Making 'true' equine IVF a reproducible success

October 13 2022, by Katherine Unger Baillie

After a decades-long quest for an efficient way to perform “true” in vitro fertilization in horses, researchers from Penn Vet have developed a new, successful technique, resulting in the birth of three healthy foals. Credit: Matheus Felix/Penn Vet

Assisted reproduction has become an invaluable technique for horse owners hoping to pass on to another generation the characteristics of cherished and successful animals. But for decades, one of the most common methods used in assisted reproduction in humans and other animals—standard in vitro fertilization (IVF)—has been stubbornly difficult to achieve.

"It's a frustrating thing," says Katrin Hinrichs, professor of reproduction at the University of Pennsylvania School of Veterinary Medicine, who, alongside her other research programs, has tried for more than three decades to tackle conventional equine IVF, in essence, convincing a sperm to fertilize an egg in a Petri dish. "When we put horse sperm with eggs, they don't even try to penetrate them. They just swim happily about ignoring the egg, leaving us with a zero-fertilization rate."

Hinrichs and others have developed techniques to produce embryos using intracytoplasmic sperm injection (ICSI), a method of fertilization that requires the technically challenging injection of a single sperm into a single oocyte, or egg, aided by a high-power microscope and manipulation equipment. However, supporting sperm to achieve "true" IVF—in which sperm incubated in a Petri dish fertilize an oocyte without further manipulation, as they would naturally inside a mare—proved elusive.

Until now. Hinrichs and colleagues report in the journal *Biology of Reproduction* a major achievement in equine reproduction: a conventional IVF technique with a 90% fertilization rate, with 74% of the fertilized eggs giving rise to blastocysts, the rapidly dividing ball of cells that develops into the embryo and placenta. The three mares into which resultant embryos were transferred each carried healthy foals to term.

"The demand for assisted reproductive technologies like IVF is getting

larger and larger in the horse breeding community," Hinrichs says. "The approach we've developed would allow more veterinary practices to offer IVF, as it doesn't require the expensive equipment and training needed to do it the way it's done now, by injecting each sperm into each egg. But for me the fun part is just nailing this down. I've been a horse person all my life, and for decades we have tried to figure out why this doesn't work in horses. And now we have a repeatable method that does work, so we can explore the 'why.'"

Threads of progress

Assisted reproduction in horses has been a focus of Hinrichs' career. Her contributions to the field have earned her numerous awards, including the honor of being inducted into the Equine Research Hall of Fame last month.

Her interest dates back to her training at Penn Vet, where she earned a Ph.D. in 1988, studying early equine pregnancy. Only a handful of years before, at Penn Vet's New Bolton Center, the first calf produced by IVF had been born. "I worked with Virgil, the first IVF calf, when I was doing my residency here. There was a culture at Penn around studying IVF," Hinrichs says. Toward the end of her doctoral studies, Hinrichs began working with unfertilized oocytes in horses, to determine how they influenced the outcome of IVF.

In 1990, a group in France led by Eric Palmer reported the first live foal born from IVF. The researchers produced one additional foal the next year, then gave up working with the technique as they could not make it efficient or repeatable. Many other groups tried to develop equine IVF procedures and failed, also unable to establish something replicable.

Other methods for assisted reproduction, producing foals from an unfertilized oocyte recovered from a donor mare, met with more success

but were laborious. In one strategy, used for valuable mares unable to produce embryos, clinicians could extract an oocyte from the mare, surgically place it in the oviduct of a recipient mare, and then inseminate the recipient mare. While a faculty member at Tufts University, Hinrichs completed the first of these procedures for clinical use. "We were able to get pregnancies from isolated oocytes," she says, "but the techniques involved were ponderous and invasive."

Another approach, which has been the most successful form of IVF in horses to date, is ICSI, using a tiny needle to pick up a sole sperm and inject it into an oocyte. In the early 2000s, Hinrichs, then at Texas A&M University, increased the efficiency of that procedure and developed methods to culture the resulting embryo in the laboratory until it could be easily transferred without surgery to a recipient mare. By around 2009, Hinrichs' clinic offered this, and specialized facilities around the world continue to do so.

Still, Hinrichs kept pursuing the development of a simplified, conventional IVF procedure. After her lab had devoted considerable energy to studying oocytes, around 2011 she turned attention to the other party involved: sperm. For sperm to fertilize an egg they must undergo a series of physiological changes in a process known as capacitation. In 2019, a researcher in Hinrichs' lab, Matheus Felix, now chief embryologist in the Penn Equine Assisted Reproduction Laboratory, began investigating how long it takes for horse sperm to capacitate and what conditions support that process.

Tying it together

The team had gathered clues that sperm from horses might need more time than that of other species, such as mice, to fertilize eggs. So they tried a longer-than-normal incubation. "Horse sperm are finicky and like to die in culture," Hinrichs says, "but we had done some previous work

that suggested factors that could prolong their life during incubation."

When Felix employed a complex medium for incubating them, which contains the compounds penicillamine, hypotaurine, and epinephrine (PHE), the team finally found a way to keep sperm alive in culture for more than a few hours. "He tried to culture the sperm overnight under these conditions, and by gosh it worked," Hinrichs says. "The sperm were alive the next day, which is a triumph."

When Felix tried again, incubating the sperm overnight and then adding an oocyte, he documented signs of fertilization. "Because typical results in the horse are zero, this one fertilized oocyte was a sign that the process could work, and we were off on our journey to develop the procedure," Hinrichs says.

"This work was really the result of an intersection of Matheus' ideas on the time needed for capacitation and all of these little bits of information that had come through unsuccessful trials that we had done dating back all the way to the 1990s," Hinrichs says. "Matheus' dedication to getting it right was the vital factor in the final success of the project."

Using the fledgling procedure as the basis for optimizing equine IVF, the research team found that pre-incubating the sperm for 22 hours in the PHE medium, then co-incubating it with oocytes for 3 hours, led to the greatest efficiency, including a 74% rate of production of blastocysts, three of which were transferred to mares that are part of the Penn Vet research herd. Three healthy foals were born as a result.

There is still room to improve on the methods, Hinrichs says. The approach only worked well with fresh sperm; frozen sperm, which is the most practical method for clinical IVF, did not result in impressive fertilization rates. And the PHE medium is cumbersome to make, meaning slight variations could compromise the procedure's success.

"For the first time, we have a method that works, and we can use it as a basis to explore what it is that makes it work and what variations are possible: how to make the procedure simpler and more applicable to practice," Hinrichs says.

Indeed, as satisfying as it is to have solved this decades-long puzzle, what excites Hinrichs most are the new questions it enables her and her group to interrogate.

"What's been driving me for 30 years are these physiological questions that we don't know the answer to," she says. "What happens in a mare during fertilization? Why are equine sperm so resistant to procedures that work in other species? What do equine sperm need to capacitate? Do they have the same changes as do sperm of other species? These are the questions I find fascinating."

More information: Matheus R Felix et al, Successful in vitro fertilization in the horse: production of blastocysts and birth of foals after prolonged sperm incubation for capacitation, *Biology of Reproduction* (2022). DOI: 10.1093/biolre/ioac172

Provided by University of Pennsylvania