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Spatial and temporal expression of spermadhesin genes in reproductive tracts of male and female pigs and ejaculated sperm

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Abstract

Spermadhesins, a novel protein family identified in the reproductive tract of ungulates, have important roles in reproduction. In this study, the expression of pig (Sus domesticus) spermadhesion genes in seminal vesicles, prostate, and bulbourethral glands from birth to sexual maturity and the spatial expression in adult male and female genital tracts and ejaculated sperm of Meishan pigs were evaluated by reverse transcription-polymerase chain reaction (RT-PCR). In general, all spermadhesin genes increased from Days 1 to 150 in the seminal vesicle and bulbourethral gland. However, their expression in the prostate was variable; it increased from Days 1 to 60 and then declined until Day 150. In adult boars, all genes had a very high level of expression in the seminal vesicle and somewhat lower (but still relatively high) in the prostate, caput and caudal epididymides, and bulbourethral gland. Expression of AQN1 and AQN3 was not detectable in the corpus epididymis. In the testis, AQN3 gene expression was not detectable, and gene expressions were weak for AQN1, PSP-I, and PSP-II, but strong for AWN. In female pigs, most spermadhesins had low expression in the cervix, uterine horn, oviduct, and ovary. Expression of AQN1 and AQN3 was very weak in the cervix and uterine horn. Signals for AQN1 in oviduct and ovary and AQN3 in ovary were not detectable, whereas AWN had high expression in the cervix and uterine horn. In ejaculated sperm, a strong mRNA signal of spermadhesins was detected. We concluded that transcripts of spermadhesins were not only distributed extensively in male and female reproductive tissues but also in ejaculated sperm. Furthermore, their dynamic changes of expression paralleled reproductive development. Seminal vesicles were the main source of spermadhesins; when the boar reached puberty, expression of spermadhesins reached very high levels.

Keywords: Gene expression; Reproductive tract; Sperm; Spermadhesins; Swine

1. Introduction

Seminal plasma, in which mammalian sperm are suspended, is a mixture of secretions from the testis, epididymis, and male accessory sexual glands (seminal vesicle, ampulla, prostate, and bulbourethral gland). Seminal plasma contains many proteins that influence both sperm and the female genital tract during sperm transport [1,2]. In boars, the major protein component of the seminal plasma is the spermadhesin family [3,4]. These proteins have a wide range of ligand-binding abilities, including saccharides, sulfated glycosaminoglycans, phospholipids, and proteinase inhibitors, consistent with diverse roles in events leading to sperm capacitation, formation of the oviductal sperm

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reservoir, gamete recognition, and binding of sperm to the ovum [4,5].

Spermadhesins represented >90% of the total boar seminal plasma proteins [6], and all five members (AQN1, AQN3, AWN, PSP-I, and PSP-II) were identified [7–12]. Spermadhesins AWN and AQN3 interacted directly with membrane phospholipids [13,14], whereas AQN1 formed a heteromer with pB1, the porcine member of the seminal Fn-2 type protein family [15]. Proteins AQN and AWN appeared to stabilize the plasma membrane over the acrosomal vesicle and were mainly released during capacitation [4,13,16]. Spermadhesins PSP-I and PSP-II were present in boar seminal plasma as a heterodimer complex (PSP-I/PSP-II) and contributed to maintaining sperm with high viability, motility, and mitochondrial activity at physiologic temperatures [17] and also may modulate immune responses in the porcine uterus [18,19].

In previous studies, the origin and localization of spermadhesins were revealed by reverse transcription-polymerase chain reaction (RT-PCR) and immunologic approaches [20]. The mRNA signals of all spermadhesins were detected in the seminal vesicle, prostate, and cauda epididymis by RT-PCR. However, only the transcript of PSP-I was detectable in the caput epididymis and rete testes, but not in all secretory tissues of boar reproductive organs. Furthermore, additional mRNA and protein signals of AWN were found in female reproductive tracts. Using RT-PCR and Western blotting, more extensive distribution of PSP-I and PSP-II was detected in reproductive organs of mature boars, including the testis, ductus epididymides (caput, corpus, and cauda), seminal vesicle, and bulbourethral gland [21].

Spermadhesin proteins have also been detected in ejaculated sperm by immunohistochemistry [22,23]. It was believed that the spermadhesin proteins on ejaculated sperm were absorbed from seminal plasma during ejaculation; however, they also could be translated from spermadhesin mRNA present on sperm.

The onset of spermatogensis and puberty in Meishan pigs occurs at much earlier stage compared with that in Western pigs [24]. Therefore, Meishan pigs offer a unique model for characterizing factors influencing male and female reproductive function. The current study included a detailed investigation of mRNA expression of all spermadhesins in ejaculated sperm and in the reproductive tracts of adult Meishan male and female pigs. Moreover, because gene expression during postnatal development in the boar has not been reported, the time course of gene expression (for all spermadhesin genes) in the seminal vesicle, prostate, and bulbourethral gland was assessed on Days 1, 30, 60, 90, and 150.

2. Materials and methods

2.1. Animal husbandry and tissue collection

Eighteen newborn Meishan male and three female piglets (Sus domesticus) were selected from five multiparity dams. The piglets were housed in the farrowing pen and weaned at 28 d and fed a standard pelleted ration. They were moved to a finishing pen at 70 d and raised to slaughter age. The protocol for animal use was approved in accordance with the University Council on Animal Care guidelines.

Seminal vesicle, prostate, bulbourethral gland, epididymides (caput, corpus, and cauda), and testis were collected from boars on Days 1 (n = 5), 30 (n = 4), 60 (n = 3), 90 (n = 3), and 150 (n = 3). In gilts, cervix, uterine horn, oviduct, and ovary were collected on Day 150 (n = 3). Pieces (0.5 cm) were dissected from all tissue samples, snap-frozen in liquid nitrogen, and stored at –80 °C pending RNA isolation. Ejaculates from Meishan boar (n = 3) were collected and centrifuged for 20 min at 300 × *g* to separate sperm and seminal plasma. The sperm pellet was washed three times with PBS, collected with centrifugation for 10 min at 300 × *g*, and stored at –80 °C pending RNA isolation.

2.2. RNA isolation

Total RNA was extracted from samples with a TRI Regent Kit (Molecular Research Center, Inc, Cincinnati, OH, USA) by following the manufacturer's instructions. Briefly, 50 mg frozen tissue sample was ground into powder on dry ice and homogenized with 1 mL Trizol reagent, whereas sperm samples were directly homogenized with 1 mL Trizol. Homogenates were treated for 8 min at room temperature to allow complete dissociation of nucleoprotein complexes. Then 0.2 mL chloroform was added and centrifuged for 20 min at 4 °C, and the aqueous phase was recovered and precipitated with isopropanol. The RNA pellets were washed with 75% ethanol, air-dried, and eluted with 20 μL RNase-free water.

2.3. RT- PCR and quantification

Total RNA was treated with DNase I for 15 min at room temperature and incubated at 70 °C for 5 min before first-strand cDNA synthesis. The concentration