

December 2014

## **EQUINE INTRACYTOPLASMIC SPERM INJECTION PROGRAM**

**Section of Theriogenology & the Equine Embryo Laboratory  
College of Veterinary Medicine & Biomedical Sciences  
Texas A&M University**

**Contact: Ms. Kindra Rader, 979-458-3894; 979-219-7543; [krader@cvm.tamu.edu](mailto:krader@cvm.tamu.edu)**

### **Websites**

**Equine Embryo Laboratory: <http://vetmed.tamu.edu/eel>**

**Large Animal Hospital Equine Theriogenology: <http://vethospital.tamu.edu/large-animal-hospital/equine-theriogenology>**

### **Purpose of the Intracytoplasmic Sperm Injection Program**

**The intracytoplasmic sperm injection (ICSI) program is offered at Texas A&M University (TAMU) as a means of establishing pregnancies from oocytes (eggs) recovered from mares. Oocytes are collected from your mare, matured, and fertilized by injecting them with individual sperm from a stallion, and the resulting embryos are allowed to develop in the laboratory for approximately one week. Developed embryos (blastocysts) are then shipped to the embryo transfer facility of your choice for transfer to a recipient mare, as for standard embryo transfer. Embryos can also be vitrified (frozen) for later transfer.**

**Note: at this time it is not feasible to vitrify (freeze) oocytes BEFORE fertilization.**

**ICSI is appropriate for mares that are unable to become pregnant themselves (e.g., mares with chronic uterine disease, cervical lacerations, or other damage to the reproductive tract that prevents the mare from conceiving or supporting an embryo). ICSI is also appropriate for stallions that have limited stores of semen, to maximize the number of foals that may be produced from this semen.**

**The oocyte recovery and ICSI procedure should only be used on mares that are not suitable candidates for routine embryo transfer (i.e., mares in which viable embryos are seldom or never recovered from standard uterine flushing), or, if done to obtain foals from a given stallion, for sperm that cannot be utilized effectively with standard insemination techniques. Because of the expense of the technology involved and the amount of labor associated with oocyte recovery and ICSI, foals**

produced from this program should be valuable enough to justify the increased effort and expense to produce offspring.

Before participating in the ICSI 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.

**Legends Auction:** Each purchased stallion season (breeding) through the Legends Premier Stallion Season Auction is for one pregnancy only. If additional pregnancies result from ICSI procedures, the associated stud fee(s) must be negotiated with stallion owner.

#### Overview of the procedure

The first step of the ICSI procedure is to recover oocytes from your mare. This is done with the mare standing, under sedation. The follicles on the ovary are punctured with a needle, using a technique known as trans-vaginal ultrasound-guided follicle aspiration (TVA). The TVA procedure is performed at the Large Animal Veterinary Medical Teaching Hospital of TAMU.

The oocytes recovered by TVA are then transported to the Equine Embryo Laboratory at TAMU, where they are cultured to induce maturation. This maturation mimics the developmental changes that would occur naturally in an oocyte within the mare, during the day or so immediately before ovulation. The maturation process generally takes 12 to 30 hours, depending upon the stage of maturation of the oocytes at the time they are recovered.

Those oocytes that mature are then subjected to ICSI, that is, injection of each oocyte with an individual sperm from the desired stallion. For this procedure, the sperm sample (fresh or frozen-thawed) is washed and prepared, and one sperm is injected into the cytoplasm of each oocyte under a high-power microscope. The resulting fertilized oocytes are cultured in the laboratory for 7 to 10 days, to allow development into blastocysts, that is, embryos suitable for transfer to a recipient mare. **Embryos will be shipped to the private embryo transfer facility of your choice for transfer to recipient mares.** If you do not wish to transfer the embryos right away, they may be vitrified for later transfer. **NOTE:** As mentioned previously, it is not currently feasible to vitrify oocytes (unfertilized eggs) prior to fertilization.

**All charges related to the transfer of resulting embryos to recipient mares will be billed to you by the embryo transfer facility performing the transfer and are not included in this contract.**

In most mares, oocytes can be recovered by TVA as often as once every two weeks. Multiple oocytes are generally obtained during each session. Alternatively, only the one large follicle preparing to ovulate may be aspirated, to recover a maturing oocyte; again, this may be performed approximately once every 2 weeks. If only the pre-ovulatory follicle is being aspirated, this may be done by placing a needle through the flank, rather than by TVA.

This contract with TAMU includes recovery of oocytes, their culture for maturation, fertilization by ICSI, embryo culture and shipment of resulting embryos.

You will receive bills from two separate entities for the work performed on your mare and on the recovered oocytes. The Veterinary Medical Teaching Hospital will bill you for charges related to the care of your mare (board and/or medical procedures) and the recovery of oocytes from your mare. The Equine Embryo Laboratory will bill you for charges related to oocyte maturation, ICSI, embryo culture and shipment of any resulting embryos.

#### **Potential risks of the TVA and ICSI procedures**

- There is the possibility of rectal tear, which can result in hospitalization or death of your mare, any time a transrectal palpation procedure is performed, including during TVA. This is a rare event and has not occurred in over 1,500 research or clinical TVAs performed at TAMU but is still always a concern.
- 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. We have not had this occur in research or clinical TVAs at TAMU; however it has been reported in one mare in the literature.
- There is the possibility of ovarian infection, including ovarian abscess, after TVA which may result in hospitalization, removal of the ovary, or death of your mare. This is a rare occurrence in our research mares (2 cases in over 1,000 aspirations) and has not occurred in our clinical program, in which mares receive antibiotics after TVA, but it is still a concern.

- There is the possibility of peritonitis (infection of the abdominal cavity) which may result in hospitalization or death of your mare. This has occurred on two occasions in our clinical program, neither resulting in death.
- TVA is a new clinical procedure and little is known about the effect of repeated TVA on the subsequent fertility of mares. We have not observed any effect of TVA on fertility of mares in our research or clinical programs, nor has it been reported, but it is still a concern.
- ICSI is a new clinical procedure and little is known of the health or performance of foals resulting from embryos produced by ICSI. There is no warranty regarding the health, merchantability, or fitness for a particular purpose or use, of foals produced by this program.

### Anticipated results

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. About 65% of these oocytes are expected to mature in the laboratory, yielding an average of 4 mature oocytes per session. Only mature oocytes can be fertilized by ICSI. After ICSI, we anticipate that about 20% of the injected oocytes will develop to blastocysts, if the sperm is from a fertile stallion.

### Overall expected results:

While overall there is an average of one blastocyst produced per aspiration session, individual sessions produce variable results: in 2014, 45% of aspiration sessions resulted in NO EMBRYO being produced; 30% resulted in one embryo being produced, and 25% resulted in two or more embryos being produced.

The average pregnancy rate per transferred embryo is about 75%; however, this can vary depending upon the conditions at the embryo transfer center to which the embryo is shipped.

An average of 25 to 30% of the pregnancies established with ICSI embryos result in pregnancy loss, typically before 30 days.

The ongoing pregnancy rate (over 90 days) after transfer of an ICSI embryo is expected to be about 50%.

**These anticipated rates may decrease markedly with:**

- **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.
- **Subfertile stallions:** The embryo development rate after ICSI is lower with some stallions.
- **Use of multiple stallions in one session:** While we are able to perform ICSI with semen from two or more stallions in one session (given an adequate number of mature oocytes obtained) we have found that embryo production rates when this is done are lower, probably because of the increased time needed for the multiple ICSI sperm preparation procedures.

**As noted above, oocytes can also be recovered from the one preovulatory follicle. This is done on occasion in mares that develop very few follicles. When a preovulatory follicle is aspirated, a 75% oocyte recovery rate is expected, and these oocytes are already mature; however, only one (or occasionally two) such follicles develop per cycle. Therefore, ICSI embryo production from aspiration of the single preovulatory follicle is about 40% per cycle.**

## **Costs for the program -- Veterinary Medical Teaching Hospital**

**Veterinary Medical Teaching Hospital:** The charge for oocyte aspiration at the VMTH at TAMU is approximately \$1,200 but can vary with the medications used for each individual mare. In addition, hospitalization of a healthy mare for reproductive procedures at the VMTH is \$27 per day. If your mare has other medical issues that warrant medical care or treatment, she may be charged the full hospitalization fee of \$63 per day.

***Non-reproductive charges:*** The client is responsible for all health costs for their mare while the mare is at Texas A&M, including vaccination, deworming, hoof care, Coggins tests, health certificates, and any medical or surgical costs related to illness or injury.

***Incidental charges:*** Costs for semen collection or shipment of semen containers are not covered by the enrollment or other fees and are charged to the client separately. Costs for routine health procedures, such as vaccination or deworming, and costs for medical or surgical treatment for illness or injury of the donor mare are also not covered by the enrollment or other fees, and are charged to the client separately.

If you have questions regarding the above HOSPITALIZATION and follicle aspiration costs for your mare in the program, please contact the Section of Theriogenology:

**Ms. Sheila Teague  
Veterinary Medical Teaching Hospital  
College of Veterinary Medicine and Biomedical Sciences  
Texas A&M University  
College Station, TX 77843-4457  
(979) 255-4245 or (979) 845-3541  
steague@cvm.tamu.edu**

## **Costs for the program-- Equine Embryo Laboratory**

**Equine Embryo Laboratory:** You will be billed directly by the Equine Embryo Laboratory for the following:

- Processing of the aspiration fluid, oocyte handling, and oocyte culture for maturation (\$350)
- Performance of ICSI on one or more oocytes (\$1250; if additional stallions are used there is a fee of \$300 for the first extra semen sample processed for ICSI, and \$500 for each additional sample)
- Embryo culture / blastocyst production (\$500 per blastocyst produced)
- If the blastocyst is shipped, there is a \$150 charge for shipment
- A surcharge (\$200) is assessed for cases that entail oocyte collection or sperm injection after hours (after 6 PM on weekdays) or on weekends or holidays
- Embryo biopsy, vitrification, or other procedures requested by you

A complete price list is available on the Equine Embryo Laboratory website at <http://vetmed.tamu.edu/eel>.

For questions about these oocyte laboratory procedures (oocyte maturation, intracytoplasmic sperm injection, embryo culture and shipment), contact:

**Ms. Kindra Rader  
Equine Embryo Laboratory  
Department of Veterinary Physiology and Pharmacology  
979-458-3894 (Lab); 979-219-7543 (Cell)  
[krader@cvm.tamu.edu](mailto:krader@cvm.tamu.edu)**

**Shipping Address:  
Equine Embryo Laboratory  
VMA Building, Room 300A (4466 TAMU)  
College of Veterinary Medicine and Biomedical Sciences  
Texas A&M University, College Station, TX 77843-4466**

**CONTRACT FOR INTRACYTOPLASMIC SPERM INJECTION** (November, 2014)

The Texas A&M University Veterinary Medical Teaching Hospital, hereafter known as VMTH, agrees to collect oocytes from the mare \_\_\_\_\_ (“donor mare”), breed \_\_\_\_\_ age \_\_\_\_\_ registration # \_\_\_\_\_. Furthermore, oocytes considered suitable by the Equine Embryo Laboratory will be subjected to intracytoplasmic sperm injection (ICSI), and any resulting blastocysts will be shipped to the private embryo transfer facility of your choice, as outlined below, for transfer to recipient mares.

**The Owner or Lessee agrees to the following:**

- 1) Pay the VMTH for each trans-vaginal oocyte aspiration performed on this mare (approximately \$1,200 per aspiration).
- 2) Pay the Equine Embryo Laboratory fees for culture of recovered oocytes, performance of ICSI on one or more oocytes, blastocyst production, embryo shipment, and any other agreed-upon manipulations, as outlined above and in our Fee Schedule. A surcharge (\$200) will be assessed for cases processed on weekends/holidays.
- 3) Pay \$27/day for hospitalization (healthy mare) of the donor mare during her stay at the VMTH.
- 4) Pay all veterinary fees associated with routine or emergency care of donor mare. In addition, the Owner/Lessee agrees to pay all veterinary fees associated with preparing donor mare for departure (eg, Coggins test and health certificates).
- 5) There is no guarantee that a blastocyst will result from these procedures or, if a blastocyst is produced, that it will yield a viable pregnancy after transfer.
- 6) Texas A&M employees will exercise reasonable care for client animals admitted to the VMTH, but neither the employees nor the University will be held responsible for injury, illness, or death of client animals while housed at University facilities.
- 7) The Owner/Lessee agrees to pay all VMTH charges in full before removing the donor mare, unless prior credit arrangements have been approved and are in the files of the VMTH.
- 8) All VMTH accounts are payable within 30 days of billing date. AFTER 30 DAYS FROM BILLING DATE, the VMTH will assess INTEREST OF 1.5% PER MONTH ON THE VMTH OUTSTANDING BALANCE.
- 9) All Equine Embryo Laboratory accounts are payable within 60 days of billing date. All Owners/Lessees must complete a Credit Card Authorization form; charges will be billed to the credit card if payment is not received within 60 days.
- 10) For accounts that are past due, A&M reserves the right to refuse service and the right to retain ownership of any vitrified blastocysts produced under this contract, until payment is received. The Owner/Lessee agrees to pay all reasonable attorney fees incurred by A&M in attempting to collect any outstanding balance.
- 11) Texas A&M University reserves the right to discontinue intracytoplasmic sperm injection services at its discretion.

**Acknowledgement:** I, \_\_\_\_\_, the owner/lessee of the above listed mare, verify that I have read all program-related information presented with this contract and that I understand the implications. I agree fully to the terms and conditions of the contract listed above.

**Signature**\_\_\_\_\_ **Date**\_\_\_\_\_

**Signature**\_\_\_\_\_ **Date**\_\_\_\_\_



## STALLION AND EMBRYO INFORMATION

The following information is important:

- A. Name, address, and phone number and EMAIL ADDRESS of primary contact regarding this mare:

---

---

---

---

- B. Name, address, and phone number of contact for collection and shipment of semen, and name of stallion:

Stallion 1: 

---

Contact 1: 

---

---

---

Stallion 2: 

---

Contact 2: 

---

---

---

- C. Desired Embryo Transfer Facility

---

---

---

---

Signature of Owner or Lessee of donor mare

---

Date

Billing Address:

---

---

---

Telephone:

---

---

**Note:** If multiple parties will be responsible for billing, then the parties involved are required to submit a formal letter, signed by all parties, outlining the specifics of the agreed-upon billing schedule, listing all related billing addresses and contact information, prior to the start of clinical work. Each person involved is also required to submit a separate Credit Card agreement form (enclosed with this contract) and to sign a separate copy of the program information and Contract.