



<p>EQUINE INTRACYTOPLASMIC SPERM INJECTION ICSI</p>
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(Modified from Texas A&M information sheet)

Purpose of the Intracytoplasmic Sperm Injection Program

The intracytoplasmic sperm injection (ICSI) program is offered at InFoal, Inc. as a means of establishing pregnancies from oocytes (eggs) recovered from mares. Oocytes are collected from your mare by Dr. Beck or your veterinarian and shipped to InFoal, Inc. 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 then shipped to the embryo transfer facility of your choice, or transferred to a recipient mare at InFoal, Inc., 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

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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 ship the oocytes to the InFoal, Inc.

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.

Overview of the procedure

The first step of the ICSI procedure is to recover oocytes from your mare. Oocytes may be recovered from small (immature) follicles, or from the one large preovulatory follicle.

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

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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.

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 Texas A&M 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.

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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. Thus,

THE ONGOING PREGNANCY RATE (OVER 90 DAYS) AFTER TRANSFER OF AN ICSI EMBRYO IS EXPECTED TO BE ABOUT 50%.

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 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.

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You will be billed for the following:

- Aspiration of the oocytes from the donor mare (\$950).
- Oocyte handling and culture for maturation (\$300)
- 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)
- Embryo transfer into recipient mare at InFoal, Inc. (\$500)
- Pregnancy fee at first positive pregnancy check (\$1500)
- Pregnancy confirmation fee and recipient mare lease (\$1500)
- Refundable recipient mare deposit, refunded when mare returned (\$1000)
- If the blastocyst is shipped, there is a \$150 charge for shipment
- Embryo biopsy, vitrification, or other procedures requested by you

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

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Hemet, Ca 92543
951-929-4533
fax 951-929-2077
Hosdok2@aol.com
InFoal-Inc.com

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CONTRACT FOR INTRACYTOPLASMIC SPERM INJECTION

(Plan B - Fee For Services - No Guarantee)

InFoal, Inc. agrees to accept oocytes from _____ ("donor mare"), breed _____ age ___ registration #____ for performance of intracytoplasmic sperm injection (ICSI). Any resulting blastocysts will be transferred into recipient mares at InFoal, Inc or shipped to the private embryo transfer facility of your choice.

The Owner or Lessee agrees to the following:

- 1) Pay InFoal, Inc. fees for culture of recovered oocytes, performance of ICSI on one or more oocytes, blastocyst production, embryo transfer or embryo shipment, and any other agreed-upon manipulations, as outlined above and in our Fee Schedule. A payment of \$2500 will accompany the mare to pay for the first aspiration, maturation and ICSI.
- 2) The Owner/Lessee acknowledges that due to the nature of the procedures, the outcome is inherently uncertain. The Owner/Lessee understands that InFoal, Inc. 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. InFoal, Inc. will exercise reasonable care in all services performed under this contract, but neither InFoal, Inc. nor its employees may be held responsible for oocyte, sperm, or embryo 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.
- 3) All InFoal, Inc. accounts are payable at time of service. All Owners/Lessees must complete a Credit Card Authorization form; charges will be billed to the credit card unless other arrangements are made.
- 4) For accounts that are past due, InFoal, Inc. 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 1 year, to discard this material. The Owner/Lessee agrees to pay all reasonable attorney fees incurred by InFoal, Inc. in attempting to collect any outstanding balance.
- 5) InFoal, Inc. reserves the right to discontinue intracytoplasmic sperm injection services at its discretion.
- 6) The client hereby releases and shall indemnify and hold InFoal, Inc. harmless from any claim, demand or loss arising from any disease, injury or death to the mare and/or pregnant recipient mare and/or in utero foal arising out of any undertaking by InFoal, Inc. under the terms of this contract. Further, Client shall exonerate, protect, indemnify, defend and hold harmless InFoal, Inc. from and against any and all liabilities, expenses, claims, fines, penalties, costs, attorney fees, and damages of any every kind for damages, injuries, losses or deaths of owners, employees or families caused by donor mares, recipient mares or resulting foals. Owner further indemnifies InFoal, Inc. against loss for any reason of semen, oocytes or embryos in the possession of InFoal, Inc. This indemnification shall extend to the successors and assigns of InFoal, Inc.

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 _____

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: NOTE: The stallion owner MUST have a contract on file for your mare, or we cannot perform ICSI. Semen must be received by the InFoal, Inc. no later than the morning of the day that ICSI is performed. Shipment of semen to InFoal, Inc. is the responsibility of the mare owner/agent.

Stallion 1: _____

Contact 1: _____

Stallion 2: _____

Contact 2: _____

- C. Desired Embryo Transfer Facility / Facilities

Signature of Owner or Lessee of donor mare

Date

Billing Address:

Telephone:



PLAN B FEE SCHEDULE - NO
GUARANTEE - CHARGE FOR EACH
PROCEDURE

Procedure	Unit price
<u>Follicle aspiration (TVA) /Ovary dissection</u>	
TransVaginal Aspiration of mare for the collection of oocytes –one Or both ovaries	\$950 per mare
Post-mortem ovary dissection with oocyte collection, oocyte culture for maturation	\$900 per mare
Processing TVA aspirate for oocyte recovery, oocyte culture for maturation	\$300 per aspirate
Oocyte processing, culture/maturation for immature oocytes recovered by referring veterinarian and shipped to laboratory	\$300 per mare
<u>ICSI</u>	
Sperm preparation, intracytoplasmic sperm injection (ICSI)	\$1250 per ICSI session (1 or more oocytes)
Processing second sperm type for ICSI in same session	\$300
Processing third (or more) sperm type for ICSJ in same session	\$500 per sperm type
ICSI-produced embryo culture with embryo production;	\$500 per transferable embryo
Packaging/consignment/shipping of embryos (ICSI, biopsied, vitrified or warmed)	\$150 per shipment
Packaging and shipment of embryos -- per client if more than 1 client's embryos are shipped in the same package	\$75 per client
Packaging and return of frozen semen/vapor shipper	\$60 per shipment (Priority Overnight)
CREDIT For each blastocyst over 3: if more than 3 blastocysts transferred per aspiration for no pregnancy at first check (-14 d)	(-) \$500 per blastocyst over 3
ICSI trial for stallion sperm (minimum 20 oocytes injected with subject and control sperm)	\$7,500
Assessment trial of shipped oocytes to establish TVA /oocyte handling protocol -maturation and ICSI, per shipment	\$500
Preparing ICSI cuts from one straw of frozen semen (includes handling, cryovials, canes, liquid nitrogen, and transport to and from storage facility)	\$100
Preparing ICSI cuts (each additional straw from the same stallion handled at the same time)	\$50 per straw
<u>Embryo Transfer</u>	
Embryo transfer into recipient mare and recipient mare availability	\$500 per embryo
Pregnancy fee at first positive pregnancy check	\$1500
Pregnancy confirmation fee and recipient mare lease	\$1500
Recipient mare deposit, refunded when recipient mare is returned	\$1000
TOTAL	\$7500
CAUTION - ALL SERVICES WILL BE CHARGED FOR EVEY IF NO PREGNANCY IS ACHIEVED OR THE PREGNANCY IS LOST	-1000 returned recipient mare = breeding-sound \$6500 PLEASE READ EXPECTED RESULTS