Vitrification Keeps Pig Embryos Viable

KEITH WELLER (K7972-10)

A confocal microscope enables animal physiologist John Dobrinsky (right) and research associate Charles Long to examine enlarged images of cryopreserved pig embryos on screen.

Consumers want high-quality food at lower prices, so producers are scurrying to meet price and quality demands of the marketplace. And interest in foods with enhanced qualities such as leaner, more tender meat is forcing livestock producers to look to science, particularly genetics, for answers.

Fortunately, Agricultural Research Service scientists at the Germplasm and Gamete Physiology Laboratory in Beltsville, Maryland, are helping to meet the challenge.

"In order to produce a better product for the consumer, we must develop strategies for maximizing the genetic potential of our domestic animals," says John R. Dobrinsky, an animal physiologist at the lab.

"Maintaining genetic diversity is the key to maintaining the most valuable genetic traits in animal populations that will provide the foundation for meeting the needs of future generations," he says.

Dobrinsky and his colleagues are working to develop technologies for preserving germplasm and embryos from today's genetically and economically superior animals. They are developing methods to preserve pig embryos indefinitely in liquid nitrogen at -320°F (-196°C). Preserved in this way, the embryos' biological activity all but ceases. After removal from storage and transplant into a surrogate mother, the embryos resume normal development.

Since the mid-1980s, the meat animal industry has been routinely cryopreserving embryos of several livestock species, especially cattle. However, the $11 billion a year swine industry has not had this technology available. Now that could change.

Scientists at the Beltsville lab are using a technique they call vitrification. They cool a liquid medium so fast that ice crystals can't form and then store pig embryos in it. For a solution to vitrify, it must be instantaneously cooled in liquid nitrogen.

Rapid cooling prevents ice crystals from forming in or around the embryos, and this is key to their safe storage and later development, Dobrinsky says. "Pig embryos are extremely sensitive to slow cooling below normal room temperatures—about 59°F (15°C). This type of slow cooling is required during conventional embryo freezing methods, and this is why pig embryo survival after cooling or cryopreservation has been so poor," says Dobrinsky.

The problem, he explains, is that embryos suffer physiological and structural changes when going from normal body temperatures to cooler temperatures. "Hypothermic conditions can change normal cell function and skeletal structure, making the embryo incapable of normal development."

Rapid cooling during vitrification is thought to outrace damaging effects evident during slow cooling. With vitrification, Dobrinsky and his colleagues achieved modest success rates—that is, about 40 percent of the embryos survived. But that was not good enough.

Dobrinsky wanted to know exactly how the embryos' cell structures were being damaged during cryopreservation. "We focused on the embryonic cytoskeleton," he says. The cytoskeleton is a network of microfilaments and microtubules that gives embryonic cells their shape and support—just as the human skeleton shapes and supports the body.

With a confocal microscope, which uses lasers, Dobrinsky took a closer look inside embryos during

and after cryopreservation. The microscope produces a digital reconstruction of all cells in the embryo.

"We could see the cell plasma membranes, and the microfilaments were being disrupted. We wanted to prevent these membrane disruptions," he says.

"Our hypothesis was this: If we dismantled the microfilaments in an orderly way before cryopreservation, they might reform normally and support the plasma membrane after cryopreservation. To do this," says Dobrinsky, "we placed from 10 to 20 embryos into small straws in a solution containing a compound called a microfilament inhibitor. We vitrified and stored those straws in a sealed canister filled with liquid nitrogen.

"We learned that they can be stored this way indefinitely," he says. "And once they are warmed, the embryos can then be further cultured or transferred to surrogate mothers, where the microfilament network will reform and normal cell development will resume."

Dobrinsky has found his system increases the survival rate to more than 80 percent in the laboratory.

"From the laboratory to the barn, we warmed vitrified embryos and transferred them to surrogate mothers, producing the first live offspring from vitrified/warmed pig embryos," says Dobrinsky. "This is a first for maternal genetics. Until now, the swine industry could only preserve sperm from select males through semen cryopreservation—processes that were developed at Beltsville in the 1970s."

This high-tech cryopreservation approach is more than just a scientific advancement: Both swine producers and consumers will benefit. It could bring a major increase in the efficiency of making pigs with important genetic traits available to breeders worldwide. Today, many pigs are transported by air freight from countries with breeder herds to those where new breeding herds are being established.

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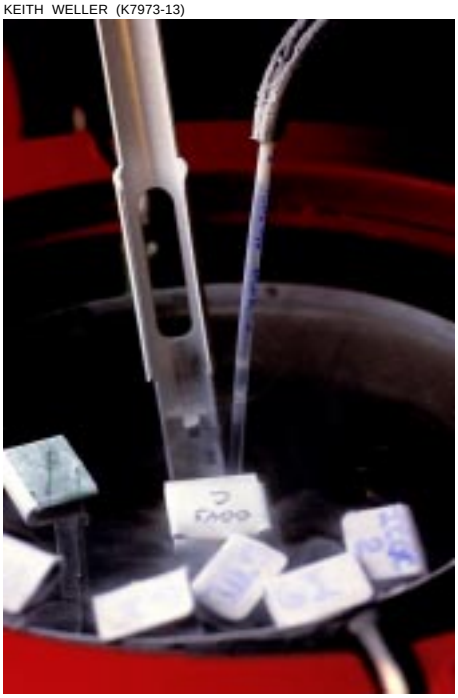

Cryopreserved pig embryos can be stored indefinitely inside special straws submerged in liquid nitrogen.

"The chance for global expansion of the swine industry is another potential advantage," says Dobrinsky. "Keeping separate breeding herds would be costly for producers. This technology will allow us to import and export valuable breeding stocks and unique germplasm—without the worry of shipping live animals. It has the potential for changing the way we produce future generations by minimizing risk of disease transmission or loss of valuable animals during transport."

Costs of caring for breeding herds and transporting animals are extremely high, because of health tests and other requirements. Shipping embryos could considerably reduce these costs and associated constraints, since embryos cost very little to maintain while in liquid nitrogen storage.

Embryo cryopreservation also allows easier regeneration of existing genetic lines or expansion of new ones. Should a disease or catastrophe wipe out an entire herd of superior or valuable pigs, a producer could regenerate a reproducing herd within 2 years.

What does this mean for the consumer?

"It will enable production of the most genetically superior swine stock that our technology and science will allow, ensuring a safe, wholesome, and healthy pork product for the consumer at reduced costs," says Dobrinsky. "We will also have permanent stocks of genetically superior animals to meet future consumer demands. This technology could revolutionize the swine industry by opening global sources of genetic stocks."—By **Tara Weaver,** ARS.

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This sow's five pigs developed from cryopreserved and surgically transferred embryos.