Equine ICSI, a private practice perspective: expectations, discussion, and thoughts
Rob Foss
Equine Medical Services, Inc., Columbia, MO

Abstract

Embryo production by ICSI, especially through the use of transported oocytes, is becoming a more common clinical procedure in equine practice. Veterinarians and in turn their clients should become familiar with not only the logistics of the procedures but also factors that can influence the outcome in either a positive or negative manner.

Keywords: Embryo, ICSI, equine, OPU, TVA, oocyte

Introduction

The veterinary practitioner collecting and shipping equine oocytes for ICSI faces many challenges. The technique of oocyte pickup (OPU) by transvaginal follicular aspiration (TVA) presents mechanical and technical demands, but these are no more important than the challenges presented by the client and the practice situation itself. Embryo production by OPU and ICSI can yield remarkable results, but not all cases are equally productive. Our practice produced 450 commercial blastocysts via ICSI in 2016 with an average of 0.7 blastocysts per TVA, with a range of 0-7 blastocysts per TVA. Unfortunately, blastocyst production is not always distributed equally among clients and mares. Client expectations may or may not be realistic; unrealistic expectations can be difficult to satisfy leading to frustrations for clients and veterinarians alike. Horse breeders are familiar with the process of artificial insemination where the major obstacle is providing sufficient sperm to populate the oviduct in a timely manner so that an ovulated oocyte will be fertilized. When both ovulation and fertilization are performed with a mechanical nature via ICSI, the uninformed client will not see further barriers to successful production of an embryo, a pregnancy, a foal, and an eventual world champion. Understanding some of the processes involved can lead to more realistic expectations and an appreciation of success.

Attrition in the process

Immediately following recovery of oocytes the numbers of potential embryos visualized by the client starts dropping. Aspiration of small antral follicles in the mare draws oocytes from a diverse pool, especially in the first aspiration of the season for a mare. The oocytes can range from not being developed enough to enter meiosis when stimulated, to atretic and degenerated oocytes that no longer retain meiotic competence. This variety of developmental stages results in only a portion of the recovered oocytes responding to in vitro maturation (IVM), regardless of the efficacy of the IVM program. Some aspirations will yield a very high percentage of oocytes that respond to maturation, up to 100%, while others can have extremely low percentages of oocytes capable of meiosis. We see an average of 60-70% of the oocytes progressing to metaphase II, but each mare and each aspiration is unique, and while a client may assume all oocytes of an individual aspirate will mature, that may not be the case.

Removal of the cumulus cells surrounding the oocyte, denuding, is performed following IVM to allow visualization of the oocyte for evaluation and for sperm injection. This takes place in a solution of hyaluronidase that breaks down some of the bonds between cumulus cells themselves as well as between the cumulus cells and the zona pellucida. Each oocyte is repetitively pipetted through pipettes of decreasing diameter to remove the cumulus cells. Occasionally this process will result in breech of the zona pellucida, possibly due to zona pellucida damage during aspiration, resulting in lysis of the oocyte. This loss of oocytes in our laboratory is usually around 2-4% but can be significantly higher in certain instances.

Not all oocytes will survive the ICSI process itself. The equine oolemma is a remarkably elastic structure and is one factor that makes equine ICSI challenging. Its extreme elasticity resists penetration with a micropipette, and even when assisted with a piezo drill, penetration can result in tearing or damage sufficient to cause lysis to the oocyte. The oocyte will appear relatively normal immediately following
ICSI, but will start to degenerate in a short period of time. Oocyte lysis following ICSI is usually in the 2-4% range for our laboratory.

Oocyte activation is the next big hurdle in the general attrition of numbers. Metaphase II oocytes remain in meiotic arrest until activated by a cytosolic sperm factor, phospholipase C zeta (PLC zeta).¹ Release of sperm factor in the oocyte results in multiphasic calcium release that is necessary for the resumption of meiosis and eventual embryogenesis.² Stallion spermatozoa possess a relatively large amount of PLC zeta activity compared to other mammals, but individual stallions vary greatly in their capacity to activate oocytes in the in vitro environment.³ While sperm cells from some individual stallions have high rates of oocyte activation others can have extremely low rates. Oocytes appear to vary in the ease of activation between mares as well, so the combination of a stallion and mare both with low activation rates can lead to extremely low activation and then cleavage of oocytes. We see an overall average of activation and then cleavage of sperm injected oocytes around 70%, but again, averages do not always hold true with individual cases.

Day 5 of embryo culture following ICSI offers the next dramatic reduction in numbers. This is the period when compaction occurs, creating the compact morula. Starting as a loose association the cells orient themselves into a compact mass with tight junctions. Cells that are not included often will lyse or be segregated out to become extruded blastomeres. Nearly all embryos that form compact morula in this phase, day 5-8 will progress to blastocysts in about two days, depending on the percentage of the cells included in the compact morula and the overall vitality of the embryo. Those that do not form compact morula will not progress. Average rates of embryos compacting at this stage are around 30%, but individual cases can vary from 0-100%. Counting on averages for individual cases can sometimes provide frustration for the client and veterinarian.

Oocyte quality/competence

Oocyte quality is probably the largest single contributor to the variability of success of equine ICSI. Meiotic competence, the ability to resume meiosis when freed from the inhibitory effect of the follicle is an important factor. Morphologic abnormalities such as vacuoles, misshapen oocytes, fragmentation, and evidence of aging such as thin zonas and large perivitteline space are negative indicators of quality. While one oocyte may appear like another, their innate potential can be quite different. The true measure of oocyte quality is developmental competence, the ability of an oocyte to not only complete meiosis but to respond to fertilization by activation, division, and development into a normal viable embryo. Depending on the individual case, the rate of developmental competence may approach that of meiotic competence or be far lower. Nuclear maturation can proceed more quickly than cytoplasmic maturation in the oocyte once placed in maturation conditions, and the inability of the cytoplasm to support the development of the resulting embryo appears to be an important factor. It also seems that there is further innate variability in the developmental competence of small follicle oocytes recovered from mare to mare.

Mare effects

Mare differences play an important role in blastocyst production, particularly antral follicle count, age, and oocyte quality. Antral follicle count will give some indication of the number of recoverable oocytes, and increased numbers of oocytes available for ICSI gives increased opportunity for successful embryo production. Antral follicle count will vary from season to season and when evaluating mares for aspiration we expect a higher oocyte recovery rate from follicles that are between 5 and 20 mm diameter with decreasing recovery from follicles larger than 20mm. One should also note that in some mares it is difficult to discern between subepithelial inclusion cysts commonly associated with the ovulation fossa and small follicles. The first aspiration session of the season has a tendency to yield higher numbers of atretic oocytes than subsequent aspirations.

Increasing mare age does have an impact on embryo production on the average, largely through decreasing antral follicle count and oocyte production. Age related oocyte quality effects seem to arise at different points for different individuals, but age 24 appears to be important in our clinical program. Each
year we have several 24-year-old mares that have good blastocyst and pregnancy production but after age 24 production falls, and early embryonic loss tends to increase.

Oocyte quality and developmental competence are quite variable from mare to mare. One factor that has been little discussed but appears to be important is the ease with which oocyte activation takes place.

**Stallion factors**

We find that some stallions’ sperm has a high rate of oocyte activation, cleavage, and blastocyst formation compared to the average and other stallions have very low rates. While results from some of the low yielding stallions can be improved with sperm preparation and artificial activation, others can prove quite difficult. The proficiency of production by the sperm of a specific stallion can only be determined by use, either on clinical cases or via trials, something for a practitioner and client to take into account before starting on a project.

While only an individual sperm cell is injected into each oocyte, the overall quality of the sperm sample can have an impact on the outcome. Some stallion managers have reserved frozen semen of non-commercial quality for use on ICSI, saving the better freezes for commercial frozen semen AI. This can be counter-productive, as higher quality semen may give better results. Since so little semen is used for ICSI, utilization of commercial quality semen should be suggested.

Refreezing previously frozen semen in ICSI-dose straws (usually around 1 million cells per straw) has become rather common and works well for some stallions. This can be a very useful procedure to increase the potential foals from some stallions with limited semen availability. Unfortunately it does not work as well for others; sometimes the sample does not retain good post-thaw motility following the refreeze and in some instances oocyte activation rates are decreased from that seen with the single-frozen sample. It is becoming more common for breeders to purchase refrozen ICSI-dose semen by the straw (especially for warmblood breeders) and practitioners should advise some caution. It would be prudent when possible to have some indication that semen from that refreeze batch has been used successfully.

**Seasonal factors**

Our practice has found the period of fall transition to be quite productive for some mares. These mares tend to have an increase in the number of small follicles available for aspiration and it appears that oocytes collected in this period can have an overall higher degree of developmental competence in some mares. Spring transition can also be useful, but the multiple follicles present do not remain small enough (5-20mm) to be high yielding for very long. As mares progress into the non-cyclic period of winter anestrus, useful oocytes can still be collected from most mares, but there appears to be a decrease in developmental competence of the oocytes collected, varying widely between individual mares.

**In vitro embryos**

Embryos produced by ICSI and in vitro culture are generally transferred to recipient mares as early blastocysts, roughly equivalent to a day 6 in vivo embryo, although this may occur between 6 and 10 days following ICSI. The embryos that form blastocysts earlier rather than later are more likely to establish viable pregnancies. Initial pregnancy rates in our practice are 5-10% lower than for in vivo produced embryos, and there is a higher rate of early embryonic death. Most of the pregnancy loss is before heartbeat detection, especially in slow-growing embryos, but there is a small group that will lose pregnancies between 30 and 45 days of gestation we do not generally see with in vivo-produced embryos.

In vitro produced blastocysts do not form an embryonic capsule until after they are transferred into the uterus. Since blastocysts that are left in culture too long will attempt to hatch by extruding trophoblast cells through the hole in the zona left from the initial ICSI, and since equine embryos not protected by a capsule or zona pellucida do not survive well in the uterus, there is a relatively small window of time in which the in vitro produced embryos are suitable for embryo transfer. The early blastocyst usually develops two days after forming the compact morula. This will often give some advance notice of an upcoming blastocyst and transfer for the practitioner, but sometimes the visually
dark nature of in vitro embryos combined with obstruction of visibility by extruded blastomeres or other cellular debris makes identification of the compact morula difficult. This means that practitioners receiving ICSI embryos for transfer might have less warning of the timing and number of embryos to be shipped than might be desirable. Once these early blastocysts are transferred, the first pregnancy examination is usually scheduled for five days later, anticipating a 2.5-5 mm vesicle as would be normal for an 11 day pregnancy.

**Conclusion**

Many factors enter into the successful outcome of OPU of equine oocytes for ICSI, but even more factors can enter into a client’s assessment of success and satisfaction for the process.

**References**