

January 2017

## **EQUINE INTRACYTOPLASMIC SPERM INJECTION PROGRAM**

**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)**

### **Website**

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

### **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 by your veterinarian then the oocytes are transported to the Equine Embryo Laboratory (EELab). At the EELab, the oocytes are matured, then 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 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.

ICSI may also be used to obtain foals from mares that suffer an untimely death. Your veterinarian can recover oocytes from the mare's ovaries post-mortem, and transport the oocytes to the EELab, or the ovaries themselves can be transported to the EELab and we can recover the oocytes from them.

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. Oocyte recovery is done by your veterinarian, or can be done by the veterinarians at the Large Animal Teaching Hospital at TAMU. Oocytes may be recovered from small (immature) follicles, or from the one large preovulatory follicle. The veterinarian will package the collected oocytes in a special medium, and transport them to the EELab.

The oocytes are then 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. For oocytes recovered from small (immature) follicles, the maturation process generally takes 30 hours. An oocyte recovered from the one preovulatory follicle after hormone stimulation is already maturing at the time it is recovered, and is cultured for 12 hours or less prior to ICSI.

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.

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

#### Anticipated results

About 65% of oocytes collected from immature (small) follicles are expected to mature in the laboratory. If your veterinarian collects the oocyte from the one dominant preovulatory follicle, after administration of an ovulation-inducing hormone, essentially 100% of these oocytes are maturing at the time of collection. Only mature oocytes can be fertilized by ICSI.

After ICSI of oocytes recovered from small follicles, we anticipate that about 20% of the injected oocytes will develop to blastocysts, if the sperm is from a normally-fertile stallion. If the oocyte is from a preovulatory follicle, the chance of the oocyte developing to a blastocyst is about 40%.

#### Overall expected results:

While overall there is an average of one blastocyst produced per aspiration session, individual sessions produce variable results: at the EELab in 2016, 39% of aspiration sessions (oocytes recovered on aspiration from one mare) resulted in NO BLASTOCYST being produced; 24% resulted in one blastocyst being produced, 17% resulted in two blastocysts being produced, and 20% resulted in 3 or more blastocysts being produced.

The average pregnancy rate per transferred embryo in 2016 was 67%; however, this can vary depending upon the conditions at the embryo transfer center to which the embryo is shipped. For example, in 2016, pregnancy rates at centers transferring 8 or more embryos varied from 50% to 87%.

**In 2016, 13% of the pregnancies established with ICSI embryos resulted in pregnancy loss, typically before 30 days. Thus,**

**The ongoing pregnancy rate (over 90 days) after transfer of an ICSI embryo is expected to be about 60%.**

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

- **Mare age: Old mares have poorer quality oocytes.**
- **Infertile mares: Some causes of infertility appear to be related to poor oocyte quality.**
- **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.**

## Costs for the program

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

- Oocyte handling and culture for maturation (\$250)
- 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).

Note that this is PER ICSI SESSION: if an oocyte from a dominant follicle is shipped at the same time as are oocytes from small follicles, the ICSI procedure must be performed twice, on two separate days, thus TWO ICSI charges will apply.

- Embryo culture / blastocyst production (\$500 per blastocyst produced)
- If the blastocyst is shipped, there is a \$165 charge for shipment
- A surcharge is assessed for cases that entail oocyte handling (\$100) or sperm injection (\$200) on weekends/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**

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**CONTRACT FOR INTRACYTOPLASMIC SPERM INJECTION – SHIPPED OOCYTES**

The Equine Embryo Laboratory at Texas A&M University agrees, subject to the terms of this contract, to accept oocytes from \_\_\_\_\_ (“donor mare”), breed \_\_\_\_\_ age \_\_\_\_\_ registration # \_\_\_\_\_ for performance of intracytoplasmic sperm injection (ICSI). Any resulting blastocysts will be shipped to the private embryo transfer facility of your choice, as outlined below, for transfer to recipient mares.

I agree to the following:

- 1) I certify that:
  - a. I am the owner, owner’s agent, or lessee of the donor mare, I have the right to enter into this contract, and this contract will not conflict with or violate the rights of any other party; and
  - b. All information on the Stallion and Embryo Information form is accurate and complete.
- 2) I will 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 the Fee Schedule. A surcharge (\$200) will be assessed for cases processed on weekends/holidays.
- 3) **I acknowledge that due to the nature of the procedures, the outcome is inherently uncertain. I understand that Texas A&M makes no representation, guarantee, or warranty, express or implied, that a blastocyst will result or, if a blastocyst is produced, that it will yield a viable pregnancy after transfer.** Texas A&M will exercise reasonable care in performing all services under this contract, but neither Texas A&M nor its employees will be responsible for oocyte, sperm, or embryo loss or for production of foals with incorrect parentage, except to the extent specified in Section 4 below. It is recommended that parentage of resulting foals be verified soon after the foal is born.
- 4) I acknowledge that I bear the sole responsibility to insure or to self-insure against damage, loss, or injury to my interests, including destruction or loss of my horse’s oocytes or embryos, loss or damage to semen stored at Texas A&M, or production of embryos with incorrect parentage not solely caused by negligent or willful misconduct of Texas A&M. If my frozen embryos or semen stored at Texas A&M should be unintentionally thawed or otherwise lost or destroyed while in storage at Texas A&M, or if embryos are produced with incorrect parentage due solely to the negligence or willful misconduct of Texas A&M, the maximum monetary value associated with these events will be:
  - a. The monetary value of each straw of semen lost or destroyed will represent the production costs to collect and process that semen, valued at a maximum of \$10 per straw.
  - b. The monetary value of each embryo will be determined by the amount invoiced to me by Texas A&M for in vitro embryo production and handling, and for vitrification (if applicable) for that embryo; this will be as per the Fee Schedule, e.g. \$500 per embryo for production and handling, \$300 per embryo for vitrification, etc.

- c. If incorrect parentage is found on genetic analysis of embryos, or foals resulting from embryos, produced by Texas A&M under this contract, the monetary value of the loss will be that for production of that embryo, determined by the amount invoiced to me by Texas A&M for in vitro embryo production and handling, vitrification (if applicable) and shipment for that embryo as per the Fee Schedule, e.g. \$500 per embryo for production and handling, \$300 per embryo for vitrification, \$165 for shipment, etc.
- 5) All Equine Embryo Laboratory accounts are payable within 60 days of billing date. I (and anyone else who will be responsible for payment) must complete a Credit Card Authorization form; charges will be billed to the credit card if payment is not received within 60 days of billing. Credit card charges will be assessed a 3.1% processing fee.
- 6) For accounts that are past due, Texas A&M reserves the right to refuse service and the right to retain ownership of any vitrified blastocysts or other material produced under this contract, until payment is received, and if payment is not received within one year, to discard this material. Texas A&M is not liable for damage to material during storage in the laboratory. I will pay all attorneys' fees and other collection costs incurred by Texas A&M in attempting to collect any outstanding balance.
- 7) If a force majeure event prevents Texas A&M from complying with any one or more obligations under this contract, that inability to comply will not constitute a breach if (a) Texas A&M uses reasonable efforts to perform those obligations, (b) Texas A&M's inability to perform those obligations is not due to its failure to (1) take reasonable measures to protect itself against events or circumstances of the same type as the force majeure event or (2) develop and maintain a reasonable contingency plan to respond to events or circumstances of the same type as the force majeure event, and (c) Texas A&M complies with its obligations under this Section 7. For purposes of this contract, "force majeure event" means any event or circumstance, whether or not foreseeable, that was not caused by Texas A&M and any consequences of that event or circumstance. If a force majeure event occurs, Texas A&M will promptly notify me of the occurrence of the force majeure event, its effect on performance, and how long Texas A&M expects it to last. Texas A&M will then update me as reasonably necessary. During a force majeure event, Texas A&M will use reasonable efforts to resume its performance under this contract.
- 8) I will use the dispute resolution process provided in Chapter 2260, Texas Government Code, and the related rules adopted by the Texas Attorney General to attempt to resolve any claim for breach of contract against Texas A&M that cannot be resolved in the ordinary course of business. I will submit written notice of a claim of breach of contract to Texas A&M's designated official, who will examine my claim and any counterclaim and negotiate with me in an effort to resolve the claim.
- 9) The substantive laws of the State of Texas (and not its conflicts of law principles) govern all matters arising out of or relating to this contract and all of the transactions it contemplates.
- 10) This contract, the Stallion and Embryo Information form, Fee Schedule, and the other written program information provided to me by Texas A&M contains our entire understanding as to ICSI and supersedes all other written and oral agreements between us as to that subject matter. We may enter into other contracts, but those will not change or alter this contract unless expressly agreed by both of us in writing. Texas A&M hereby objects to any different or additional contract terms on any purchase order, acknowledgement, or other form.
- 11) Texas A&M reserves the right to discontinue intracytoplasmic sperm injection services at its discretion.

Acknowledgement: I, \_\_\_\_\_, the owner/ owner's agent / 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 \_\_\_\_\_

Donor Mare \_\_\_\_\_

## STALLION AND EMBRYO INFORMATION

The following information is important:

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

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- B. Name, address, and phone number of contact for collection and shipment of semen, and name of stallion: **NOTE: The stallion owner MUST have a contract on file for your mare, or we cannot perform ICSI. Semen must be received by the Equine Embryo Laboratory no later than the morning of the day that ICSI is performed. Shipment of semen to the Equine Embryo Laboratory is the responsibility of the mare owner/agent.**

Stallion 1: \_\_\_\_\_

Contact 1: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Stallion 2: \_\_\_\_\_

Contact 2: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

- C. Desired Embryo Transfer Facility / Facilities

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\_\_\_\_\_  
Signature of Owner or Lessee of donor mare

\_\_\_\_\_  
Date

Billing Address:

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Telephone:

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**Note:** If multiple parties will be responsible for payment, 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. **IF NO LETTER OF AGREEMENT IS ON FILE, THEN THE PERSON SIGNING THIS CONTRACT IS RESPONSIBLE FOR PAYMENT OF ALL CHARGES.**