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Use of phosphoproteomics to study tyrosine kinase activity in capacitating boar sperm Kinase activity and capacitation

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Abstract

It is generally accepted that sperm capacitation is associated with the protein kinase A-mediated appearance of tyrosine phosphoproteins, although the substrates and kinase(s) involved have not been identified. We described a Mr 32,000 tyrosine phosphoprotein, "p32", appearing in porcine sperm coincident with capacitation. We also discovered a tyrosine kinase-like enzyme in boar sperm of Mr 32,000 ("TK-32") with enhanced activity during capacitation. The present work was conducted to further characterize and to identify these capacitation-related protein(s). Fresh porcine sperm were incubated to induce capacitation then immunoprecipitation, immunoblotting and proteomic analysis revealed seven tyrosine-phosphorylated proteins aligned in the range of Mr 30,000 with different isoelectric pH values (p*I*). Therefore, p32 may be composed of several tyrosine phosphoproteins. Three were identified as acrosin-binding sp32 (p*I* 6.5), and two triosephosphate isomerase isoforms

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(p*I* 7.1 and 7.9). At present, however, proteonomic analysis has not revealed any kinase at Mr 32,000. Immunoprecipitation experiments show that p32 and TK-32 are different molecules, as TK-32 activity remains in the supernatant of the antiphosphotyrosine precipitates. Finally, in-gel renaturation and immunoblotting suggest that TK-32 is a mitogen-activated protein kinase (MAPK). The discovery of p32 and the MAPK-like TK-32 provides new insight regarding the mechanisms underlying capacitation in the pig.

Keywords: Capacitation; Phosphoprotein; Bicarbonate; Calcium; MAPK

1. Introduction

1.1. Description of sperm capacitation

Capacitation can be defined as the ensemble of cellular modifications that allow mammalian sperm to bind to the oocyte zona pellucida and undergo the acrosome reaction. It is, therefore, an event that is necessary for successful fertilisation in vivo and in vitro, perhaps with the exception of intracytoplasmic sperm injection (ICSI), although this procedure is certainly not routine in animal production.

First discovered independently by Austin and Chang in 1951 [1,2], capacitation is known to be accompanied by an altered pattern of sperm respiration [3,4] and modified membrane architecture [5–7]. Related reports of increased membrane destabilisation [8] are thought to be related to increased sperm permeability possibly by altered channel or pump function [9,10], thereby leading to elevations in intracellular calcium [11] and pH [12,13]. It is only recently that capacitation has been accepted to be a signal transduction-mediated phenomenon, although it has been speculated to be regulated by intracellular events involving second messengers [14,15]. Firstly, Visconti et al. [16] demonstrated that capacitation of mouse sperm is associated with the appearance of a group of tyrosine phosphoproteins, implying the presence of tyrosine kinase activity. Furthermore, these tyrosine-phosphorylated proteins appear in mouse sperm in a cAMP-dependent manner [17], which suggested a novel signal transduction pathway: activation of a tyrosine kinase by cAMP. These findings were additionally intriguing given the notion that a cascade of signalling events is apparently triggered in the absence of a ligand on the sperm.

1.2. Practical importance of understanding pig sperm capacitation

Compared to other species, the mouse and human capacitation systems are better characterised. In contrast, pig sperm capacitation is relatively poorly elucidated. Our laboratory, however, has been interested in the porcine for both fundamental and practical reasons. Understanding capacitation is important for optimizing boar semen preservation protocols, as the sperm must be conserved in a state that does not prematurely promote capacitation in a non-regulated manner. It is well-established that cooling and cryopreservation induces non-regulated capacitation-like modifications in a subpopulation of sperm from various species (reviewed in [18,19]). Since incubation in conditions known to support capacitation dramatically increases sperm fragility [11], it is unlikely that those