In order to perform assisted reproductive techniques, it is imperative to be familiar with the estrous cycle of the mare and how to manipulate it. To be successful in obtaining pregnancies when performing these procedures, equipment, facilities and owner’s expectations should be researched and determined. In private practice, numerous assisted reproductive techniques can be offered to clients. These include; insemination with cooled or frozen semen, low dose or deep horn insemination, preparing cooled or frozen semen, embryo transfer, trans-vaginal oocyte aspiration and transfer, Gamete intra-fallopian transfer (GIFT), and Intra-cytoplasmic sperm injection (ICSI).

Artificial insemination consists of the manual deposition of the correct number of live motile spermatozoa into the mare’s uterus at the optimal time. This can include fresh, cooled, or frozen semen. The advantages of artificial insemination include; breeding large number of mares with one ejaculate, transport semen from distant places using frozen or chilled semen, adding extenders and antibiotics to semen to increase the fertility of some stallions, not having to transport the mare to the stallion, breeding more mares to a stallion, reduce the risk of disease transmission, have greater access to stallions, earlier detection of fertility problems, decrease the risk of injury to mare, stallion and personnel, and easier to breed the mare at the proper time. The disadvantages of artificial insemination include; needing increased knowledge and skills in mare and stallion management, increased equipment needed, decreased revenues for vanning companies and boarding facilities, and a decreased genetic pool. With this known, artificial insemination has become a widely accepted and used technique in the non-thoroughbred populations.

**Semen Handling**

Methods of preserving stallion sperm by cooling the sperm to 5 °C has been developing for greater than 20 years.1-2 Ideally an insemination dose that is packaged, cooled and shipped starts with a dose of 1 billion progressively motile sperm that contains a dose of at least 500 million progressively motile sperm when it reaches its destination. Incubating sperm with high levels of seminal plasma for an increased duration has been determined to be detrimental to spermatozoa. Therefore techniques such as diluting the sperm at least 1:3 with cooling extender or centrifugation prior to mixing with cooling extender has decreased seminal plasma concentration to < 25% of the fluid volume.3 If centrifugation is necessary the use of a cushion in the bottom of the centrifuge tube allows the sperm to be centrifuged with greater force and/or for longer times which allows increased sperm recovery during the process and limits the damage done to the spermatozoa.3 Modification to the original skim-milk formula have produced a number of commercial cooling extenders that have improved sperm survival after cooling. Researchers have investigated adding different antioxidants to the mixtures which have helped sperm longevity significantly compared to classic Kenney extenders.2,4 Some of these additives are milk casein proteins including phosphocasienate.2

**Frozen Semen**

Success with frozen semen requires that the veterinarian be familiar with the techniques for properly thawing, evaluating and handling of frozen semen as well as the breeding strategies employed to maximize fertility. When a mare enters into a breeding program in which frozen semen will be used, there are many factors that will influence the pregnancy rate. These include; the integrity and fertility or quality of the semen, the insemination dose, the timing of the insemination, the site of insemination, the reproductive status of the mare and the management of the mare during her estrous cycle.5,6 It is imperative that the owner of the mare understands the impact of these factors and their economic ramifications so
that they can decide which stallions to breed to, how many doses of frozen semen to have available and whether their mare is a good candidate.

One of the most important reasons why frozen equine semen has not been used on a broader scale is the great variability of sperm from different stallions to tolerate the freezing and thawing process.\(^7\) It is thought that only 25% of stallions will have pregnancy rates comparable to fresh semen or natural cover when inseminated into healthy mares at the proper time.\(^8\) This stallion variability leads to the pregnancy rate per cycle ranging from 0-100% with most falling between 30-70%.\(^5\)\(^6\)\(^9\) This increases the number of cycles (1.8-3) needed to obtain a pregnancy per season compared to fresh or cooled semen.\(^10\) It is therefore imperative that mares bred with frozen semen be chosen wisely so that there are no compounding factors.\(^11\) In a study in which records of the insemination of 578 mares between the 1991-2000 breeding seasons resulted in a 56% first cycle pregnancy rate (range 41-72%) depending on the mare population. Mare status had the biggest effect on 7-14 day pregnancy rates with young maiden mares (<7 years old), older maiden mares (>8 years of age), barren mares and mares with foal at foot having an average first cycle pregnancy rate of 68.8, 37.2, 56.2 and 59.3% respectively.\(^6\) In addition, mares that were diagnosed with endometritis based on culture and cytology had an overall first cycle pregnancy rate of 38.5% in the cycle post-treatment.\(^6\)

**Preparing the mare**

Since pregnancy rates per cycle are decreased when mares are bred with frozen semen, it is critical that the mare is in optimum condition when ready to be bred. History and knowledge of the previous estrous cycles is important. All mares should be examined by trans-rectal palpation and ultrasound early in estrus and depending on the mare’s age and history a culture and cytology performed. Young maiden mares <7 years of age may not need to have this procedure performed unless intra-luminal fluid is present, while older barren mares may need to have a low volume lavage performed in order to get a representative sample. It is important that endometritis due to infection or inflammation be treated with the appropriate antibiotics and anti-inflammatories in the cycle prior to the breeding cycle, so that precious doses of semen are not wasted. Repeat culture and cytology is warranted prior to breeding the following estrus.

Once the mare is in estrus, she can be followed daily until a 35 mm follicle is evident with the appropriate uterine edema and cervical dilation. Administration of an ovulatory inducing agent such as Human chorionic gonadotropin or the GnRH analog deslorelin (Ovuplant or Sucromate) is imperative in order to maximize the use of semen by minimizing the number of insemination doses per cycle as well as potentially reduce the number of times palpated and the intensity of management. Ovulation occurs in the next 48 hours with a range between less than 12 and greater than 48 hours.\(^12\) Due to the wide range in ovulation times mares are palpated every 6-8 hours so that insemination can occur when ovulation is imminent or immediately after. Deslorelin on the other hand has a smaller window between 36-48 hours during which ovulation will occur. This is the author’s preference, usually identifying ovulation 40-42 hours after deslorelin (Sucromate) administration. Timing of insemination relative to ovulation is so important because the freezing and thawing process causes membrane and osmotic change as well as changes in the intracellular and extracellular calcium levels of the thawed spermatozoa.\(^13\) This has an impact on the number of sperm capable of attaching to the oviductal epithelium as well as delaying the spermatozoa uterine transit time and longevity.\(^14\)

There is controversy between studies as to whether timed breeding at 24 and 40 hours post ovulatory inducing agent yields higher pregnancy rates than breeding once prior or within 12 hours post ovulation.\(^15\)\(^16\) If only one dose is available per cycle then intensive management with palpation/ultrasonography every 6-8 hours is believed to be necessary and breeding within 6 hours post ovulation provides pregnancy rates comparable to mares bred within 12 hours prior to ovulation.\(^17\)\(^20\) However, in a practical situation with long distances involved palpation every 6-8 hours may not be possible. Newcombe et al 2011
found in a retrospective study of 867 cycles that there was no statistical difference in either the pregnancy rate or early loss rate in a postovulatory insemination regime with routine post-insemination treatment when the examination of mares was at intervals of any less than 12-15 hours. Therefore, if management warrants, mares can be checked twice daily and bred within 12-15 hours of ovulation satisfactorily (44-56% pregnancy rates).21

Insemination

Dose and technique potentially affect one another. In addition, the number of spermatozoa per insemination dose can vary depending on the number of times a mare is bred per cycle. The standard accepted insemination dose for cooled semen is 500 million progressively motile spermatozoa deposited within the body of the uterus every 48 hours until ovulation occurs.22 Based on numerous studies, it appears that insemination doses for frozen-thawed semen exceeding 250 progressively motile spermatozoa, again placed within the uterine body is likely to optimize fertility.3,15 Depending on the progressive motility of the individual stallion’s spermatozoa an insemination dose of 600-800 million total spermatozoa may be necessary. Using these numbers Barbachini and Ioomis found pregnancy rates were higher for mares inseminated twice with 800 million total sperm (76% per cycle) compared to those inseminated once with 800 million total sperm (57%). For those inseminated twice with 400 million total sperm, pregnancy rates were 80% per cycle. Pregnancy rates were similar for those inseminated with 400 million total sperm at 24 and 40 h after hCG (21/35; 60%/cycle) compared to those inseminated twice with 800 million total sperm (4/13; 31%/cycle).16 Sieme et al. reported similar pregnancy rates for mares inseminated once 30 h after hCG with either 100 or 800 million total frozen-thawed sperm.23 Based on these studies, one can conclude that when using good quality frozen semen acceptable pregnancy rates can be obtained when mares are examined with ultrasound daily after hCG administration and inseminated at 24 and 40 h with 400 million total sperm. Pressure from owners to split doses due to the high cost of frozen semen/dose, especially if it is imported, as well as the poor quality or infertility of some stallion’s spermatozoa when cryopreserved, a limited supply of frozen semen from a dead horse and emerging technologies such as sex-sorted semen has led to the search for the answer to the question, How low can you go?24 This question has led to the development of different techniques; deep intrauterine horn insemination manually or via hysteroscopy, or gamete intrafallopian transfer, minimizing the dose of semen by allowing spermatozoa to gain access to the oviductal papilla in greater numbers and more efficiently.24 With either method of low-dose insemination, the inseminate dose has been reduced to 1 – 25 x 10^6 PMS in volumes ranging from 20 to 1000 microliters.25 Pregnancy rates for manual deep horn insemination of 5 – 25 x 10^6 fresh spermatozoa ranged from 30 to 56%,26 Pregnancy rates for hysteroscopic insemination of 0.001 – 10 x 10^6 fresh spermatozoa range from 10 to 75%.26,27 Debate continues over which technique offers higher pregnancy rates, and comparisons between studies is hampered by potential differences in the fertility of the mares, stallions, and expertise of personnel. Direct comparison of these techniques with the same population of mares and stallions showed no difference in pregnancy rates when 5 x 10^6 PMS cooled for 24 h were inseminated.28 If the insemination dose is decreased to 1 x 10^6, it has been suggested that pregnancy rates may be higher with hysteroscopic versus manual deep horn insemination.27

The inflammatory component of low-dose insemination techniques is also controversial. Some studies have found a significant interaction between pregnancy rate, method of insemination and reproductive history, with problem mares having lower pregnancy rates when inseminated deep horn verses inseminated in the body. The opposite was found in “normal” mares.23 While other studies have revealed that mares with delayed uterine clearance accumulated more fluid 24 and 48 h after insemination than normal mares, there was no difference in the percentage of leukocytes among groups or treatments. There did seem to be an association between the duration of the hysteroscopy and the concentration of leukocytes in normal, but not in DUC mares. Regression analysis of the data determined that if hysteroscopy extended beyond 7 min, endometritis is likely to
persist 48 h after the procedure.\textsuperscript{24} Gu¨ven et al compared uterine inflammatory reactions of mares inseminated with low and high numbers of frozen – thawed semen into the tip of the uterine horn (UH) ipsilateral to the preovulatory follicle with those of mares inseminated into the uterine body (UB). The variables used to assess inflammation were: presence of ultrasonically detectable intrauterine fluid, PMN numbers, lysozyme concentration, and TIC in uterine fluid. Mares inseminated with $20 \times 10^6$ using deep horn technique, accumulated less intrauterine fluid than those in the other groups, which had similar amounts. No significant differences in PMN numbers were detected in either tampon or lavage fluid. Enzyme levels between groups did not differ statistically, except for TIC, which was lowest in the uterine horn $200 \times 10^6$ group. Therefore, they concluded that deep uterine horn AI caused no greater inflammation or irritation than uterine body AI in normal mares 24 h after insemination.\textsuperscript{29}

**Novel techniques**

Interest in the use of sex-sorted semen and the associated technology has increased over the past few years. A new technique developed by Herman Ramirez in Chile uses nanoparticles to separate female from male spermatozoa.\textsuperscript{30} These cooled sexed spermatozoa should be inseminated within 12 hours of sexing. This technique is being used with increased frequency in polo ponies in Brazil, Chile and Argentina and becoming a commercial reality. The first sexed sorted semen inseminated mares produced their first foals this year. Freezing sex sorted semen after this technique has not been investigated. Another technique that is gaining popularity is utilizing epididymal spermatozoa as a frozen semen resource. Stallions that need to be euthanized due to unforeseen circumstances or young stallions that are castrated but the owners want to preserve their genetic material are candidates for this procedure. Several methods of sperm recovery from cauda epididymis have been described, including aspiration, flotation – in which sliced epididymis is placed on gel medium for some time, and retrograde flush of the cauda - in which pressure is generated by a syringe attached to the vas deferens and sperm is carried by the extender and expelled through a cut on the junction of cauda and corpus.\textsuperscript{31-33} Martinez-Pastor compared the retrograde flush and flotation techniques for the recovery of epididymal sperm and they have obtained a higher number of sperm using retrograde flush.\textsuperscript{34} In addition, the sample obtained by this technique did not present other cell types, which may be considered an important advantage of this technique. It has also been demonstrated that spermatozoa obtained from stallion epididymis after dilution with suitable extender present similar progressive motility when compared to ejaculated sperm obtained by artificial vagina.\textsuperscript{35} Epididymal sperm presents very poor motility, which is probably due to the lack of exposure to activating factors present in the seminal plasma.\textsuperscript{36} Studies have been investigating the influence of seminal plasma and sperm motility factors such as caffeine, progesterone, PHE (penicillamine, hypotaurine and epinephrine) and heparine.\textsuperscript{36,37}

Pentoxifylline is a substance that promotes the increase of the AMPc, which is responsible for spermatic motility, being able to stimulate recently recovered immobile spermatozoa. The use of a skim-milk extender containing pentoxifylline, Botu-Turbo (BT) for equine epididymal semen increased total and progressive motility in comparison to the skim-milk extender Botu-Semen (B).\textsuperscript{38} A recent study was conducted by Monteiro et al. (2009b) in order to compare post thaw fertility of ejaculated sperm (Group 1), epididymal sperm frozen immediately after castration (Group 2) and epididymal sperm frozen after 24h refrigeration at 5 °C (Group 3).\textsuperscript{39} All inseminations were performed with $800 \times 10^6$ viable sperm in the tip of uterine horn. Conception rates were, respectively, 61.5%, 92.3% and 61.5% (for groups respectively).\textsuperscript{38} In addition epididymal spermatozoa have been demonstrated to be suitable for ICSI procedures in horses.\textsuperscript{38}
Embryo transfer

The first successful embryo transfer in the horse was reported in 1972. Since then numerous new assisted reproductive techniques have been developed to help mares produce viable progeny. Embryo transfer is a process that removes an embryo from a donor mare and transfers it into a recipient mare, whom will continue the pregnancy and foal the offspring of the donor mare. This procedure has become popular for numerous reasons: one donor mare can produce multiple offspring in one breeding season; donor mares that are in training or athletic competition can continue their program without interruption or having to carry a foal to term; younger mares can be used as donors (ie. two year olds) without suffering the consequences of birth; mares that have reproductive problems or physical disabilities (fractured pelvis, chronic laminitis etc.) that make it difficult to carry a foal to term; perpetuation of endangered species and finally, research purposes.

Numerous factors have been identified to influence the success of an embryo transfer program. Keys to success are attention to detail, optimal reproductive management of donor mares, careful selection and management of recipient mare, adherence to guidelines for embryo recovery, evaluation and handling, plus a gentle transcervical transfer technique. Most importantly, donor mares need to be reproductively sound, stallions of good fertility and recipient mares of good quality. If normal donor mares are bred to fertile stallions an embryo can be recovered approximately 70% of the time an attempt is made, and about 50-60% of recovered embryos result in a pregnant recipient. This is an incredibly important factor for both the client and veterinarian to be aware of so that all expectations can be met and the financial implications recognized ahead of time.

Mares entering the embryo transfer program at the McGee Fertility Unit usually can be divided into two groups. The first are performance mares that are either in the middle of their show carrier or active polo ponies. The second are mares that have physical or fertility problems or older maiden mares whom have just finished their athletic careers. Some of the problems encountered in the donor mare population include; poor oocyte quality associated with age; anestrus; chronic endometritis (fungal/yeast, bacterial); decreased uterine clearance with persistent post-mating induced endometritis, cervical failure to dilate and abdominal hernias/prepubic tendon rupture. Some mares will have embryo flushes performed to try and differentiate early embryonic loss from fertilization and oviductal issues. Additionally, all donor mares are not necessarily under the direct management of the veterinarian performing the flushes and transfers, precise ovulation dates, breeding procedures and post breeding examinations are often equivocal, not optimal or not known. This can be challenging and frustrating at times.

The success of an embryo transfer program originates with the donor mare. If her reproductive issues are not addressed prior to breeding, the semen with which she is bred is suboptimal, she is not bred and ovulates within an appropriate time period or her post breeding management is not sufficient, performing the embryo flush will most likely be unsuccessful. A complete breeding soundness evaluation should be performed on the donor mare initially, to include; rectal palpation and ultrasound, culture and cytology, +/- biopsy depending on the mare’s age and reproductive history. A manual evaluation of her cervix both in diestrous and estrus can give insight to potential problems that may occur when breeding. Determining if the mare has regular estrus and diestrus periods and a normal interovulatory period can also rule out potential problems. Control of donor and recipient estrous cycle management, breeding and post-ovulation treatments will potentially increase embryo yield and decrease frustration and confusion.

Recipient mares should be selected based on age, history and cervical and uterine tone. To some clients, size and temperament of the mare matters so management of client expectations is imperative. Uterine culture/cytology and biopsies are suggested to make sure pregnancy maintenance once established should not be a problem. Donor mares should be cycling normally and in good physical condition before attempting embryo recovery. Having the donor, recipient and semen on the premises allows careful planning
and synchronization by the management and veterinarian. Ideally recipient mares will be placed under lights the beginning of December so that when needed they are already cycling and can be synchronized accordingly with the donor. If however they are in deep anestrus or transition mares can be treated with 6.6mg of estradiol 17B for two consecutive days followed by 5-7 days of short-acting progesterone preparation. (200mg IM q 24hrs).  

Three recipients for each donor mare is optimum, however two recipients will usually be successful if synchronized appropriately. Synchrony between donor and recipient has expanded recently so from the recipient ovulating one day ahead of the donor to 3 or 4 days after the donor.  

This can be accomplished most effectively by using progesterone and estradiol (P&E) or altrenogest in combination with prostaglandin and an ovulatory inducing agent such as deslorelin or HCG. Hagyard Equine Medical Institute does not support a recipient herd anymore due to cost prohibitive expenses of managing the farm and horses and land needed for enough recipients (30-50). Therefore, all embryos are shipped to a larger recipient herd or the owners provide their own screened recipients. Donors should be mated as close to ovulation as possible with 500 million progressively motile spermatozoa via artificial insemination from a fertile stallion.  

Participants in clinical practice are usually performed 6.5 - 9 days after ovulation. The equine embryo does not descend into the uterus until about day six after ovulation, therefore earlier attempts may not be as successful. Collections performed on day 6.5 or early day 7 can be done to procure a small (<300 micrometers) embryo for cryopreservation. The majority of mares in the United States are flushed day 7.5 - 8 after ovulation because the embryo recovery rates are high and the majority of embryos are blastocysts or expanded blastocysts which are larger and easily found under the microscope. The author prefers to perform an embryo flush day 8 post ovulation. This allows for easier searching of a larger expanded blastocyst verses a day seven morula or smaller blastocyst. Day 9 post ovulation embryos usually result in recovery of large embryos (1 – 2 mm in diameter). Embryo recovery is done by passing a bivona catheter with a cuff through the cervix into the uterus. The cuff is then inflated with flush media. Options for flush media include a variety of complete flush media which contain a Zwitterion buffer system, antibiotics and purified albumen or polyvinyl alcohol as a surfactant or lactated ringers solution or Hartman’s solution without additives. A recent study showed no difference in embryo recovery rate or pregnancy rate after transfer using a complete flush medium or Hartmann’s solution.  

Care must be taken if using a non-complete flush to recognize that embryos have a higher propensity to stick to the collection or searching dishes. A Y shaped closed system with individual tubes flushing in and out are connected to a filter. The uterus is then flushed with one to four liters depending on the size of the uterus. This solution is then allowed to drain out of the uterus into the filtered container where the embryo is recovered. This process is repeated several times, so that a total of six to eight liters of fluid has been used. The embryo is then searched for under a dissecting microscope. It has been demonstrated that if an embryo is not recovered following an initial series of lavages 1-2 additional liters can be infused and 20IU of oxytocin administered. A recent report showed initial recovery rates were 42.8% which increased to 57.2% with “reflush” attempts. Once the procedure is finished the donor mare receives prostaglandin. The embryo should be evaluated for stage of development, grade and size. These criteria can determine which recipient is the best match for the embryo as well as which embryos may be successfully cryopreserved. 

Embryos should be transferred into recipients as quickly as possible once they are recovered or placed in holding media in an equitainer if they are to be shipped. Non-surgical transfer entails depositing the embryo into the uterus via the cervix similar to the artificial insemination technique. This method is less traumatic to the recipient and less time consuming. The ability to maintain the viability of the equine embryo for 24 hours has allowed for the embryos to be shipped from breeding farm or reproductive facility to embryo transfer stations. This has resulted in very little decrease in pregnancy rates compared to
immediate transfer. These stations usually have large numbers of recipient mares and receive embryos from all over the country. Once embryos have been transferred recipients are examined for pregnancy, potentially day 11 or 12, 14, 16, 25 and 35. Early identification of a pregnancy allows for notification of an owner and facilitates subsequent breeding decisions and management of the donor mare. Pregnancy rate after transfer, pregnancy loss rate and live foal rate are key statistics used to evaluate the success of an embryo transfer program. These will vary according to the fertility of your donor mares, recipient mares and technical abilities. Guidelines for evaluation of success of an equine embryo transfer program presented by Colorado State University conclude that 70-75% pregnancy rate is good with > 95% being outstanding. 

Unfortunately, cryopreservation of equine embryos has not been as successful as in cattle. Freezing embryos that are less than 300 microns have resulted in a 50% pregnancy rate. This means that donor mares need to be flushed at day 6 after ovulation, when recovery rates are lower. It appears that between day 6 and 7 the capsule forms around the embryo that prevents cryoprotectants from penetrating the embryo. Commercial vitrification kits are now available from different manufacturers that make the process easier to perform although timing of manipulation is essential for success. Identifying which breed registries accept this technique is imperative prior to embarking on this procedure. Practices need to have the facilities and capabilities to maintain liquid nitrogen tanks for embryo preservation.

**Oocyte transfer**

Oocyte transfer is a technique that collects oocytes from the donor mare’s follicles using transvaginal ultrasound guided approach. This procedure is an alternative for mares in which embryo transfer is not an option. Mares that may have cervical problems, chronic infection/inflammation/yeast, oviductal or uterine adhesions or failure of ovulation are good candidates. The success rate in oocyte retrieval is about 75-80% in these mares. The oocyte is then placed into the oviduct or a recipient mare that has previously been inseminated. The recipient mare needs to be anestrous or have their dominant follicle aspirated so only the donor mare’s oocyte will be fertilized. Oocyte transfer is done by a flank incision with visualization of the oviduct and placement of the oocyte into the ampulla. Pregnancy rates after the transfers have been performed are determined by the age of the mare. Older mares have been shown to be less viable and more prone to fertilization problems or early embryonic death resulting in only 25-40% pregnancy rates. Younger healthy mares when oocytes are transferred into the same recipients had pregnancy rates as high as 60-80%.

**GIFT**

Another option with sub-fertile stallions is gamete intrafallopian tube transfer (GIFT). This technique is similar to oocyte transfer however, both the oocyte and spermatozoa are placed into the oviduct of the recipient mare. This allows the use of low numbers of sperm or frozen semen with a limited supply. Only 200,000 spermatozoa are needed. Pregnancy rates are similar to oocyte transfer.

**Other assisted reproductive techniques**

Newer assisted reproductive techniques have become more common over the last ten years with varying results depending on the laboratory. Intracytoplasmic sperm injection (ICSI) is a procedure that helps with stallions that have limited quantity, extremely low sperm numbers or poor semen quality since only one healthy spermatozoa is needed. The procedure includes the following steps:

1) recovery of oocytes from immature or dominant stimulated follicles via transvaginal ultrasound guided aspiration (TVA)
2) maturation (in vitro for immature, in-vivo for mature) of the oocyte to a point when metaphase (II&III) of meiosis(intact oolema and visible polar body) has occurred and
3) fertilization (ICSI) performed by injection and an immobilized blastocyst stage is reached and
4) transfer of the embryo in the blastocyst stage into the recipient.43

Several commercial ICSI centers in the United States are available and have demonstrated reasonable efficiency of the procedure. Current and realistic expectations should be relayed to the clients to prevent unnecessary disappointment. Oocyte aspiration in clinical practice has been described as having a 50-70% recovery rate of immature follicles aspirated and an 80% recovery rate for dominant stimulated follicles.42 This however comes with a lot of practice. For immature follicles in normal mares in vitro maturation rate to the MII stage has been reported to be approximately 65%,42,44 Following ICSI, blastocyst formation has been reported from 12 -23% depending on the laboratory.52,44,45 Once the embryo is transferred there is a reported increase in pregnancy loss in ICSI derived embryos with an expected 50 - 65% live foal rate.43 Again, realistic expectations for clients include necessitating recovery of 8 - 12 oocytes to result in a live offspring in normal mares.43 This may be accomplished by performing TVA every 2 weeks for potentially 8 sessions. This may take numerous oocyte aspiration sessions depending on follicular activity of the mare. It is important to inform the clients that the costs associated with the procedure are more expensive than traditional embryo transfer. However practitioners that are able to provide oocyte aspiration locally, decrease the costs associated with shipping and boarding donor mares, which will also separate the cost of the ICSI procedure and the recipient mare fees.

The decision of whether or not oocyte aspiration is right for your practice is dependent on the individual practice. The benefit of maintaining mares and providing the service locally needs to be weighed against the expense of the equipment required and the time needed to master TVA and achieve acceptable oocyte recovery rates as well as access to mares on which to practice. It is also important to have a good working relationship with a center that has proven blast development and pregnancy rates.

While assisted reproductive techniques can expand your general equine practice it is important for everyone involved to have realistic expectations about success rates and costs so that there are no surprises or disappointments.

References:
43. Schnobrich MR. How to add oocyte collection to your equine reproductive practice. *Clin Therio* 2017;9;3: 377-383