Department of Large Animal Clinical Sciences 4475 TAMU



Jan 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

<u>Websites</u> Equine Embryo Laboratory: <u>http://vetmed.tamu.edu/eel</u> Large Animal Hospital Equine Theriogenology: <u>http://vethospital.tamu.edu/large-animal-hospital/equine-theriogenology</u>

Purpose of the Intracytoplasmic Sperm Injection Program

The intracytoplasmic sperm injection (ICSI) program is offered at Texas A&M University 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.

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 prevent the mare from conceiving or supporting an embryo). ICSI is also appropriate in the case of a stallion with limited sperm supplies, to maximize the number of foals that may be produced from this sperm.

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.

Texas Veterinary Medical Center College Station, TX 77843-4475

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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.

<u>Legends Auction</u>: 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 Texas A&M University (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: injection with 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 11 days, to allow development into blastocysts, that is, embryos suitable for transfer to a recipient mare. <u>Embryos will be shipped to the private embryo transfer facility of your choice for transfer to recipient mares</u>.

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

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 preovulatory 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 <u>Veterinary Medical Teaching Hospital</u> will bill you for charges related to the care of your mare and the recovery of oocytes from your mare. The <u>Equine Embryo Laboratory</u> will bill you for charges related to oocyte maturation, ICSI, embryo culture and shipment of any resulting embryos.

Anticipated results

When TVA of all follicles is performed, we typically recover oocytes from about 50% of the follicles aspirated. In 2013 this averaged 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 normally fertile stallion.

Overall, this means:

We expect an average of approximately 0.8 transferrable embryos per aspiration session in mares up to about 20 years of age. However, some mares will provide no embryos and some will have multiple embryos.

In 2013, the pregnancy rate per transferred embryo was 79%, however, 20% of these pregnancies resulted in embryo loss, typically before 30 days. Thus,

The ongoing pregnancy rate after embryo transfer is expected to be about 65%.

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.
- Subfertile stallions: The embryo development rate after ICSI is lower with some stallions.

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.

Costs for the program -- Veterinary Medical Teaching Hospital

<u>Veterinary Medical Teaching Hospital</u>: 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 \$26 per day. If your mare has other medical issues that warrant medical care or treatment, she may be charged the full hospitalization fee of \$60 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

<u>Equine Embryo Laboratory</u>: 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 per extra semen sample processed for ICSI)
- 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 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 <u>http://vetmed.tamu.edu/eel</u>.

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

<u>Shipping Address:</u> 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

<u>CONTRACT FOR INTRACYTOPLASMIC SPERM INJECTION</u> (January, 2014)

The Texas A&M University Veterinary Medical Teaching Hospital, hereafter known as A&M, agrees to collect oocytes from the mare _______ ("donor mare"), breed ______ age _____ registration # ______. Furthermore, oocytes considered suitable by A&M will be subjected to intracytoplasmic sperm injection (ICSI), and any resulting blastocyts 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 A&M for each transvaginal oocyte aspiration performed on this mare (approximately \$1,200 per aspiration).
- 2) Pay the Equine Embryo Laboratory fees for culture of recovered oocytes (\$350); performance of ICSI on one or more oocytes (\$1,250); blastocyst production (\$500 per embryo); embryo shipment (\$150 per shipment); and sperm preparation for ICSI from additional stallions if performed (\$300 each) and for any other agreed-upon embryo manipulations. A surcharge (\$200) will be assessed for cases processed on weekends/holidays.
- 3) Pay \$26 per day for hospitalization (healthy mare) of the donor mare during her stay at A&M.
- 4) Pay all veterinary fees associated with routine or emergency care of donor mare. In addition, the Owner or 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) A&M employees will exercise reasonable care for client animals admitted to the University, 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 charges in full before removing the donor mare, unless prior credit arrangements have been approved and are in the files of the Veterinary Medical Teaching Hospital and the Equine Embryo Laboratory.
- 8) A&M reserves the right to discontinue intracytoplasmic sperm injection services at its discretion.
- 9) All 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 OUTSTANDING BALANCE. A&M reserves the right to refuse service when the Owner's/Lessee's account is past due. The Owner/Lessee agrees to pay all reasonable attorney fees incurred by A&M in attempting to collect any outstanding balance.

STALLION AND EMBRYO INFORMATION The following information is important:

	mare:			
,	Name, address stallion:	ss, and phone number of contact for collection and shipment of semen, and name o		
	Stallion 1:			
	Contact 1 :			
	Stallion 2:			
	Contact 2 :			
	Desired Emb	sired Embryo Transfer Facility		
gna	ture of Owner	or Lessee of donor mare Date		
lling	g Address:			
len	hone:			