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"In vitro" capacitation and subsequent acrosome reaction are related to changes in the expression and location of midpiece actin and mitofusin-2 in boar spermatozoa

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Abstract

The induction of "in vitro" capacitation (IVC) and subsequent, progesterone-induced "in vitro" acrosome reaction (IVAR) was concomitant with an increase in actin polymerization, also showing an increase in actin presence at the apical area of the midpiece. The presence of mitofusin-2, a protein involved in the regulation of the coordinated mitochondrial function, expanded from midpiece to the principal piece after IVC and IVAR. All of these results indicate that the increase of boar sperm mitochondrial activity during IVC and the first minutes of IVAR is concomitant with changes in the expression and location of both actin and mitofusin-2. Our results suggest that both actin and mitofusin-2 play important roles in the modulation of boar sperm mitochondrial function, both by originating changes in the protein membrane environment and by changes in the mitochondrial structure itself.

Keywords: Boar sperm; Capacitation; Acrosome reaction; Mitochondria; Actin; Mitofusin-2

1. Introduction

Capacitation has been defined as the overall changes that the sperm undergoes after being ejaculated that allow it to fertilize the oocyte [1–3]. Capacitation results in altered plasma membrane architecture and permeability, which ultimately modulates flagellar activity and renders the sperm apical head plasma membrane fusogenic [4]. Physiologically, spermatozoa acquire fertilization competence in the female reproductive tract, but capacitation should also be achieved in a defined media, the composition of which approximates the environment of the female reproductive tract [5].

Several intracellular changes are known to occur, including increases in membrane fluidity, cholesterol efflux, intracellular Ca^{+2} and cAMP concentrations, protein tyrosine phosphorylation, and changes in swimming patterns and chemotactic motility [6]. Hyperactivated motility is one of the best characterized phenomena associated with capacitated sperm. Despite this, little is known regarding the energy sources from which spermatozoa transform their motion parameters to hyperactivation during capacitation. In fact, the source of

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ATP that supports sperm motility has long been debated in the field of gamete research. In mammalian sperm there are two pathways for ATP production: glycolysis, which occurs along the entire length of the principal piece of the flagellum, and mitochondrial respiration, centered on mitochondria of the midpiece. Mitochondrial respiration is the most efficient source of ATP and, in this way, it has been inferred that, under normal conditions, the ATP required for sperm motility is mainly obtained through mitochondrial respiration [7]. Hence, mitochondrial status has been related not only to sperm motility in bull [8], horse [9], ram [10], and mouse [7] but also to fertilization ability in humans [11]. However, several works strongly indicate that mitochondria are not the only energy source for sperm motility and capacitation, with glycolysis having also been related to these phenomena. In fact, the gene knockout of the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) caused the appearance of nonmotile sperm and a significant reduction of the ATP content (10% of the total) despite having no deficiency in oxygen consumption [12]. Accordingly, Marin et al. [13] reported that glycolysis plays a significant role as an energy source in boar sperm. This is in accordance with the presence of an active and specific glycolytic activity in mammalian sperm [7]. In addition, several studies have documented the relationship between glycolysis and capacitation-dependent cell signaling [14]. Previous results published by our laboratory would be in agreement with this proposal. In this sense, the achievement of "in vitro" capacitation (IVC) was not accompanied by a significant increase in the rhythm of O_2 consumption, which is a direct marker of Krebs cycle activity [15]. On the contrary, the achievement of subsequent, progesterone-induced acrosome reaction (IVAR) was concomitant with a rapid and transient peak of O_2 consumption [15], suggesting that the increase of the Krebs cycle rhythm may be instrumental in the correct achievement of IVAR. All of these data suggest that the maintenance of sperm function, and specially the achievement of capacitation, would be the result of the equilibrium between the energy obtained from glycolysis and the Krebs cycle, depending on the precise functional status of sperm.

Centering on mitochondrial function, it has been described that capacitation is related to a notorious loosening and distension of mitochondria forming the sperm mitochondrial sheath [16]. We do not know the molecular processes that induce these changes, although several possibilities can be expressed. One of the most interesting would be based on changes in the actin-constituted cytoskeletal network that surrounds mitochondria. In this way, the achievement of the "in vitro" capacitation in boar sperm has been related to a protein kinase A (PKA)-dependent increase in actin polymerization [17,18]. Another possibility is the modulation of mitochondrial activity through specific actions of mitochondria-modulator proteins like mitofusin2 (MFN2). The presence of MFN2 has been described in boar sperm, where changes in its expression and location have been related to the alterations of mitochondrial function during freezing-thawing [19]. MFN2 is especially interesting, because this mitochondria membrane protein participates in mitochondrial fusion in mammalian cells, thus contributing to the maintenance and operation of the actin-constituted mitochondrial network [20]. The mitochondrial network controls the coordinated action of all mitochondria in a cell. This allows mitochondria to control cellular processes, such as apoptosis [21], intracellular Ca^{+2} signaling [22], energy metabolism [20,23], and sperm capacitation [24].

The main aim of this work is to study the expression and location of actin and MFN2 in boar sperm subjected to "in vitro" capacitation (IVC) and subsequent "in vitro", progesterone-induced acrosome reaction (IVAR). For this purpose, we determined the changes in the expression and midpiece location of both actin and MFN2 through Western blot analyses and immunocytochemistry. Our results indicate that IVC and subsequent IVAR are related to specific changes in the expression and midpiece location of both actin and MFN2.

2. Materials and methods

2.1. Boar semen collection

All procedures described within were approved by the Autonomous University of Barcelona Animal Care and Use Committee and were performed in accordance with the Animal Welfare Law issued by the Catalan Government (Generalitat de Catalunya, Spain).

Commercial artificial insemination (AI) doses from boars of proven fertility were obtained from a commercial farm (Servicios Genéticos Porcinos, S.L.; Roda de Ter, Spain). The sperm was manually collected, maintained at 37 °C in a water bath and diluted at 2×10^7 sperm per mL in a commercial dose extender for refrigerated semen (MR-A Extender; Kubus, S.A.; Majadahonda, Spain) and distributed in 100-mL commercial doses. Six of the 100-mL doses obtained, chosen at