Purpose of the Intracytoplasmic Sperm Injection Program

The intracytoplasmic sperm injection (ICSI) program is offered at Texas A&M as a means of establishing pregnancies from oocytes (eggs) recovered from donor mares. Using ICSI, oocytes are injected with individual sperm from a donor stallion, and the resulting embryos are allowed to develop in the laboratory for approximately one week. Developed embryos are then shipped to a private embryo transfer facility for transfer to a recipient mare, as for standard embryo transfer.

This advanced reproductive technology is appropriate for mares that are unable to become pregnant themselves (i.e., mares with chronic uterine disease, cervical lacerations, or other damage to the reproductive tract that prohibit the mare from conceiving or supporting an embryo in the uterus for any length of time). The procedure can also be attempted when pregnancies are sought from limited sperm supplies.

The 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 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 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.
Overview of the procedure

Intracytoplasmic sperm injection is a recently developed technique in which oocytes (eggs) of a valuable broodmare (donor mare) are fertilized in the laboratory. The oocytes are recovered from the mare’s ovarian follicles, and then cultured to induce maturation, mimicking the developmental changes that would occur in an oocyte during the day or so immediately before ovulation. This maturation process generally takes 12 to 30 hours, depending upon the stage of maturation of the recovered oocytes.

Matured oocytes are injected with individual sperm from the desired stallion. For this procedure, 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 a private embryo transfer facility for transfer to recipient mares.

In donor mares, oocytes are typically removed from all follicles on the ovary once every two weeks, using a transvaginal ultrasound-guided technique with the mares under sedation. This procedure is performed at the Large Animal Teaching Hospital of Texas A&M University (TAMU). 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.

This contract with TAMU includes recovery of oocytes, fertilization, embryo culture and shipment of resulting embryos.

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

Anticipated results

When transvaginal aspiration of all follicles is performed, we typically recover oocytes from 40 to 50% of the follicles aspirated; in fertile mares, this averages 4 to 5 oocytes per aspiration session. About 60% of these oocytes may mature in the laboratory and be injected with sperm (average of 3 per session). We anticipate a 25% rate of blastocyst development if the sperm is from a fertile stallion, thus approximately 75% chance of a transferrable embryo per aspiration session.
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.

When oocytes are recovered from the one preovulatory follicle, a 75% recovery rate is expected; however, only one (or occasionally two) such follicles develop per cycle.

**Benefits of the program**

This program (fertilization and embryo development in the laboratory) has some benefits over oocyte transfer (surgical transfer of matured oocytes to the oviducts of inseminated recipient mares). The major benefit is that it avoids the need for surgery on the recipient mare, as embryos develop in the laboratory to the stage that they may be transferred to recipients by standard embryo transfer. Because of this, when multiple oocytes are recovered, all oocytes that form embryos may be transferred to separate recipient mares, and thus have the potential to produce a foal. In addition, the ICSI program can utilize equally frozen, fresh, or cooled, transported semen, and sperm of low numbers or low quality.

**Costs for the program**

**Enrollment fee**: When a donor mare is brought to A&M for management of the estrous cycle and for oocyte aspiration, a non-refundable enrollment fee of $1,100 is charged when the intracytoplasmic sperm injection contract is signed. The enrollment fee covers all examination expenses related to oocyte aspiration for the donor mare for 1 oocyte-aspiration session.

**Additional cycles**: Charges for additional oocyte aspiration sessions are $1,100 per session.

**Equine Embryo Laboratory fees**: Fees are assessed for oocyte recovery from the aspiration fluid and oocyte maturation in the laboratory ($200); performance of ICSI on one or more oocytes ($950; if additional stallions are used there is a fee of $150 per extra semen sample processed for ICSI); and embryo culture with blastocyst production ($500 per blastocyst produced). If the blastocyst is shipped,
there is a $100 charge for shipment. A surcharge ($200) is assessed for cases that entail oocyte collection or sperm injection on weekends/holidays. These fees are charged directly from the laboratory.

**Hospitalization:** A hospitalization charge for the donor mare is in addition to the enrollment or other fees, and is $25 per day.

**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.

**Non-reproductive charges:** The client is responsible for all health costs for the donor 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.

If you have questions regarding the intracytoplasmic sperm injection (ICSI) program, please contact:

Section of Theriogenology  
Veterinary Medical Teaching Hospital  
College of Veterinary Medicine and Biomedical Sciences  
Texas A&M University  
College Station, TX 77843-4457  
(979) 845-3541
CONTRACT FOR INTRACYTOPLASMIC SPERM INJECTION  (March, 2011)

The Texas A&M University Veterinary Medical Teaching Hospital, hereafter known as A&M, agrees to collect oocytes from the donor mare ______________________________________ breed __________________ age __________ registration # ________________. Furthermore, oocytes considered suitable by Texas A&M will be subjected to intracytoplasmic sperm injection (ICSI), and any resulting blastocysts will be transported to a private embryo transfer facility for transfer to recipient mares.

The Owner or Lessee__________________________________ agrees to the following:

1)  Pay A&M an $1,100 non-refundable fee to cover expenses for the first transvaginal oocyte aspiration.

2)  Pay A&M $1,100 for each additional oocyte-aspiration session.

3)  Pay the Equine Embryo Laboratory fees for culture of recovered oocytes ($200); performance of ICSI on one or more oocytes ($950); blastocyst production ($500 per embryo); embryo shipment ($100); and sperm preparation for ICSI from additional stallions ($150 each). A surcharge ($200) will be assessed for cases processed on weekends/holidays.

4)  Pay $25 per day for hospitalization of the donor mare during her stay at A&M.

5)  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).

6)  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.

7)  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.

8)  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.

9)  A&M reserves the right to discontinue intracytoplasmic sperm injection services at its discretion.

10)  All accounts are payable within 30 days of billing date. AFTER 30 DAYS FROM BILLING DATE, INTEREST OF 1.5% PER MONTH ON THE OUTSTANDING BALANCE WILL BE ASSESSED. 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.
The following information is important:

a. How many ICSI pregnancies do you want from this donor mare? _________________

b. Name, address, and phone number of primary contact regarding this mare.
___________________________________________________________________________________
___________________________________________________________________________________
___________________________________________________________________________________
___________________________________________________________________________________

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

Stallion 1: ____________________________________________
Stallion 2: ____________________________________________

Contact 1: ____________________________________________
___________________________________________________________________________________
___________________________________________________________________________________

Contact 2: ____________________________________________
___________________________________________________________________________________
___________________________________________________________________________________

Signature of Owner or Lessee of donor mare ___________________________ Date ____________

Billing Address:
___________________________________________________________________________________
___________________________________________________________________________________

Telephone: ________________________________________________

Social Security Number (for billing purposes): ____________________________

MasterCard/Visa (circle one) account number: ____________________________
Expiration Date: __________________________

Signature Veterinary Medical Teaching Hospital Date ____________

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