Grading



Notes:

Hatching blastocyst is when trophectoderm starts to herniate through the zona.

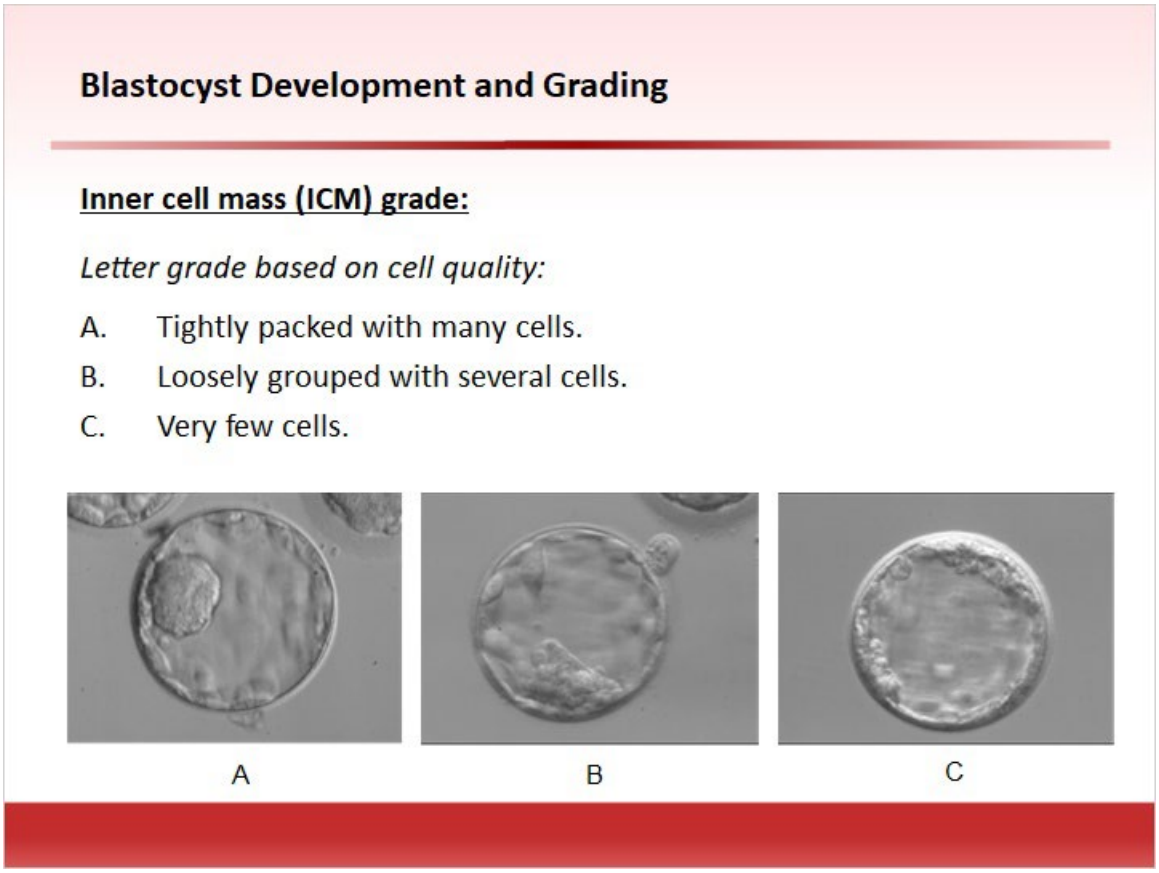
1.25 Blastocyst Development and Grading



Notes:

When the blastocyst has completely escaped from the zona, it is termed a hatched blastocyst.

1.26 Blastocyst Development and Grading

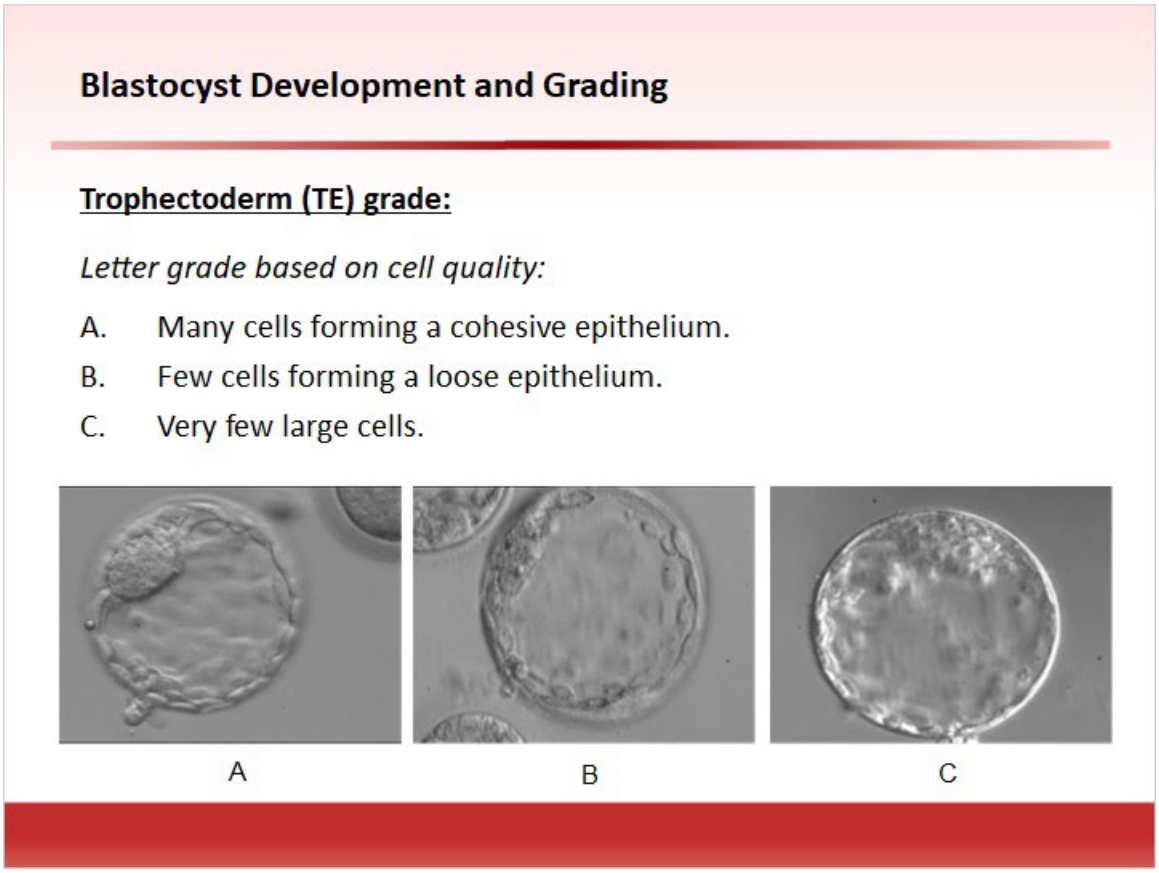


Notes:

Inner cell mass is evaluated and given a letter grade based on cell quality:

"A" grade is assigned if the ICM is tightly packed with many cells.
"B" grade is assigned if the ICM is loosely grouped with several cells.
A "C" grade is assigned if the ICM has very few cells.

1.27 Blastocyst Development and Grading

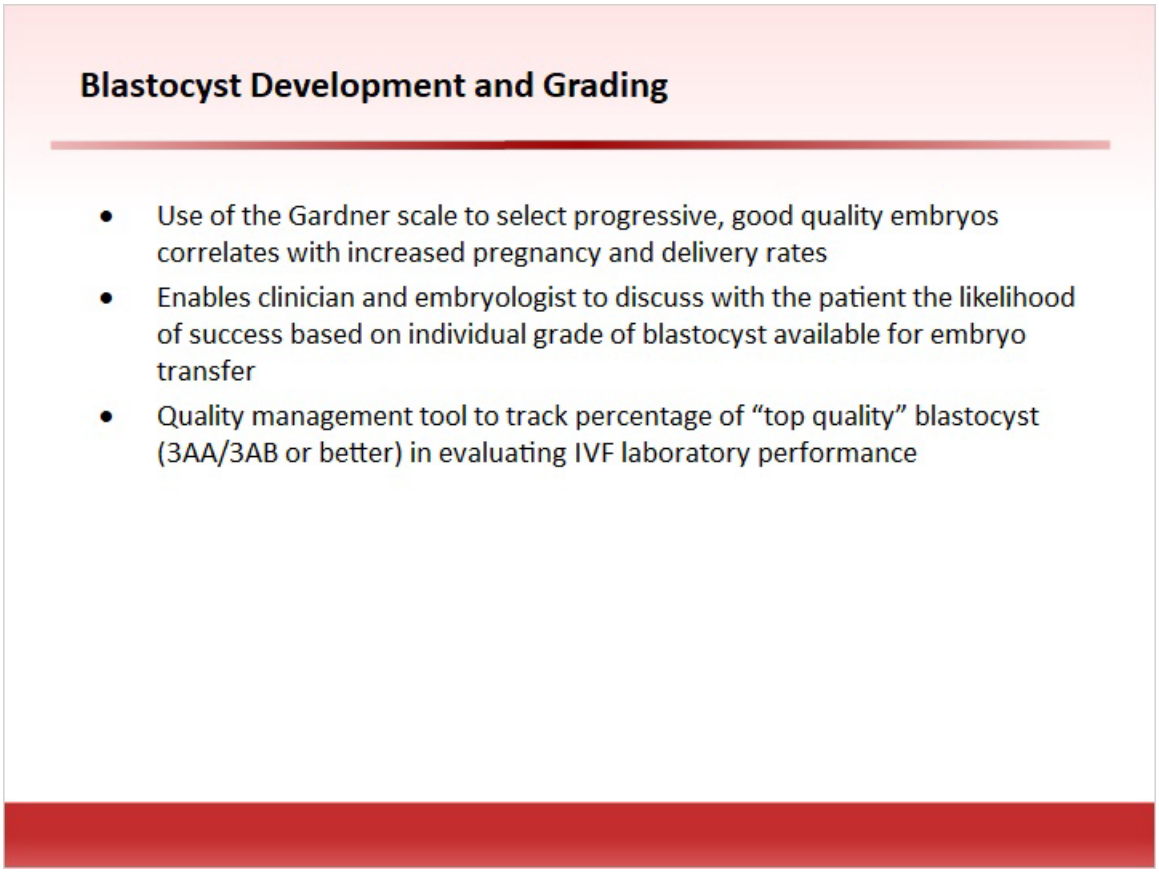


Notes:

Trophectoderm cell grade is also assigned a letter grade based on cell quality:

"A" grade is given when many cells forming a cohesive epithelium are observed.
A "B" grade is given when fewer cells are observed forming a loose epithelium.
Lastly, a "C" grade is given if very few large cells are seen.

1.28 Blastocyst Development and Grading



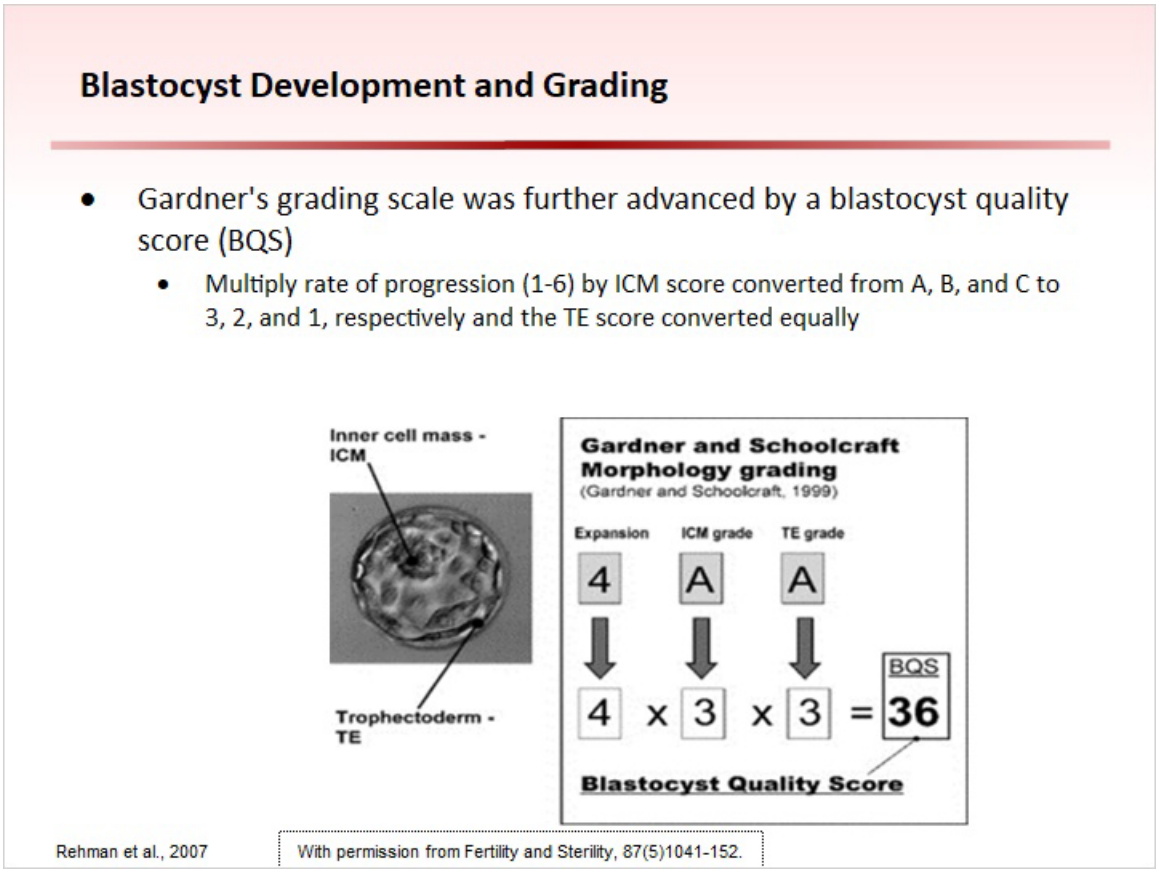
Notes:

Use of the Gardner scale to select progressive, good quality embryos correlates with increased pregnancy and delivery rates. However, no grading scale has been shown to be predictive of a positive outcome.

The scale enables the clinician and embryologist to discuss with the patient the likelihood of success based on the individual grade of blastocyst available for embryo transfer.

It has also been a useful quality management tool in many practices to track the percentage of "top quality" blastocysts (3AA/3AB or better) in evaluating IVF laboratory performance.

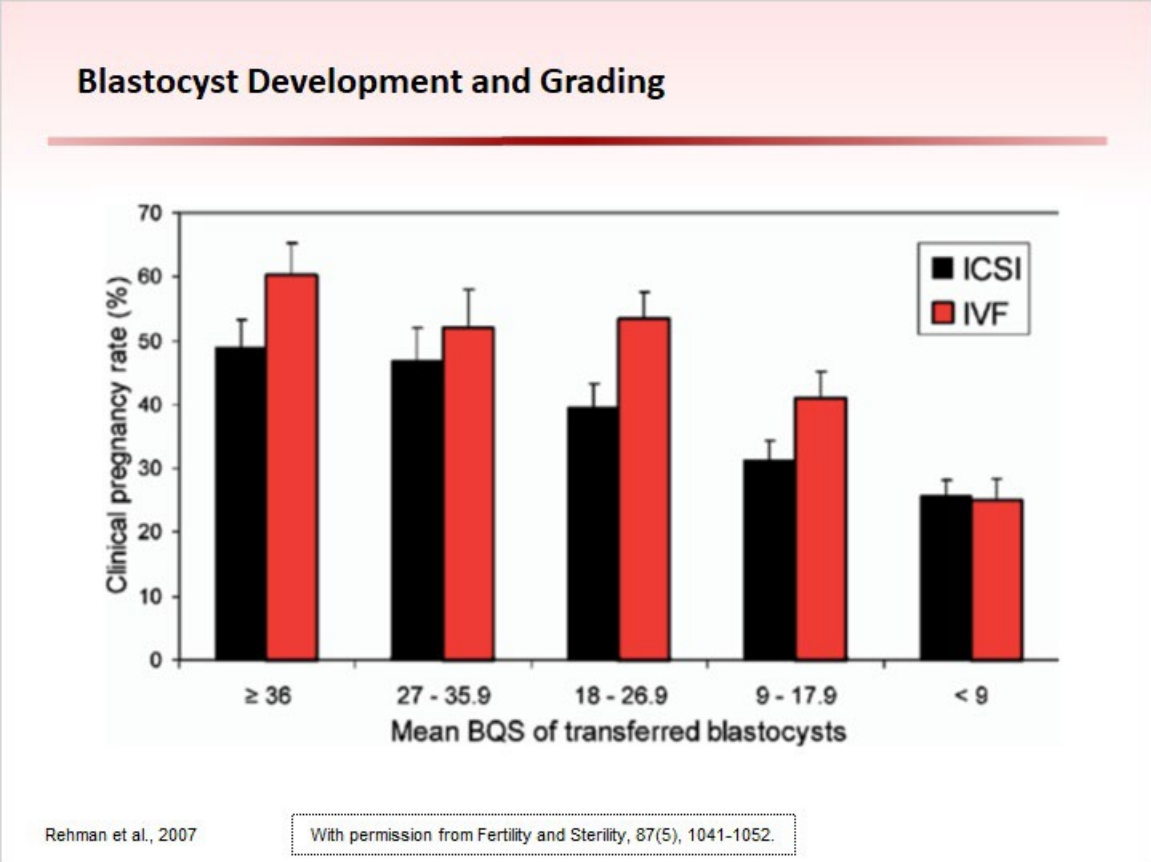
1.29 Blastocyst Development and Grading



Notes:

Gardner's grading scale was further advanced by developing a blastocyst quality score (BQS) by multiplying the rate of progression (1-6) by the ICM score converted from A, B, and C to 3, 2, and 1, respectively, and the TE score converted the same.

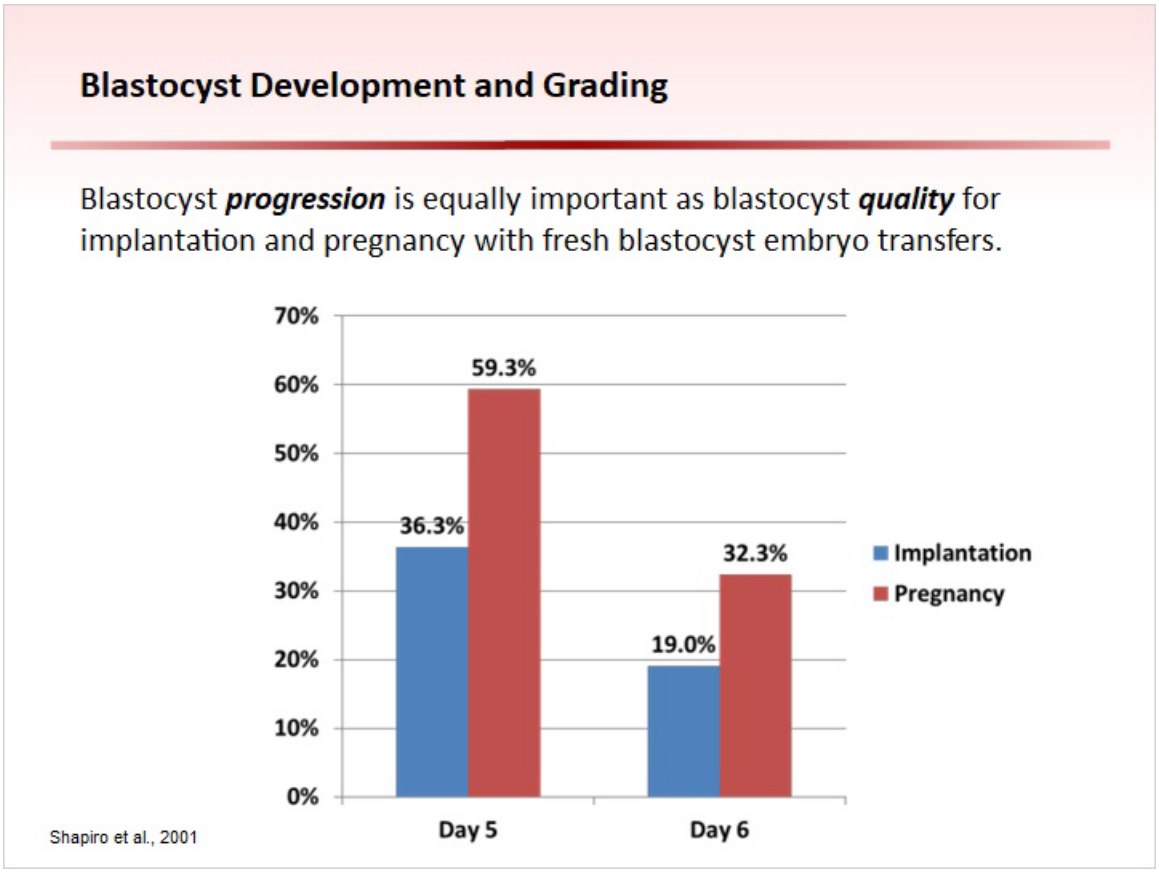
1.30 Blastocyst Development and Grading



Notes:

Applying this grading scale, Rehman showed that mean clinical pregnancy rates for ICSI and IVF declined from 48.8% and 60.2%, respectively, with the highest mean BQS equivalent to a 4AA or higher morphology, to 25.6% and 25.0% with the lowest mean BQS transferred, equivalent to embryos averaging below 1AA morphology in both ICSI and IVF cycles, respectively. The graph shows a definite trend toward lower pregnancy rates for both IVF and ICSI cycles as mean BQS of blastocyst-stage embryos transferred decreases.

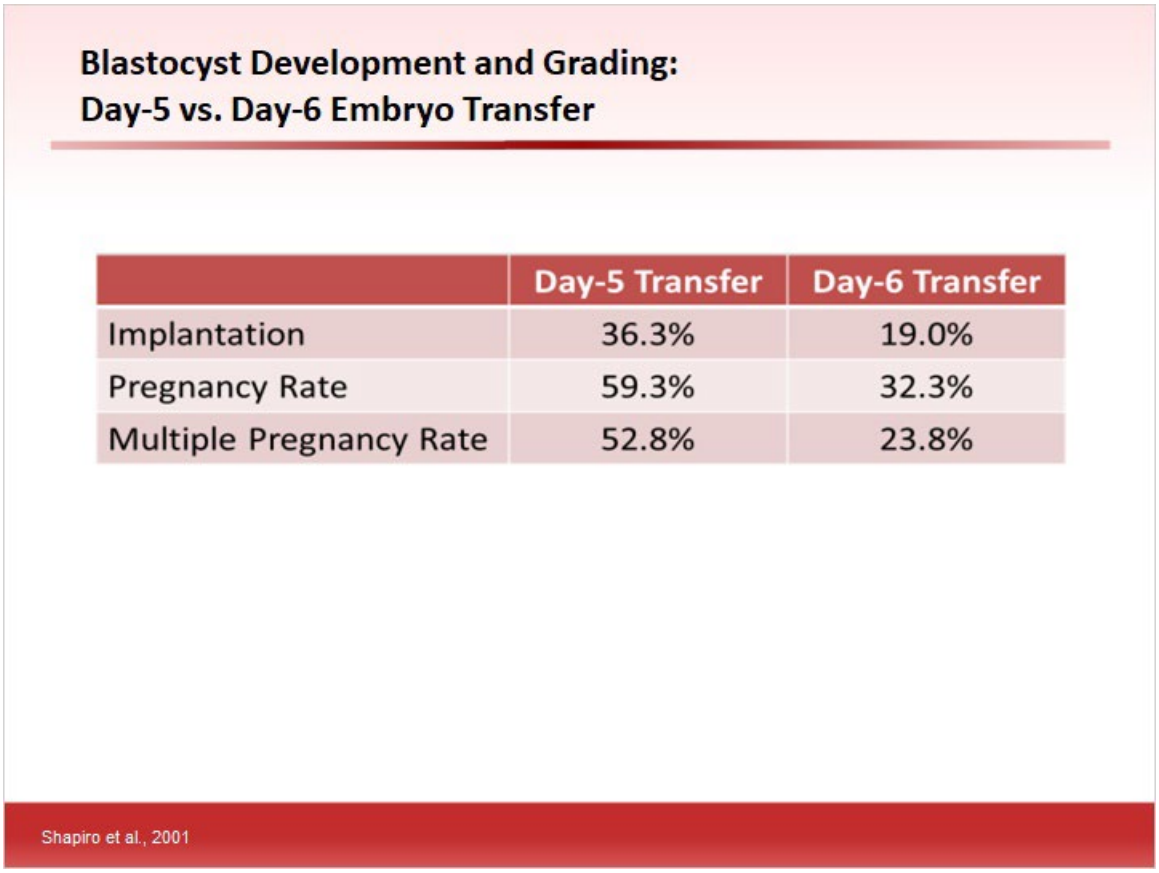
1.31 Blastocyst Development and Grading



Notes:

Shapiro and colleagues demonstrated that with fresh blastocyst embryo transfers, blastocyst progression was equally as important as blastocyst quality for implantation and pregnancy. Implantation and pregnancy rates decreased from day-5 embryo transfer to day-6.

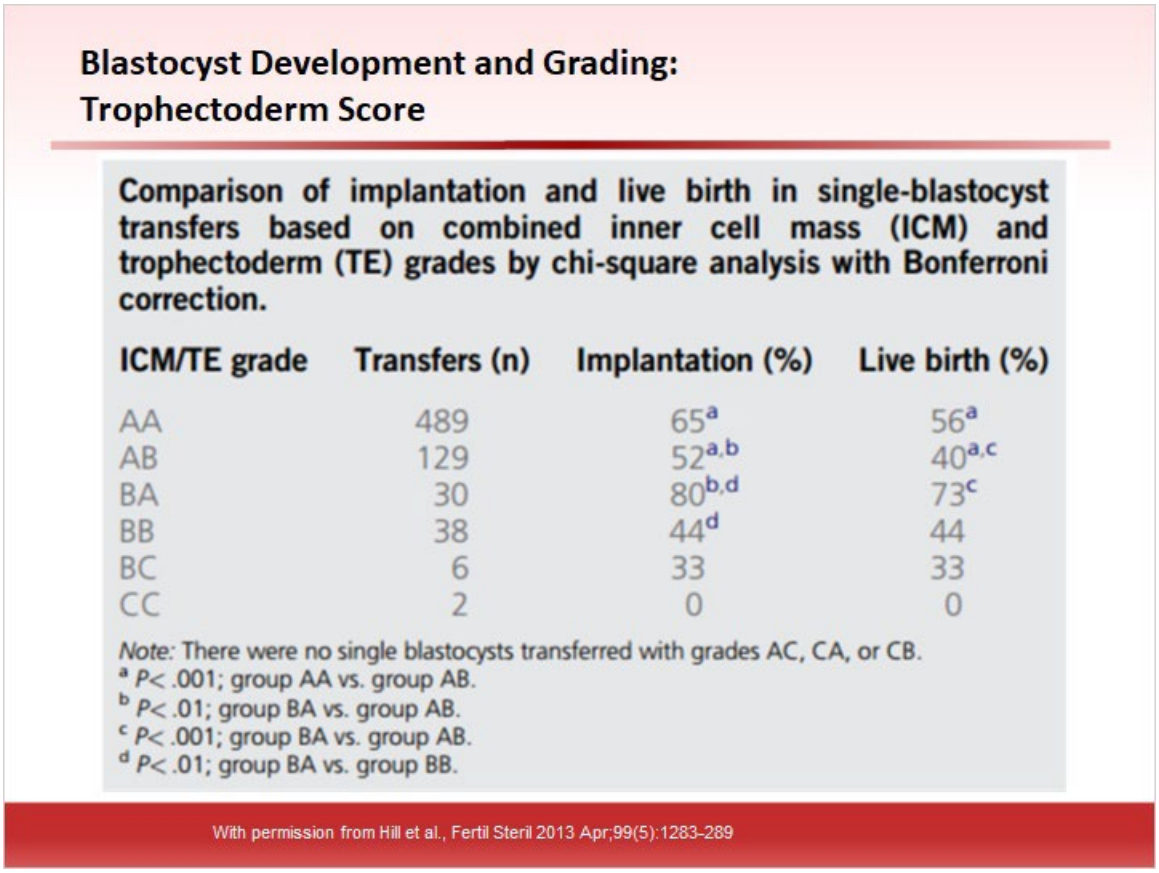
1.32 Blastocyst Development and Grading:



Notes:

In that report, blastocysts transferred on day 5 implanted at nearly twice the rate of blastocysts transferred on day 6 (36.3% vs. 19.0%). Pregnancy rates were also almost twice as high among the day-5 transfer patients (59.3% vs. 32.3%). Multiple pregnancies were more than twice as common for day-5 compared with day-6 transfers (52.8% vs. 23.8%).

1.33 Blastocyst Development and Grading:



Blastocyst Development and Grading: Trophectoderm Score

Comparison of implantation and live birth in single-blastocyst transfers based on combined inner cell mass (ICM) and trophectoderm (TE) grades by chi-square analysis with Bonferroni correction.

ICM/TE grade	Transfers (n)	Implantation (%)	Live birth (%)
AA	489	65[a]	56[a]
AB	129	52[a,b]	40[a,c]
BA	30	80[b,d]	73[c]
BB	38	44[d]	44
BC	6	33	33
CC	2	0	0

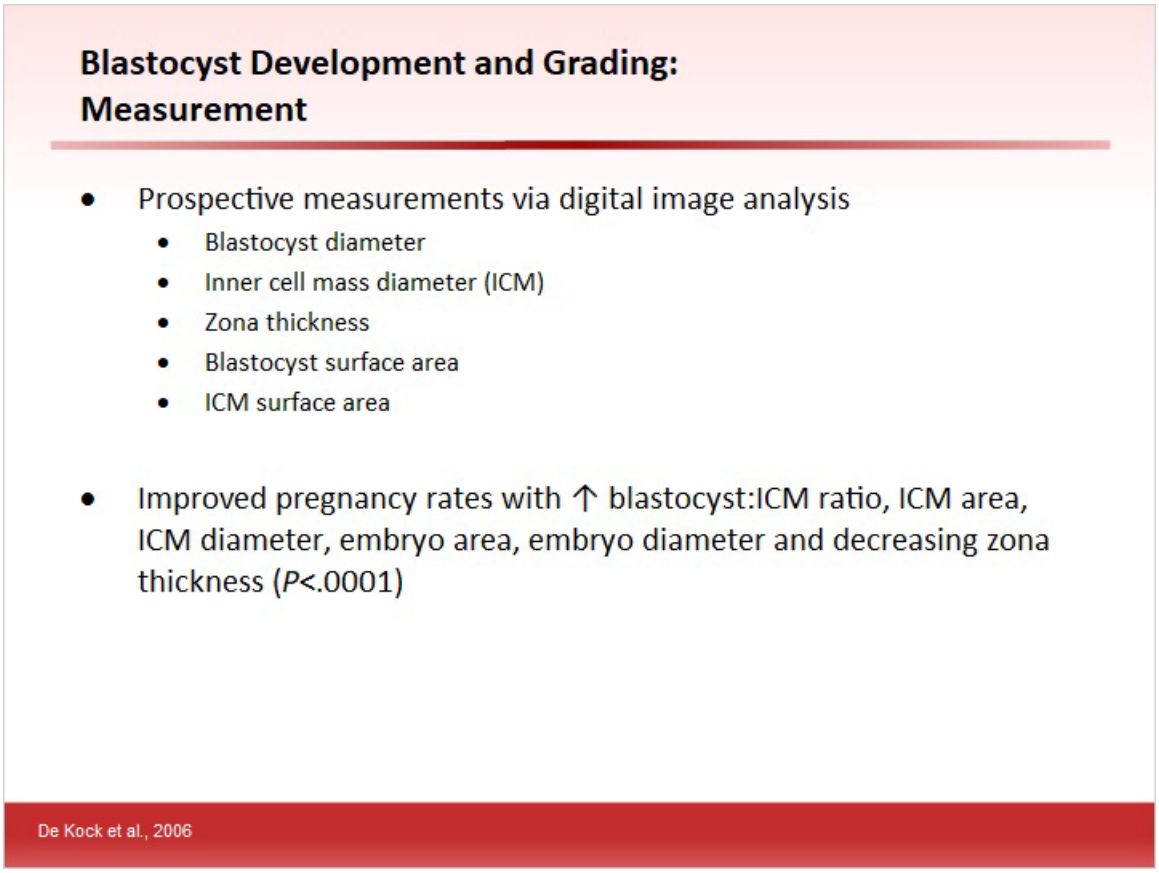
Note: There were no single blastocysts transferred with grades AC, CA, or CB.
[a] $P < .001$; group AA vs. group AB.
[b] $P < .01$; group BA vs. group AB.
[c] $P < .001$; group BA vs. group AB.
[d] $P < .01$; group BA vs. group BB.

With permission from Hill et al., Fertil Steril 2013 Apr;99(5):1283-289

Notes:

While most publications give priority to blastocysts with higher inner cell mass (ICM) grades, Hill and colleagues demonstrated that the trophectoderm (TE) score is a good indicator of outcomes of single blastocyst transfers. Live birth rates were 57%, 40%, and 25% for TE grades A, B, and C, respectively.

1.34 Blastocyst Development and Grading:



Notes:

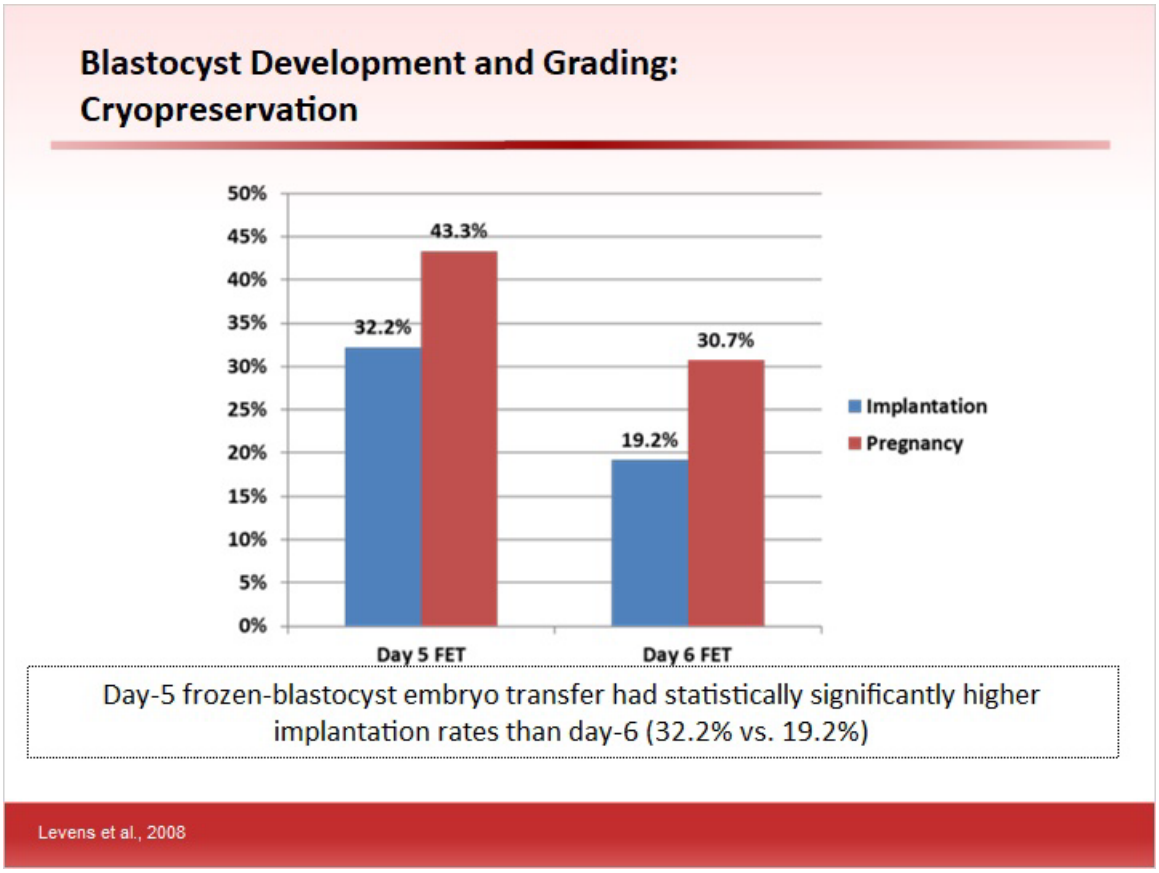
Digital image analysis software has been used to prospectively measure blastocyst diameter, inner cell mass diameter (ICM), zona thickness, blastocyst surface area, and ICM surface area. Improved pregnancy rates were observed with increasing blastocyst:ICM ratio, ICM area, ICM diameter, embryo area, embryo diameter, and decreasing zona thickness (P<.0001).

1.35 Blastocyst Development and Grading

Notes:

Similar implantation and pregnancy rates have been reported for cryopreserved blastocyst embryos.

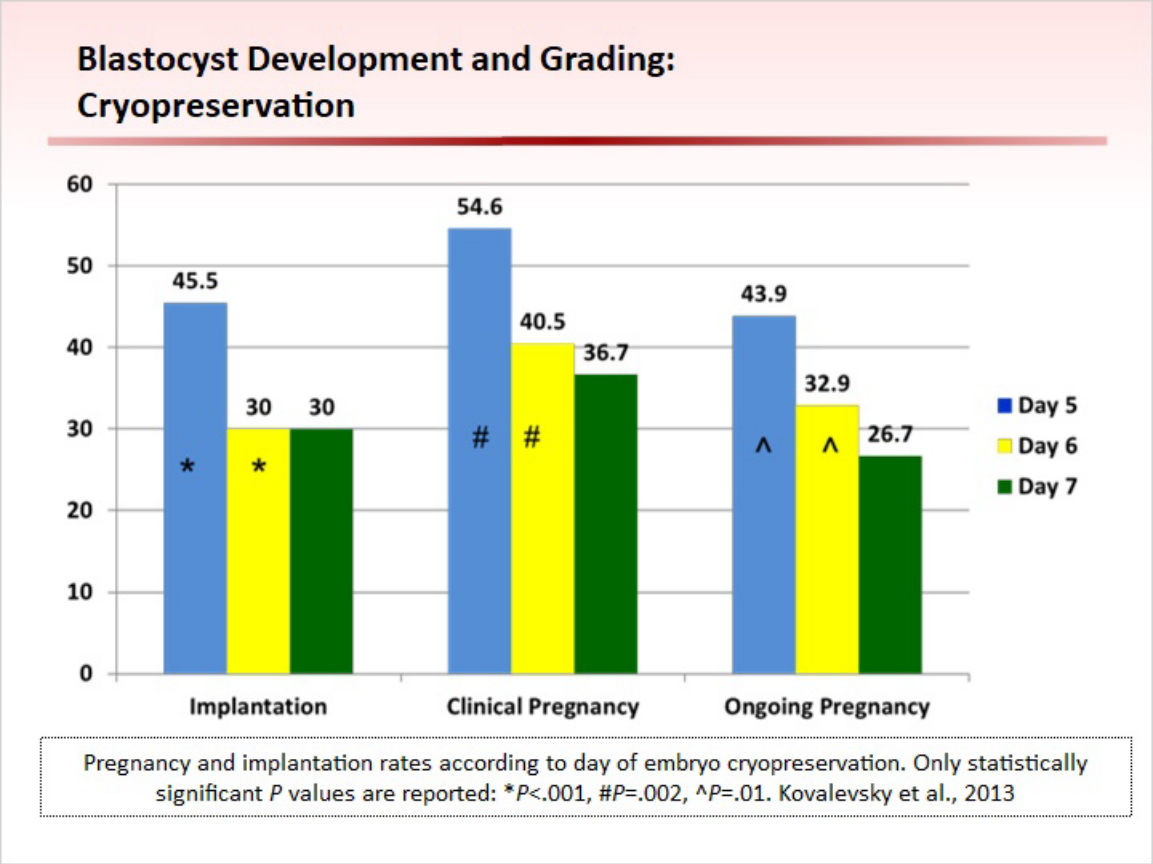
1.36 Blastocyst Development and Grading:



Notes:

As with fresh-blastocyst transfer, cryopreserved day-5 blastocysts have higher implantation rates and trend toward improved pregnancy outcomes when compared with cryopreserved day-6 blastocysts. It can be surmised that the development rate may, in part, predict implantation and subsequent frozen-blastocyst embryo transfer outcomes, although embryos not achieving the blastocyst stage until day 6 still demonstrate acceptable outcomes.

1.37 Blastocyst Development and Grading:

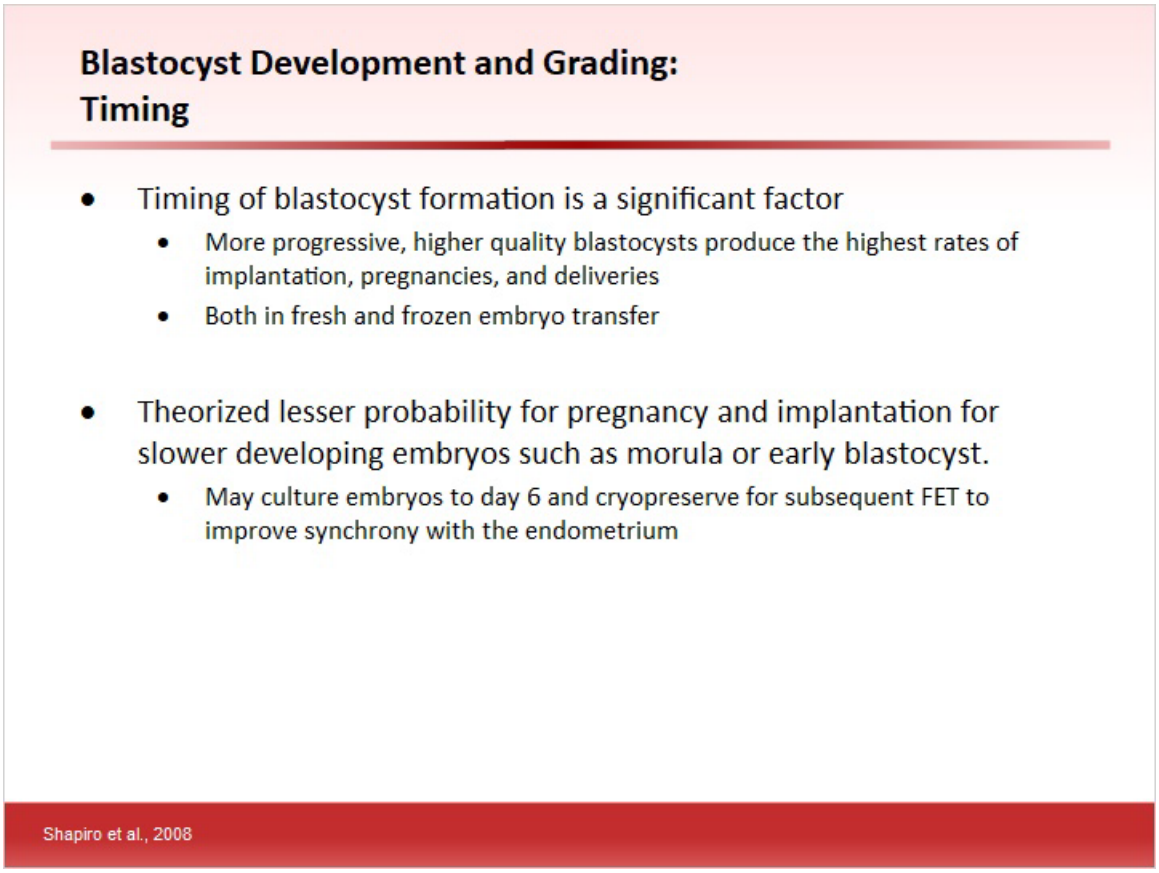


Pregnancy and implantation rates according to day of embryo cryopreservation. Only statistically significant *P* values are reported: *$P<.001$, #$P=.002$, ^$P=.01$. Kovalevsky et al., 2013

Notes:

Others demonstrated similar decreases in implantation and pregnancy rates with later frozen-blastocyst embryo transfer dates.
Interestingly, albeit a small sample size of 48 day-7 transfers, the researchers showed that slow-developing blastocysts that require an additional 48 hours of culture beyond day 5 produce acceptable implantation and pregnancy rates.
It should be noted that blastocyst culture to day 7 is not universally adopted. Future investigation using more rigorous prospective study design and a larger sample size is warranted.

1.38 Blastocyst Development and Grading:

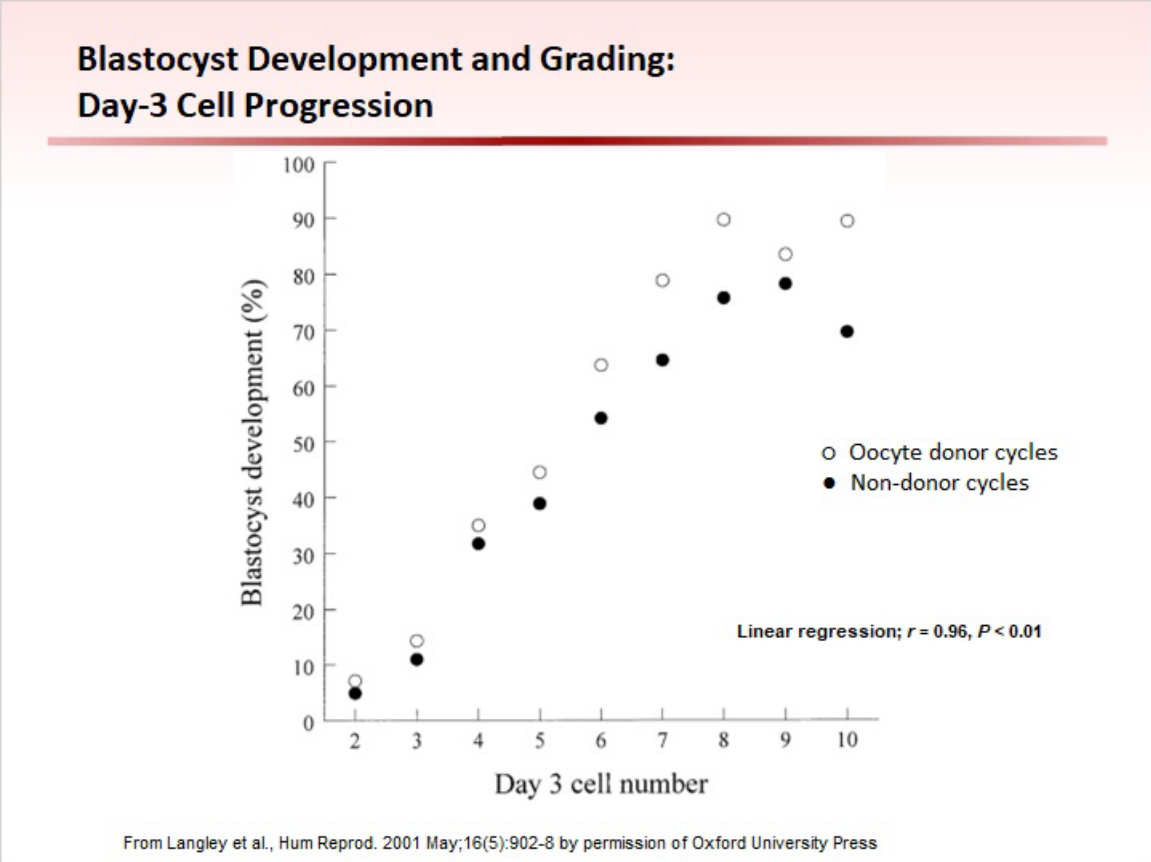


Notes:

Timing of blastocyst formation is a significant factor, as the more progressive, higher-quality blastocysts produce the highest rates of implantation, pregnancies, and deliveries, both in fresh and frozen embryo transfer.

Shapiro theorized a lesser probability for pregnancy and implantation for slower developing embryos such as morula or early blastocysts. It may be advised that these embryos are cultured to day 6 and cryopreserved for subsequent FET to improve synchrony with the endometrium.

1.39 Blastocyst Development and Grading:

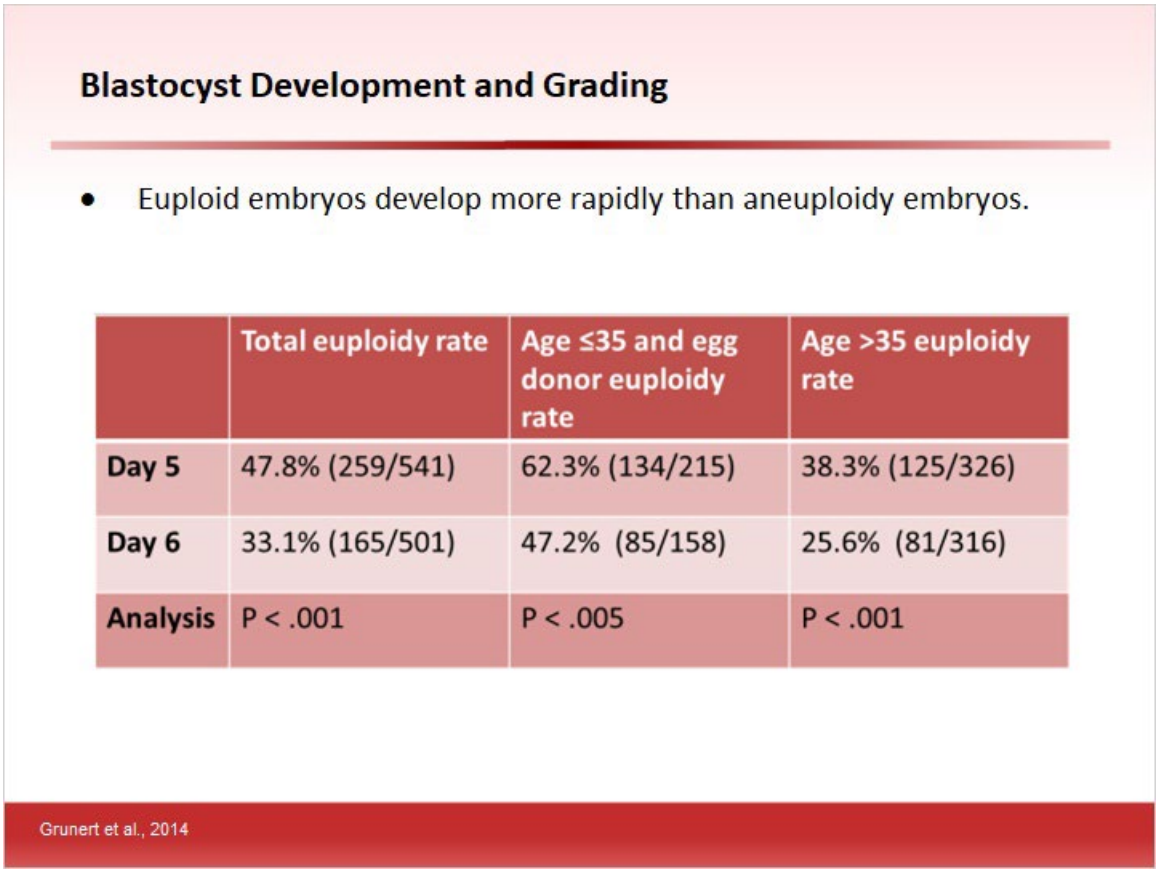


Notes:

Day-3 cell progression can be predictive of blastocyst formation. A proportional relationship exists between the number of blastomeres present on day 3 and rates of blastocyst formation. As day-3 blastomere numbers increase, a significant increase in blastocyst formation can be observed in both donor and non-donor cycles.

Although higher blastulation rates were observed in embryos that had progressed to at least 8 cells by 72 hours post-insemination, a significant number of embryos with initially retarded development were able to progress to the blastocyst stage by 120 to 144 hours post-insemination. These data suggest that blastocyst culture may be a viable option for all patients, regardless of the number of day-3 rapidly progressing embryos.

1.40 Blastocyst Development and Grading



Notes:

It has also been shown that euploid embryos develop faster than do aneuploid embryos. A significant difference was found in the euploidy rate for day-5 vs. day-6 blastocysts.

1.41 Blastocyst Development and Grading:

Blastocyst Development and Grading: Summary

- Evidence supports transfer of the most rapidly developing, highest quality blastocyst on day 5.
- Top quality day-5 blastocysts with high ICM and TE scores tend to have the highest implantation potential and the lowest rates of aneuploidy.
- Day 3 cell stage is proportional to day-5 blastulation; progressive cleavage-stage embryos blastulate at the highest rate. A significant number with slower development are able to progress to the blastocyst stage.
- Euploid embryos reach blastocyst faster than aneuploid embryos.

Notes:

To summarize, evidence supports transfer of the most rapidly developing, highest quality blastocyst on day 5.
Top quality day-5 blastocysts with high ICM and TE scores tend to have the highest implantation potential and the lowest rates of aneuploidy.
Day-3 cell stage is proportional to day-5 blastulation; progressive cleavage-stage embryos blastulate at the highest rate. In addition, a significant number with slower development are able to progress to the blastocyst stage.
Evidence supports that euploid embryos develop faster than aneuploid ones.

1.42 Thank you!

Notes:

Thank you for participating in this educational activity.