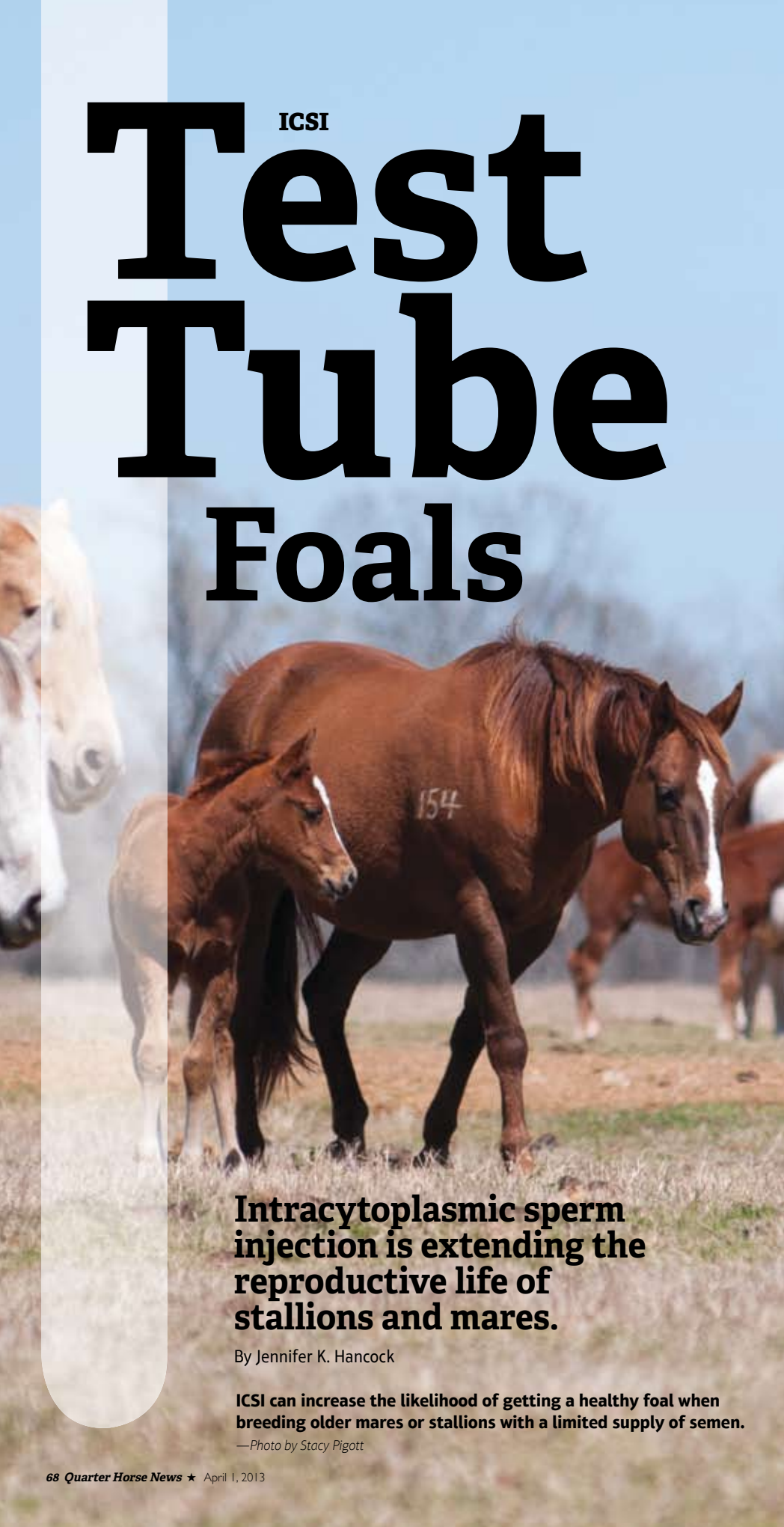


# ICSI Test Tube Foals



**Intracytoplasmic sperm injection is extending the reproductive life of stallions and mares.**

By Jennifer K. Hancock

**ICSI can increase the likelihood of getting a healthy foal when breeding older mares or stallions with a limited supply of semen.**

—Photo by Stacy Pigott

**R**esearchers at Colorado State University produced their first intracytoplasmic sperm injection (ICSI) foal in 1996, and in 2003, the university began offering ICSI on a commercial basis. Texas A&M University has also been at the forefront of advancements in ICSI technology over the past 17 years. While the technology is not new, its laboratory restrictions and associated costs have kept it out of reach for many breeders. That is beginning to change as the number of facilities offering the service continues to increase and the cost of the procedure decreases. ICSI has also become a more popular topic thanks, in part, to the recent sale of High Brow Cat and the announcement that ICSI will be used to extend the stallion's frozen semen supply.

As the name implies, intracytoplasmic sperm injection involves inserting an individual sperm into an egg removed from a mare to establish pregnancy. The resulting embryo is allowed to continue to mature in the laboratory for a variable length of time and is then placed in a recipient mare to develop.

ICSI, like most cutting-edge technologies, is gaining popularity and the costs associated with the procedure are beginning to decrease. At Colorado State University and Texas A&M University, they refer to ICSI as one of their assisted reproductive technologies (ART).

"It's not a procedure that is on the margin anymore," explains Elaine M. Carnevale, a veterinarian and associate professor at Colorado State University's Department of Biomedical Sciences Animal Reproduction and Biotechnology Laboratory. "It's a very well-documented, very reliable procedure. You are not going to get a pregnancy as fast as you might with an embryo transfer with a normal mare and normal stallion, but you will get it a lot faster than you will with an embryo transfer if you have a problem mare or problem stallion. It's not an experimental type of procedure any more — we've just done way, way too many of them."

"The procedure can be used for a couple of different reasons," says Dickson Varner, Professor and Pin Oak Stud Chair of Stallion Reproductive Studies in the Department of Large Animal Clinical Sciences at Texas A&M University. "It can be used for mares that are unable to become pregnant themselves if they



**JoAnne Stokes performing ICSI at Colorado State University's Equine Reproduction Laboratory.**

—Photo courtesy of Colorado State University

have uterine disease, cervical lacerations, or other damage to the reproductive tract such that they cannot conceive or support an embryo for any length of time for standard embryo transfer. For these mares, aspiration of the eggs from the follicles is a likely approach for estab-

or 1,000 straws, or maybe even more than that, from one ICSI dose," he adds. "So you can create almost a limitless supply of semen from stallions if it is used for ICSI purposes."

According to Carnevale, there are advantages to using ICSI for stallion owners besides having

## **ICSI involves inserting an individual sperm into an egg removed from a mare.**

lishment of pregnancies by ICSI using semen from any stallion.

"The other option is to obtain foals from a stallion who has a very limited supply of semen and the sperm can't be utilized with standard insemination techniques. So you have two approaches. It can be used for subfertile mares or stallions with a limited supply of semen."

One major advantage to stallion owners that have a limited supply of frozen semen is the ability to not only stretch that supply, but also to possibly multiply a single breeding dose by 1,000 times.

"For stallions that have a limited supply of semen, that supply is generally frozen semen," Varner says. "For ICSI purposes, you can thaw one of those straws, and refreeze it in a diluted fashion such that you can get 100 to 200 straws from one standard straw for ICSI purposes. You just need a very few sperm for injection of oocytes.

"So essentially, a standard insemination dose for a stallion would be eight 0.5-mL straws, which is 4 cc, and one could probably make 800

frozen semen stored from a sterile or deceased stallion and wanting to extend that supply.

"For stallions that just have poor or bad quality semen, then you can still pull good sperm out of a lot of bad ones so you can still get pregnancies from stallions that have a lot more challenges like testicular degeneration or an injury that is really limiting the number of sperm that they produce," she says. "That was one of the main uses that we thought of initially for it. It is used a lot in human reproductive medicine at fertility clinics. It's a very common procedure there."

Chad Bushaw of Crown Ranch in Weatherford, Texas, breaks down the process like this: "I breed a lot of horses myself, and it seems to me that what we have done is instead of relying on Mother Nature to either work or not, what we are doing is adding some modern technology along with Mother Nature. Instead of doing a traditional insemination and hoping that the sperm from those eight straws is going to find its way to where it needs to be, we're taking the egg from the mare and using science to

## **Necessary Advancements**

**An old English proverb says that necessity is the mother of invention. Difficult situations often encourage inventive solutions and that is also the case with intracytoplasmic sperm injection, or ICSI.**

"At Texas A&M there are certain regulations regarding horses that are used that belong to the state of Texas," explains Dickson Varner, a professor of theriogenology in the department of Large Animal Clinical Sciences at Texas A&M University. "In essence, one surgery is allowed, and the second surgery has to coincide with euthanasia of the animal. You only have one surgical attempt on a horse if it's owned by the state of Texas."

This presented a problem in regards to ICSI, which as an innovative new reproductive procedure almost 20 years ago relied exclusively on surgery to insert fertilized eggs into a recipient mare.

"At this point in time, we do not have a recipient herd for that purpose," Varner says. "Drs. Hinrichs and Choi grow all of the embryos up in the laboratory and then transfer those embryos in a container to an embryo transfer facility that then does the embryo transfer. It reduces the headaches and the additional invasive surgeries that were typically done in the past, even for standard embryo transfer. In the early days, it was thought that surgical embryo transfers were more successful than nonsurgical embryo transfers. And lo and behold in the years following, we found that the nonsurgical technique worked just as well and it didn't create as much problem in the recipient mares."

fertilize the egg in a Petri dish scenario."

That's not only good news for the stallion owner, but also for mare owners that want to continue to have access to popular bloodlines or extend a mare's reproductive years.

### **Egg hunt**

When ICSI was a fledgling technology, oocytes, or eggs, were harvested from the mare and surgically inserted back into the mare, or a recipient, after it had been injected with a sperm cell. That technology is still utilized, but advancements now allow for blastocysts [an embryo cultured for approximately one week, until two cell types have developed] to be transferred back through a mare's oviduct similar to how embryo transfers are performed.

"If you have a fertile mare and are interested in breeding to a stallion with a limited semen supply, you would essentially have that mare managed by shipping her to a facility to aspirate the oocytes," Varner says. "The oocytes can be aspirated one of two ways – one can aspirate a mature, dominate follicle from the ovary that's nearing maturation itself and inject it, or the more popular technique is to aspirate all of the immature follicles on the ovaries and allow them to mature in the laboratory. The likelihood of getting a pregnancy on a cycle is going to be increased because you might get four, five, six, eight or more oocytes from that mare as opposed to one if you just aspirate a dominate follicle."

"I think the more people know and understand, the more they will realize that it actually does work and it isn't as invasive to mares as people used to think when they used to have to go through the mare's flank," Bushaw says. "With some of the perceptions out there, people just don't understand what it is, and it sounds scary. The reality is that it's a game changer because now we can go breed to stallions that have frozen semen stored. There's a lot of these great horses that we are going to be able to extend their production years almost indefinitely if the technology keeps increasing."

Varner says data collected by Dr. Katrin Hinrichs, Professor and Pin Oak Stud Chair of Mare Reproductive Studies at Texas A&M University in 2012, shows that the likelihood of establishing a pregnancy with a non-aged mare is probably about 50 percent per cycle.

"Based on the work of Drs. Hinrichs and Young Ho Choi in 2012 for 50 oocyte aspirations, if mares were up to 19 years of age, it took about 1.8 cycles to establish a pregnancy. You are looking at about a 50 percent pregnancy rate per session."

That same 2012 data shows if a mare was between 20 and 23 years of age, it's an average of four cycles to establish a pregnancy, and if they are older than 23, it took 16.7 cycles to establish a pregnancy. Varner says that in old subfertile mares, it's a last-ditch effort to obtain pregnancy.

"We work with so many problem mares and problem stallions so the results vary when compared to doing an embryo transfer on a young fertile mare where you are going to have a 78 percent success rate," Carnevale says. "If you do an embryo transfer on an older mare with a problem stallion you might have a 10 percent rate or less. We still deal with those same issues with ICSI. For a young mare with a healthy oocyte and a fertile stallion, you are looking at about a 40 percent pregnancy rate – it's higher with an embryo. With an older mare, it would be lower than that. I usually consider that it's going



**An ICSI procedure – an oocyte is being held in place by gentle suction from the pipette on the left, while a sperm will be injected using the small pipette.** —Photo courtesy of Colorado State University

## The reality is that it's a game changer, because now we can go breed to stallions that have frozen semen stored.

to take most mares probably three cycles to get a pregnancy with ICSI, but we've had mares come in here and give us two oocytes and get two pregnancies in one cycle. As with all breeding, it can vary a lot."

"The other thing that is a game changer is that historically the horses that we bred with ICSI were 20- and 30-year-old mares that we couldn't get bred normally," Bushaw adds.

"Those were the only ones that were even ICSI candidates so the success rates were fairly iffy. It worked and created a lot of pregnancies that otherwise wouldn't have been, but it was basically in these mares that were in the geriatric ward or were problem mares. What we have done now is we have people sending in their most fertile young mares because mare owners are being forced to use ICSI to breed to High Brow Cat. There might be some extra expense, but anyone who has done this long enough – if you have a mare that you have trouble breeding and you have two or three shipments of semen and you pay couriers or you pay flight

expenses or you pay for flushes that aren't successful – you can pay a lot more money using traditional breeding methods than what ICSI costs. Now that we are breeding young, fertile mares, I think that it is going to really work well, and I don't think that it's going to cost any more at the end of the day than if I have a breeding or two at a traditional farm."

Texas A&M's research veterinarians are working to increase the odds of success by harvesting as many oocytes – whether mature or not – as possible.

"The interesting thing about the approach that Drs. Hinrichs and Choi are using at Texas A&M is that the oocytes are retrieved from the mare's follicles, and they are matured in the laboratory because when they are aspirated from the follicles they may not be mature yet," Varner says. "They are put in the laboratory in a certain type of media, allowed to mature. The oocytes that have matured are injected with the sperm and then they are allowed to continue to grow in the laboratory in another type of media

that allows the fertilized embryo to grow into a blastocyst stage.

“Drs. Hinrichs and Choi have developed a technique such that embryo can be grown in the laboratory for a week or so following sperm injection and the resulting blastocyst can then be transferred through the cervix,” Varner adds. “It eliminates the need to do flank surgery or laparotomy on the recipient mare to put those fertilized eggs in the oviduct. They are allowed to grow up in the laboratory, those that reach blastocyst stage are transferred similar to what you would do with a standard embryo transfer.”

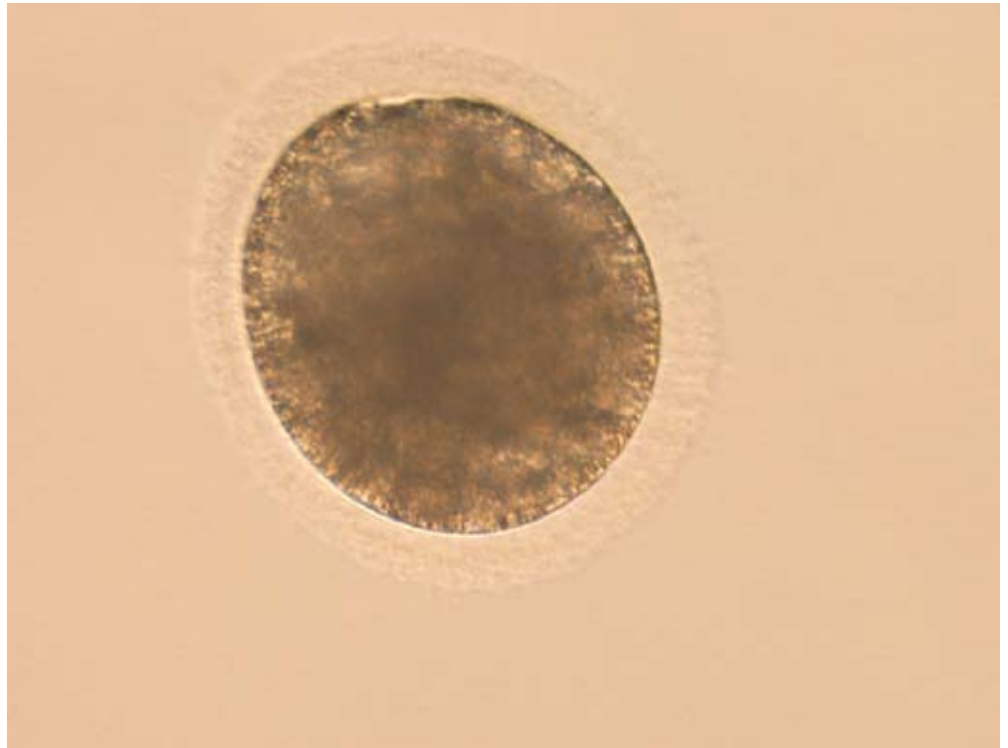
Other advancements involving the technology allow for a mare to stay close to home instead of traveling to an ICSI facility while her harvested oocytes travel to a lab to be injected.

“The other thing that we have done is have people ship oocytes to us, and we have shipped the ICSI produced embryo somewhere else to transfer,” Carnevale says. “There are all kinds of things that you can do that make the costs different and gives the procedure flexibility if needed.”

### **Cost of Technology**

The latest gadgets and technologies usually come with the highest price tags and that is no different in the world of equine reproduction.

“There has been some pushback, but I



**A blastocyst produced by ICSI ready for transfer into a recipient's uterus**

—Photo courtesy of Colorado State University

think that once people talk to the veterinarians at CSU or Texas A&M, they find that the cost is just a fraction of what it was a couple of years ago," Bushaw says. "Each year you've got dozens of vet students that come through these great universities that are not focused on anything but this, and they are going out and doing this for a living in real life. It's like computers or cell phone minutes or anything else, the cost is continuing to come down every year. The people who are pushing back the most are the ones that had experiences five years ago and the expenses are a fraction of what they were then."

"Transferring that really early stage embryo – like a two- to four-cell embryo – back to a recipient mare's oviduct – and that's done surgically – would be the most expensive," Carnevale explains. "You can culture out that embryo in an incubator for a week and wait until it's a little blastocyst and transfer back into a recipient's uterus – like a standard embryo transfer – and that's less expensive because you don't have the surgery. The price range is going to go from \$2,000 to almost \$10,000, depending on what procedure you use and how difficult it is for that mare. There's a big variation."

Texas A&M does not maintain a recipient herd, so mare owners must work with an embryo

transfer facility in addition to the university.

"It costs in the neighborhood of \$1,250 per session if no oocytes are recovered," Varner says. "If you wind up getting a blastocyst that develops and is sent to an embryo transfer facility, the total cost is about \$2,850. If you have a session where you have two blastocysts develop

### **Other advancements involving the technology allow for a mare to stay close to home instead of traveling.**

and they are sent to an embryo transfer facility on separate days, it's about \$3,450. You can see that if it takes an average of two sessions, one can easily invest \$6,000 or \$7,000 for the ICSI procedure and that doesn't include the cost of embryo transfer. In essence, one could have \$8,000, \$10,000 or \$12,000 tied up in these mares for this purpose. It's a technique that probably needs to be used with mares that have

high value and, of course, stallions that have high value as well because it's not inexpensive."

Other costs to consider are breeding fees. If more than two viable embryos are created, one can be frozen for future use. But mare owners should know the conditions of their breeding contracts.

"Arrangements need to be solidified with the stallion owner because you don't know when the fee is going to be assessed for additional pregnancies," Varner advises.

"It's more expensive, and you have to collect the egg from the mare, so there are not as many places where that can be done," Carnevale says. "It's a little more difficult than flushing out an embryo, so it's more technically demanding and it requires a special facility. It's also more expensive – it's like anything with more technology in it that it's a different ballgame. The primary advantage from a clinical standpoint is that you can get fertilization when we couldn't get it because of a mare problem or a stallion problem otherwise."

"I think in a year or two, if we have this conversation, we won't even be differentiating between ICSI and other breeding methods," Bushaw says. "We'll just be talking about breeding mares, and it'll be one of those things that we don't even think about. ★