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Case Report

Case Study on Role of Zona Pellucida Modifications during Meiotic Maturation in the Sperm-Egg Interactions of Poricne Oocytes

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Abstract

The oocyte maturation includes nuclear maturation and cytoplasmic maturation before fertilization. Moreover, the oocyte nucleus, the cytoplasm and other cell organelles occur changes, zona pellucida (ZP) synchronously undergoes biochemical and structural modifications in the final maturation phase of oocyte prior to fertilization (ZP maturation). It is reported that ZP modifications, such as glycosylation, sialylation, sulfation, glucosaminylation, fucosylation and galactosylation of glycoproteins, have been implicated in various events during fertilization. N-glycosylation of ZP glycoproteins during the meiotic maturation played a critical role in ZP acquiring the capacity to accept sperm in porcine cumulus oocyte complexes (COCs). In addition, the time course of sialylation is correlated with the induction of germinal vesicle breakdown (GVBD) during oocyte maturation. The sialylation of ZP glycoproteins during oocytes maturation is responsible for sperm-ZP interactions. Moreover, Sulfation in ZP glycoproteins during oocyte maturation is critically important in regulating the fundamental steps of sperm-ZP interactions. Although there is a correlation between the cumulus cells during meiotic maturation of porcine oocytes and N-glycosylation of ZP glycoproteins, the cumulus cells are not essential in sialylation and sulfation of ZP glycoproteins that responsible for sperm-ZP interactions.

INTRODUCTION

Although the techniques for IVF have proceeded very rapidly during the past decade, polyspermic penetration still remains a persistent obstacle to porcine IVF systems. Despite of that the embryo production by IVF had been developed successfully in many other species, their developmental potential is very low in pigs [1-5]. The poor developmental competence might be caused by the lack of cytoplasmic maturation in *in vitro* matured porcine oocytes, even though they undergo normal nuclear maturation [3,5-7].

Recently, it would be known that oocyte maturation is mediated by not only nuclear and cytoplasmic maturation but also zona pellucida (ZP) maturation. The ZP, a transparent envelope surrounding the plasma membrane of mammalian oocyte, is a highly glycosylated extracellular matrix. The porcine ZP is composed of three glycoprotein families, ZP1, ZP3α and ZP3β [8,9]. ZP1 is split into two smaller molecules, ZP2 and ZP4, under reducing conditions [10]. The ZP3 families have been shown to comprise approximately 60% of the total glycoprotein content [8]. The ability of sperm to bind ZP has been detected in ZP glycoproteins, and it is generally accepted that this activity is ascribed to the carbohydrate moieties in ZP glycoproteins [11-15]. ZP glycoproteins are considered to have multiple sperm-binding sites, because it has been shown in mice that ZPC glycoprotein binds to the plasma membrane of acrosome-intact sperm and induces the acrosome reaction (AR), while ZPA glycoprotein binds to acrosome-reacted sperm [16].

The early events of mammalian fertilization involve the initial binding of acrosome-intact sperm to ZP glycoproteins. The sperm-ZP binding induces AR, after which the acrosome-reacted sperm binds transiently to ZP before penetrating the zona matrix. Presumably, the sperm penetration through ZP is facilitated by hydrolytic enzymes released from the sperm acrosome.

Following penetration into perivitelline space, the sperm fuses with oolemma and activates the egg, triggering the ZP block to polyspermy. Although these cellular events are well described in many species, their underlying molecular mechanisms are less well understood [17]. In rodents, the initial sperm-ZP recognition is mediated by the binding of sperm surface β1,4-galactosyltransferase (GalTase) to terminal GlcNAc residues on ZP3 [18-20]. Moreover, Huang et al. [21], reported that the initial sperm-ZP recognition was severely affected by fucose. The preincubation of hamster sperm with GlcNAc had an inhibitory effect on the sperm-ZP attachment [22]. The participation of terminal GlcNAc residues on ZP in human sperm function has been studied with results of the sperm-ZP binding and the AR induction [23-25]. In short, GalNAc residues of ZP glycoproteins play pivotal roles in the sperm-ZP interactions during fertilization of the mammalian species [26].

It is generally accepted that the specific interaction between sperm and ZP is a carbohydrate mediated event in different species including human [11,12,27-30]. Due to a critical involvement of carbohydrate in the sperm-ZP interactions, a detailed description of the carbohydrate composition in ZP is necessary.

CASE STUDY

The increase in terminal GlcNAc residues in ZP glycoprotein through new *N*-glycosylation during the first 24 h of meiotic maturation played a critical role in ZP acquiring the capacity to accept sperm in porcine COCs [31]. There is a correlation between the cumulus cells during meiotic maturation of porcine oocytes and *N*-glycosylation of ZP glycoproteins responsible for sperm-ZP interactions [32]. The first 36 h of *N*-glycosylation of GlcNAc residues in ZP during IVM was indispensible for sperm-ZP interactions of porcine denuded oocytes (DOs). Since the longer culture period in the absence of *N*-glycosylation inhibitor after the onset of IVM culture periods was needed to obtain the sperm penetration at the same levels of untreated oocytes in DOs rather than COCs. The cumulus cells are partly involved in ZP glycosylation during oocyte maturation [31].

The time course of the sialylation in ZP glycoproteins is correlated with the induction of germinal vesicle breakdown (GVBD) during oocyte maturation, and the cumulus cells during oocyte maturation are not essential in the sialylation of ZP glycoproteins responsible for sperm-ZP interactions [33].

Moreover, the sulfation of ZP glycoproteins during meiotic maturation plays an important role in sperm-ZP interactions [33]. As indicated by 2D gel electrophoresis, the increase of acidity was consistent with the sulfation of ZP glycoproteins during oocyte maturation, and the ZP acidification was prevented in the oocytes treated with $NaClO_3$. The blocking of ZP sulfation by $NaClO_3$ treatment during IVM in COCs and DOs markedly abolished the incidence of polyspermy with no inhibitory effect on penetration, however the number of sperm bound to ZPs and the rate of AR-inducing sperm were decreased, and the time course of ZP sulfation was related to the induction of GVBD, irrespective of the presence of cumulus cells. These results support the hypothesis that sulfation in ZP glycoproteins during oocyte maturation is critically important in regulating the fundamental steps of sperm-ZP interactions.

DISCUSSION

Porcine IVF is remarkably low normal fertilization rates, resulting from a high rate of polyspermy. This high polyspermic rate that exceeds more than 50% remains to be solved [34-37]. Polyspermic penetration *in vitro* was not due to delayed or incomplete cortical granule exocytosis [37] but more likely to a delayed ZP reaction and/ or simultaneous sperm penetration [37].

The polyspermic block resides either at ZP, or the egg plasma membrane, or both depend on species. Polyspermy is primarily blocked by ZP changes in hamster, goat, ovine, bovine oocytes, by oolema changes in rabbit oocytes and by both in mouse, rat, guinea pig, and cat oocytes [38]. Moreover, proteinases, ovoperoxidase, *N*-acetylglucosaminidase and neuraminidase are thought to bring about changes in the ZP. After sperm-egg fusion, cortical granules (CG) release into perivitelline spaces (cortical reaction), causing the ZP to become refractory to sperm binding and penetration (zona reaction). The CG proteinase exerts the ZP sperm receptor modification and catalyzes the proteolysis of ZP2 as a consequence of a decrease in solubility of the ZP (zona hardening) [39]. Interestingly, according to the findings reported by Velásquez et al. [40], the neuraminidase released from the CG during cortical reaction of bovine oocytes and its neuraminidase would participate in polyspermic block by removing sialic acid from ZP. The desialylation of ZP glycoproteins during oocyte maturation decreased the sperm penetration, sperm binding to ZP and AR induction.

Apparently, GlcNAc residues in ZP were important for the initial steps in fertilization of porcine oocytes. Similar observations on the involvement of zona GlcNAc in the fertilization of hamster and human oocytes have been reported [41-43,24]. As reported by Rath et al. [44], ZP glycoproteins underwent biochemical changes, such as *N*-glycosylation, during final maturation of porcine oocytes. The *N*-glycosylation of ZP glycoproteins is involved in the sperm-ZP interactions in porcine COCs [31]. Similarly, *N*-linked carbohydrates chains of ZP3α play a major role in the sperm binding to ZP [45]. Conversely, the *O*-linked carbohydrate chain of mouse ZP3 is involved in mediating the sperm binding to ZP [46].

Velásquez et al., [40] reported that the α-2,3-linked, but not α-2,6-linked, sialic acids residues in bovine ZP glycoproteins were necessary for the binding between gametes. Thus, it seems that there is a difference between the two species in the sialylated oligosaccharide form associated with sperm-ZP interactions.

These findings strongly suggest that porcine ZP glycoproteins undergo the sialic modifications in the final maturation phase of oocytes prior to fertilization, as reported by Rath et al., [44]. A similar phenomenon is observed in bovine oocytes, and the number of sperm bound to ZPs and the rate of penetration were remarkably decreased in oocytes treated with neuraminidase compared with untreated oocytes [40]. These findings indicate that the sialylation of ZP glycoproteins occurred in accordance with GVBD during oocyte maturation in both COCs and DOs [33]. Rath et al. [44], reported that porcine ZP glycoproteins undergo the acidic modifications elicited by sulfation in the final maturation phase of oocytes prior to fertilization. The

modifications of ZP glycans responsible for sperm AR induction are established by glycosylation, sulfation, sialylation and fucosylation during oocyte growth and maturation [47].

The amount of sulfate is high in the basic structures of *N*-linked oligosaccharides in the porcine ZP, and the sulfated glycans of ZP glycoproteins play important roles in the binding of boar sperm to eggs and penetration by the sperm [48]. It is likely that the blocking sulfation of ZP glycoproteins had a specific influence on the secondary binding of AR sperm to the ZP during the initial stages of fertilization, thus resulting in a decrease in polyspermic fertilization [33].

CONCLUSION

Porcine oocytes undergo ZP modifications during maturation, and *N*-glycosylation, sialylation and sulfation of ZP glycoproteins during meiotic maturation is essential in sperm-ZP interactions. *N*-glycosylation of GlcNAc residues in ZP glycoproteins was indispensable for sperm-ZP interactions, and such *N*-glycosylation occurred during the first 24 and 36 h of IVM of porcine COCs and DOs, respectively. ZP acidifications elicited by sialylation and sulfation of ZP glycoproteins during oocyte maturation contributed to the porcine ZP acquiring the capacity to accept sperm. These ZP acidifications were temporally compatible with the induction of GVBD during oocyte maturation, but did not require for the presence of cumulus cells. The inhibition of *N*-glycosylation and the removal of sialic acid residues in ZP glycoproteins during oocyte maturation dramatically decreased in the incidences of sperm penetration and polyspermy, the number of sperm bound to ZP and percentage of AR-inducing sperm. By contrast, it is very interesting that the blocking sulfation of ZP glycoproteins during oocyte maturation significantly suppressed in polyspermic fertilization with no detrimental effect on sperm penetration and MPN formation.

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