Medical Xpress

Reproductive biologists move in vitro fertilization knowledge forward

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Two new papers from reproductive biologists at the University of Massachusetts Amherst, with international partners, report advances in understanding the basic processes of sperm capacitation that may one day improve success rates of in vitro fertilization (IVF) by providing a shortcut to bypass problems, and may eventually lead to a male contraceptive.

A "pill for men" may be a long way down the road, says Pablo Visconti, lead UMass Amherst author, but this new fundamental knowledge of how sperm acquire the ability to fertilize an egg, letting scientists either block or enhance the process, is at the heart of being able to control it. Findings by the international research teams appear in early online editions this month of *Proceedings of the National Academy of Sciences* (*PNAS*) and the *Journal of Biological Chemistry* (*JBC*), which named one study a finalist for "Paper of the Week."

As Visconti recalls, it was the discovery in the 1950s of sperm capacitation that made IVF possible. Sperm are not fertile until they spend time in the specialized environment of the female reproductive tract, moving through a series of biochemically delicate stages known as capacitation. In the past 50 years it has become clear not only that this signal transduction cascade for capacitation involves many stages, but that each mammalian species has its own different and specific requirements for success.

That is, IVF requirements are not universal, and species-specific requirements need to be established on a case-by-case approach. Visconti says there is great interest in using IVF in horses, for example, but despite years of research this is prohibitively difficult because too little is known about requirements for sperm capacitation in horses. Many biologists are also interested now in using IVF to help preserve endangered species, but the hurdles are immense because each requires very specific procedures, the biochemist adds.

Any better understanding of the cascade of events, where mistakes in IVF often occur, would be useful, Visconti says. Conducting experiments with mouse sperm in vitro, he and colleagues at UMass Amherst, with others at Weill Cornell Medical College, University of Hawaii Medical School, Asahikawa Medical University Japan, Universidad Nacional de Rosario and Facultad de Medicina Argentina and Universidad Nacional Autónoma de México made two discoveries that should help.

In the *PNAS* study, the researchers experimented with increasing intracellular calcium using the calcium ionophore, A23187. Calcium is known to play a role late in the sperm capacitation process, after many earlier steps in pathways that depend on soluble adenylyl cyclase, cyclic adenosine monophosphate (cAMP), protein kinase A (PKA) and other enzyme cascades have taken place.

A major drawback of adding calcium ionophore, however, is that it overwhelms the sperm with too much and quickly immobilizes them, Visconti notes. But he and colleagues found that they could get around this by simply washing the excess away. Thus they basically bypass early capacitation stages by applying the calcium ionophore, wash it away, and the sperm proceed with capacitation.

Under these conditions, the authors report, ionophore-treated sperm fertilized 80 percent of eggs, which developed into normal offspring.

Their data indicate that ionophore-treated mouse sperm can fertilize ova even when the cAMP/PKA signaling pathway is inhibited.

Visconti credits his co-authors Hiroyuki Tateno in Japan and Ryuzo Yanagimachi in Hawaii with the idea of washing ionophore away after use. He points out, "Until they conceived it, no one had thought of this trick. They did the first experiments," Visconti explains. "Later, our experiments demonstrated that by treating the sperm with calcium ionophore, we were activating these cells far downstream of the normal biological process. And when the ionophore is washed away, the sperm retain just the calcium they need. They self-regulate the optimal calcium concentration and are ready to go on."

He adds, "Our laboratory is mainly interested in the basic science of how sperm acquire fertilizing capacity, but one interesting idea here is that this shortcut offers some translational possibilities for calcium ionophore use in IVF. This shortcut may address many of the difficult situations we encounter in IVF, in many species."

In the *JBC* finalist for "Paper of the Week," Visconti and colleagues including Ana Maria Salicioni address a long-standing question about the presence or not of transmembrane adenylyl cyclases in the highly compartmentalized sperm head and tail and their functional pathways.

Salicioni says, "We show that the rules for producing cAMP in head and tail are different and the targets are different. The main target in the tail is PKA. In the head the target is not clear yet, but it certainly is not PKA. So the cAMP pathways in head and tail are completely different, which is entirely new."

Using biochemical analysis methods in knockout mice, Visconti and colleagues show that while the soluble adenylyl cyclase (sAC) is present in the tail, transmembrane adenylyl cyclases (tmAC) are present in the head sperm compartment. These enzymes are involved in the synthesis of cAMP and, unexpectedly, the signaling pathways using this second messenger are different in the head and tail.

Provided by University of Massachusetts Amherst