

# LATEST ADVANCEMENTS IN EQUINE REPRODUCTION

*Now available to breeders*

by Cindy Reich\* ■ photos by Sexing Technologies (USA), Equine Reproduction Innovations, Inc.

**A**s the breeding season approaches, there are several astonishing new techniques available to breeders to increase the production from valuable or previously infertile mares and stallions.

Additionally, for the first time commercially, breeders also have the ability to have semen from stallions sorted into X or Y chromosome bearing cells, thus creating a confirmed filly or colt, depending on the sample used.

Many breeders have mares that are genetic treasures, but due to various reasons are no longer producing foals. Usually due to advanced age, or fertility problems such as fluid in the uterus, scar tissue or a damaged cervix, these wonderful mares have been retired from the breeding arena.

Some of these mares can continue to produce with the assistance of embryo transfer, but others cannot even produce viable embryos.

Thanks to an amazing new technology known as Intracytoplasmic Sperm Injection (ICSI) these mares can now go back into production with often fantastic results. While a mare may develop several follicles on her ovaries during

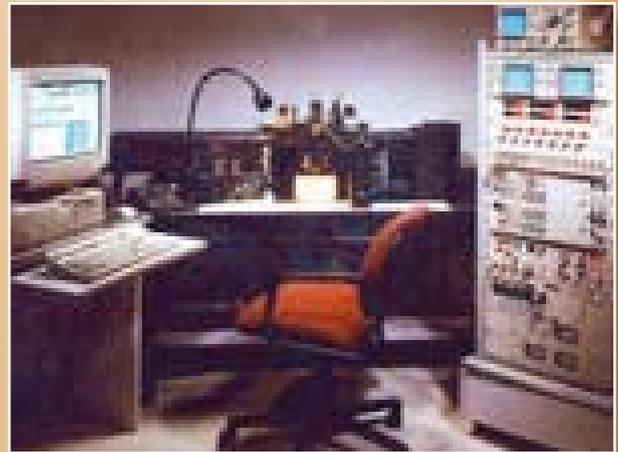
her estrus cycle, usually one becomes dominant and goes on to ovulate, releasing the egg into the oviduct, where it is then fertilized by a single sperm cell when the mare is bred.

Although millions of sperm cells are deposited into the mare's reproductive tract when she is bred, once a single sperm cell has penetrated the egg, a reaction occurs which blocks all other sperm cells from being able to fertilize the egg. ICSI takes this procedure out of the mare and does it under a microscope, mechanically fertilizing the egg by injecting a single sperm cell into the egg.

ICSI is a mechanical form of in-vitro fertilization, whereby the sperm is mechanically injected into the egg. In routine in-vitro fertilization, the egg and sperm are put into a dish and the egg is fertilized by one of the sperm cells. However, for reasons not understood at this time, regular in-vitro fertilization does not work in horses. It is necessary to manually inject the egg to cause fertilization.

## EGG RECOVERY

The egg is recovered from the donor mare by using a thin,



Flow cytometer. ©Sexing Technologies, USA

*ultrasound-guided needle that is inserted into the follicle. The fluid, containing the egg (oocyte) is then drained into a dish and the egg located under a microscope. This procedure is done with the donor mare standing in a set of stocks and does not require a general anesthesia.*

### **EGG INJECTION**

*The egg is put into a special solution and placed in a dish under the microscope. A single sperm cell from the desired sire is injected with a micro-pipette into the egg. Once fertilization occurs, the egg will start dividing and becomes an early embryo. This is a very delicate procedure and requires a very highly skilled technician as well as a very specialized microscope and equipment. Once the egg has been fertilized, it is then surgically transferred into a recipient mare.*

### **BENEFITS OF ICSI FOR MARES**

*Mares can have a variety of problems that may cause them to become infertile. Aging, damage to the uterus or cervix, scar tissue in the oviduct, fluid in the uterus, infections and many other problems may cause a mare to fail to become pregnant when bred. Some of these mares can continue to produce offspring with embryo transfer, but others cannot. In fact, some mares become highly sensitive to repeated flushing of the uterus such as what is used for embryo transfer and start to have negative responses in the uterus. These*

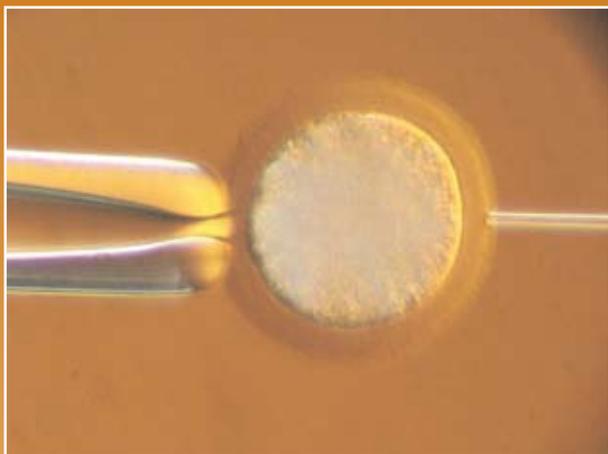
*mares then cannot produce foals even with the help of embryo transfer.*

*In ICSI the uterus is bypassed completely and the egg is recovered from the follicle on the ovary. Therefore, mares that previously could not produce foals because of uterine or cervical problems can produce foals from ICSI.*

### **BENEFITS OF ICSI FOR STALLIONS**

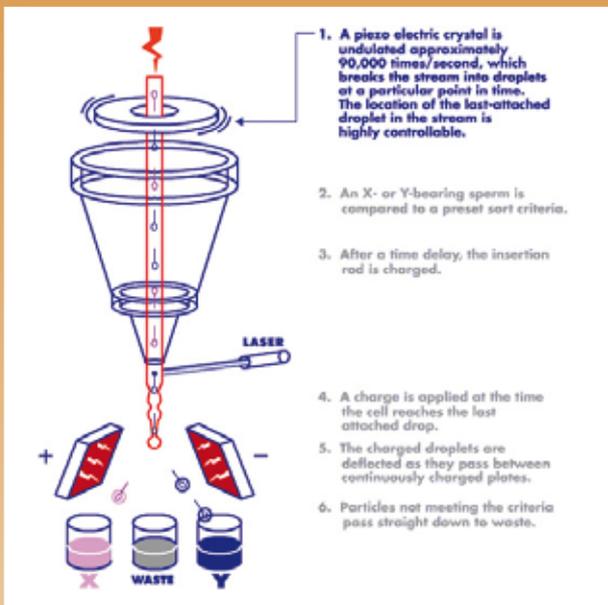
*Stallions can benefit from the ICSI procedure as well. For example, if a breeder has a limited amount of semen from a stallion that has died, multiple foals can be produced from one straw of semen. Without the use of ICSI, it would take 4-12 straws per mare to achieve a pregnancy, depending on the stallion and the quality of the semen. In this way, stallion owners can maximize the production of foals from limited amounts of frozen semen. For example, veterinarians at Equine Reproduction Innovations were able to produce 13 pregnancies from one straw of frozen semen in 2008.*

*Stallions with limited fertility can also benefit from ICSI. Some stallions may not have been frozen until they were quite old, and their semen quality was not the best. However, with ICSI, the technician is doing the job a vital sperm cell would normally do, and that is fertilize the egg. All that is required is a sperm cell with viable DNA.*

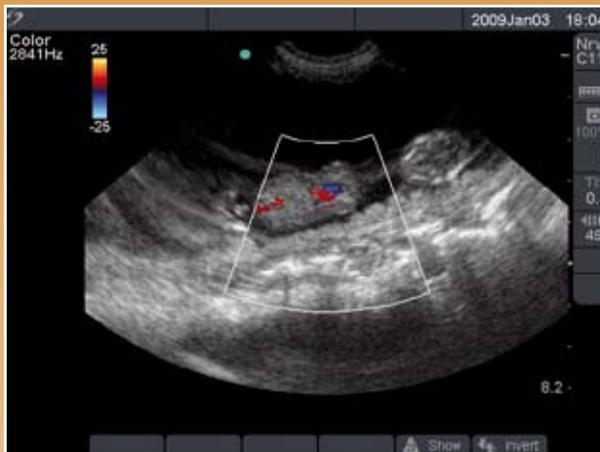


ICSI procedure with pipette and egg  
 @Equine Reproduction Innovations, Inc., USA

Ultrasound photo of a early fetus produced by the ICSI procedure.  
 @Equine Reproduction Innovations, Inc.



Schematic of sex semen sorting. @Sexing Technologies, USA



Low motility is not an issue, as the only reason a sperm cell needs to be motile is to get to the egg. With ICSI, this job is done without high motility being necessary. Stallions with sperm quality that is not adequate for breeding mares normally can produce offspring using ICSI.

For stallions who have died and the breeder has only a limited amount of frozen semen, ICSI is often the only way to truly maximize the number of foals that can be produced with limited amounts of valuable semen. In that regard, in 2010, Equine Reproduction Innovations in Colorado, USA will be standing \*Aladdin, \*Muscat, \*Nariadnai Bey Shah, Khemosabi and Brass at stud with the use of ICSI. These remarkable genetics would not be accessible for use without the advantage of the ICSI procedure.

**SEX SELECTED SEMEN**

All foals receive one sex chromosome from each parent. The mare will always contribute an X chromosome, and the stallion will contribute either a Y or an X chromosome. If the stallion contributes an X chromosome, the resulting

foal will be XX and a filly. If the stallion contributes a Y chromosome, then the foal will be XY and a colt. Using a specialized cell counter called a flow cytometer; the sperm cells can be treated with a fluorescent dye that allows the machine to sort the X from the Y bearing sperm cells. Using ICSI along with a single dose of sex selected semen gives the breeder a greater than 95% chance of producing the desired sex foal. Therefore, with the use of sorted semen, breeders can choose the sex of the foal prior to breeding the mare.

The use of these advanced reproductive techniques will allow breeders access to genetics that have not been available for decades. The chance to cross a modern bloodline with some of the legendary sires of the past is an exciting proposition for those who are looking to create a new dynasty in the Arabian breed. Furthermore, for breeders who require a specific sex foal-for sales, or for quickly building up their own breeding program, sex selected semen may be a useful tool. □

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