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Maturation conditions and boar affect timing of cortical reaction in porcine oocytes

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Abstract

The cortical reaction induces changes at the egg's Zona pellucida (ZP), perivitelline space and/or oolemma level, blocking polyspermic fertilization. We studied the timing of sperm penetration and cortical reaction in pig oocytes matured under different conditions and inseminated with different boars. Immature (germinal vesicle stage) and *in vitro* matured (IVM) (metaphase II stage) oocytes were inseminated and results assessed at different hours post insemination. Penetrability and polyspermy rates increased with gamete coincubation time and were higher in IVM oocytes. A strong boar effect was observed in IVF results. Cortical reaction (assessed as area occupied by cortical granules) and galactose-β(1-3)-Nacetylgalactosamine residues on ZP (area labeled by peanut agglutinin lectin, PNA) were assessed in IVM and *in vivo* matured (IVV) oocytes at different hours post insemination. After maturation, IVM and IVV oocytes displayed similar area occupied by cortical granules and it decreased in fertilized oocytes compared to unfertilized ones. Cortical reaction was influenced by boar and was faster in polyspermic than in monospermic oocytes, and in IVM than in IVV oocytes. The outer ZP of inseminated oocytes appeared stained by PNA and the labeled area increased along with gamete coculture time. This labeling was also observed after insemination of isolated ZP, indicating that this modification in ZP carbohydrates is not induced by cortical reaction. The steady and maintained cortical reaction observed at 4 to 5 h post insemination in IVV monospermic oocytes might reflect the physiological time course of this important event in pigs. Both maturation conditions and boar affect cortical granules release.
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Keywords: Pig oocytes; Cortical reaction; Epididymal sperm; Monospermy

1. Introduction

The release of cortical granule (CG) contents into the perivitelline space, the so-called "cortical reaction" prevents polyspermy by inducing changes at the zona pellucida (zona reaction), perivitelline space and/or oolemma level [1]. The CG are unique oocyte organelles. They migrate to the peripheral oolemma during maturation and once they are released by appropriate stimulus their content is not synthesized again [1,2]. Thus, it could be assumed that an efficient block against polyspermic penetration implies that once the spermatozoon sperm fuses with the oolemma, the oocyte displays a temporary adequate cortical reaction (CR) with a complete release of the CG contents followed by a homogeneous distribution into the perivitelline space.

In pigs, the high incidence of polyspermy is still an unresolved problem (reviewed by [3]), which has been associated with "failures" in the CR, such as i) delayed

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and incomplete CR [4], ii) lack of distribution of released CG contents into the perivitelline space [5], iii) delayed zona reaction [6], iv) undefined differences between *in vitro* (IVM) and *in vivo* (IVV) matured oocytes [5] such as incomplete zona pellucida maturation in the former ones.

In rodents CR has been described as a fast phenomenon; after a spermatozoon fuses with the oolemma it takes 9 min for completion in hamster [7] or 10 to 60 min in mouse [8]. In pigs there is still a lack of data about the physiological basis and timing of the CR. Most studies are conducted by inducing the CR with chemicals [6,9] or assessing the CR only at one or two specific time points after insemination [9–11] rather than in a wide time frame. *In vitro*, an important decrease in CG density is observed about 3 h post insemination (hpi) [11], parallel to sperm penetration [12]. It has been hypothesized that CR in pigs could not be as fast as in other mammals since the majority of *in vitro* matured (IVM) oocytes showed no CR up to 6 hpi [4]. Comparison between the different reports on porcine CR timing is difficult because both IVF medium [10] and IVF conditions [13] affect CG density. The information about CR timing of IVM and *in vivo* (IVV) matured oocytes is controversial. Studies from the same group report that IVM and ovulated oocytes display similar CR pattern [12] but that IVM oocytes have a delayed CR that causes polyspermy [14]. Later studies from the same group and others [5,6] reported that high incidence of polyspermy was not due to a delayed CR but differences in CG contents distribution in the perivitelline space between IVM and IVV oocytes [5,6].

The temporal dependence between sperm penetration and establishment of the zona-mediated block to polyspermy might vary significantly. The specific modifications of the zona pellucida (ZP) induced by CR have not been fully described so far, but it is known that released CG-enzymes induce changes to the ZP carbohydrate, inducing enzymatic removal of glycan ligands [15]. Under physiological conditions the fusion of the penetrating spermatozoon triggers CG release and, despite the boar effect on IVF results being well documented [16,17], its direct role on the CR has, to our knowledge, never been investigated thoroughly. It is also unknown whether sperm cells from different boars would trigger CR timing and patterns differently. Epididymal spermatozoa have some advantages over ejaculated ones as they have never been in contact with seminal plasma and its decapacitation molecules, thus resulting in consistent IVF rates with lower variability [11,18]. Therefore, a study of the time frame of sperm-induced CR in differently matured oocytes after insemination with spermatozoa from different boars would broaden the knowledge about the role of CR in fertilization.

The objective of this work was to study the effect of maturation conditions and boar on oocyte penetrability and CR after insemination with epididymal spermatozoa. Modifications at galactose-β(1-3)-Nacetylgalactosamine residues on ZP were measured by the degree of peanut agglutinin (PNA) binding.

2. Materials and methods

2.1. Experimental design

Two experiments were performed to study the effects of maturation conditions and boar on penetrability and the cortical reaction. In Experiment 1, penetrability data were obtained by doing homologous *in vitro* penetration assays both with immature (germinal vesicle stage) and *in vitro* matured (metaphase II) oocytes. Both kinds of oocytes were inseminated *in vitro* with epididymal spermatozoa from three boars. At 2, 3, 4, 5, 6, 7 and 18 h post insemination (hpi), samples were fixed (15 oocytes per group) and penetrability results assessed. Four and five replicates were done with immature and *in vitro* matured oocytes, respectively. In Experiment 2, *in vitro* and *in vivo* matured oocytes; and zona pellucidae collected from *in vitro* matured oocytes were inseminated *in vitro* with the same boars and IVF conditions as in Experiment 1. After adding spermatozoa, samples (4–6 oocytes per group) were processed at 0.5, 1, 2, 4, 5, 6, 7 and 18 hpi to assess cortical granule density and zona pellucida labeling with fluorescein isothiocyanate-conjugated peanut agglutinin (FITC-PNA). Seven and five replicates were carried out with *in vitro* (IVM) and *in vivo* (IVV) matured oocytes, respectively.

2.2. Culture media

Unless otherwise indicated, all the chemicals used in this study were purchased from Sigma-Aldrich (Munich, Germany).

Oocyte maturation medium was NCSU-37 supplemented with 0.57 mM cysteine, 1 mM dibutyryl cAMP, 5 μg/mL insulin, 50 μM β-mercaptoethanol, 1 mM glutamine, 10 IU/mL eCG (Folligon, Intervet International B.V., Boxmeer, Holland), 10 IU/mL hCG (Chorulon, Intervet International B.V., Boxmeer, Holland), 10 ng/mL EGF and 10% (v/v) porcine follicular fluid. The fertilization medium was modified TALP [19] with

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