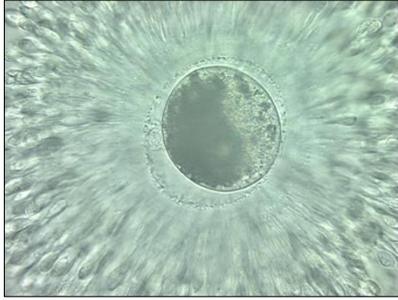




The Assisted Reproductive Techniques (ART) Program at the Equine Reproduction Laboratory



The ART Program at CSU is one of the most established laboratories of its kind within the equine industry in the United States. Dr. Elaine Carnevale and her team pioneered many of the procedures utilized for equine assisted reproductive techniques, and they continue to provide both cutting-edge clinical and research opportunities for horse owners. The use of traditional IVF techniques has not been successful in the horse; therefore, intracytoplasmic sperm injection (ICSI) is the best option. It is important for horse owners and breeders to understand the regulations established within their respective breed registries prior to pursuing these procedures. Enrollment within the ART program at CSU is appropriate for

subfertile mares that are unable to become pregnant themselves or donate embryos, as well as stallions with a limited amount of frozen semen. Below is a step-by-step overview of the process:

- 1. Initial Consultation with Client Coordinators:** Initially, you may want to discuss your mare's reproductive history to determine her potential success within the ART program with one of our veterinarians, and our client coordinators can give you an overview of the procedures and financial aspects of the program. A contract for services will be emailed/mailed for your review and signature. The list of fees for services will be included. This contract should be completed to the best of the owner's/breeder's ability, paying special attention to the feeding and maintenance sections. No services will be performed without a signed contract on file.
- 2. Establishing Individual Care:** Once your mare arrives, an ART veterinarian will perform a physical examination and contact you to review any medical problems, nutritional requirements, and exercise/turn out options during her stay. Each mare will have an individual wellness plan, overseen by the veterinarian and barn manager, to optimize her health while residing at CSU. The mares are housed in individual stalls with attached runs.
- 3. Monitoring Cycles:** A veterinarian will monitor your mare's ovarian activity using transrectal ultrasonography to determine the optimal timing of oocyte collections. We will recommend laboratory testing and/or uterine health evaluations as needed.
- 4. Harvesting Oocytes (Eggs):** In general, we can collect two different types of oocytes from mares. Immature oocytes are often collected from smaller follicles and must be artificially matured within an incubator. We can also collect an oocyte from a large follicle just prior to ovulation; in this case, the oocyte has begun to mature and prepare for fertilization within your mare's follicle. Each situation is different, and the decision to collect either oocyte-type is based upon consultation with one of the ART veterinarians. An ultrasound probe positioned within the vagina of the sedated mare will allow us to image the follicles and allow us to properly position the ovary. For the oocyte collection, a needle is punctured through the vaginal wall and into the follicle, and gentle suction applied to collect the oocyte. After collection, oocyte(s) are kept in an incubator until fertilization 12-30 hours later. A report will be emailed to the owner indicating that this procedure has taken place.
- 5. The ICSI Process:** Upon maturation, the ICSI procedure is performed by carefully injecting a single sperm into each oocyte by a highly trained technician. The fertilized oocyte is returned to an incubator and allowed to develop into an embryo, which usually occurs within 6-8 days. Because the preservation of semen is essential in many situations, we can talk to you about options for maximizing the use of semen. These options include refreezing sperm in small numbers in straws specifically designed for ICSI or cutting a small portion of a straw under liquid nitrogen.
- 6. Embryo Transfer:** Once a viable embryo has been produced, it can be handled in several ways; which should be determined prior to this step:
 - a. Uterine transfer into a CSU managed recipient mare through Mare Services
 - b. Shipment to another facility for uterine transfer into a recipient mare





- c. Uterine transfer back into the donor mare (autotransfer), which is only an option for young/reproductively healthy oocyte donors that are capable of carrying their own foal
 - d. Uterine transfer into the owner's own recipient which has been properly synchronized for the embryo
 - e. Cryopreservation of the embryo, which can be stored & transferred at a later date
7. **Pregnancy Exams:** If transferred to a recipient mare at CSU, the Mare Services program will provide updates regarding pregnancy examinations. The first pregnancy ultrasound will occur 5-6 days following the transfer. Additional pregnancy examinations will be scheduled based on the results.

Anticipated Results (based upon past 2016 data):

Oocyte Recovery Rates Per Follicle Aspirated:

	Preovulatory Follicle	Secondary Follicles (small follicles collected at same time as preovulatory follicle)	Immature (all small follicles)
Young Donor (2-14 yr)	83%	58%	79%
Middle-Aged Donor (15-19 yr)	80%	72%	None aspirated
Old Donor (20-27 yr)	86%	79%	100%

- We anticipate about 20% of sperm-injected oocytes to develop into an embryo suitable for transfer, but this will vary with mare and stallion.

Pregnancy Rates Per Embryos Transferred:

	16 day	35 day	Sent home
Young Donor (2-14 yr)	50%	43%	43%
Middle-Aged Donor(15-19 yr)	71%	57%	57%
Old Donor (20-27 yr)	33%	17%	17%

It is important to understand there are many factors that affect success. Older mares have decreased oocyte quality, similar to women, and may be more difficult to establish and maintain successful pregnancies. Additional factors such as semen quality and medical conditions of either the mare or stallion will also affect the success. Our goal is to keep you as involved as possible throughout the process in order to make informed breeding decisions.