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## INVITED RESEARCH HIGHLIGHT

Male Fertility

# From PAWP to “Pop”: opening up new pathways to fatherhood

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**I**nfertility remains a significant problem for many couples. Approximately one in seven couples who attempt to conceive will fail to do so within 1 year. In about 65% of these cases, there is a male component of infertility.<sup>1</sup> Despite normal semen parameters, the etiology of infertility remains uncertain in more than 50% of couples.<sup>2</sup> Defects in sperm proteins and/or structures may underlie certain cases of male infertility. Although many men would like to be called “Pop”, “Dad”, or “Papa”, those who are classified with idiopathic male infertility have few options for becoming fathers. Recent studies by Aarabi *et al.*<sup>3</sup> may open the door to new therapies.

Fertilization occurs when a metaphase II-arrested oocyte is activated by sperm-egg fusion. A transient rise and subsequent oscillations in intracellular calcium levels are key features of this activation, involving cortical granule exocytosis, completion of meiosis, decondensation of the sperm nucleus, and pronuclei formation. The complete process culminates in cleavage of the one-celled zygote to the two-cell embryo. The release of intracellular calcium is initiated by the sperm-borne oocyte-activating factor (SOAF) delivered by the fertilizing sperm, which triggers the phosphoinositide signaling pathway and propagates calcium oscillations in the fertilized oocyte.<sup>4</sup> Without this step, meiotic resumption

does not occur, and the egg does not develop further. Important to the understanding of egg activation is the elucidation of the sperm factor responsible for triggering calcium oscillations in the fertilized oocyte.

Over the years, several promising candidates have been proposed as the SOAF. Currently, two major SOAF candidates are under investigation: phospholipase C-zeta (PLC $\zeta$ )<sup>5–8</sup> and the male germ cell-specific postacrosomal WW binding protein or PAWP<sup>3,9</sup> encoded in the mouse by the WW domain binding protein 2, N-terminal-like (*Wbp2nl*) gene. Although it is still uncertain which factor(s) in sperm is (are) responsible for this rise in intracellular

calcium, the investigators in this study detail three criteria by which a candidate protein could be classified as a SOAF: microinjection of the recombinant protein into an oocyte mimics oocyte activation induced by intracytoplasmic sperm injection, relevant antibodies and/or competitive peptides block sperm-induced egg activation, and the candidate protein resides in the postacrosomal sheath subcompartment of the sperm perinuclear theca. This study is a further examination of these criteria.

In their recent publication, Aarabi *et al.*<sup>3</sup> focused on PAWP as a major SOAF responsible for calcium oscillations occurring in oocytes after activation. The authors confirmed their previous findings in *Xenopus* and porcine systems by demonstrating that PAWP also triggers calcium oscillations and pronuclear formation in human and mouse oocytes. They found that expression of recombinant PAWP protein in oocytes mimics egg activation initiated by intracytoplasmic sperm injection. To provide further evidence that PAWP acts as a SOAF, sperm-induced calcium oscillations were blocked by co-injection of a competitive inhibitor peptide derived from the WW domain-binding motif of PAWP.

A significant advancement that this manuscript offers is that these studies are the first to show a candidate protein that successfully fulfills all three criteria required for a protein to be accurately classified as a SOAF. Specifically, PAWP is present in the perinuclear theca of the sperm and is present in elongating spermatids.<sup>10</sup> This ensures that the protein is deposited into the ooplasm at fertilization. We would also like to draw attention to the eloquent experimentation showing inhibition of the sperm-induced calcium oscillations by PAWP competitive peptides. When PAWP is blocked by competitive inhibition in human or mouse oocytes, activation fails to initiate, indicating a potential dominant role for PAWP in egg activation and subsequent embryo development.

Aarabi *et al.*<sup>3</sup> discuss the potential of treating male infertility using a synthetic version PAWP. The research is promising for the diagnosis and

treatment of male factor infertility cases where a patient's spermatozoa are unable to initiate or induce activation of the egg to form an early embryo. Regardless of whether PAWP is the sole SOAF, these findings may provide clinicians with a therapeutic approach for egg activation in cases where intracytoplasmic sperm injection alone is not sufficient to activate the egg.

Further investigations need to be performed to decide more definitively if PAWP is an essential SOAF and whether it acts in concert with other factors to elicit calcium oscillations and oocyte activation. For example, future experiments should determine if the PAWP competitive peptides interfere the egg activation observed by others with PLC $\zeta$ . It will also be important to ascertain whether the mice nullizygous for the *Wbp2nl* gene exhibit an infertile phenotype and, if so, at what level of the reproductive process the defect appears (e.g., egg activation or earlier steps). Finally, these studies produced the recombinant PAWP protein within the egg from cRNA, a method whereby it is difficult to control the levels of protein expression. Although it is technically demanding to do so, studies to characterize a SOAF should determine the amount of the candidate protein in a single sperm and demonstrate that microinjection of an equivalent quantity into an unfertilized egg is sufficient for activation.

The studies by Aarabi *et al.*<sup>9</sup> emphasize the importance of understanding the molecular mechanisms of infertility. Although idiopathic infertility can oftentimes be overcome by intracytoplasmic sperm injection, this is not always the case. The authors of this manuscript have recently shown a correlation between PAWP levels in sperm and fertilization rates in humans.<sup>9</sup> Low levels of PAWP in sperm as detected by immunofluorescence and flow cytometry resulted in decreased rates of normal embryonic development after intracytoplasmic sperm injection. These studies indicate a need for a better understanding of the molecular mechanisms underlying male infertility. This clarity could

result in drug discovery to treat couples suffering from idiopathic infertility as well as help identify targets for male contraception. The studies detailed in this manuscript represent very important steps forward in our understanding of egg activation.

## COMPETING INTERESTS

The authors declare no competing interests.

## REFERENCES

- 1 Niederberger C, Joyce G, Wise M, Meacham R. In: Litwin M, Saigal CS, editors. Urologic Diseases in America. Washington, DC: US Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (US Government Printing Office, 2007); 2007.
- 2 Shukla KK, Mahdi AA, Rajender S. Apoptosis, spermatogenesis and male infertility. *Front Biosci (Elite Ed)* 2012; 4: 746–54.
- 3 Aarabi M, Balakier H, Bashar S, Moskovtsev SI, Sutovsky P, *et al.* Sperm-derived WW domain-binding protein, PAWP, elicits calcium oscillations and oocyte activation in humans and mice. *FASEB J* 2014; 28: 4434–40.
- 4 Whitaker M. Calcium at fertilization and in early development. *Physiol Rev* 2006; 86: 25–88.
- 5 Ito J, Parrington J, Fissore RA. PLC $\zeta$  and its role as a trigger of development in vertebrates. *Mol Reprod Dev* 2011; 78: 846–53.
- 6 Yoon SY, Eum JH, Lee JE, Lee HC, Kim YS, *et al.* Recombinant human phospholipase C zeta 1 induces intracellular calcium oscillations and oocyte activation in mouse and human oocytes. *Hum Reprod* 2012; 27: 1768–80.
- 7 Swann K, Lai FA. PLC $\zeta$  and the initiation of Ca (2+) oscillations in fertilizing mammalian eggs. *Cell Calcium* 2013; 53: 55–62.
- 8 Lee HC, Arny M, Grow D, Dumesic D, Fissore RA, *et al.* Protein phospholipase C zeta 1 expression in patients with failed ICSI but with normal sperm parameters. *J Assist Reprod Genet* 2014; 31: 749–56.
- 9 Aarabi M, Balakier H, Bashar S, Moskovtsev SI, Sutovsky P, *et al.* Sperm content of postacrosomal WW binding protein is related to fertilization outcomes in patients undergoing assisted reproductive technology. *Fertil Steril* 2014; 102: 440–7.
- 10 Wu AT, Sutovsky P, Xu W, van der Spoel AC, Platt FM, *et al.* The postacrosomal assembly of sperm head protein, PAWP, is independent of acrosome formation and dependent on microtubular manchette transport. *Dev Biol* 2007; 312: 471–83.

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