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Post-Mortem Oocyte Recovery and EQUINE INTRACYTOPLASMIC SPERM INJECTION (ICSI) PROGRAM

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Purpose of the Intracytoplasmic Sperm Injection Program

The intracytoplasmic sperm injection (ICSI) program is offered at Texas A&M as a means of producing embryos from oocytes (eggs) recovered from a mare's ovaries after her untimely death.

Ovaries removed from mares post-mortem may be shipped directly to the Equine Embryo Laboratory for dissection and recovery of oocytes; alternatively, oocytes may be harvested from the ovaries at the mare's location by a skilled veterinarian and the isolated oocytes shipped to our laboratory for maturation culture and ICSI.

Using ICSI, oocytes that mature in culture are injected with individual sperm from a desired stallion, and the resulting fertilized eggs are allowed to develop in the laboratory for 7- 10 days. ***It is important to know that the results with oocytes recovered post-mortem are variable***, due to many factors, including mare age, underlying cause of the mare's death, treatments that she may have received, the conditions under which the ovaries are removed and transported, and the length of time between the mare's death and receipt of the ovaries by the Equine Embryo Laboratory (*see Anticipated results*). In addition, stallion factors can affect the results. These effects can be seen at essentially every step of the procedure, they can impact:

- The number of oocytes recovered from the ovaries
- The percentage of these oocytes that mature
- The percentage of oocytes that start embryo development (cleave) after ICSI
- The percentage of embryos that continue to grow to the blastocyst stage
- The pregnancy rate after transfer of the blastocysts produced
- The rate of pregnancy loss after pregnancies are established

Overview of the procedure

The ovaries should be shipped to the laboratory by the fastest method possible. There is an airport (CLL) in College Station that is served by American Airlines and United Airlines. For best results, ovaries should be received within 6 hours of the mare's death; longer times can be associated with a lower rate of embryo development, and a higher rate of pregnancy loss after transfer of embryos.

DO NOT place ovaries on ICE or put them in the refrigerator! Ovaries should be shipped at room temperature or a little cooler (50 to 70 °F).

Oocytes recovered from the ovaries by a referring veterinarian may be shipped to the Equine Embryo Laboratory via FedEx Priority Overnight – oocytes should be shipped at **room temperature**.

Semen (fresh or frozen) from the desired stallion should arrive the day after the ovaries/oocytes have been received. Typically, sperm injection is conducted in the afternoon of the second day following receipt of the ovaries/oocytes.

If blastocysts, that is, embryos suitable for transfer to the uterus of a recipient mare, develop, owners have the option of shipping the blastocyst to an embryo transfer center for transfer to recipient mares, or vitrifying the blastocyst for warming and transfer at a later date. **It is important to know that vitrification may lower the chance of successful pregnancy by ~10%.**

Oocytes (unfertilized eggs) unfortunately cannot be frozen or vitrified successfully at this time.

Because of the expense of the technology involved, and the amount of labor associated with ICSI, foals from the deceased mare should be valuable enough to justify the 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.

Anticipated results

Typically, 10 to 15 oocytes may be recovered from one pair of ovaries post-mortem; however, this number is variable and is typically lower if the mare is old or has been chronically ill. **Again, even a large number of oocytes recovered does not guarantee that an embryo will be produced.**

From our past results, on average, 50% of the recovered oocytes should mature in culture, and these will be fertilized by ICSI. After fertilization, 20% of fertilized oocytes are expected to

develop to the blastocyst stage in the laboratory. However, because of individual mare, ovary/oocyte transport, and stallion factors, the number of blastocysts produced from individual mares post-mortem in our laboratory has varied from 0 to 11.

After transfer, approximately 60% of the transferred blastocysts are expected to establish pregnancies, but some of these pregnancies will be lost. About 50% of transferred blastocysts are expected to continue to develop normally and produce a foal. Taken altogether, this gives us **about a 50% chance of normal pregnancy for every 10 oocytes recovered**. These percentages will decrease markedly if the mare is aged, has been ill or debilitated for an extended period of time, or if the ovaries have not been packaged properly or are delayed in arriving. *Results can also vary depending on the quality of the sperm used for the procedure.*

Costs for the program

Removal of ovaries: If the mare is at Texas A&M at the time of death, there is a fee for removal of the ovaries. This will be charged by the Veterinary Teaching Hospital.

Please refer to the Fee Schedule available on our website <http://vetmed.tamu.edu/eel> for a complete listing of all associated costs and fees, which may include:

- Ovary dissection, oocyte collection, and oocyte culture for maturation
- Performance of sperm injection (ICSI) on all oocytes that have matured
- Preparation of semen from first **additional** stallion desired for ICSI
- Production and handling of each blastocyst (transferrable embryo) produced
- Shipment of blastocysts for transfer (at cost)
- Vitrification of blastocysts
- Surcharges if ovaries must be processed after working hours (after 6:00 PM) or on weekends/holidays due to time of receipt of tissues. An additional surcharge is also assessed in cases which ICSI must also be performed outside of working hours or on weekends/holidays.

Blastocysts can be vitrified in the case that the mare dies outside of the normal embryo transfer season. Embryos are stored at another facility until they are ready to be transferred.

Incidental charges: Any costs for semen collection or shipment of semen containers or other charges not covered by the above information are charged to the client separately.

All charges related to the transfer of resulting embryos to recipient mares will be billed to you, the client, by the veterinary practice performing the transfer and are not included in our services.