

Evaluation of in vitro-produced bovine embryos

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At the 2014 joint meeting of the American and Canadian Embryo Transfer Associations, I presented a pre-conference symposium on bovine embryo grading in an interactive forum where participants provided real-time feedback on how they would grade embryos of various stages and qualities. One aspect of grading touched on was the difference between in vitro-produced (IVP) and in vivo-derived (IVD) embryos. When asked whether the attendees believed we need separate grading systems for these two types of embryos, the crowd was split with 47% of respondents supporting the development of a new system and 53% believing it is not needed. Here I will review some of the challenges involved with grading IVP and IVD embryos, the implications of these challenges, and propose a method by which researchers and practitioners could collaborate to gather data that can be easily shared and used to develop a consensus on the best guidelines for grading IVP embryos.

Morphological evaluation of embryos

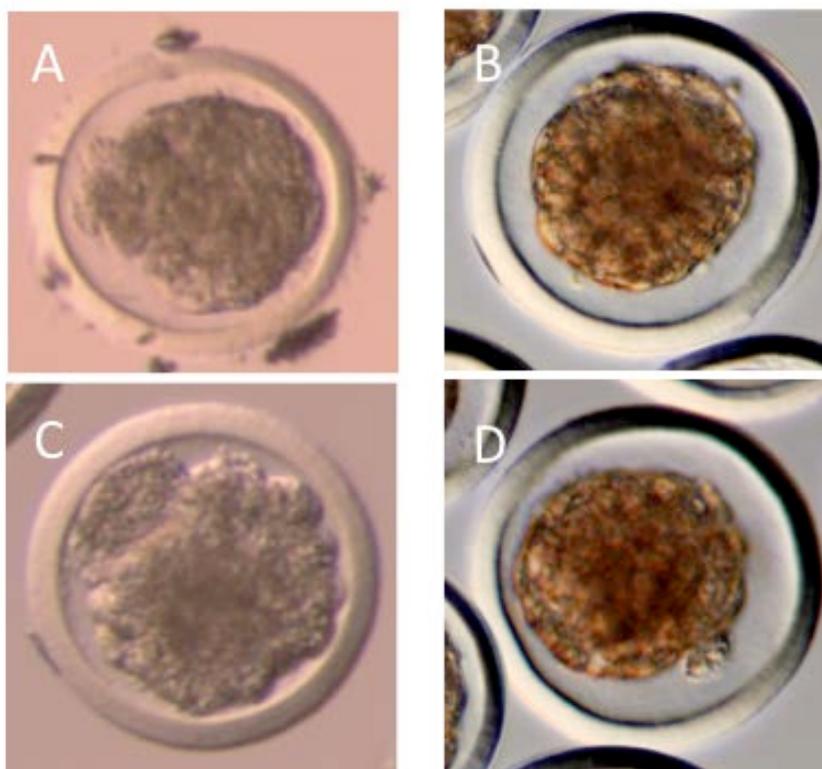
The only way to truly know if an embryo is viable is to transfer it to a recipient and see if a healthy offspring results. Of course the impracticality of transferring every embryo leaves practitioners with the difficult task of determining the likelihood that an embryo can generate a pregnancy just by looking at it. While most practitioners get good results grading in vivo-produced embryos using the standards set by the International Embryo Transfer Society (IETS Manual, 4th edition), it has been noted that IVP embryos have different morphologies than IVD embryos. Pregnancy rates after transfer of IVP embryos are lower than IVD embryos (Hasler 2000) and IVP embryos do not survive freezing as well as IVD embryos (Pollard and Leibo 1993). Clearly there are differences in these two types of embryos; differences from mitochondrial function and metabolism to developmental rates and gene expression. That body of literature will not be reviewed here. Rather I will focus on the observable differences between IVD and IVP embryos.

Most practitioners and embryologists know a good embryo when they see one, but when it comes to intermediate quality embryos (the 2s and 3s) they can be difficult to distinguish. Many have suggested that IVP embryos have more variable morphologies, and therefore are more difficult to grade. Embryos are graded on their conformity to a number of physical characteristics including shape, color/density of cytoplasm, number and compactness of cells, area of perivitelline space, number of extruded or degenerate cells, frequency and size of cytoplasmic vesicles and stage of development relative to age (Wright and Ellington 1995). Variation from the norm or what is considered characteristic of an excellent quality embryo seems to be more common for IVF embryos than IVP embryos. Below are some of the most notable differences.

The perivitelline space and poor compaction

Several studies have demonstrated differences in IVP and IVD embryos when it comes to the size of the perivitelline space, which is often related to how well the embryos have compacted during the formation of the morula. In one study by Van Soom and de Kruif (1992), in vivo-produced zygotes and 2-cell embryos were cultured in the same system as slaughterhouse-derived, in vitro-produced zygotes. A diminished perivitelline space was observed at the morula stage in IVF embryos. In addition, IVP zygotes consistently had a larger perivitelline space than their IVP counterparts. This feature is still seen in IVP embryos today, despite 24 years of research since this was documented (Figure 1).

Figure 1. An IVP (A and C) and IVD (B and D) bovine embryo at the morula stage of development. Note the smaller perivitelline space in the IVP embryos in addition to the poor compaction. Embryo C also has some extruded material on the upper left edge of the embryo. The perivitelline space of the IVD embryo is more uniform as is the compaction of the morula.



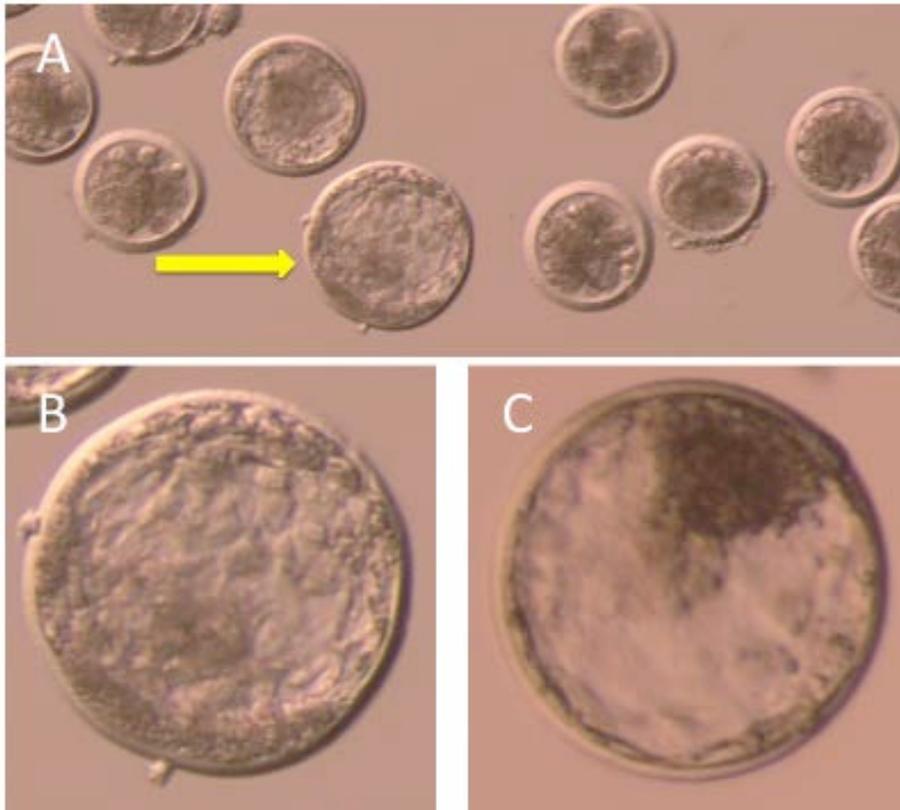
Debris and extruded cells

Many practitioners and embryologists have noted an increase in the amount and frequency of cellular debris associated with IVP embryos. This has been confirmed in ultrastructure analysis of IVP and IVD embryos. Rizos and colleagues (2002) found cellular debris in the perivitelline space of embryos, and also noted it around the ICM in the blastocoele cavity. This can make grading IVP embryos particularly difficult, especially at the blastocyst stage, when the amount of debris may be difficult to assess as it gets compressed between the zona pellucida and the expanding embryo. IETS guidelines for grading embryos state that a grade 1 embryo (top quality) has more than 85% of the cell mass within the zona pellucida as part of the viable embryo, grade 2 must contain 50%, grade 3 25%, and grade 4 <25%. Being able to evaluate the amount of living cells in the embryo effectively is important for assigning the proper grade to an embryo. Figures 2 and 3 provide examples of IVP and IVD embryos with varying amounts of debris at different stages of development. Sometimes expanded blastocysts collapse, which can allow evaluators to better assess the amount of debris associated with the embryo.

Figure 2. Two IVP embryos (A and B) and one IVD embryo (C) with varying amounts of extruded cells or debris. A is a morula that has a minimal amount of cellular debris so by that standard could be categorized as a grade 1 embryo but the poor compaction and minimal perivitelline space would drop this embryo to a grade 2. Panel B is an early IVP blastocyst with a significant amount of debris that would drop it into the grade 2 category. Panel C is a day 7 IVD embryo with an acceptable amount of debris such that it would still be considered a low grade 1 or high grade 2.



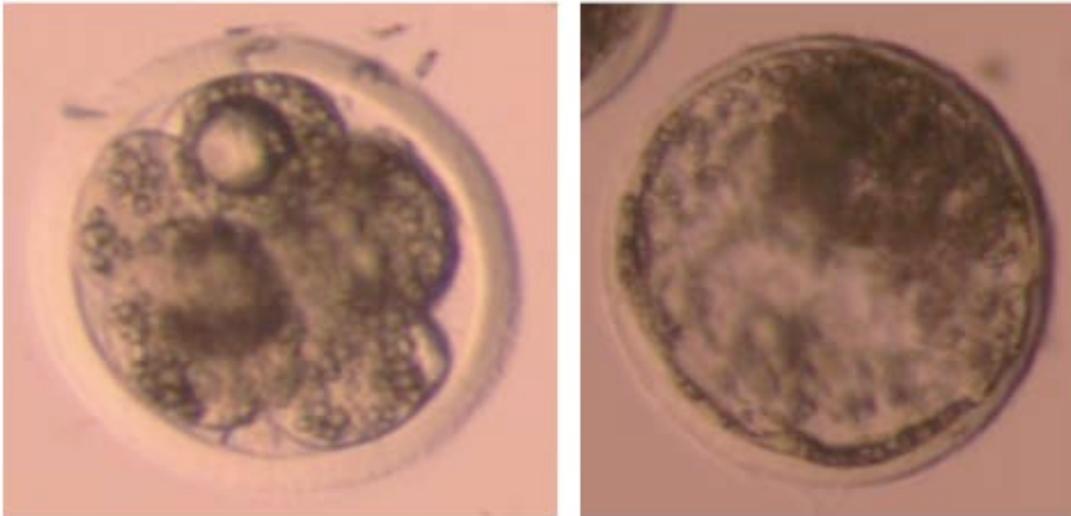
Figure 3. If scanning this group of embryos under fairly low power, one may quickly assume that the embryo indicated by the yellow arrow in panel A is a grade 1 embryo. Upon higher magnification, one can more readily appreciate the extruded cells compressed against the zona pellucida that could be mistaken for the inner cell mass. This is an easy mistake to make if not looking at embryos at the proper magnification when grading (at least 60x). Unorganized or poor ICM formation is another common feature of IVP embryos. Panel C is an IVP embryo with a well-defined ICM.



Vacuoles

Some embryos have what appear to be bubbles or vacuoles in their cells, which can give them a grainy appearance. This occurs in both IVP and IVD embryos, though the incidence appears to be higher in IVP embryos (Crosier et al., 2000, Shamsuddin et al., 1992). In vitro-produced embryos exhibit more vacuolization within the cytoplasm of blastomeres than in vivo-derived ones. The size and abundance varies widely (Figure 4). There are no known causes of vacuolization, but it is generally associated with poor embryo quality.

Figure 4. Two IVP embryos with extensive vacuolization. The left panel has a 6-cell embryo with vacuoles of a variety of sizes. The right panel has a blastocyst with a number of very small vacuoles visible in the trophoblast and ICM cells.



Cell shape, opacity, and lipid

Ultrastructure analysis of IVP and IVD embryos has also revealed differences related to the amount of lipid (Crosier et al., 2000). Morulae from superovulated cows were compared to morulae produced from slaughterhouse ovaries. IVF embryos had a greater volume density of lipid than IVP embryos. IVF embryos cultured in 10% serum throughout culture had a higher volume density of lipid than with culture systems with restricted serum during the first 72 h culture, no serum, or in vivo-produced embryos. Other groups have also noted a higher incidence of lipid droplets in IVF embryos over IVP embryos, confirming what has been noted in other studies and related to poor cryotolerance of embryos (Rizos et al., 2002, Pollard and Leibo 1994). Higher lipid content can make embryos appear to be darker and the cells more opaque. As with vacuoles, the embryos can appear grainy. Van Soom and de Kruif (1992) noted that unlike IVD embryos, IVP embryos had more square, swollen blastomeres and irregular, opaque cells in the blastocyst.

Implications of morphological differences

Given these morphological differences, are IVP embryos really more difficult to grade? In 1995, Farin and colleagues tried to tackle this question with a study in which six experienced embryologists were asked to grade the same IVP and IVD embryos. They found that when it came to stage of embryo development agreement between technicians was higher for IVD (85%) than for IVP embryos (72%). The challenge appeared to be distinguishing morula from blastocysts. When it came to quality, agreement was similar for IVD (61%) and IVP embryos (58%) with the most challenging differentiation being between grade 2 and 3 IVP embryos. It is likely that poor organization of the embryos, excessive debris and extruded cells and opacity of the embryos contributed to the differences between evaluators.

Many of the characteristics noted in IVP embryos suggest that they are inferior in quality, and indeed that has been realized with lower pregnancy rates when these embryos are transferred (Hasler 2000). While the gap between pregnancy rates from IVD and IVP embryos may be closing as our in vitro culture systems improve, we are still working with a suboptimal environment compared to the oviduct or uterus. During the in vitro embryo production process, embryos sit in a static environment where their resources within the media are being depleted; waste products also can be concentrated around the embryo, something that is not likely to happen in vivo. This environment can alter embryo metabolism and affect development of the embryo.

Many of the studies that have documented morphological differences in IVP and IVD embryos were conducted well over a decade ago when using co-culture systems and high concentrations (10% or greater) of serum in maturation or early culture stage media were more common. Serum and co-culture have been linked to the increase in lipid that is seen in IVP embryos (Crosier et al., 2000 as an example) and also to large calves when those embryos have been transferred (Hasler 2000). Some IVF systems still incorporate serum but often at much lower concentrations particularly during maturation and early embryo development.

The App

So where does all of this leave us? It is hard to gauge the quality of embryos being produced through IVF industry wide as most practitioners and companies do not have the means and/or desire to share their data. We also don't know which of the noted differences in IVP embryos are affecting pregnancy outcomes, though it is likely a combination of factors. These data have been hard to gather traditionally as they require practitioners to keep detailed records and then send them in or input them into a computer – a task the requires time – something that most practitioners do not have in abundance. With the ubiquity of handheld smart phones and tablets with high quality cameras we now have a tool that could be used on the fly to document embryos that are being produced and transferred.

I propose the development of an application (or app) for AETA/CETA where practitioners, using adapters for their smart phones and tablets on their microscopes, could take pictures and videos of embryos before they transfer them. Additional data could be included such as the type of embryo (IVP vs IVD), whether the embryo came from a superstimulated cycle, day, stage, etc. and ultimately pregnancy result. These data would be gathered by the app and could be presented annually at the meeting.

As users upload photos and videos of embryos, they could ask for immediate feedback from other users on how they would grade specific embryos. An open resource where people could go through hundreds, maybe thousands, of photos and videos would provide an excellent training tool and would likely result in more uniform grading across the industry. It would also be a valuable resource for students and new practitioners. A well designed, easy-to-use app would provide a large amount of information that could move us towards better understanding how IVP embryos are performing in the industry. Anonymity through self-assigned user names would likely increase the responsiveness of participants both in providing information and pictures/videos of embryos to be included in the database as well as being willing to grade embryos submitted by others. The power of the app would grow with greater participation.

Conclusion

Embryo grading systems allow practitioners and embryologist to make educated decisions on which embryos to transfer and which to discard. It is clear that IVP embryos have morphologies that differ from IVD embryos. As IVP embryos become more integrated in cattle reproduction practices, knowing how the various morphologies impact pregnancy rates is critical in helping practitioners make decisions that lead to pregnancy rates that are comparable to (or better than) IVD embryos. Without improvements to current in vitro systems, it may be that stricter culling of IVF embryos compared to IVD embryos is necessary to meet that goal (e.g. no transfers of grade 3 embryos). We now have the technology to generate data necessary to begin to answer some of these questions through the potential development of an app for handheld and tablet devices. This app would provide near real-time feedback for questions regarding embryo grading, a valuable training tool for new and experienced embryologists, and a database of information that allows us to better understand how IVP embryos (including those with the aforementioned morphologies) are performing in the industry on a global scale.

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