



FOLLICLE ASPIRATION FOR OOCYTE RECOVERY

Select Breeders Services Veterinary Services (SBS-VS) is proud to offer transvaginal follicle aspiration for oocyte recovery from your mare. While standard in vitro fertilization (IVF) techniques do not work well in the horse, ICSI (Intracytoplasmic Sperm Injection), where an individual sperm cell is microscopically injected into each oocyte, can be quite successful. This technique is useful for mares that cannot produce embryos on their own or for situations where supplies of semen are limited.

Before participating in the TVA program, it is important for each owner/lessee to know the regulations of their breed registry regarding the possibility of registering any resulting foals. It is also important for the mare owner to discuss with the stallion owner the stud fee and other charges associated with multiple ICSI-produced foals.

Overview of the follicle aspiration procedure

The first step of the TVA/ICSI procedure is to recover oocytes from your mare. The oocytes are recovered by transvaginal follicular aspiration (TVA) with the mare standing, under sedation. The follicles on the ovary are visualized with an ultrasound probe placed in the mare's vagina. The ovaries are held by trans-rectal palpation, and each follicle is punctured with a needle passed through the vaginal wall. The contents of the follicle are then aspirated, and the follicle is flushed. The TVA takes 15-60 minutes depending on how many follicles are being aspirated.

In most mares, oocytes can be recovered by TVA *as often as once every two weeks*. All the visible follicles can be aspirated. Each follicle contains one single oocyte, thus the number of oocytes that can be recovered from a mare is dependent on the number of follicles present on her ovaries.

Multiple oocytes are generally obtained during each session, but since these oocytes did not come from follicles ready to ovulate, they are "immature" and will need to be matured in vitro before they can be fertilized.

Once the oocytes have been collected, they are carefully processed, packaged, and sent to a reputable equine ICSI lab for maturation and fertilization.

Oocyte processing and ICSI

Oocytes recovered by SBS-VS will be shipped to an ICSI lab. In the ICSI lab, the oocytes are placed into maturation culture. Only mature oocytes can be fertilized by ICSI.

ICSI requires a small amount of semen that can be fresh or frozen depending on availability. We have strong working relationships with top equine ICSI labs in the country and can assist in coordinating semen delivery so that it arrives when it is needed.

Once ICSI fertilization has been completed and a healthy embryo is cultured at the laboratory (process takes 7-9 days) the resulting embryos will need to be transferred into recipient mares or vitrified (frozen) for storage and future transfer. Oocytes cannot be cryopreserved in mares, so embryo vitrification is the best means of storing genetic material from your mare. We work closely with the ICSI labs to coordinate shipping the vitrified embryos back to SBS-VS for transfer, or to the recipient facility of your choice. If ICSI is performed in the off-season, or if excess embryos develop, the embryos can be vitrified, and the shipping is coordinated at a later time.

Anticipated results

Every donor mare is different, and results can vary considerably

When TVA of all follicles is performed, we typically recover oocytes from about 50% of the follicles aspirated. This averages about 7 oocytes per aspiration session. Statistically there is a much higher chance of achieving pregnancies via the immature aspirations, but this is dependent on the mare having a large number of follicles.

However, since these are immature oocytes, not all of them will be able to mature when worked with in the embryo laboratory. About 65% of immature oocytes are expected to mature in the laboratory (in vitro).

Oocytes (eggs) fertilized through ICSI will divide and grow approximately 70-80% of the time, of those 20-30% will grow in culture to the blastocyst stage for non-surgical transfer. Oocyte quality and vigor decline with the donor mare's age so that older donors tend to have lower success rates than young mares.

The number and quality of oocytes recovered from follicle aspiration can be affected by:

- Mare age: Old mares have fewer follicles, and oocyte recovery rates are lower.
- Infertile mares: Some causes of infertility appear to be related to poor oocyte quality, which will affect embryo production by ICSI.

Potential risks of the TVA procedure

While statistically there is very little risk associated with TVA, it is not a benign procedure and should be performed on systemically healthy mares. Potential risks include:

-Rectal tear: Tears can result in hospitalization or death of your mare and are possible any time a transrectal palpation procedure is performed, including during TVA. This is a rare event, but is always a concern.

-Bleeding: There is the possibility of bleeding from a major vessel as a result of TVA, which may result in hospitalization or death of your mare

- Ovarian infection/abscess: Again this complication may result in hospitalization, removal of the ovary, or death of your mare. This is a rare occurrence (in research mares at Texas A&M- only 2 cases in over 1,000 aspirations). Mares undergoing TVA receive antibiotics after each aspiration session, but it is still a concern.

-Peritonitis (infection of the abdominal cavity)- Peritonitis may result in hospitalization or death of your mare. Mares are monitored closely post-TVA so that medical intervention can be started should the mare show any signs of peritonitis.

-Effect on fertility- TVA is a relatively new clinical procedure and little is known about the effect of repeated TVA on the subsequent fertility of mares. No negative effect of TVA on fertility of mares has been found or reported in other research or clinical programs, but it is still a concern.

Anticipated Costs

Every case is different but in general you can expect to pay \$2,000-\$3,500 to produce a viable embryo from one aspiration to transfer into a recipient (not including recipient lease or transfer). This estimate includes: aspiration, packaging and shipping recovered oocytes to an ICSI lab, culture of oocytes at the ICSI lab, ICSI fertilization of mature oocytes, culture of embryos, and shipment of embryos back to SBS-VS.

Please note this does not include the price of a pregnant recipient, which will be dependent on the transfer facility you choose. For specific prices please refer to the SBS-VS Fee Schedule and the ICSI contract for the ICSI lab being used.

If you have any questions on the above information or would like to discuss the potential of TVA as a part of your mare's breeding management plan, please contact Dr. Martinez de Andino (elenamartinezdeandino@selectbreeders.com).



2020 OOCYTE ASPIRATION CONTRACT

Select Breeders Services- Veterinary Services, hereinafter SBS-VS, agrees to perform transvaginal follicle aspiration for oocyte recovery for the donor mare listed below. All collected oocytes will be shipped to an appropriate ICSI lab for further processing of oocytes in an attempt to create a viable embryo.

Owner: _____ Donor Mare: _____

Billing Address: _____ Primary Contact: _____

_____ Primary Phone Number: _____

By entering into this contract, the Owner agrees to the following:

1. To pay SBS-VS for each transvaginal oocyte aspiration performed on this mare (see Fee Schedule)
2. To pay for shipment of oocytes to the ICSI lab of owner's choice (see Fee Schedule)
3. To pay all veterinary fees associated with routine or emergency care of donor mare and/or foal.
4. A 50% deposit for estimated charges is due at time of admission.
5. SBS-VS makes no guarantees, warranties, or representations as to the success or outcome of the transvaginal oocyte aspiration procedures herein contemplated. The Owner understands that SBS-VS makes no representation, guarantee, or warranty, express or implied, that oocytes will be recovered or that a blastocyst (transferrable embryo) will result from the subsequent ICSI procedure. SBS-VS will exercise reasonable care in all services performed under this contract, but neither SBS-VS nor its employees may be held responsible for oocyte loss or for production of foals with incorrect parentage. It is recommended that parentage of resulting foals be verified soon after the foal is born.
6. Transvaginal oocyte aspiration is not without some risk to the mare. The owner accepts all potential procedural risks including any complications that may develop as a result of this procedure and accept that such complications may incur additional fees. Owner has been made aware of common potential complications of this procedure and acknowledges that post-procedural care may be required and will be undertaken as deemed necessary by SBS-VS.
7. The Owner will release and hold SBS-VS harmless from any claim, demand or loss arising from any disease, injury or death to the Donor Mare and/or foal and/or in utero foal arising out of any undertaking by SBS-VS. Further, Owner shall exonerate, protect, indemnify, defend, and hold harmless SBS-VS and its Lessors(s) of the mare(s), from and against any and all liabilities, expenses, claims, fines, penalties, costs, attorney's fees, and damages of every kind including, without limitation, those arising out of or attributed, directly or indirectly, to or

resulting from any and all negligent acts or omissions of Donor Mare Owner, or of any person while the mare(s) are under Donor Mare Owner's possession or control.

8. The person signing this Contract represents and warrants that he/she is the true and lawful owner of the Donor Mare, or if not the owner, then has actual authority to act as Owner's agent for purposes of entering into this Contract, and to make any and all decisions regarding the animal and its veterinary care.

9. All charges will be due within 30 days of receipt of invoice and if account becomes delinquent, interest at a rate of 1.5% per month will be applied to account and all further services can be terminated at the discretion of SBS-VS until account is settled.

10. Owner agrees to pay all charges in full before removing the donor mare.

11. SBS-VS reserves the right to discontinue follicle aspiration services at its discretion.

Signature of Donor Mare Owner or Agent: _____ Date: _____



ICSI MARE INFORMATION SHEET

1. Primary person to contact regarding this mare:

Name: _____

Address: _____

Phone: _____

Email: _____

2. Stallion to be used for ICSI:

Stallion 1: _____

Stallion 2: _____

3. Desired embryo transfer facility:

SBS-VS

Other: _____

4. On arrival please provide copy of:

- Completed SBS-VS TVA Contract
- Completed ICSI Contract for lab of your choice
- Credit Card Authorization Form
- Current Coggins
- Mare intake form

*Please note that semen must be shipped to the ICSI lab of your choice for injection. Most stallion owners/stations will not authorize the use of semen for your mare's oocytes without a completed stallion breeding contract and paid stud fee. Please ensure the stallion owner/station has all necessary documents on file to avoid delays in injecting your mare's oocytes.