EQUINE INTRACYTOPLASMIC SPERM INJECTION PROGRAM – Post-Mortem Oocyte Recovery

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Purpose of the Intracytoplasmic Sperm Injection Program

The intracytoplasmic sperm injection (ICSI) program for Post-Mortem Oocyte Recovery is offered at Texas A&M as a means of establishing pregnancies from oocytes (eggs) recovered from the ovaries after a mare's death. Using ICSI, oocytes are injected with individual sperm from a 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.

Embryos may also be vitrified (frozen) to be transferred at a later date; this lowers the chance of successful pregnancy by about 25%. 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.

Overview of the procedure

Intracytoplasmic sperm injection is a recently developed technique in which a mare's oocytes are fertilized in the laboratory (in vitro). When the Equine Embryo Laboratory receives the ovaries from the deceased mare, the ovaries are dissected, and all visible follicles on the ovaries are processed to recover oocytes. The oocytes are then cultured in a hormone-containing medium, to induce maturation; this mimics the natural events that occur 1-2 days prior to ovulation. Our in-vitro maturation process generally takes 24 to 30 hours, depending upon the quality of the recovered oocytes.

After this culture period, oocytes that have matured 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 to

allow development into blastocysts, that is, embryos suitable for transfer to the uterus of a recipient mare. Owners then have the option of shipping any developed blastocyst to a private veterinary practice for transfer to recipient mares or vitrifying the produced blastocyst for warming and transfer at a later date.

Method for Ovary Collection and Shipment

Ovaries are removed from the mare by the referring veterinarian and should be shipped to TAMU by the fastest method possible. The recommended method to obtain the ovaries is to anaesthetize the mare with xylazine and ketamine, remove the ovaries, and then perform euthanasia.

Once removed, the ovaries should be placed in a plastic bag, either in normal saline or, if none is available, just in the bag. **Do not refrigerate the ovaries.**

If transport time is less than 2 hours, ovaries should be kept around body temperature (90-98 ºF or 32-37 ºC). This can be accomplished by placing them in a styrofoam container with ballast in the container (e.g. a liter bag of saline or water) at 37 ºC.

For transport times of 2 to 8 hours, ovaries should be cooled to room temperature, or a little below room temperature. Ship in an Equitainer® or in well-insulated Styrofoam packaging with room temperature coolant cans and/or ballast.

For transport times over 8 hours, ovaries should be cooled, but no lower than 12 ºC (54

This can be done by placing the ovaries in an Equitainer® with one frozen coolant can (on the bottom) and one room temperature coolant can, then packing the ovaries and ballast in the isothermalizer cup at room temperature. A Styrofoam box or semen-shipper can also be used: place a frozen pack in the box or shipper with good insulation between the pack and the bagged ovaries, with ballast at room temperature.

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 or removal of the ovaries from a live mare; longer times can be associated with a lower rate of embryo development, and a higher rate of pregnancy loss after transfer of embryos.

Semen (fresh or frozen) from the desired stallion should arrive the day after the ovaries have been received. Typically, sperm injection is conducted in the morning two days after the ovaries have been received.

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.

Anticipated results

All visible follicles on the donor mare's ovaries will be processed for oocyte recovery. Typically, 10 to 15 oocytes will be recovered from one pair of ovaries post-mortem; however, this number is quite variable and is typically lower if the mare is old or has been chronically ill.

From our past results, on average, 50% of the recovered oocytes should mature in culture, and be fertilized by ICSI. After fertilization, 20% of fertilized oocytes are expected to develop to the blastocyst stage in the laboratory. After transfer, approximately 60% of the transferred blastocysts are expected to establish pregnancies, and 75% of these are expected to continue to develop normally. Taken altogether, this gives us **about a 50% chance of normal pregnancy for every 10 oocytes recovered**. These percentages will decrease if the mare is old, has been ill or debilitated, if the ovaries are delayed in arriving, or have not been packaged properly. 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 $250 fee for removal of the ovaries. This will be charged by the Veterinary Teaching Hospital.

Ovary dissection and oocyte maturation: $500. This covers dissection of the ovaries to recover oocytes, classification of the oocytes, and their maturation in culture.

If oocytes are found to have matured after culture, the fee for performance of ICSI on one or more oocytes is $1250; sperm preparation for ICSI from additional stallions is $300 each.

The fee for blastocyst production and handling is $500 per blastocyst produced. Blastocysts can be vitrified in the case that the mare dies outside of the normal embryo transfer season for $300 and are stored at another facility until they are ready to be transferred. Clients are strongly encouraged to have their embryos warmed at our lab before having them sent to their desired transfer facility.

Cost for each shipment of blastocysts to an embryo transfer service is $150.

A surcharge ($200) is assessed for cases in which ovaries are processed after 6:00 pm or on weekends/holidays, and an additional surcharge ($200) is assessed in cases in which ICSI must be performed on weekends/holidays.

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.