



Porcine model for the study of sperm capacitation, fertilization and male fertility

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Received: 27 August 2019 / Accepted: 28 January 2020 / Published online: 5 March 2020
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Abstract

Mammalian fertilization remains a poorly understood event with the vast majority of studies done in the mouse model. The purpose of this review is to revise the current knowledge about semen deposition, sperm transport, sperm capacitation, gamete interactions and early embryonic development with a focus on the porcine model as a relevant, alternative model organism to humans. The review provides a thorough overview of post-ejaculation events inside the sow's reproductive tract including comparisons with humans and implications for human fertilization and assisted reproductive therapy (ART). Porcine methodology for sperm handling, preservation, in vitro capacitation, oocyte in vitro maturation, in vitro fertilization and intra-cytoplasmic sperm injection that are routinely used in pig research laboratories can be successfully translated into ART to treat human infertility. Last, but not least, new knowledge about mitochondrial inheritance in the pig can provide an insight into human mitochondrial diseases and new knowledge on polyspermy defense mechanisms could contribute to the development of new male contraceptives.

Keywords Pig · Male fertility · Capacitation · Zona pellucida · Fertilization

Introduction – domestic pig as a biomedical model

The use of domestic pigs as a model animal for medicine/surgery dates all the way back to ancient Greek physician-philosopher Galen (Zuidema and Sutovsky 2019; this issue of CTR). Physiologically and genetically, the domestic pig bridges the gap between laboratory rodents and humans. At the gamete level, pig spermatozoa are similar to human (e.g.,

centrosomal contribution) as well as zygotic and pre-embryo development (timing of paternal mitophagy and major zygotic genome activation (MZGA), sperm-zona interactions in terms of sustained sperm binding and anti-polyspermy defense) when compared to the rodent model (Fig. 1). Boar ejaculates are plentiful and physiologically relevant as spermatozoa have full contact with seminal plasma at the time of semen collection and are often collected naturally using the gloved hand technique, without the use of electroejaculation or surgical removal from epididymides as often done in rodents (Geisert et al. 2019).

Domestic boars are conducive to studies that require a large number of spermatozoa or seminal plasma as they produce high volume ejaculates (up to 500 ml) in three distinct fractions (pre-sperm, sperm-rich and post-sperm rich, with the gel fraction intermittent through the ejaculation process). The boar ejaculate is rich in seminal plasma produced mostly by large vesicular (major portion) and bulbourethral (gelling portion) glands with a small contribution by the prostate. Boar ejaculates are easy to process for artificial insemination (AI), intrauterine insemination (IUI), in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Additionally, embryo transfer technology is already well developed and semen

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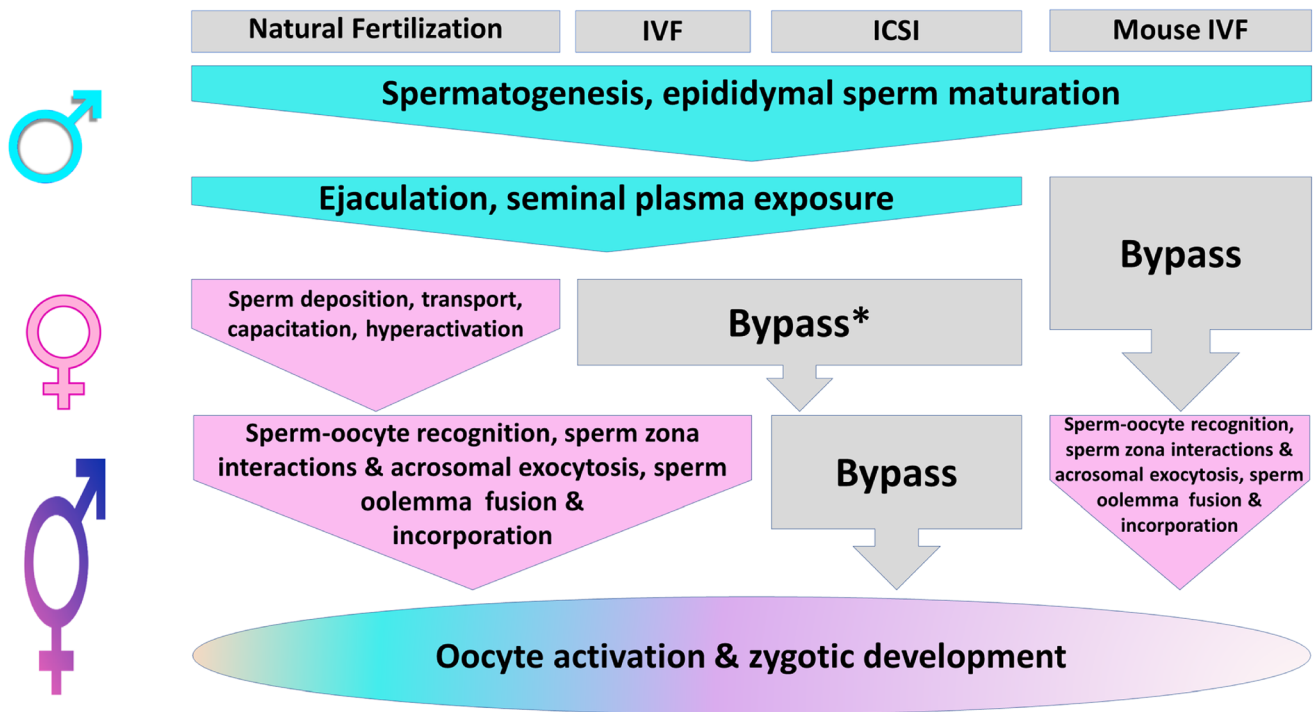
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The Male Side of Fertilization Cascade



* *Capacitation & hyperactivation may occur to some extent in the IVF medium*

Fig. 1 Comparative flowchart of major fertilization events during natural and assisted (IVF, ICSI) fertilization in the domestic pig, humans and rodents, represented by the mouse. The similarities between porcine and human fertilization are contrasted with the mouse as a most common animal model extrapolated to humans, without the intent to distract from the significance and impact of rodent models. One major difference between humans and pigs on one side and rodents on the other is the lack of physiological sperm exposure to seminal plasma in rodent studies where

semen collection is difficult and epididymal spermatozoa never exposed to seminal plasma are used to study sperm capacitation and gamete interactions in vitro, which do involve the seminal plasma originated sperm surface proteins during rodent gamete interactions in situ. Also obvious is the difference between ICSI vs. IVF and ICSI vs. natural fertilization, wherein multiple steps of gamete transport and gamete interactions are bypassed by direct microinjection of a single spermatozoon in the oocyte cytoplasm

freezing and sexing technologies are feasible and likely to improve in the future. Significant for biomarker discovery and validation, data from single sire AI are available in boars that can be correlated with the expression of sperm quality/fertility biomarkers. On the female side, the acquisition of oocytes is relatively easy in pigs as compared to other mammalian model species. The harvesting of gilts for meat is a standard procedure, resulting in an excess of ovaries that are typically discarded in industry settings. This provides an opportunity for researchers to gain access to many ovaries from which oocytes may be extracted. While gilts are often not cycling at the time of slaughter, hormones in oocyte maturation media help subside this issue or cycling sow ovaries can be requested from sow-specific slaughter times (Yuan et al. 2017). As pigs are a litter bearing species, each ovary contains many follicles from which oocytes can be aspirated. This allows researchers to conduct large-scale IVF studies with

markedly less hassle than other mammalian models provide. It also removes the ethical dilemmas and costs associated with using human or primate gametes outright.

The present review takes inventory of current research using the domestic pig as a biomedical model for male fertility research, focusing on the early events of the reproductive process starting with semen deposition and transport in female reproductive, through capacitation and fertilization and concluding with thoughts on early zygotic development and peculiarities of assisted fertilization.

Semen deposition and transport

In pigs, semen is deposited directly into the uterus of a sow (Fig. 2a) from where it is transported through the isthmus to ampulla through the aid of uterine peristaltic contractions (Fig.

2b, b'). Sows ovulate once every 21 days and natural mating is only allowed when a sow is in estrus (commonly referred to as standing heat). The average duration of estrus in weaned sows expressing estrus on day 4 has been reported to be 54 ± 15 h, with ovulation occurring 54–86% hours after initial onset (reported in Soede et al. 1994; recently reviewed in Knox 2015). The mechanism guaranteeing that spermatozoa stay viable with fertilization potential in the female reproductive tract by the time of ovulation is crucial since mating can occur up to 2 days prior to ovulation. Ejaculated spermatozoa traversing the oviduct are decelerated by the mucoidal environment and captured by cilia epithelial cells in the isthmic region (Fig. 2c, c'), forming a functional oviductal epithelium sperm reservoir (SR) (Suarez et al. 1991). The effects of oviductal secretion on the reproductive process is thought to be a result of the dynamically balanced combined action (inhibitory and stimulatory) of multiple factors present in the oviductal lumen at different stages of the ovulatory cycle and in the presence of gametes or embryos; see for review Ghersevich et al. (2015). Changes in the distribution of intraluminal mucus in the porcine oviductal reservoir during estrus were studied in Johansson et al. (2000). In most mammalian species, contact of spermatozoa with the oviduct is mediated by sperm oviduct-binding proteins with affinity to the apical surface of the oviduct lining epithelial cells (Suarez 1987). Binding of spermatozoa to the oviduct is carbohydrate-mediated and therefore, the binding molecule should have the ability to bind to the sperm surface while having the ability to interact with carbohydrates (Green et al. 2001). Proteins in the apical region of the plasma membrane of the sperm head bind to the oviduct and increase in vitro sperm survival (Fazeli et al. 2003). By

being preserved within the SR, surface-bound spermatozoa are able to maintain their motility much better than those that float freely in the oviductal fluid in both pig and bovine (Fazeli et al. 1999; Gualtieri and Talevi 2000). The SR may also serve a sperm selection function by releasing waves of the most motile, functionally and morphologically intact spermatozoa, ensuring the selection of the best quality spermatozoa and thus lowering the probability of polyspermic fertilization while prolonging sperm lifespan before oocyte ovulation. The SR is also the place where sperm capacitation and hyperactivation occurs (Fig. 2d, d'), which is a prerequisite for sperm detachment from it (Suarez 1998). Although SR is assumed to exist in the human oviduct, there is, for obvious reason, limited knowledge of how it is established and regulated. Due to its reproductive organ size and reproductive mechanism similarities to humans, domestic pig offers a useful animal model to address such essential questions.

In pigs, the sperm-oviduct interactions are based on the high affinity of spermatozoa to oligomannose-containing binding sites, terminal mannosyl and galactosyl residues and hybrid N-glycan types (Green et al. 2001; Wagner et al. 2002). The mannose-binding sites are localized in the apical region of the sperm head and are lost during capacitation (Ekhlesi-Hundrieser et al. 2005). The main oviduct-binding protein on the boar sperm head, AQN1, has been described (Ekhlesi-Hundrieser et al. 2005). This spermadhesin, originating from seminal plasma, closely binds to the sperm surface (Dostalova et al. 1994; Sanz et al. 1992). AQN1 showed a broad carbohydrate-binding pattern as it recognizes galactose as well as mannose structures. AQN1 inhibits in vitro sperm binding to fallopian explants depending on its concentration

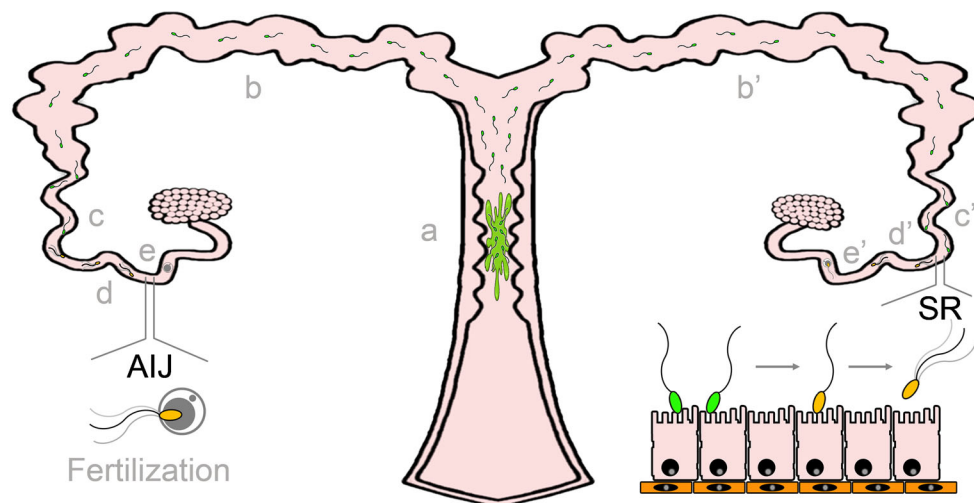


Fig. 2 Gamete transport in sow reproductive tract. Ejaculation to fertilization: the path to fertilization in the pig. (a) Spermatozoa are activated by seminal plasma factors upon ejaculation in the cervix. (b, b') Spermatozoa pass through the uterine horns, aided by peristaltic muscle contractions. (c, c') Upon passing through the uterotubal junction (UTJ), spermatozoa reach the oviductal sperm

reservoir (SR) where some spermatozoa are capable of binding glycans and remain until the time of ovulation. (d, d') Hyperactivated spermatozoa released from the SR in response to ovulatory cues undergo sperm capacitation and head toward the ampulla-isthmus junction (AIJ) where ovulated M2 oocytes are prepared for fertilization (e, e')

(Ekhlasi-Hundrieser et al. 2005). DQH/pB1/BSP1 is another protein on the boar sperm head that binds to mannose (Jelinkova et al. 2004). This surface protein localizes to the apical part of the ejaculated sperm head where it can mediate sperm binding to the SR (Manaskova et al. 2007). A recent study demonstrated that porcine spermatozoa recognize carbohydrate structures containing Lewis X motifs with high affinity (Silva et al. 2017). Lewis X-containing glycans are considered to be among the most important receptors for sperm-oviduct binding in pigs. It is a trisaccharide antigen that interacts with glycolipids. Lewis X glycans were found in the porcine isthmus (Machado et al. 2014). The same group tested 377 glycans, where spermatozoa showed a high affinity to Lewis X trisaccharide and biantennary structures containing a mannose core with 6-sialylated lactosamine (Kadirvel et al. 2012). Later, ADAM5 and MFGE8 (also referred to as lactadherin, P47, and SED1) were identified as proteins on the sperm head binding sulfated Lewis X (Silva et al. 2017).

Additional studies aimed at finding spermatozoa-binding proteins in the porcine oviduct focused on the unique sperm-oviduct binding protein in pigs; a sperm-binding glycoprotein (SBG), also known as Deleted in Malignant Brain Tumor 1 (DMBT1), isolated from oviductal epithelial cells containing an O-linked Gal 1–3 GalNAc disaccharide (Marini and Cabada 2003). Boar spermadhesin AQN1 recognizes galactose in this disaccharide. Evidence that SBG/DMBT1 is a sperm-binding partner of AQN1 was presented when SBG/DMBT1 was localized in portions of the oviductal tube where sperm clusters have been detected (Talevi and Gualtieri 2010; Teijeiro et al. 2008). A recent study demonstrated that the main scavenger receptor cysteine-rich (SRCR) domain in DMBT1 promoted sperm binding to form the SR in the oviduct and this function is probably mediated by the polypeptide itself (Roldan et al. 2018). Additionally, annexins were isolated from porcine oviductal cells based on their affinity for sperm membrane proteins. One of the oviductal annexins reported is annexin A2 (ANXA2), localized on the apical surface of oviductal epithelial cells. It is the major candidate as a receptor for boar spermatozoa to form the SR, most likely through the interaction with AQN1 spermadhesin (Marini and Cabada 2003; Teijeiro et al. 2009). ANXA2 may exist in a bound form with S100 calcium-binding protein A10 (S100A10) as well as separately. However, this binding distinctly differs from the biological function of ANXA2 (Teijeiro et al. 2016). It was found that ANXA2 is bound to S100A10 in oviducts of pigs and cows, as well as mice, humans, cats, dogs and rabbits. In sows, it localizes in the outer layer of the apical plasma membrane of oviductal epithelial cells (Teijeiro et al. 2016). At least one other protein on the apical membrane of oviductal cells that maintains the fertilizing ability was reported. This was shown to be heat shock 70 kDa protein 8 (HSPA8), which mediates sperm-oviduct binding (Elliott et al. 2009). The ensuing biological activity of this protein is most likely responsible to prolong and maintain sperm

viability in the oviduct (Fazeli et al. 2003). Unlike pigs, humans deposit spermatozoa in the anterior vagina near the cervical opening, as the anatomy of both male and female genitalia differs from pigs; however, transport of semen to the site of fertilization is similar in these two species. For an extensive comparison of gamete transport, we recommend the review by Suarez (2015). The existence of the SR in humans was indicated *in vitro* (Kervancioglu et al. 1994; Murray and Smith 1997) and the data are not conclusive to postulate a unified model for sperm transport and storage in humans (Williams et al. 1993). Suarez (2015) suggested that fertilization in humans is a relatively inefficient and an unregulated process as coitus took on an additional role of promoting long-term couple bonding.

Spermatozoa are released from the SR during sperm capacitation (Suarez 1998) (Fig. 2d, d'), timed to coincide with ovulation and controlled by endocrine signals originating from the ovulating follicle(s) and ovulation products (oocyte cumulus complexes) of the ipsilateral ovary (Hunter 1996; Hunter and Rodriguez-Martinez 2004). Two theories have been considered regarding sperm release from SR: (i) by the capacitation-induced removal of proteins from the sperm surface that terminates sperm binding to oviduct cells (Topfer-Petersen et al. 2008) and/or (ii) by cleavage of carbohydrate residues on the epithelial surface of the oviduct through glycolytic enzymes present in the oviductal fluid after ovulation (Carrasco et al. 2008). A contributing force for sperm release is the increased frequency and amplitude of sperm flagellar movement brought about by sperm hyperactivation (Suarez 2008, 2016). Capacitated, hyperactivated spermatozoa are then translocated to the site of the fertilization, the ampulla (Fig. 2e, e'). The human cervix has been hypothesized to be the SR with no robust evidence to support it. Furthermore, human spermatozoa may not form a distinct SR in the oviduct (Williams et al. 1993) and spermatozoa seem to be stored for longer periods of time purely by their deceleration by (i) obstacles formed by increasing oviductal lumen complexity toward the ovary, (ii) oviductal mucus (Jansen 1980) and (iii) spermatozoa adhering with low affinity to the oviductal epithelium (Pacey et al. 1995a, b). To our knowledge, there is no conclusive research to indicate what happens to human spermatozoa at the time of ovulation.

Seminal plasma and acquisition of sperm surface proteins involved in gamete transport and fertilization

The cell-free portion of ejaculate, human and animal seminal plasma is a complex mixture of secretions originating mainly from the epididymis and accessory sex glands, which provides a supportive environment for ejaculated spermatozoa (Calvete et al. 1997). Reflective of a widespread misunderstanding of seminal plasma is the belief that it is a relatively homogenous

fluid, like that of blood plasma, a fluid with well-regulated homeostasis. Contrarily, seminal plasma is anything but this, thus referred to as seminal fluids by some (Bjorndahl and Kvist 2003). The basic function of SP is to modulate the post-testicular maturation process that includes an attachment/rearrangement of the sperm surface proteins/glycoproteins secreted throughout the male genital tract. Sperm-binding secretory proteins have been shown to contribute to the formation of an oviductal sperm reservoir in the pig (Petrunikina et al. 2001) as well as controlling sperm maturation by inhibiting capacitation (Vadnais and Althouse 2011; Vadnais et al. 2005; Vadnais and Roberts 2007). The SP contains decapacitation factors that prevent premature acrosomal exocytosis and the proteins that bind to the sperm surface increase fertilization potential (Centurion et al. 2003). Inactivation and/or removal of these factors may affect in vivo capacitation (Calvete et al. 1997); they are also necessary for events leading to successful fertilization such as sperm-zona pellucida interactions and sperm-oocyte fusion, as reviewed in Rodriguez-Martinez et al. (2009). Additionally, SP has been shown to modulate the immune response in the uteri of pigs, humans and other mammals (Kelly and Critchley 1997; O’Leary et al. 2004; Rodriguez-Martinez et al. 2010; Rozeboom et al. 1999; Schubert et al. 2008; Veselsky et al. 2000) by modifying gene expression affecting local processes of immune defense at the oviductal sperm reservoir (Alvarez-Rodriguez et al. 2019; Sharkey et al. 2012); also reviewed in Schjenken and Robertson (2014). Properties of seminal plasma such as the ability to maintain uncapacitated sperm state and to immune-suppress the female reproductive tract are widely exploited in pig semen handling/processing, storage/extension/preservation and AI (Rodriguez-Martinez et al. 2009; Rodríguez-Martínez and Peña Vega 2013). Such know-how could be translated into human reproductive medicine, to benefit intrauterine insemination, IVF and sperm prepping for intracytoplasmic sperm injection (ICSI).

Extensive proteomic studies of SP proteins with interspecies comparisons have been performed (De Lazari et al. 2019; Druart et al. 2013; Gonzalez-Cadavid et al. 2014; Perez-Patino et al. 2016a; Perez-Patino et al. 2016b). Identified proteins range from various hormones, enzymes, proteinase inhibitors and growth factors to proteins and glycoproteins with various function. Furthermore, the effects of SP composition on sperm fertilization capacity varies depending on the fertility of individual animals (De Lazari et al. 2019; Gonzalez-Cadavid et al. 2014). The most extensive proteomic study to date (Perez-Patino et al. 2016b) identified 536 proteins in boar SP, 409 of them annotated in *S. scrofa* taxonomy, with only 20 specifically implicated in reproductive processes. The nature of involvement of the majority of SP proteins in reproduction thus remains unclear, in animals and in humans. An electrophoretic profile of boar seminal plasma revealed the predominance of

proteins (85.3%) with MW below 25 kDa with a high predominance of fibronectin and spermadhesins (AQN1, AQN3, AWN, PSPI and PSPII) (Druart et al. 2013). This is in accordance with another study (Gonzalez-Cadavid et al. 2014) where spermadhesins represented at least 45.28% of the total intensity of all spots. Only a limited number of studies have focused on the human seminal plasma proteome, with a total of 2064 non-redundant proteins identified. For a thorough review of the human seminal plasma proteome, we recommend the following reviews Gilany et al. (2015) and Jodar et al. (2017). Alterations of semen proteome including sperm and seminal plasma proteins from asthenozoospermic, oligozoospermic and teratozoospermic patients were noted, compared to normozoospermic individuals (Jodar et al. 2017), which could be targeted for the discovery of sub-/in-fertility biomarkers (Bieniek et al. 2016; Drabovich et al. 2014).

Many SP proteins have been studied extensively and their function established. Spermadhesins, a group of glycoproteins of 12–16 kDa, predominate in boar SP. Spermadhesins, as well as protein containing fibronectin type II (Fn2) domains, DQH sperm surface protein/binder of sperm 1 (BSP1), are adhesive proteins that bind to the surface of boar sperm during ejaculation. All spermadhesins with BSP1 and their structures, biochemical features and binding properties were characterized and are reviewed in detail (Jonakova et al. 2007; 2010; Jonakova and Ticha 2004; Topfer-Petersen et al. 1998). Posttranslational modifications, such as glycosylation, determine the variety of functional properties of boar spermadhesins (Calvete et al. 1995). Collectively, AQN1, AQN3 and AWN are heparin-binding proteins that form the base sperm-coating layer (mostly AWN and AQN3) covering predominantly the acrosomal region of the sperm head (Fig. 2) to which other spermadhesins aggregate thus forming outer layers. Their function is to stabilize the membrane covering the acrosome and to participate in the formation of the oviductal sperm reservoir (mainly AQN1) (Ekhlesi-Hundrieser et al. 2005; Liberda et al. 2006). Most AQN and AWN spermadhesins adsorbed onto ejaculated spermatozoa are released from the sperm surface during capacitation (Fig. 3), indicating that a large subpopulation of each boar spermadhesin is loosely associated to the sperm surface and functions as decapacitation factors (Dostalova et al. 1994). This removal event is essential for detachment of spermatozoa from the oviductal epithelium. A sperm-oocyte binding test and other experimental data demonstrated that intact AQN1, AWN and DQH proteins on the sperm surface are required for the primary binding of spermatozoa to the zona pellucida (ZP) (Dostalova et al. 1995; Ensslin et al. 1995; Manaskova and Jonakova 2008; Manaskova et al. 2000; Rodriguez-Martinez et al. 1998; Veselsky et al. 1992, 1999). PSP-I and II are the major SP proteins (more than 50% of all proteins), forming heparin-non-binding heterodimers of glycosylated spermadhesins (Calvete et al. 1995; Manaskova et al. 2000),

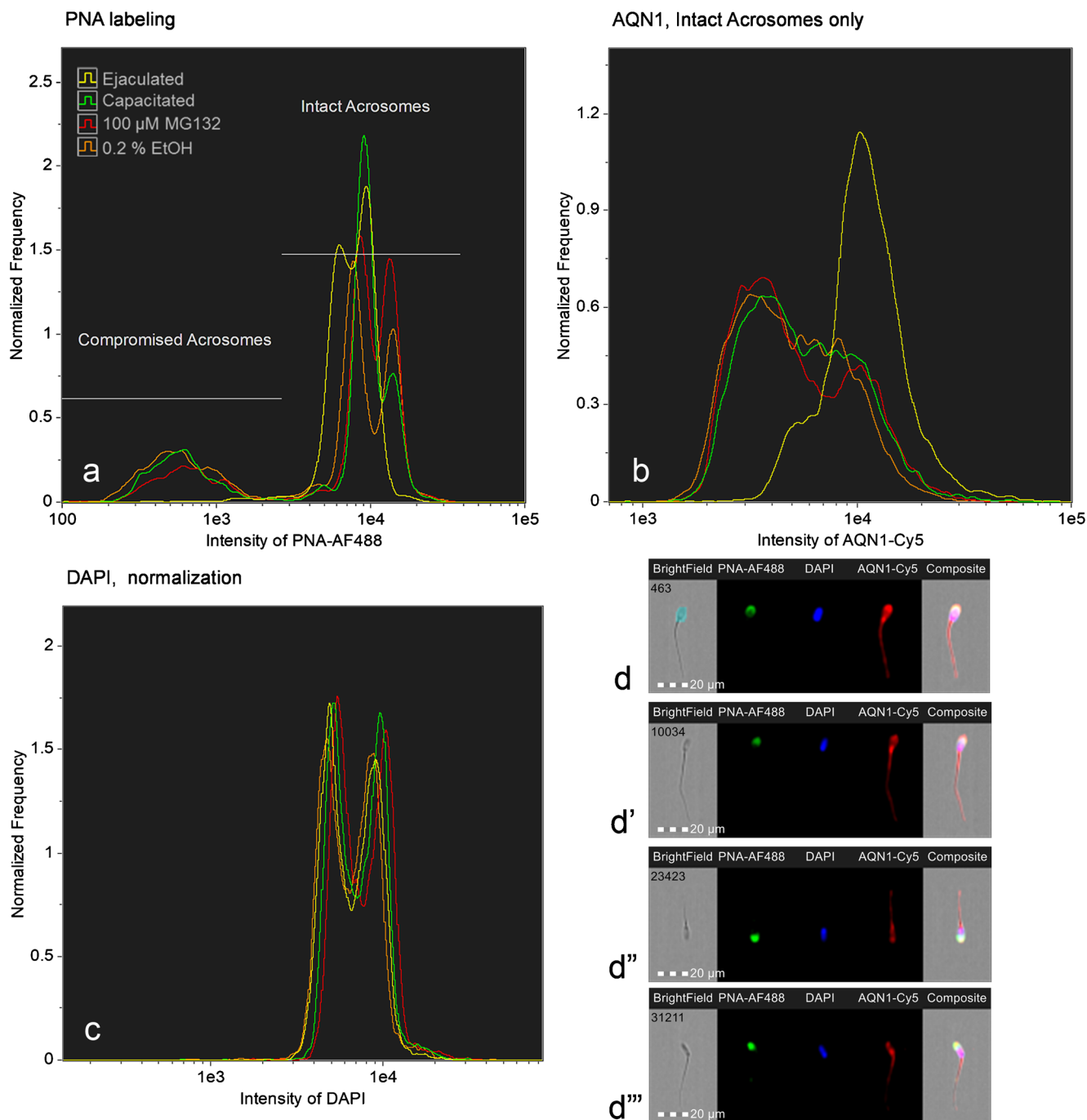


Fig. 3 Image-based flow cytometric measurements of spermadhesin AQN1 shedding during sperm in vitro capacitation (IVC) under proteasome permissive/inhibiting conditions (100 μ M MG132) and vehicle control (0.2% EtOH), marrying fluorometry with epifluorescence imaging of AQN1 localization in the ejaculated and capacitated spermatozoa. Spermatozoa were fixed with acetone and labeled with green fluorescent peanut agglutinin (PNA) lectin to monitor acrosomal integrity (a), red fluorescent rabbit polyclonal anti-AQN1 antibody (Jonakova et al. 1998) to monitor AQN1 shedding from the sperm during IVC (b) and blue fluorescent DNA stain 4',6-diamidino-2-phenylindole (DAPI) applied for normalization purpose (c). Representative images of ejaculated (d), capacitated–non-inhibited (d'), as well as proteasomally inhibited (d''),

spermatozoa including vehicle control (d''') are presented below fluorescence histograms. A mask is shown in the brightfield image of the ejaculated spermatozoon (d) that was utilized to calculate fluorescence intensities of AQN1. From epifluorescence images, one can see the shedding of AQN1 from the acrosomal region, which participates in the formation of the oviductal epithelium sperm reservoir (prior to capacitation) and ZP interaction after capacitation, also represented by the lower intensity peak in histogram b. Proteasomal inhibition had no effect on this shedding event as we reported previously (Zigo et al. 2019a). AQN1 is also localized to the connecting piece and flagellum, however, functions of AQN1 in these regions remains to be elucidated. Every flow cytometric run represents 10,000 DAPI-defined sperm events

the pro-inflammatory and immune-stimulatory activity of which is believed to modulate immune response in the uterus (O’Leary et al. 2004; Rodriguez-Martinez et al. 2010; Rozeboom et al. 1999). Furthermore, it has been reported that the addition of spermadhesins PSP-I/PSP-II to sperm medium results in an incremental, concentration-dependent increase of sperm viability/longevity, implying potential use for sperm preservation in reproductive technology (Centurion et al. 2003). Similar to pigs, ten most abundant human SP proteins represent > 80% of human SP proteome (Drabovich et al. 2014), including semenogelins I and II (accounting for up to 30% of total SP proteome), fibronectin, kallikrein-like protease, lactoferrin, laminin and serum albumin (Pilch and Mann 2006). The BSP1 homolog (BSPH1) was described in humans (Plante et al. 2014), solely expressed in epididymal tissue; it shares many biochemical and functional features with angulates’ BSPs secreted by seminal vesicles.

Under physiological conditions, boar seminal plasma proteins form variable aggregates (homo- and hetero-oligomers), differing in relative molecular mass, ratio of individual spermadhesins and DQH/BSP1 (most abundant proteins of boar SP) and in their interactive properties (Calvete et al. 1997; Jelinkova et al. 2003; Jonakova et al. 2000; Manaskova et al. 2000, 2003). Such aggregates are formed and bound to the sperm surface during ejaculation. The interaction of aggregated forms with polysaccharides of glycosaminoglycans of oviductal epithelial cells occurs leading up to sperm capacitation. The aggregates of DQH, AQN and AWN proteins interact with cholesterol and may be important acceptors of cholesterol released from the spermatozoa’s membrane during capacitation. It is apparent that SP interactions with spermatozoa could be beneficial in the short term for normal maintenance of sperm viability after ejaculation/semen deposition (decapacitating factors) but detrimental in the long-term condition of semen preservation (cholesterol extraction from sperm plasma membrane). The PSP spermadhesins are present in boar seminal plasma as a heterodimer complex (PSP I/PSP II). Very little is known about the fate of spermadhesins after sperm capacitation. We know that AQN1 with adhered SPINK2 (Davidova et al. 2009, Jonakova et al. 1992) is ubiquitinated. Furthermore, AQN1 and SPINK2 interact with ubiquitin C terminal hydrolase UCHL3 and with the PSMD8 and PSMD4 subunits of the 19S regulatory complex of sperm proteasome. This suggests that the activity and turnover of these seminal plasma proteins may be controlled by the ubiquitin-proteasome system (UPS) (Yi et al. 2007, 2010a, b). Recently, we demonstrated that UPS is involved in seminal plasma protein de-aggregation during *in vitro* capacitation by targeting and degrading DQH/BSP1, which is the major component of high-molecular aggregates (Zigo et al. 2019a). It is also known that proteasomes in both the human and boar spermatozoa become activated/phosphorylated during sperm capacitation (Morales et al. 2007; Zigo et al. 2018).

Sperm capacitation

Although spermatozoa acquire the potential to fertilize an oocyte within the epididymides, the expression of this functional competence is suppressed until after ejaculation and sperm detachment from the oviductal sperm reservoir. Spermatozoa must first spend a period of time within the female reproductive tract before acquiring the competency to fertilize, a process that is collectively termed capacitation (Austin 1951; Chang 1951), during which they undergo a series of biochemical and biophysical changes. These changes include (i) surface properties, such as peripheral membrane protein desorption, integral plasma membrane redistribution; (ii) plasma membrane properties, such as lipid composition and transmembrane phospholipid asymmetry, lateral diffusion of phospholipids, loss of cholesterol and reorganization of detergent-resistant domains; (iii) accelerated metabolism; (iv) internal pH and cytosolic activities of calcium and other ions; (v) a strong hyperpolarization of membrane potential; (vi) altered cyclic nucleotide metabolism; and (viii) protein phosphorylation through regulation of both protein kinases and phosphatases (Florman and Fissore 2015). These events take place independently, in a compartmentalized manner in both the sperm head and flagellum. Capacitated spermatozoa express at least three of the following features: (i) hyperactivated motility of the flagellum, (ii) signal transduction regulation allowing spermatozoa to respond to chemoattractant and (iii) the ability to interact with an oocyte and undergo acrosomal exocytosis.

The purpose of this section is to focus on the aspects of capacitation that were described in pigs rather than to give an in-detail review of sperm capacitation. For a comprehensive review of sperm capacitation in mammals including mice, pigs, bulls, rams, stallions and humans, we recommend the following reviews; Aitken and Nixon (2013), Bailey (2010), Buffone et al. (2014), Florman and Fissore (2015) Gadella and Boerke (2016), Gangwar and Atreja (2015), Gervasi and Visconti (2016) Harayama (2018), Ickowicz et al. (2012), Leemans et al. (2019), Puga Molina et al. (2018), Santi et al. (2013), Visconti and Florman (2010) and Visconti et al. (2011).

Capacitation is linked with the functional reprogramming of spermatozoa within the female reproductive tract over a period of at least 3–4 h (Hunter and Rodriguez-Martinez 2004). However, spermatozoa may begin to capacitate as soon as they are mixed with seminal plasma containing HCO_3^- by direct stimulation of soluble adenylyl cyclase ADCY 10 (a.k.a. sAC or SACY) (Okamura et al. 1985). Capacitation is generally believed to initiate with cholesterol efflux from the plasma membrane (PM) (Davis 1981) and the loss of decapacitation factors from the PM surface (Fraser 1984). However, the literature is ambiguous whether these events happen concomitantly or one is a consequence of the other. It was shown that the addition of de-capacitating factors can

partially reverse capacitation and render spermatozoa incapable of recognizing and fertilizing an oocyte, at least in mice (Fraser et al. 1990). These factors originate in the epididymides and accessory sex glands and their removal from non-capacitated spermatozoa results in a rapid increase in their fertilizing ability (Fraser 1984). In pigs, discussed above, these were found to be spermadhesins, reviewed in Jonakova et al. (2010) and binder of sperm proteins (BSPs), reviewed in Plante et al. (2016), while in humans, these are considered to be semenogelins and their degradation products, including seminal plasma motility inhibitor (Yamasaki et al. 2017) and intrinsic platelet-activating factor acetylhydrolase (Zhu et al. 2006). Another powerful de-capacitation factor in both pig and human semen is cholesterol (Cross 1998; Davis 1981). The ubiquitin-proteasome system plays a key role in sperm capacitation (for review see Kerns et al. 2016). Relatedly, we recently reported that the UPS plays a role in spermadhesins and DQH/BSP1 de-aggregation during boar sperm capacitation as an important step of the detachment of spermatozoa from the oviductal epithelium (Zigo et al. 2019a) as well as other proteins' compartmentalization such as lactadherin MFGE8, disintegrin ADAM5 and acrosomal matrix protein ACRBP (Zigo et al. 2019b). Cholesterol efflux from the plasma membrane has also been correlated with an influx of bicarbonate ions, the activation of ADC 10 and a rise in intracellular Ca^{2+} into the spermatozoon (Flesch and Gadella 2000; Gadella et al. 2008). Besides its key role in the initiation of critical signal transduction cascades, the bicarbonate ion plays a direct role in sperm surface remodeling via stimulation of phospholipid scramblase activity (Gadella and Harrison 2000, 2002). These functional membrane changes allow for lipid raft reorganization at the apical ridge regions of sperm head (Boerke et al. 2008; van Gestel et al. 2005) that were found to possess ZP-binding complexes (van Gestel et al. 2007). The same group showed a redistribution of phospholipids to play a role in the formation of SNARE complexes that allow for close apposition and docking of the PM and outer acrosomal membrane (OAM), important for acrosomal exocytosis (Tsai et al. 2010, 2012).

Hyperactivated motility is a consequence of capacitation, enabling spermatozoa to detach from the oviductal epithelium, migrate through the viscous lumen of the oviduct and penetrate through the cumulus cell layer and ZP. Quiescent epididymal spermatozoa upon contact with seminal plasma start expressing symmetrical, low amplitude flagellar beating also known as “pro-hook” or “non-full” type hyperactivation. During capacitation, they start to express asymmetrical, high-amplitude beating also known as “anti-hook” or “full type” hyperactivation (Chang and Suarez 2011; Harayama et al. 2012). The onset of sperm hyperactivation is associated with an influx of Ca^{2+} ions into the sperm tail cytosol (Suarez et al. 1992, 1993), shown to stimulate the cAMP pathway and activate protein kinase A (PKA), resulting in protein tyrosine

phosphorylation of target proteins in the tail connecting principal pieces (Harayama 2003; Harayama et al. 2004, 2012; Harayama and Nakamura 2008). The calcium/calmodulin pathway was proposed as another signaling pathway regulating sperm motility (Hurtado de Llera et al. 2014) and these two pathways seem to be mutually independent (Litvin et al. 2003). It was shown that the MAPK pathway and ROS regulation of capacitation also occur in pig spermatozoa (Awda and Buhr 2010). For a more in-detail overview of signal transduction pathways in the pig, we recommend a review by Hurtado de Llera et al. (2016). Irrespective of signal transduction pathways, targets of protein tyrosine phosphorylation in ejaculated boar spermatozoa have been reported (Dube et al. 2005; Flesch et al. 1999; Katoh et al. 2014; Tardif et al. 2001, 2003) and their number is limited when compared to mouse spermatozoa (Visconti et al. 1995).

In vivo capacitation conditions may be easily mimicked in vitro and, as obvious from the previous text, three components are vital for capacitation-supporting media: HCO_3^- , Ca^{2+} and a cholesterol acceptor such as bovine serum albumin (Flesch and Gadella 2000; Tardif et al. 2003). It was previously shown that hyperactivation can be induced highly and synchronously in laboratory animals such as mouse and hamster by simple incubation in this capacitation-supporting medium (Chang and Suarez 2011; Li et al. 2015; Suzuki et al. 2010; Tateno et al. 2013). In contrast, hyperactivation of boar spermatozoa is difficult to induce in the same medium (Harayama 2013; Harayama et al. 2012; Katoh et al. 2014). This suggests that parts of the cAMP/protein phosphorylation signaling pathways are more suppressed in boar ejaculated spermatozoa than in mouse and hamster epididymal spermatozoa. Instead, replacement of HCO_3^- with a cAMP analog cBiMPS and supplementation of protein phosphatase 1 and 2A inhibitors, greatly improve the capacity of a capacitation-supporting medium to induce hyperactivation of boar ejaculated spermatozoa (Harayama et al. 2012). Spermatozoa capacitate in vitro in an unregulated manner, which can lead to “over-capacitation” resulting in spontaneous acrosomal exocytosis that is undesirable for AI. Several molecules such as fertilization promoting peptide, adenosine, calcitonin and adrenaline found in SP have been shown to have capacitation-regulating effects (Fraser 2008). These molecules initially accelerate capacitation but then inhibit acrosome loss, thus maintaining sperm fertilization potential.

Previous markers of sperm capacitation have included hyperactivation, Ca^{2+} influx, protein tyrosine phosphorylation, change in plasma membrane integrity and acrosomal modifications and exocytosis. Recently, we described the importance of Zn^{2+} efflux for the spermatozoa to gain fertilization competency (Kerns et al. 2018a). This is marked by four distinct zinc localization patterns (zinc signatures) that are associated with key markers of sperm capacitation (hyperactivation, change in plasma membrane integrity, acrosomal

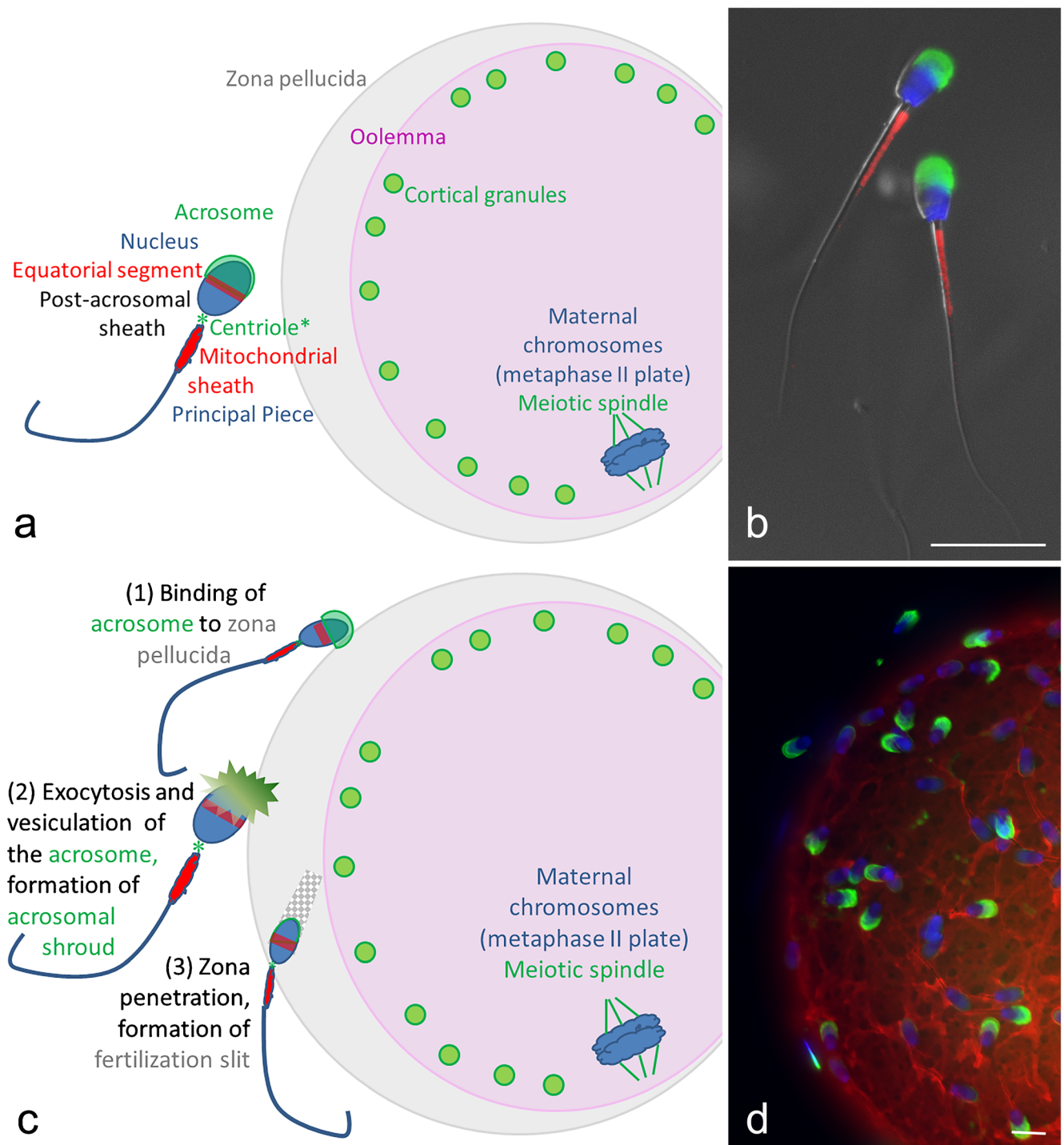


Fig. 4 Summary of porcine gamete structure and early sperm-oocyte interactions. **(a)** Initial gamete contact occurs between the sperm acrosome and oocyte zona pellucida, upon which the sperm acrosome undergoes exocytosis, commonly referred to as the acrosome reaction. At this time, the major sperm head (equatorial segment, post-acrosomal sheath) and tail structures (centriole, mitochondrial sheath, principal piece) remain intact, although they have already been primed during sperm capacitation to facilitate the subsequent fertilization events. Similarly, the oocyte is quiescent, having reached cell cycle arrest at the metaphase of the second meiotic division. Cortical granules are primed for exocytosis near the inner face

of the oolemma and the oocyte chromosomes are arranged in a metaphase plate anchored by the meiotic spindle. **(b)** The boar sperm mitochondrial sheath is highlighted by immunolabeling of PACRG protein (red). The acrosome is labeled green with lectin PNA and sperm DNA is counterstained blue with DAPI. **(c)** Following acrosomal exocytosis, the spermatozoa remain motile in order to penetrate the zona pellucida, digesting a fertilization slit in it. **(d)** Zona pellucida (red, anti-ZPC antibody labeling) bound spermatozoa at the onset of acrosomal exocytosis (green, lectin PNA). Blue DNA is counterstained by DAPI

Table 1 An overview of ZP protein nomenclature with the respective UniProt references (<https://www.uniprot.org/>) for each ZP glycoprotein in mouse, human, pig and bovine species. Rows show ZP orthologues across the selected species while ZP1 and ZP4 are paralogous, which is indicated by the superscripts in ZPB genes subfamilies. While humans express all four ZP glycoproteins, in mouse, pig and cow, one of the ZPB paralogues is not expressed. Theoretical masses are calculated from nucleotide sequences and may vary from apparent masses depending on posttranslational modifications, mainly glycosylation

Glycoprotein	Mouse			Human			Pig			Bovine			ZP genes subfamilies			
	Aliases	Mass/ kDa	UniProt	Aliases	Mass/ kDa	UniProt	Aliases	Mass/ kDa	UniProt	Aliases	Mass/ kDa	UniProt	Aliases	Mass/ kDa	UniProt	
ZP1	ZP1	68.72	Q62005	ZP1	70.05	P60852	Protein is not expressed	Protein is not expressed	P42099	Protein is not expressed	Protein is not expressed	ZP2, ZPA	79.55	Q9BHI0	ZPB ¹	
ZP2	ZP2, Zpa	80.21	P20239	ZP2, ZPA	80.36	Q05996	ZP2, ZPA, PZPL	79.73	P42099	ZP2, ZPA	79.55	Q9BHI0	ZPA			ZPA
ZP3	ZP3, Zpc	46.30	P10761	ZP3, ZP3A, ZP3B, ZPC	47.02	P21754	ZP3, ZPC, ZP3-β	46.24	P42098	ZP3, ZPC	46.55	P48830	ZP3, ZPC			ZPC
ZP4	Protein is not expressed			ZP4, ZPB	59.40	Q12836	ZP4, ZPB, ZP3-α	59.33	Q07287	ZP4, ZPB	59.20	Q9BHI1	ZP4, ZPB			ZPB ²

modification, ability to detect the oocyte, bind to ZP and undergo acrosomal exocytosis). For further review of zinc's role in sperm capacitation, see the review by Kerns et al. (2018b).

Zona pellucida binding and associated sperm surface molecules

Sperm interactions with the oocyte ZP (Fig. 4) include several phases such as loose attachment to the ZP glycoproteins, primary binding of spermatozoa to the ZP, induction of the acrosomal exocytosis by the ZP, secondary binding of spermatozoa to the ZP and final penetration through the ZP (Yanagimachi 1994). Binding of spermatozoa to the glycoprotein coat is a receptor-mediated event that involves sperm surface protein interactions with the complementary ZP glycoconjugates. A number of identified sperm receptors possess a lectin-like affinity for a specific sugar residue on ZP that is responsible for the primary binding. Carbohydrate structures on ZP3 that mediate primary sperm-ZP interaction are well documented in the mice model (McLeskey et al. 1998; Ryu and Lee 2017; Suarez 1996; Topfer-Petersen 1999). Spermatozoa bind to O-linked oligosaccharides of ZP3 by their acrosomal region of the plasma membrane, causing aggregation of male cell receptor molecules to ZP3 and initiation of acrosomal exocytosis in mice (Reid et al. 2011).

Mammalian ZP glycoproteins are coded by three genes, namely ZPA, ZPB and ZPC (Harris et al. 1994). Due to the fact that the sequencing of ZP genes was done much later than the ZP glycoproteins were described (Bleil and Wassarman 1980), this caused confusion in nomenclature as more than three ZP proteins were detected by electrophoretic analysis in pig (Menino and Wright 1979). The following nomenclature of porcine ZP (pZP) glycoproteins can be found in the older literature: pZP1/PZPL (90 kDa, ZPA), pZP3α (55 kDa, ZPB), pZP3β (55 kDa, ZPC), while proteins designated pZP2 (65 kDa) and pZP4 (25 kDa) are in fact proteolytic products of PZPL (Hedrick and Wardrip 1986, 1987; Nakano et al. 1987; Wardrip and Hedrick 1985; Yurewicz et al. 1987). The overview of the ZP glycoprotein HUGO nomenclature for mouse, human, pig and bovine is presented in Table 1. Two names for ZP glycoproteins are used interchangeably: ZPA or ZP2, ZPB or ZP1 and ZPC or ZP3; however, this nomenclature has become questionable when a paralogue to mouse ZP1 was identified in humans as ZP4 (Hughes and Barratt 1999). A thorough phylogenetic analysis (Spargo and Hope 2003) proposes a unified system of nomenclature for the ZP gene family that removes ambiguities. In this regard, pigs are similar to humans in which four genetically distinct ZP proteins exist. The primary sperm receptor activity in pig has been mapped to O- and N-linked glycans on PZP3β (ZPC), a binding homolog of mouse ZP3 (Topfer-Petersen et al. 1993; Yonezawa et al. 1995; Yurewicz et al. 1991). The tri- and tetra-

antennary N-glycans localized in the N-terminal region of PZP3 α (ZPB) mediate the sperm binding to the ZP whereas the structurally identical tri- and tetra-antennary N-glycans of ZP3 β (ZPC) appear to play no role in gamete recognition (Kudo et al. 1998; Yonezawa et al. 1999). It was proposed that both β -galactosyl and α -mannosyl residues of porcine ZP are involved in sperm binding (Song et al. 2007; Yonezawa et al. 2005). Additionally, the increasing sialylation and sulfation of ZP during maturation of the porcine oocyte is indispensable for the sperm-ZP binding, induction of acrosomal exocytosis and sperm-zona penetration (Lay et al. 2011).

Sperm molecules involved in the primary ZP binding are localized to the apical region of the capacitated, acrosome-intact sperm head; while the ones involved in secondary ZP binding are localized to the inner acrosomal membrane (IAM) and/or acrosomal matrix. The main candidates responsible for the sperm-ZP binding in the pig model are AWN, AQN1 and AQN3 spermadhesins (Calvete et al. 1997; Dostalova et al. 1995; Ensslin et al. 1995; Jonakova et al. 1991, 1998; Petrunkina et al. 2000; Topfer-Petersen et al. 1998; van Gestel et al. 2007), which belong to the heparin-binding protein group (Jonakova et al. 1998). These three spermadhesins identically bind to Gal β (1–3)-GalNAc and Gal β (1–4)-GlcNAc carbohydrate structures of ZP glycoproteins (Topfer-Petersen et al. 1998); AQN1 binds to the plasma membrane by an indirect lipid-binding mechanism. AWN and AQN3 stabilize the plasma membrane over the acrosomal cap and the majority are released from the surface during capacitation, while the few retained spermadhesins are thought to play a role in gamete recognition and binding (Dostalova et al. 1994). DQH/pB1/BPS1 is another seminal plasma protein described as a sperm-ZP receptor (Jonakova et al. 1998; Manaskova et al. 2007). DQH is homologous to BSPs that are abundantly present in bull seminal plasma (Calvete et al. 1997).

Sperm-borne primary ZP receptors that have been studied in detail are as follows: ZAN/zonadhesin (Bi et al. 2003; Hardy and Garbers 1995; Hickox et al. 2001; Lea et al. 2001), a major sperm membrane protein with the ZP binding ability; B4GALT1/ β -1,4-galactosyltransferase/EC:2.4.1.22 (Larson and Miller 1997; Rebeiz and Miller 1999), the first described primary ZP binding receptor; ACRBP/SP32 (van Gestel et al. 2007); hyaluronidase/PH-20/SPAM1/EC:3.2.1.35 (Yoon et al. 2014); and angiotensin-converting enzyme/ACE/EC:3.4.15.1 (Williams et al. 1992; Zigo et al. 2013). Sperm primary ZP binding receptor glycan that is introduced to the sperm surface during epididymal transit is α -D-mannosidase that was also shown to be the primary ZP receptor in mice (Cornwall et al. 1991); however, speculations abound whether it can serve the same purpose in pig (Jin et al. 1999; Kuno et al. 2000; Okamura et al. 1995). Some primary ZP binding receptors like arylsulphatase A/ARSA/P68/SLIP1/EC:3.1.6.8 (Carmona et al. 2002; Tanphaichitr et al. 1998) and MFGE8/SED1/P47/lactadherin (Ensslin et al.

1998; Petrunkina et al. 2003; van Gestel et al. 2007; Zigo et al. 2015) are expressed in both the testis and epididymis. Multiple proteins with ZP binding affinity were reported in pigs (van Gestel et al. 2007) such as ADAM2/fertilin β /PH-30, DCXR/L-xylulose reductase/dicarbonyl reductase/EC:1.1.1.10/P26h/P34H/P31m, KCNC4/potassium voltage-gated channel subfamily C member 4, PTPN13/protein tyrosine phosphatase non-receptor type 13, PRDX5/Peroxiredoxin-5; furthermore, ADAM3 (Kim et al. 2009), ADAM20-like and ADAM5 (Mori et al. 2012), PKDREJ (Zigo et al. 2013), RAB2A (Zigo et al. 2015) and an uncharacterized, non-annotated adhesion protein z/APz (Peterson and Hunt 1989; Zayas-Perez et al. 2005). These, however, need to be studied further to elucidate their function. With the identification of multiple ZP binding receptors, the assumption that the sperm ZP receptor was a single molecule was disproved. Multiple studies involving KO mice for certain ZP binding receptors were unable to obtain infertile offspring, suggesting a redundant function of these receptors. Newer evidence shows that these receptors associate together in high-molecular (0.75–1.3 MDa) multi-protein complexes and thus mediating the interaction with the ZP (Kongmanas et al. 2015; Redgrove et al. 2011). Intriguingly, these complexes in both species prominently feature proteasomes, also known to accelerate their enzymatic activities at capacitation (Kerns et al. 2016; Zapata-Carmona et al. 2019), perhaps in preparation for sperm-zona binding and zona penetration. Other components of these complexes, implicated in sperm-oocyte interaction include chaperones, cytoskeletal proteins, epididymal fluid/seminal plasma proteins and various enzymes (Kongmanas et al. 2015; Redgrove et al. 2011).

The most frequently studied secondary ZP binding receptor in pig is a fucose-binding protein (Topfer-Petersen et al. 1985) that was subsequently N-terminal sequenced as ACR/acrosin/EC 3.4.21.10 (Topfer-Petersen and Henschen 1987) and later shown to play the function of a secondary ZP binding receptor (Tesarik et al. 1988; Topfer-Petersen and Calvete 1995). We recently reported acrosin on the boar sperm surface that may have a mediator function in primary sperm-zona binding (Zigo et al. 2013, 2015). Another well-documented protein is zona pellucida binding protein (ZBPB a.k.a. ZBPB1/Sp38/IAM38) (Mori et al. 1993, 1995; Zigo et al. 2013; Tardif et al. 2010; Zigo et al. 2013; Yu et al. 2006). Proteins with known intra-acrosomal localization with ZP binding affinity are sperm acrosomal protein SP-10 (ACRV1/ASPX) (Herr et al. 1990) that was shown to be involved in secondary ZP-binding affinity at least in bovine (Coonrod et al. 1996); and ZAN. Interestingly, ZAN was initially thought to be participating in the secondary ZP-binding due to its intra-acrosomal localization (Tanphaichitr et al. 2007); however, it was later shown that a portion is translocated to the sperm surface during sperm capacitation (Tardif and Cormier 2011). ZAN may thus serve a dual purpose. Other intra-acrosomal proteins were reported on the surface of capacitated

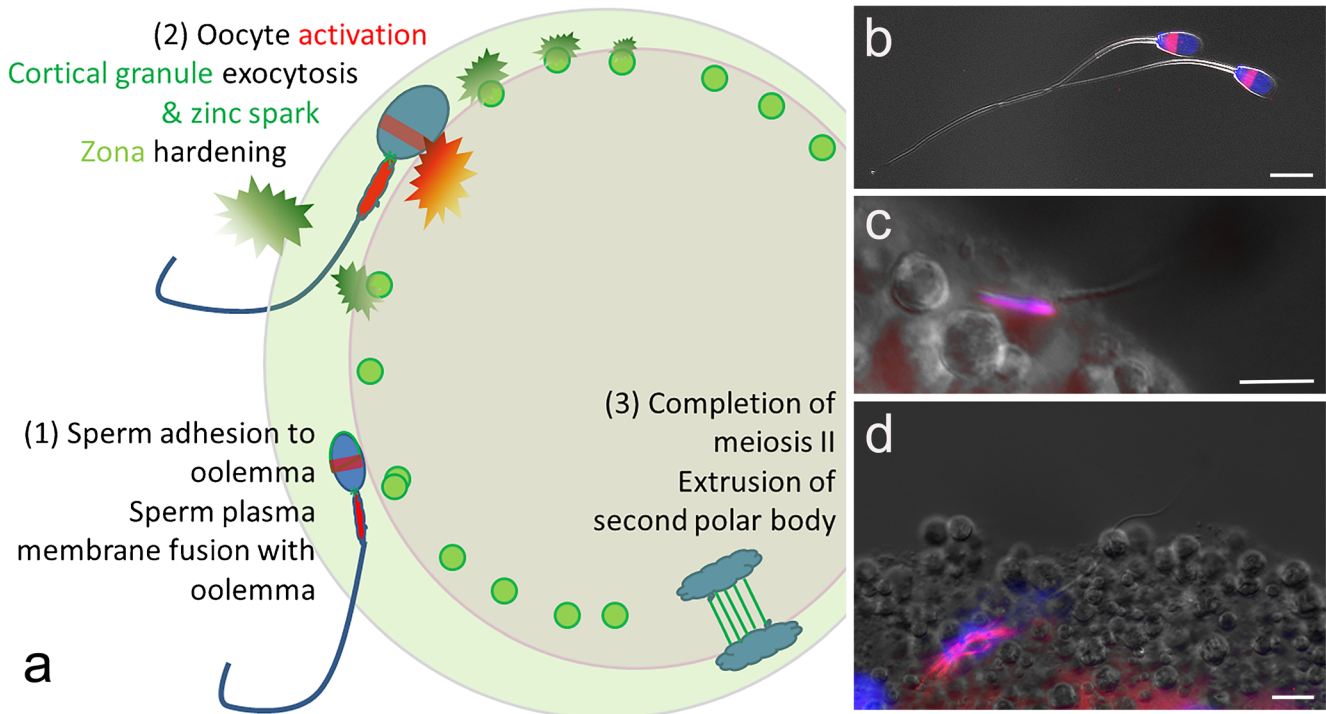


Fig. 5 Oocyte activation. (a) Once the sperm head reaches the perivitelline space between the zona and the oolemma (1), its equatorial segment adheres to and fuses with the oolemma, at which time the sperm tail movement ceases. Upon sperm-oocyte plasma membrane fusion (2), the post-acrosomal sheath of the sperm head releases the oocyte activating factors that utilize oocyte's intrinsic calcium signaling pathways to trigger the reactivation of the oocyte meiotic cycle and activate oocyte anti-polyspermy defense by zona pellucida modification through cortical

granule exocytosis (cortical reaction and zona hardening) and zinc ion release (the zinc spark). (b-d) Signaling protein WBP2NL (red), a putative component of the sperm-borne oocyte activating factor (SOAF) is immunolabeled in the intact post-acrosomal sheaths of boar spermatozoa (d) and during the early (e) and late (f) stages of SOAF release, coinciding with the onset of sperm chromatin decondensation and formation of the nascent paternal pronucleus

spermatozoa in boar as well as in other species (Kongmanas et al. 2015; Zigo et al. 2013; Tanphaichitr et al. 2015; Zigo et al. 2013; Wassarman 2009). Altogether, the sperm surface protein complexes implicated in early steps of porcine fertilization share similarities with those of human spermatozoa.

Fertilization

It was long believed that only capacitated, acrosome-intact spermatozoa can bind to ZP of an oocyte, undergo acrosomal exocytosis and penetrate ZP. This model has been challenged in mice where spermatozoa that seemingly already underwent acrosomal exocytosis were reaching ZP (Hino et al. 2016; Jin et al. 2011; La Spina et al. 2016; Muro et al. 2016). A similar observation was made in the pig (Mattioli et al. 1998). Furthermore, mouse acrosome-exocytosed spermatozoa recovered from the perivitelline space were able to fertilize other oocytes (Inoue et al. 2011). Irrespective of the place of acrosomal exocytosis, the inner acrosomal membrane on the sperm head becomes exposed and able to bind to the ZP, also known as secondary ZP binding. Furthermore, acrosomal proteases implicated in sperm penetration through ZP, such as the

26S proteasome and matrix metalloproteinase MMP2 remain associated with IAM after acrosomal exocytosis (Ferrer et al. 2012; Yi et al. 2010b; Zimmerman et al. 2011). After the passage through the ZP (Fig. 5), this region closely associates with the oolemma prior to fusion (Huang and Yanagimachi 1985). However, it is the sperm head equatorial segment and later the posterior head regions that closely adhere to and fuse with the oolemma (Myles et al. 1987; Yanagimachi 1994). Oolemma fuses with the sperm equatorial segment rather than with the inner acrosomal membrane and the spermatozoon is completely engulfed by the oocyte (Moore and Bedford 1983; Shalgi and Phillips 1980).

Binding of the spermatozoon to the oolemma is mediated by adhesion molecules that are localized to the equatorial segment. Four boar sperm plasma membrane proteins (62, 39, 27 and 7 kDa estimated molecular mass) have been suggested as the predominant binders of the porcine oolemma (Ash et al. 1995; Berger et al. 2011). Another study showed significantly greater relative binding of the porcine oocyte plasma membrane to the 14- and 10-kD porcine sperm plasma membrane proteins (Sartini and Berger 2000). Members of the ADAM ("a disintegrin and a metalloprotease") family proteins on spermatozoa and integrin $\alpha 6 \beta 1$ receptors on the oocyte were

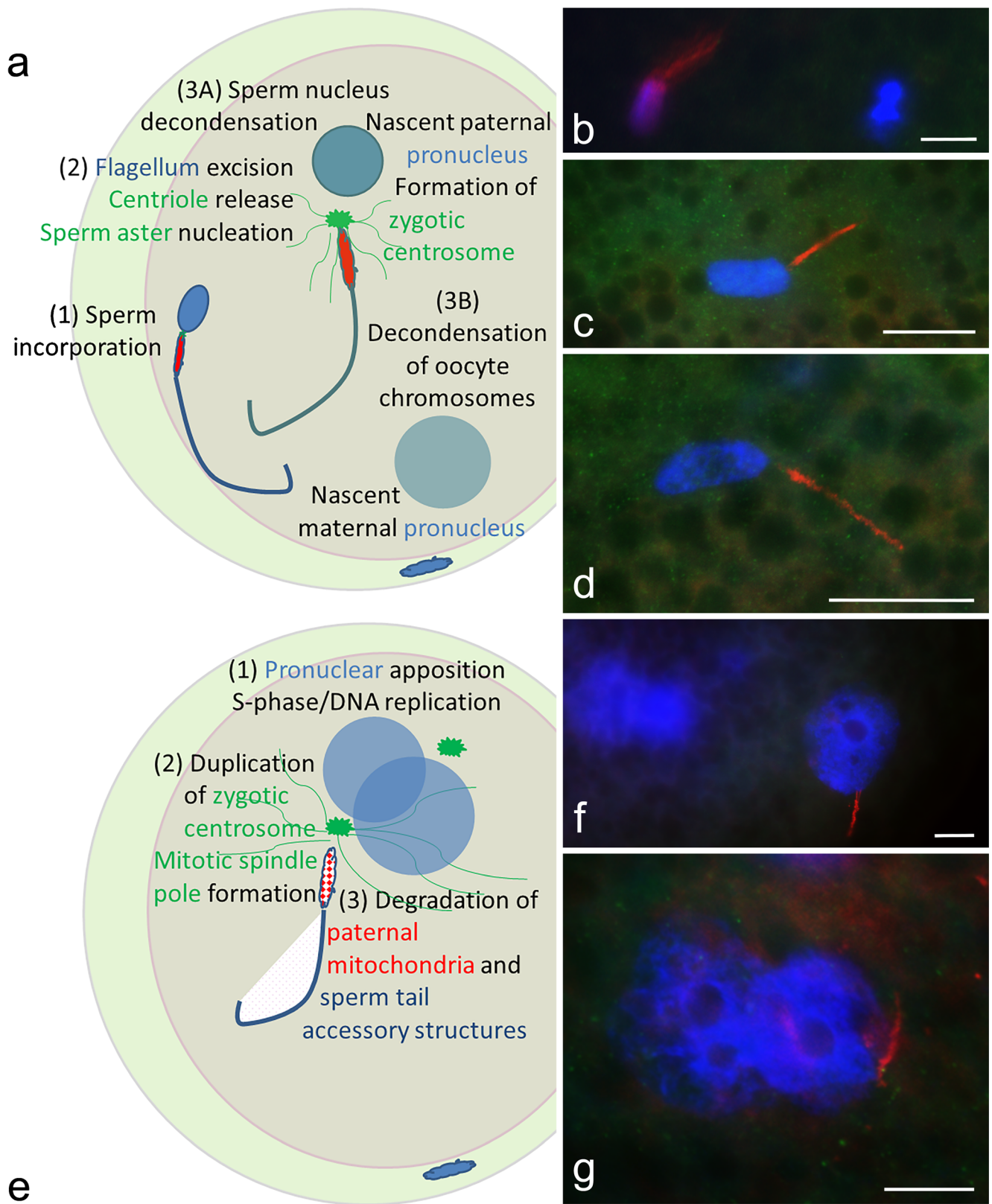
implicated as the adhesion partners in mice (McLeskey et al. 1998; Snell and White 1996; Wassarman 1999). Two mouse-sperm ADAM protein complexes, the heterodimers fertilin- α (ADAM1)/fertilin- β (ADAM2) and ADAM1/cyritestin (ADAM3) interact with integrin in the oolemma through their disintegrin domains (Blobel 1999; Primakoff and Myles 2000; Schlondorff and Blobel 1999). In the ADAM1/ADAM2 complex, the function of fertilin- β is to support sperm-oolemma binding, whereas fertilin- α has been implicated in the subsequent fusion step of sperm and oocyte (Bigler et al. 1997; Huovila et al. 1996; Wassarman 1999). Findings support that ADAM1/ADAM2 and ADAM1/ADAM3 complexes are not essential in the gamete-fusion pathway (Frayne and Hall 1999; Kim et al. 2006). The expression of porcine fertilin- β (ADAM2) is limited to the testis (Day et al. 2003). The study of Fabrega et al. (Fabrega et al. 2011) described proteolytic processing for boar sperm ADAM1 occurring mainly in the testis and in addition throughout the caput epididymis for ADAM2. Immunolocalization of ADAM1 showed that fertilin- β migrates from the acrosomal region to the acrosomal ridge during the sperm transit throughout the epididymis (Fabrega et al. 2011) and may suggest that fertilins are rather involved in the primary binding to the ZP as is the case of porcine ADAM2 (van Gestel et al. 2007). CRISP (cysteine-rich secretory proteins) family proteins, originating in the epididymis, are other adhesion/fusion proteins. The first reported CRISP1, also referred to as DE, was found to initially associate with the dorsal region of the rat sperm head, with subsequent migration to the equatorial segment upon acrosomal exocytosis (Ellerman et al. 2002) with the posterior region of the sperm head localized in other mammals. The majority of DE is lost during capacitation; however, the remaining DE is involved in gamete fusion rather than adhesion (Cohen et al. 2000). A human orthologue has also been reported (Cohen et al. 2001). In the pig, CRISP-1 has been found to express in the epididymis and CRISP2 in testicular tissue (Vadnais et al. 2008), however, sperm localization of CRISP proteins has not been reported yet.

While oolemma integrins and sperm disintegrins may play a supporting role in sperm-oolemma adhesion within the oolemma's tetraspanin web (Sutovsky 2009), the only gene ablation-proven protein-protein interaction essential for sperm-oolemma adhesion is between sperm head IZUMO1 (OBF13) and oolemma IZUMO1R (JUNO/FOLR4), a mechanism that is likely conserved in all mammals, including humans and pigs (Bianchi et al. 2014; Chalbi et al. 2014). IZUMO1 is a testis-specific member of the immunoglobulin superfamily (IgSF), firstly reported by Inoue et al. (2005) in mouse and later shown to be present in humans and pigs (Hayasaka et al. 2007; Kim et al. 2013). Tanihara et al. (2014) suggested that functional exposure of IZUMO by porcine spermatozoa after their acrosomal exocytosis and passage through the ZP may result in the acceleration of sperm

incorporation in the ooplasm. Furthermore, the molecular architecture of the IZUMO1-JUNO fertilization complex approximates interaction between the two molecules during gamete adhesion (Aydin et al. 2016). The deletion of IZUMO1 gene results in infertile male offspring; however, the precise function is still to be determined. Similar to IZUMO, SPACA6 gene encodes a immunoglobulin-like protein and the disruption of this gene causes a fertilization block associated with a failure of gametes fusion (Lorenzetti et al. 2014). The binding on the oolemma partner is not known for SPACA6. The IZUMO binding partner CD9 belongs to the tetraspanin family. At fertilization, CD9 associates with IZUMO1, as well as with a subset of β 1 integrins, including integrin α 6 β 1 (Hemler 1998; Porter and Hogg 1998). Oocytes of mice with a targeted disruption of the CD9 gene rarely fused with wild-type spermatozoa and are subfertile (Miyado et al. 2000). Furthermore, double-ablated mice lacking CD9 and related CD81 tetraspanins are completely infertile (Rubinstein et al. 2006). The importance of CD9 in the mouse sperm-oocyte interaction is clearly established, while the exact function(s) still needs to be determined (Evans 2012). CD9 together with CD81 localize to the oolemma and membrane and vesicles in the perivitelline space of porcine oocytes and embryos and may likely participate in membrane reorganization facilitating the protein-protein interactions and protein network interaction resulting in successful fertilization (Jankovicova et al. 2019). Anti-CD9 antibody-treated porcine oocytes showed reduced sperm binding to oolemma and sperm incorporation (Li et al. 2004). Integrins α 5 β 1 and β 1 were suggested to be the gamete adhesion molecules in the pig as well, as the antibody to an extracellular domain of the β 1 integrin subunit reduced pig sperm-oocyte binding (Linfor and Berger 2000).

Oocyte activation and anti-polyspermy defense

While the essential role of JUNO-IZUMO binding in sperm-oolemma adhesion is now well established, the molecule involved in the actual fusion between plasma membranes of the respective gametes are yet to be discovered and few candidates have been proposed (Sutovsky 2009). Significantly more progress has been made in the study of sperm factors causing oocyte activation. Upon sperm-oolemma fusion, the post-acrosomal perinuclear theca quickly dissolves in the ooplasm (Fig. 5), releasing signaling proteins collectively termed SOAF, for the sperm-borne oocyte activating factor(s) (reviewed in Oko et al. 2017). These factors, studied in detail in the pig and to a lesser extent in human spermatozoa, directly or indirectly induce phospholipase/inositol-3-phosphate-dependent oscillatory release of calcium from the oocyte endoplasmic reticulum, acting as second messenger, to trigger a



multi-pronged signaling cascade that forces the completion of oocyte meiosis, expulsion of the second polar body, activation of anti-polyspermy defense, induction of pronuclear

development and formation of the zygotic centrosome. These early events culminate in pronuclear apposition, zygotic DNA replication and first embryo cleavage. The mingling of

Fig. 6 Pronuclear development and sperm mitophagy. (a) Following sperm incorporation in the ooplasm (1), the tail is excised from the head (2), which starts to unravel and form the paternal pronucleus (3a) concomitantly with the completion of oocyte meiosis and formation of the nascent maternal pronucleus (3b). Head-tail excision enables the release of the sperm-borne centriole and consequent formation of the zygotic centrosome and sperm aster. (b–d) Blue DNA labeling (DAPI) reveals the progression of the sperm nucleus decondensation early after sperm incorporation. (e) Pronuclei are brought to apposition by sperm aster microtubules (1) as the process of paternal and maternal DNA replication commences. Simultaneously, the zygotic centrosome duplicates (2) and migrates to form the poles of the future mitotic spindle. Meanwhile, the sperm mitochondrial sheath and other tail structures are degraded (3). (f, g) DNA labeling shows the progression of pronuclear apposition while the red MitoTracker labeling highlights the progressive deterioration of the sperm mitochondrial sheath, the early stage of which is already visible in panel d

chromosomes (syngamy) is generally considered as the end of fertilization and the beginning of embryonic development (Yanagimachi 1994). At present, the preferred SOAF molecule is the sperm-borne, albeit not germline-specific, phospholipase PLCZ1 (Saunders et al. 2002). The alternative or perhaps complementary SOAF factor is the male germline/spermatid specific WW-domain signaling protein WBP2NL (alias PAWP; Wu et al. 2007). Though the sperm content of these respective proteins consistently correlates with fertility in men (e.g., Azad et al. 2018; Tavalaei et al. 2017), genetic ablation of neither *Plcz1* nor *Wbp2nl* renders male mice completely infertile (Hachem et al. 2017). A possibility of cross-compensation has been discussed, affirmed by increased *Wbp2nl* gene expression in *Plcz1* null mice (Hachem et al. 2017; Nozawa et al. 2018). Furthermore, somatic homolog WBP2, present in mouse but not in phylogenetically higher mammalian spermatozoa, could compensate for lack of WBP2NL in the null spermatozoa (Hamilton et al. 2018).

Multiple lines of anti-polyspermy defense are triggered by oocyte activation to prevent embryo-lethal polyspermy. Depolarization of oolemma occurs instantly after binding of a spermatozoon to the oolemma thus preventing polyspermic fertilization, also known as the primary/fast block to polyspermy in invertebrates (Jaffe and Gould 1985) but little is known about such event in mammals. The aspects of oocyte activation are directly or indirectly dependent upon a Ca^{2+} -driven signaling pathway and downstream regulation of specific protein kinase activities (Florman and Ducibella 2006). The induction of cortical granules exocytosis is the result of the Ca^{2+} -driven signaling pathway. These lysosome-like organelles cause hardening of the ZP after their exocytosis, as they secrete the zona-cleaving protease ovastacin (Burkart et al. 2012). The ZP becomes modified rendering it impermeable to other spermatozoa also known as the secondary/slow block to polyspermy (Yanagimachi 1994). Post-fertilization shedding of JUNO from oolemma, discovered in the mouse (Bianchi et al. 2014), is yet to be examined as a possible anti-polyspermy contributor in pig and human oocytes. Recently, we suggested that there might be yet another possible

mechanism to prevent polyspermy – through the zinc shield (Kerns et al. 2018a, b; Sutovsky et al. 2019) generated by the oocyte activation-induced zinc spark (Duncan et al. 2016; Que et al. 2017), which, based on our studies of zinc release during sperm capacitation (Kerns et al. 2018a), could at least temporarily decapacitate accessory spermatozoa in the perivitelline space or on the zona surface.

Post-fertilization sperm mitophagy and zygotic development

Mitochondrial inheritance has been explored using many different animal models including *C. elegans* (Sato and Sato 2011), *Drosophila* (Politi et al. 2014; Wolff and Gemmill 2013), mice (Rojansky et al. 2016; Shitara et al. 2000, 2001), bovine (Sutovsky et al. 1996, 2003) and porcine (Song et al. 2016; Sutovsky et al. 2003, 2004). Though all these models have their advantages and disadvantages, the porcine model and porcine IVF system have some unique features that set it apart as an ideal model animal for the study of mitochondrial inheritance and furthermore, connecting those discoveries to human health and fertility outcomes.

Specifically, the porcine IVF system further sets itself apart because of the timing of post-fertilization sperm mitophagy in pigs that occurs very early in the porcine zygote (Fig. 6), at one-cell stage, as compared to the 2–4 cell stage in rodents, ruminants, and primates (Sutovsky et al. 2003, 2004; Zuidema and Sutovsky 2019). This rapid post-fertilization sperm mitophagy is a result of an interplay between VCP protein-dependent dislocation and proteasomal degradation of mitochondrial membrane proteins and bulk digestion of the weakened sperm mitochondrial ghosts by ubiquitin-dependent autophagy/mitophagy (Song et al. 2016). Consequently, we do not have to worry about interfering with ubiquitin-regulated elements of cell cycle machinery during the first embryo mitosis, which is affected by the treatments targeting sperm mitophagy such as proteasomal inhibition, lysosome quenching and blocking of autophagy (Glotzer et al. 1991; Song et al. 2016). This allows us to probe and interpret post-fertilization sperm mitophagy without compromising early fertilization/zygotic development events. In the context of human health, such animal model exploration is likely to be reinvigorated with the recently discovered evidence of multi-generational, familial biparental mitochondrial inheritance in humans (Luo et al. 2018), a phenomenon that previously has only been documented in one other human case (Schwartz and Vissing 2002). This discovery has implications for human health regarding heteroplasmy and mitochondrial diseases but it also may have implications within our livestock species, as well as, wild animal species. A deeper understanding of how biparental mitochondrial inheritance is enforced, in the pig model will help to breach the gaps between humans and less suitable animal models.

Parallel to the onset of sperm mitophagy, the porcine sperm head with hypercondensed, protamine-packaged DNA has to be unraveled to promote paternal pronucleus development (see Fig. 6a–d). Protamines are specialized, arginine-rich male germline proteins that replace histones during spermatid elongation in the testis (Balhorn 2007); held together by disulfide bonds and zinc bridges, making the sperm nucleus a highly stable and sperm DNA transcriptionally silent until after fertilization (Bjorndahl and Kvist 2010). Pig spermatozoa are naturally resilient to DNA decondensation as shown by Lee et al. (2003) where the failure of paternal pronucleus formation was the major cause for the failure of fertilization in activated ICSI zygotes. Intact or partially decondensed sperm heads were found in unfertilized oocytes and pre-blastocyst embryos. Such a feature can be related to relatively high boar sperm chromatin integrity determined by sperm chromatin structure assay (SCSA) as the percentage DNA fragmentation index (%DFI). Multiple studies have shown the statistical threshold of 2–6%DFI to have a significant negative effect on the farrowing rate and average number of total pigs born; such DNA fragmentation levels might probably be the lowest of domestic animals and humans (Boe-Hansen et al. 2008; Didion et al. 2009; Martinez 2005; Rybar et al. 2004; Waberski et al. 2002). The sperm head is stabilized in these ways to prevent DNA damage during storage and sperm transport via the male and female reproductive tracts. Such stabilization must be removed after fertilization through zinc bridge removal (Bjorndahl and Kvist 2010) and disulfide bond reduction mediated by oocyte glutathione (Perreault et al. 1984; Sutovsky and Schatten 1997) and by the sperm perinuclear theca-released glutathione-S-transferase GSTO2 (Hamilton et al. 2019). Once the sperm chromatin begins to unravel, the protamines that provided the highly condensed structure are replaced by histones (Kopečný and Pavlok 1975). The chromatin recondenses around these new histones (Borsuk and Manka 1988; Wright and Longo 1988) and a second decondensation process takes place. The male/paternal pronucleus then takes form. This process must occur in order to make the paternal chromatin permissive to DNA replication and transcription and compatible with the oocyte chromatin (Adenot et al. 1991; McLay and Clarke 2003). The paternal and maternal pronuclei can then undergo the process of apposition, aided by the sperm-released centriole-turned zygotic centrosome (see Fig. 6e–g). This event is a prelude to syngamy (maternal and paternal genetic mixing) and starts the process of mitosis and embryogenesis (Sun and Nagai 2003). Human and porcine zygotes seem to undergo these genomic processes in a similar timeframe (Mao et al. 2018). Additionally, human and porcine embryos reach the blastocyst stage within a similar timeframe, at which point the difference between the two species begins to increase with more dramatic differences in implantation and placental development. However, as far as early embryonic

development is involved, humans and pigs seem to share many conserved processes.

Pig as a model for assisted reproductive therapy

A wide-scale of assisted reproductive technologies has been developed in the domestic pig, both for production and research. Many, if not most are relevant to human-assisted reproductive therapy (ART) and have been used to better understand and safeguard clinical procedures such as IVF, ICSI and in vitro embryo culture. Gene editing by CRISPR/Cas9 has also taken root in pig research laboratories (Mao et al. 2018; Ryu and Lee 2017; Whitworth et al. 2014). The ease of gamete acquisition, as well as the physiological and genomic similarities between pigs (Archibald et al. 2010; Day 2000) and humans make the porcine biomedical model continue to grow in popularity. This is especially true in the realm of ART and the study of early fertilization events, including mitochondrial inheritance studies in which oocytes can be preinjected with antibodies or non-permeant inhibitors of autophagic events (Song et al. 2016). Contrary to human fertilization, IVF in the pig has had issues with high polyspermy, which can be mitigated by optimization of sperm concentration, fertilization media/conditions and addition of the recombinant homologs of the polyspermy mitigating factor naturally present in female oviductal fluid such as osteopontin (Hao et al. 2006) or ubiquitin C-terminal hydrolases UCHL1 and UCHL3 (Mtango et al. 2011; Yi et al. 2007). Intracytoplasmic sperm injection (ICSI) in the domestic pig is complicated by high disulfide bond crosslinking of sperm head structures, which can be disrupted by piezo drill actuated ICSI or relieved by the addition of culture media components supporting glutathione synthesis during oocyte maturation (Katayama et al. 2005, 2007). Both approaches promote sperm nucleus conversion into the paternal pronucleus once the intact (i.e., fully covered) sperm head is deposited into the ooplasm by microinjection. While there is no evidence as of now for ICSI promoting heteroplasmy in mammals, it is possible that skipping sperm head and tail (including midpiece with mitochondrial sheath) demembration that occurs at sperm-oolemma fusion during natural fertilization could impede timely recognition and disposal of paternal mitochondria after ICSI. Recent studies in fish suggest this could indeed be happening in vertebrate zygotes (Peng et al. 2018).

Conclusions and perspectives and implications for human and animal medicine

Domestic pig use in biomedical research will likely continue to increase, using both wild-type and transgenic pigs.

Transgenic pig models specifically designed for the study of male fertility could be developed. There is already the pig model of cystic fibrosis (CF), which replicates human patients' male-infertile phenotype by the KO of the CF-transmembrane receptor (CFTR), with male pigs being infertile due to CF-associated absence of the vas deferens. Of note, such infertile phenotypes, or other clinical symptoms of CF, are not observed in a similarly engineered mouse model. Also, CFTR is expressed by the animal (PS, unpublished) and human spermatozoa (Yefimova et al. 2019). Another model useful for the study of sperm function and fertilization has been our own GFP-proteasome pig (Miles et al. 2013), allowing us to identify a number of proteasome-interacting sperm proteins including seminal plasma proteins discussed in the present review. With regard to seminal plasma, new methods for the management of sperm capacitation, viability and fertilizing potential after semen collection, developed for boar, could translate into improved protocols for human sperm processing prior to IUI, IVF and ICSI. Work on mitochondrial inheritance is significant for livestock fitness and productivity while having implications for human medicine. New documented cases of paternal heteroplasmy support the link with mitochondrial disease in humans. Although the notion of paternal mtDNA leakage in humans and the chimpanzee population has been around since the 1990s, patients with mitochondrial disease are not routinely or even occasionally, screened for it. What is the true incidence of it in human populations and if it is prevalent, is it the root cause of certain mitochondrial diseases? Could this be managed in human ART, wherein the prevalent ICSI-sperm injection method might delay mitophagy by introducing a spermatozoon with intact membranes (they are removed as the spermatozoon enters the oocyte during natural fertilization process)? How about the practice of oocyte rejuvenation by mitochondrial donation in female infertility patients of advanced reproductive age? Those and other questions can be answered with the help of relevant large animal models such as the domestic pig.

Acknowledgments We would like to thank to our past and present colleagues, trainees, associates and collaborators who inspired and facilitated our studies reviewed in this article. In particular, we appreciate the support from Dr. Richard Oko (Queen's University, Kingston, ON) and Dr. Randall Prather (National Swine Research Resource Center, University of Missouri). Logistic, technical and clerical support by Ms. Miriam Sutovsky and Ms. Kathy Craighead is gratefully acknowledged.

Funding information This review is supported by the National Institute of Food and Agriculture (NIFA), U.S. Department of Agriculture (USDA) grant number 2015-67015-23231 (P.S.); the USDA NIFA Agriculture and Food Research Initiative's Post-Doctoral Fellowships program, grant number 2019-67012-29714 (K.K.); grant number 5 R01 HD084353-02 from National Institutes of Health (NIH) National Institute of Child and Human Development (P.S.); and seed funding from the Food for the twenty-first Century Program of the University of Missouri (P.S.); and by the project BIOCEV (CZ.1.05/1.1.00/02.0109) from the European Regional Development Fund (P.M.-P., V.J.), by the Grant Agency of the

Czech Republic No. GA-18-11275S (P.M.-P.), by the Institutional support of the Institute of Biotechnology, Czech Academy of Sciences RVO: 86652036 (P.M.-P., V.J.), CellFit COST Action CA16119, Ministry of Education, Youth and Sports, Czech Republic INTER-COST LTC 18059 (P.M.-P., L.T.), Internal Grant Agency of Czech University of Life Sciences in Prague (SV18-08-21230) (P.M.-P., L.T., F.B.).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed. All studies involving vertebrate animals were completed under the strict guidance of an Animal Care and Use protocol approved by the Animal Care and Use Committee (ACUC) of the University of Missouri. This article does not contain any studies with human participants performed by any of the authors.

References

- Adenot PG, Szollosi MS, Geze M, Renard JP, Debey P (1991) Dynamics of paternal chromatin changes in live one-cell mouse embryo after natural fertilization. *Mol Reprod Dev* 28(1):23–34
- Aitken RJ, Nixon B (2013) Sperm capacitation: a distant landscape glimpsed but unexplored. *Mol Hum Reprod* 19(12):785–793
- Alvarez-Rodriguez M, Atikuzzaman M, Venhoranta H, Wright D, Rodriguez-Martinez H (2019) Expression of immune regulatory genes in the porcine internal genital tract is differentially triggered by spermatozoa and seminal plasma. *Int J Mol Sci* 20(3):513
- Archibald AL, Bolund L, Churcher C, Fredholm M, Groenen MA, Harlizius B, Lee KT, Milan D, Rogers J, Rothschild MF, Uenishi H, Wang J, School LB, Swine Genome Sequencing C (2010) Pig genome sequence—analysis and publication strategy. *BMC Genomics* 11:438
- Ash K, Berger T, Horner CM, Calvert CC (1995) Identification of porcine sperm plasma membrane proteins that may play a role in sperm-egg fusion. *Zygote* 3(2):163–170
- Austin CR (1951) Observations on the penetration of the sperm in the mammalian egg. *Aust J Sci Res B* 4(4):581–596
- Awda BJ, Buhner MM (2010) Extracellular signal-regulated kinases (ERKs) pathway and reactive oxygen species regulate tyrosine phosphorylation in capacitating boar spermatozoa. *Biol Reprod* 83(5):750–758
- Aydin H, Sultana A, Li S, Thavalingam A, Lee JE (2016) Molecular architecture of the human sperm IZUMO1 and egg JUNO fertilization complex. *Nature* 534(7608):562–565
- Azad N, Nazarian H, Nazari L, Ghaffari Novin M, Piryaei A, Heidari MH, Masteri Farahani R, Sadjadpour SS (2018) Evaluation of PAWP and PLC γ Expression in infertile men with previous ICSI fertilization failure. *Urol J* 15(3):116–121
- Bailey JL (2010) Factors regulating sperm capacitation. *Syst Biol Reprod Med* 56(5):334–348
- Balhorn R (2007) The protamine family of sperm nuclear proteins. *Genome Biol* 8(9):227
- Berger T, Nitta BJ, Ducolomb Y, Betancourt M (2011) Interaction of potential porcine sperm ligands with the oocyte plasma membrane. *Reprod Domest Anim* 46(1):15–20
- Bi M, Hickox JR, Winfrey VP, Olson GE, Hardy DM (2003) Processing, localization and binding activity of zonadhesin suggest a function in sperm adhesion to the zona pellucida during exocytosis of the acrosome. *Biochem J* 375(Pt 2):477–488

- Bianchi E, Doe B, Goulding D, Wright GJ (2014) Juno is the egg Izumo receptor and is essential for mammalian fertilization. *Nature* 508(7497):483–487
- Bieniek JM, Drabovich AP, Lo KC (2016) Seminal biomarkers for the evaluation of male infertility. *Asian J Androl* 18(3):426–433
- Bigler D, Chen M, Waters S, White JM (1997) A model for sperm-egg binding and fusion based on ADAMs and integrins. *Trends Cell Biol* 7(6):220–225
- Bjorndahl L, Kvist U (2003) Sequence of ejaculation affects the spermatozoon as a carrier and its message. *Reprod BioMed Online* 7(4):440–448
- Bjorndahl L, Kvist U (2010) Human sperm chromatin stabilization: a proposed model including zinc bridges. *Mol Hum Reprod* 16(1):23–29
- Bleil JD, Wassarman PM (1980) Structure and function of the zona pellucida: identification and characterization of the proteins of the mouse oocyte's zona pellucida. *Dev Biol* 76(1):185–202
- Blobel CP (1999) Roles of metalloprotease-disintegrins in cell-cell interactions, in neurogenesis, and in the cleavage of TNF α . *Adv Dev Biochem* 5:165–198
- Boe-Hansen GB, Christensen P, Vibjerg D, Nielsen MB, Hedeboe AM (2008) Sperm chromatin structure integrity in liquid stored boar semen and its relationships with field fertility. *Theriogenology* 69(6):728–736
- Boerke A, Tsai PS, Garcia-Gil N, Brewis IA, Gadella BM (2008) Capacitation-dependent reorganization of microdomains in the apical sperm head plasma membrane: functional relationship with zona binding and the zona-induced acrosome reaction. *Theriogenology* 70(8):1188–1196
- Borsuk E, Manka R (1988) Behavior of sperm nuclei in intact and bisected metaphase II mouse oocytes fertilized in the presence of colcemid. *Gamete Res* 20(3):365–376
- Buffone MG, Wertheimer EV, Visconti PE, Krapf D (2014) Central role of soluble adenylyl cyclase and cAMP in sperm physiology. *Biochim Biophys Acta* 1842(12 Pt B):2610–2620
- Burkart AD, Xiong B, Baibakov B, Jimenez-Movilla M, Dean J (2012) Ovastacin, a cortical granule protease, cleaves ZP2 in the zona pellucida to prevent polyspermy. *J Cell Biol* 197(1):37–44
- Calvete JJ, Mann K, Schafer W, Raida M, Sanz L, Topfer-Petersen E (1995) Boar spermadhesin PSP-II: location of posttranslational modifications, heterodimer formation with PSP-I glycoforms and effect of dimerization on the ligand-binding capabilities of the subunits. *FEBS Lett* 365(2–3):179–182
- Calvete JJ, Raida M, Gentzel M, Urbanke C, Sanz L, Topfer-Petersen E (1997) Isolation and characterization of heparin- and phosphorylcholine-binding proteins of boar and stallion seminal plasma. Primary structure of porcine pB1. *FEBS Lett* 407(2):201–206
- Carmona E, Weerachayanukul W, Soboloff T, Fluharty AL, White D, Promdee L, Ekker M, Berger T, Buhr M, Tanphaichitr N (2002) Arylsulfatase a is present on the pig sperm surface and is involved in sperm-zona pellucida binding. *Dev Biol* 247(1):182–196
- Carrasco LC, Romar R, Aviles M, Gadea J, Coy P (2008) Determination of glycosidase activity in porcine oviductal fluid at the different phases of the estrous cycle. *Reproduction* 136(6):833–842
- Centurion F, Vazquez JM, Calvete JJ, Roca J, Sanz L, Parrilla I, Garcia EM, Martinez EA (2003) Influence of porcine spermadhesins on the susceptibility of boar spermatozoa to high dilution. *Biol Reprod* 69(2):640–646
- Chalbi M, Barraud-Lange V, Ravaux B, Howan K, Rodriguez N, Soule P, Ndzoudi A, Boucheix C, Rubinstein E, Wolf JP, Ziyat A, Perez E, Pincet F, Gourier C (2014) Binding of sperm protein Izumo1 and its egg receptor Juno drives Cd9 accumulation in the intercellular contact area prior to fusion during mammalian fertilization. *Development* 141(19):3732–3739
- Chang MC (1951) Fertilizing capacity of spermatozoa deposited into the fallopian tubes. *Nature* 168(4277):697–698
- Chang H, Suarez SS (2011) Two distinct Ca(2+) signaling pathways modulate sperm flagellar beating patterns in mice. *Biol Reprod* 85(2):296–305
- Cohen DJ, Ellerman DA, Cuasnicu PS (2000) Mammalian sperm-egg fusion: evidence that epididymal protein DE plays a role in mouse gamete fusion. *Biol Reprod* 63(2):462–468
- Cohen DJ, Ellerman DA, Busso D, Morgenfeld MM, Piazza AD, Hayashi M, Young ET, Kasahara M, Cuasnicu PS (2001) Evidence that human epididymal protein ARP plays a role in gamete fusion through complementary sites on the surface of the human egg. *Biol Reprod* 65(4):1000–1005
- Coonrod SA, Herr JC, Westhusin ME (1996) Inhibition of bovine fertilization in vitro by antibodies to SP-10. *J Reprod Fertil* 107(2):287–297
- Cornwall GA, Tulsiani DR, Orgebin-Crist MC (1991) Inhibition of the mouse sperm surface alpha-D-mannosidase inhibits sperm-egg binding in vitro. *Biol Reprod* 44(5):913–921
- Cross NL (1998) Role of cholesterol in sperm capacitation. *Biol Reprod* 59(1):7–11
- Davidova N, Jonakova V, Manaskova-Postlerova P (2009) Expression and localization of acrosin inhibitor in boar reproductive tract. *Cell Tissue Res* 338(2):303–311
- Davis BK (1981) Timing of fertilization in mammals: sperm cholesterol/phospholipid ratio as a determinant of the capacitation interval. *Proc Natl Acad Sci U S A* 78(12):7560–7564
- Day BN (2000) Reproductive biotechnologies: current status in porcine reproduction. *Anim Reprod Sci* 60–61:161–172
- Day AE, Quilter CR, Sargent CA, Mileham AJ (2003) Chromosomal mapping, sequence and transcription analysis of the porcine fertilin beta gene (ADAM2). *Anim Genet* 34(5):375–378
- De Lazari FL, Sontag ER, Schneider A, Moura AAA, Vasconcelos FR, Nagano CS, Mattos RC, Jobim MIM, Bustamante-Filho IC (2019) Seminal plasma proteins and their relationship with sperm motility and morphology in boars. *Andrologia* 51(4):e13222
- Didion BA, Kaspersom KM, Wixon RL, Evenson DP (2009) Boar fertility and sperm chromatin structure status: a retrospective report. *J Androl* 30(6):655–660
- Dostalova Z, Calvete JJ, Sanz L, Topfer-Petersen E (1994) Quantitation of boar spermadhesins in accessory sex gland fluids and on the surface of epididymal, ejaculated and capacitated spermatozoa. *Biochim Biophys Acta* 1200(1):48–54
- Dostalova Z, Calvete JJ, Topfer-Petersen E (1995) Interaction of non-aggregated boar AWN-1 and AQN-3 with phospholipid matrices. A model for coating of spermadhesins to the sperm surface. *Biol Chem Hoppe Seyler* 376(4):237–242
- Drabovich AP, Saraon P, Jarvi K, Diamandis EP (2014) Seminal plasma as a diagnostic fluid for male reproductive system disorders. *Nat Rev Urol* 11(5):278–288
- Druart X, Rickard JP, Mactier S, Kohnke PL, Kershaw-Young CM, Bathgate R, Gibb Z, Crossett B, Tsikis G, Labas V, Harichaux G, Grupen CG, de Graaf SP (2013) Proteomic characterization and cross species comparison of mammalian seminal plasma. *J Proteome* 91:13–22
- Dube C, Leclerc P, Baba T, Reyes-Moreno C, Bailey JL (2005) The proacrosin binding protein, sp32, is tyrosine phosphorylated during capacitation of pig sperm. *J Androl* 26(4):519–528
- Duncan FE, Que EL, Zhang N, Feinberg EC, O'Halloran TV, Woodruff TK (2016) The zinc spark is an inorganic signature of human egg activation. *Sci Rep* 6:24737
- Ekhlas-Hundrieser M, Gohr K, Wagner A, Tsolova M, Petrunkina A, Topfer-Petersen E (2005) Spermadhesin AQN1 is a candidate receptor molecule involved in the formation of the oviductal sperm reservoir in the pig. *Biol Reprod* 73(3):536–545

- Ellerman DA, Da Ros VG, Cohen DJ, Busso D, Morgenfeld MM, Cuasnicu PS (2002) Expression and structure-function analysis of de, a sperm cysteine-rich secretory protein that mediates gamete fusion. *Biol Reprod* 67(4):1225–1231
- Elliott RM, Lloyd RE, Fazeli A, Sostaric E, Georgiou AS, Satake N, Watson PF, Holt WV (2009) Effects of HSPA8, an evolutionarily conserved oviductal protein, on boar and bull spermatozoa. *Reproduction* 137(2):191–203
- Ensslin M, Calvete JJ, Thole HH, Sierralta WD, Adermann K, Sanz L, Topfer-Petersen E (1995) Identification by affinity chromatography of boar sperm membrane-associated proteins bound to immobilized porcine zona pellucida. Mapping of the phosphorylethanolamine-binding region of spermadhesin AWN. *Biol Chem Hoppe Seyler* 376(12):733–738
- Ensslin M, Vogel T, Calvete JJ, Thole HH, Schmidtke J, Matsuda T, Topfer-Petersen E (1998) Molecular cloning and characterization of P47, a novel boar sperm-associated zona pellucida-binding protein homologous to a family of mammalian secretory proteins. *Biol Reprod* 58(4):1057–1064
- Evans JP (2012) Sperm-egg interaction. *Annu Rev Physiol* 74:477–502
- Fabrega A, Guyonnet B, Dacheux JL, Gatti JL, Puigmule M, Bonet S, Pinart E (2011) Expression, immunolocalization and processing of fertilins ADAM-1 and ADAM-2 in the boar (*Sus domesticus*) spermatozoa during epididymal maturation. *Reprod Biol Endocrinol* 9: 96
- Fazeli A, Duncan AE, Watson PF, Holt WV (1999) Sperm-oviduct interaction: induction of capacitation and preferential binding of uncapacitated spermatozoa to oviductal epithelial cells in porcine species. *Biol Reprod* 60(4):879–886
- Fazeli A, Elliott RM, Duncan AE, Moore A, Watson PF, Holt WV (2003) In vitro maintenance of boar sperm viability by a soluble fraction obtained from oviductal apical plasma membrane preparations. *Reproduction* 125(4):509–517
- Ferrer M, Rodriguez H, Zara L, Yu Y, Xu W, Oko R (2012) MMP2 and acrosin are major proteinases associated with the inner acrosomal membrane and may cooperate in sperm penetration of the zona pellucida during fertilization. *Cell Tissue Res* 349(3):881–895
- Flesch FM, Gadella BM (2000) Dynamics of the mammalian sperm plasma membrane in the process of fertilization. *Biochim Biophys Acta* 1469(3):197–235
- Flesch FM, Colenbrander B, van Golde LM, Gadella BM (1999) Capacitation induces tyrosine phosphorylation of proteins in the boar sperm plasma membrane. *Biochem Biophys Res Commun* 262(3):787–792
- Floman HM, Ducibella T (2006) Fertilization in mammals. In: Neill JD (ed) *Knobil and Neill's physiology of reproduction*, vol. 1. Elsevier Academic Press, Inc., New York, pp 55–112
- Floman HM, Fissore RA (2015) Fertilization in mammals. In: Plant TM, Zeleznik AJ (eds) *Knobil and Neill's physiology of reproduction*, vol. 1. Academic Press, New York, pp 149–196
- Fraser LR (1984) Mouse sperm capacitation in vitro involves loss of a surface-associated inhibitory component. *J Reprod Fertil* 72(2):373–384
- Fraser LR (2008) The role of small molecules in sperm capacitation. *Theriogenology* 70(8):1356–1359
- Fraser LR, Harrison RA, Herod JE (1990) Characterization of a decapacitation factor associated with epididymal mouse spermatozoa. *J Reprod Fertil* 89(1):135–148
- Frayne J, Hall L (1999) Mammalian sperm-egg recognition: does fertilin beta have a major role to play? *Bioessays* 21(3):183–187
- Gadella BM, Boerke A (2016) An update on post-ejaculatory remodeling of the sperm surface before mammalian fertilization. *Theriogenology* 85(1):113–124
- Gadella BM, Harrison RA (2000) The capacitating agent bicarbonate induces protein kinase A-dependent changes in phospholipid transbilayer behavior in the sperm plasma membrane. *Development* 127(11):2407–2420
- Gadella BM, Harrison RA (2002) Capacitation induces cyclic adenosine 3',5'-monophosphate-dependent, but apoptosis-unrelated, exposure of aminophospholipids at the apical head plasma membrane of boar sperm cells. *Biol Reprod* 67(1):340–350
- Gadella BM, Tsai PS, Boerke A, Brewis IA (2008) Sperm head membrane reorganisation during capacitation. *Int J Dev Biol* 52(5–6): 473–480
- Gangwar DK, Atreja SK (2015) Signalling events and associated pathways related to the mammalian sperm capacitation. *Reprod Domest Anim* 50(5):705–711
- Geisert RD, Sutovsky P, Lucy M, Bartol FF, Meyer AE (2019) Reproductive physiology of swine. In: Bazer FW, Lamb GC, Wu G (eds) *Animal agriculture 1st edition: challenges, innovations, and sustainability*. Academic Press, Cambridge, pp 263–282
- Gervasi MG, Visconti PE (2016) Chang's meaning of capacitation: a molecular perspective. *Mol Reprod Dev* 83(10):860–874
- van Gestel RA, Brewis IA, Ashton PR, Helms JB, Brouwers JF, Gadella BM (2005) Capacitation-dependent concentration of lipid rafts in the apical ridge head area of porcine sperm cells. *Mol Hum Reprod* 11(8):583–590
- van Gestel RA, Brewis IA, Ashton PR, Brouwers JF, Gadella BM (2007) Multiple proteins present in purified porcine sperm apical plasma membranes interact with the zona pellucida of the oocyte. *Mol Hum Reprod* 13(7):445–454
- Ghersevich S, Massa E, Zumoffen C (2015) Oviductal secretion and gamete interaction. *Reproduction* 149(1):R1–r14
- Gilany K, Minai-Tehrani A, Savadi-Shiraz E, Rezaadoost H, Lakpour N (2015) Exploring the human seminal plasma proteome: an unexplored gold mine of biomarker for male infertility and male reproduction disorder. *J Reprod Infertil* 16(2):61–71
- Glotzer M, Murray AW, Kirschner MW (1991) Cyclin is degraded by the ubiquitin pathway. *Nature* 349(6305):132–138
- Gonzalez-Cadavid V, Martins JA, Moreno FB, Andrade TS, Santos AC, Monteiro-Moreira AC, Moreira RA, Moura AA (2014) Seminal plasma proteins of adult boars and correlations with sperm parameters. *Theriogenology* 82(5):697–707
- Green CE, Bredl J, Holt WV, Watson PF, Fazeli A (2001) Carbohydrate mediation of boar sperm binding to oviductal epithelial cells in vitro. *Reproduction* 122(2):305–315
- Gualtieri R, Talevi R (2000) In vitro-cultured bovine oviductal cells bind acrosome-intact sperm and retain this ability upon sperm release. *Biol Reprod* 62(6):1754–1762
- Hachem A, Godwin J, Ruas M, Lee HC, Ferrer Buitrago M, Ardestani G, Bassett A, Fox S, Navarrete F, de Sutter P, Heindryckx B, Fissore R, Parrington J (2017) PLCzeta is the physiological trigger of the Ca(2+) oscillations that induce embryogenesis in mammals but conception can occur in its absence. *Development* 144(16):2914–2924
- Hamilton LE, Suzuki J, Acteau G, Shi M, Xu W, Meinsohn MC, Sutovsky P, Oko R (2018) WBP2 shares a common location in mouse spermatozoa with WBP2NL/PAWP and like its descendent is a candidate mouse oocyte-activating factor. *Biol Reprod* 99(6): 1171–1183
- Hamilton LE, Suzuki J, Aguila L, Meinsohn MC, Smith OE, Protopoulos N, Xu W, Sutovsky P, Oko R (2019) Sperm-borne glutathione-S-transferase omega 2 accelerates the nuclear decondensation of spermatozoa during fertilization in mice. *Biol Reprod* 101(2):368–376
- Hao Y, Mathialagan N, Walters E, Mao J, Lai L, Becker D, Li W, Critser J, Prather RS (2006) Osteopontin reduces polyspermy during in vitro fertilization of porcine oocytes. *Biol Reprod* 75(5):726–733
- Harayama H (2003) Viability and protein phosphorylation patterns of boar spermatozoa agglutinated by treatment with a cell-permeable cyclic adenosine 3',5'-monophosphate analog. *J Androl* 24(6):831–842

- Harayama H (2013) Roles of intracellular cyclic AMP signal transduction in the capacitation and subsequent hyperactivation of mouse and boar spermatozoa. *J Reprod Dev* 59(5):421–430
- Harayama H (2018) Flagellar hyperactivation of bull and boar spermatozoa. *Reprod Med Biol* 17(4):442–448
- Harayama H, Nakamura K (2008) Changes of PKA and PDK1 in the principal piece of boar spermatozoa treated with a cell-permeable cAMP analog to induce flagellar hyperactivation. *Mol Reprod Dev* 75(9):1396–1407
- Harayama H, Muroga M, Miyake M (2004) A cyclic adenosine 3',5'-monophosphate-induced tyrosine phosphorylation of Syk protein tyrosine kinase in the flagella of boar spermatozoa. *Mol Reprod Dev* 69(4):436–447
- Harayama H, Noda T, Ishikawa S, Shidara O (2012) Relationship between cyclic AMP-dependent protein tyrosine phosphorylation and extracellular calcium during hyperactivation of boar spermatozoa. *Mol Reprod Dev* 79(10):727–739
- Hardy DM, Garbers DL (1995) A sperm membrane protein that binds in a species-specific manner to the egg extracellular matrix is homologous to von Willebrand factor. *J Biol Chem* 270(44):26025–26028
- Harris JD, Hibler DW, Fontenot GK, Hsu KT, Yurewicz EC, Sacco AG (1994) Cloning and characterization of zona pellucida genes and cDNAs from a variety of mammalian species: the ZPA, ZPB and ZPC gene families. *DNA Seq* 4(6):361–393
- Hayasaka S, Terada Y, Inoue N, Okabe M, Yaegashi N, Okamura K (2007) Positive expression of the immunoglobulin superfamily protein IZUMO on human sperm of severely infertile male patients. *Fertil Steril* 88(1):214–216
- Hedrick JL, Wardrip NJ (1986) Isolation of the zona pellucida and purification of its glycoprotein families from pig oocytes. *Anal Biochem* 157(1):63–70
- Hedrick JL, Wardrip NJ (1987) On the macromolecular composition of the zona pellucida from porcine oocytes. *Dev Biol* 121(2):478–488
- Hemler ME (1998) Integrin associated proteins. *Curr Opin Cell Biol* 10(5):578–585
- Herr JC, Wright RM, John E, Foster J, Kays T, Flickinger CJ (1990) Identification of human acrosomal antigen SP-10 in primates and pigs. *Biol Reprod* 42(2):377–382
- Hickox JR, Bi M, Hardy DM (2001) Heterogeneous processing and zona pellucida binding activity of pig zonadhesin. *J Biol Chem* 276(44):41502–41509
- Hino T, Muro Y, Tamura-Nakano M, Okabe M, Tateno H, Yanagimachi R (2016) The behavior and acrosomal status of mouse spermatozoa in vitro, and within the oviduct during fertilization after natural mating. *Biol Reprod* 95(3):50
- Huang TT Jr, Yanagimachi R (1985) Inner acrosomal membrane of mammalian spermatozoa: its properties and possible functions in fertilization. *Am J Anat* 174(3):249–268
- Hughes DC, Barratt CL (1999) Identification of the true human orthologue of the mouse *Zp1* gene: evidence for greater complexity in the mammalian zona pellucida? *Biochim Biophys Acta* 1447(2–3):303–306
- Hunter RH (1996) Ovarian control of very low sperm/egg ratios at the commencement of mammalian fertilisation to avoid polyspermy. *Mol Reprod Dev* 44(3):417–422
- Hunter RH, Rodriguez-Martinez H (2004) Capacitation of mammalian spermatozoa in vivo, with a specific focus on events in the Fallopian tubes. *Mol Reprod Dev* 67(2):243–250
- Huovila AP, Almeida EA, White JM (1996) ADAMs and cell fusion. *Curr Opin Cell Biol* 8(5):692–699
- Hurtado de Llera A, Martin-Hidalgo D, Gil MC, Garcia-Marin LJ, Bragado MJ (2014) The calcium/CaMKK α /beta and the cAMP/PKA pathways are essential upstream regulators of AMPK activity in boar spermatozoa. *Biol Reprod* 90(2):29
- Hurtado de Llera A, Martin-Hidalgo D, Gil MC, Garcia-Marin LJ, Bragado MJ (2016) New insights into transduction pathways that regulate boar sperm function. *Theriogenology* 85(1):12–20
- Ickowicz D, Finkelstein M, Breitbart H (2012) Mechanism of sperm capacitation and the acrosome reaction: role of protein kinases. *Asian J Androl* 14(6):816–821
- Inoue N, Ikawa M, Isotani A, Okabe M (2005) The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. *Nature* 434(7030):234–238
- Inoue N, Satouh Y, Ikawa M, Okabe M, Yanagimachi R (2011) Acrosome-reacted mouse spermatozoa recovered from the perivitelline space can fertilize other eggs. *Proc Natl Acad Sci U S A* 108(50):20008–20011
- Jaffe LA, Gould M (1985) Polyspermy-preventing mechanisms. In: Metz CB, Monroy A (eds) *Biology of Fertilization*. Academic Press, New York, pp 223–250
- Jankovicova J, Secova P, Manaskova-Postlerova P, Simonik O, Frolikova M, Chmelikova E, Horovska L, Michalkova K, Dvorakova-Hortova K, Antalikova J (2019) Detection of CD9 and CD81 tetraspanins in bovine and porcine oocytes and embryos. *Int J Biol Macromol* 123:931–938
- Jansen RP (1980) Cyclic changes in the human fallopian tube isthmus and their functional importance. *Am J Obstet Gynecol* 136(3):292–308
- Jelinkova P, Manaskova P, Ticha M, Jonakova V (2003) Proteinase inhibitors in aggregated forms of boar seminal plasma proteins. *Int J Biol Macromol* 32(3–5):99–107
- Jelinkova P, Liberda J, Manaskova P, Ryslava H, Jonakova V, Ticha M (2004) Mannan-binding proteins from boar seminal plasma. *J Reprod Immunol* 62(1–2):167–182
- Jin YZ, Dacheux F, Dacheux JL, Bannai S, Sugita Y, Okamura N (1999) Purification and properties of major alpha-D-mannosidase in the luminal fluid of porcine epididymis. *Biochim Biophys Acta* 1432(2):382–392
- Jin M, Fujiwara E, Kakiuchi Y, Okabe M, Satouh Y, Baba SA, Chiba K, Hirohashi N (2011) Most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the zona pellucida during in vitro fertilization. *Proc Natl Acad Sci U S A* 108(12):4892–4896
- Jodar M, Soler-Ventura A, Oliva R (2017) Semen proteomics and male infertility. *J Proteome* 162:125–134
- Johansson M, Tienthai P, Rodriguez-Martinez H (2000) Histochemistry and ultrastructure of the intraluminal mucus in the sperm reservoir of the pig oviduct. *J Reprod Dev* 46(3):10
- Jonakova V, Ticha M (2004) Boar seminal plasma proteins and their binding properties. *Collect Czechoslov Chem Commun* 69:461–475
- Jonakova V, Sanz L, Calvete JJ, Henschen A, Cechova D, Topfer-Petersen E (1991) Isolation and biochemical characterization of a zona pellucida-binding glycoprotein of boar spermatozoa. *FEBS Lett* 280(1):183–186
- Jonakova V, Calvete JJ, Mann K, Schafer W, Schmid ER, Topfer-Petersen E (1992) The complete primary structure of three isoforms of a boar sperm-associated acrosin inhibitor. *FEBS Lett* 297(1–2):147–150
- Jonakova V, Kraus M, Veselsky L, Cechova D, Bezouska K, Ticha M (1998) Spermadhesins of the AQN and AWN families, DQH sperm surface protein and HNK protein in the heparin-binding fraction of boar seminal plasma. *J Reprod Fertil* 114(1):25–34
- Jonakova V, Manaskova P, Kraus M, Liberda J, Ticha M (2000) Sperm surface proteins in mammalian fertilization. *Mol Reprod Dev* 56(2 Suppl):275–277
- Jonakova V, Manaskova P, Ticha M (2007) Separation, characterization and identification of boar seminal plasma proteins. *J Chromatogr B Anal Technol Biomed Life Sci* 849(1–2):307–314
- Jonakova V, Jonak J, Ticha M (2010) Proteomics of male seminal plasma. In: Jiang Z, Ott TL (eds) *Reproductive genomics in domestic animals*. Blackwell Publishing, Oxford, UK, pp 339–368

- Kadirvel G, Machado SA, Korneli C, Collins E, Miller P, Bess KN, Aoki K, Tiemeyer M, Bovin N, Miller DJ (2012) Porcine sperm bind to specific 6-sialylated biantennary glycans to form the oviduct reservoir. *Biol Reprod* 87(6):147
- Katayama M, Sutovsky P, Yang BS, Cantley T, Rieke A, Farwell R, Oko R, Day BN (2005) Increased disruption of sperm plasma membrane at sperm immobilization promotes dissociation of perinuclear theca from sperm chromatin after intracytoplasmic sperm injection in pigs. *Reproduction* 130(6):907–916
- Katayama M, Rieke A, Cantley T, Murphy C, Dowell L, Sutovsky P, Day BN (2007) Improved fertilization and embryo development resulting in birth of live piglets after intracytoplasmic sperm injection and in vitro culture in a cysteine-supplemented medium. *Theriogenology* 67(4):835–847
- Katoh Y, Takebayashi K, Kikuchi A, Iki A, Kikuchi K, Tamba M, Kawashima A, Matsuda M, Okamura N (2014) Porcine sperm capacitation involves tyrosine phosphorylation and activation of aldose reductase. *Reproduction* 148(4):389–401
- Kelly RW, Critchley HO (1997) Immunomodulation by human seminal plasma: a benefit for spermatozoon and pathogen? *Hum Reprod* 12(10):2200–2207
- Kerns K, Morales P, Sutovsky P (2016) Regulation of sperm capacitation by the 26S proteasome: an emerging new paradigm in spermatology. *Biol Reprod* 94(5):117
- Kerns K, Zigo M, Drobnis EZ, Sutovsky M, Sutovsky P (2018a) Zinc ion flux during mammalian sperm capacitation. *Nat Commun* 9(1):2061
- Kerns K, Zigo M, Sutovsky P (2018b) Zinc: a necessary ion for mammalian sperm fertilization competency. *Int J Mol Sci* 19(12)
- Kervancioglu ME, Djahanbakhch O, Aitken RJ (1994) Epithelial cell coculture and the induction of sperm capacitation. *Fertil Steril* 61(6):1103–1108
- Kim E, Yamashita M, Nakanishi T, Park KE, Kimura M, Kashiwabara S, Baba T (2006) Mouse sperm lacking ADAM1b/ADAM2 fertilin can fuse with the egg plasma membrane and effect fertilization. *J Biol Chem* 281(9):5634–5639
- Kim E, Park KE, Kim JS, Baek DC, Lee JW, Lee SR, Kim MS, Kim SH, Kim CS, Koo DB, Kang HS, Ryoo ZY, Chang KT (2009) Importance of the porcine ADAM3 disintegrin domain in sperm-egg interaction. *J Reprod Dev* 55(2):156–162
- Kim E, Kim JS, Lee Y, Song BS, Sim BW, Kim SU, Saitoh T, Yazawa H, Nunoya T, Chang KT (2013) Molecular cloning, characterization of porcine IZUMO1, an IgSF family member. *Reprod Domest Anim* 48(1):90–97
- Knox RV (2015) Recent advancements in the hormonal stimulation of ovulation in swine. *Vet Med (Auckl)* 6:309–320
- Kongmanas K, Kruevaisayawan H, Saewu A, Sugeng C, Fernandes J, Souda P, Angel JB, Faull KF, Aitken RJ, Whitelegge J, Hardy D, Berger T, Baker MA, Tanphaichitr N (2015) Proteomic characterization of pig sperm anterior head plasma membrane reveals roles of acrosomal proteins in ZP3 binding. *J Cell Physiol* 230(2):449–463
- Kopečný V, Pavlok A (1975) Incorporation of Arginine-3H into chromatin of mouse eggs shortly after sperm penetration. *Histochemistry* 45(4):341–345
- Kudo K, Yonezawa N, Katsumata T, Aoki H, Nakano M (1998) Localization of carbohydrate chains of pig sperm ligand in the glycoprotein ZPB of egg zona pellucida. *Eur J Biochem* 252(3):492–499
- Kuno M, Yonezawa N, Amari S, Hayashi M, Ono Y, Kiss L, Sonohara K, Nakano M (2000) The presence of a glycosyl phosphatidylinositol-anchored alpha-mannosidase in boar sperm. *IUBMB Life* 49(6):485–489
- La Spina FA, Puga Molina LC, Romarowski A, Vitale AM, Falzone TL, Krapf D, Hirohashi N, Buffone MG (2016) Mouse sperm begin to undergo acrosomal exocytosis in the upper isthmus of the oviduct. *Dev Biol* 411(2):172–182
- Larson JL, Miller DJ (1997) Sperm from a variety of mammalian species express beta1,4-galactosyltransferase on their surface. *Biol Reprod* 57(2):442–453
- Lay KM, Oshiro R, Arasaki C, Ashizawa K, Tatemoto H (2011) Role of acidification elicited by sialylation and sulfation of zona glycoproteins during oocyte maturation in porcine sperm-zona pellucida interactions. *J Reprod Dev* 57(6):744–751
- Lea IA, Sivashanmugam P, O'Rand MG (2001) Zonadhesin: characterization, localization, and zona pellucida binding. *Biol Reprod* 65(6):1691–1700
- Lee JW, Tian XC, Yang X (2003) Failure of male pronucleus formation is the major cause of lack of fertilization and embryo development in pig oocytes subjected to intracytoplasmic sperm injection. *Biol Reprod* 68(4):1341–1347
- Leemans B, Stout TAE, De Schauwer C, Heras S, Nelis H, Hoogewijs M, Van Soom A, Gadella BM (2019) Update on mammalian sperm capacitation: How much does the horse differ from other species? *Reproduction* 157(5):R181–R197
- Li YH, Hou Y, Ma W, Yuan JX, Zhang D, Sun QY, Wang WH (2004) Localization of CD9 in pig oocytes and its effects on sperm-egg interaction. *Reproduction* 127(2):151–157
- Li H, Hung PH, Suarez SS (2015) Ejaculated mouse sperm enter cumulus-oocyte complexes more efficiently in vitro than epididymal sperm. *PLoS One* 10(5):e0127753
- Liberda J, Manaskova P, Prelovská L, Ticha M, Jonakova V (2006) Saccharide-mediated interactions of boar sperm surface proteins with components of the porcine oviduct. *J Reprod Immunol* 71(2):112–125
- Linfor J, Berger T (2000) Potential role of alpha and beta1 integrins as oocyte adhesion molecules during fertilization in pigs. *J Reprod Fertil* 120(1):65–72
- Litvin TN, Kamenetsky M, Zarifyan A, Buck J, Levin LR (2003) Kinetic properties of “soluble” adenylyl cyclase. Synergism between calcium and bicarbonate. *J Biol Chem* 278(18):15922–15926
- Lorenzetti D, Poirier C, Zhao M, Overbeek PA, Harrison W, Bishop CE (2014) A transgenic insertion on mouse chromosome 17 inactivates a novel immunoglobulin superfamily gene potentially involved in sperm-egg fusion. *Mamm Genome* 25(3–4):141–148
- Luo S, Valencia CA, Zhang J, Lee NC, Slone J, Gui B, Wang X, Li Z, Dell S, Brown J, Chen SM, Chien YH, Hwu WL, Fan PC, Wong LJ, Atwal PS, Huang T (2018) Biparental inheritance of mitochondrial DNA in humans. *Proc Natl Acad Sci U S A* 115(51):13039–13044
- Machado SA, Kadirvel G, Daigneault BW, Korneli C, Miller P, Bovin N, Miller DJ (2014) LewisX-containing glycans on the porcine oviductal epithelium contribute to formation of the sperm reservoir. *Biol Reprod* 91(6):140
- Manaskova P, Jonakova V (2008) Localization of porcine seminal plasma (PSP) proteins in the boar reproductive tract and spermatozoa. *J Reprod Immunol* 78(1):40–48
- Manaskova P, Liberda J, Ticha M, Jonakova V (2000) Aggregated and monomeric forms of proteins in boar seminal plasma: characterization and binding properties. *Folia Biol (Praha)* 46(4):143–151
- Manaskova P, Balinova P, Kraus M, Ticha M, Jonakova V (2003) Mutual interactions of boar seminal plasma proteins studied by immunological and chromatographic methods. *Am J Reprod Immunol* 50(5):399–410
- Manaskova P, Peknicova J, Elzeinova F, Ticha M, Jonakova V (2007) Origin, localization and binding abilities of boar DQH sperm surface protein tested by specific monoclonal antibodies. *J Reprod Immunol* 74(1–2):103–113
- Mao J, O'Gorman C, Sutovsky M, Zigo M, Wells KD, Sutovsky P (2018) Ubiquitin A-52 residue ribosomal protein fusion product 1 (Uba52) is essential for preimplantation embryo development. *Biol Open* 7(10):bio035717

- Marini PE, Cabada MO (2003) One step purification and biochemical characterization of a spermatozoa-binding protein from porcine oviductal epithelial cells. *Mol Reprod Dev* 66(4):383–390
- Martinez FA (2005) Studies on the interaction of chromatin-unstable boar sperm with the female reproductive tract. In: Institute of Reproductive Medicine. University of Veterinary Medicine Hannover, Hannover, Germany, p 119
- Mattioli M, Lucidi P, Barboni B (1998) Expanded cumuli induce acrosome reaction in boar sperm. *Mol Reprod Dev* 51(4):445–453
- McLay DW, Clarke HJ (2003) Remodelling the paternal chromatin at fertilization in mammals. *Reproduction* 125(5):625–633
- McLeskey SB, Dowds C, Carballada R, White RR, Saling PM (1998) Molecules involved in mammalian sperm-egg interaction. *Int Rev Cytol* 177:57–113
- Menino AR Jr, Wright RW Jr (1979) Characterization of porcine oocyte zonae pellucidae by polyacrylamide gel electrophoresis. *Proc Soc Exp Biol Med* 160(4):449–452
- Miles EL, O’Gorman C, Zhao J, Samuel M, Walters E, Yi YJ, Sutovsky M, Prather RS, Wells KD, Sutovsky P (2013) Transgenic pig carrying green fluorescent proteasomes. *Proc Natl Acad Sci U S A* 110(16):6334–6339
- Miyado K, Yamada G, Yamada S, Hasuwa H, Nakamura Y, Ryu F, Suzuki K, Kosai K, Inoue K, Ogura A, Okabe M, Mekada E (2000) Requirement of CD9 on the egg plasma membrane for fertilization. *Science* 287(5451):321–324
- Moore HD, Bedford JM (1983) The interaction of mammalian gametes in the female. In: Hartmann JF (ed) Mechanism and control of animal fertilization. Academic Press, New York, pp 453–497
- Morales P, Diaz ES, Kong M (2007) Proteasome activity and its relationship with protein phosphorylation during capacitation and acrosome reaction in human spermatozoa. *Soc Reprod Fertil Suppl* 65:269–273
- Mori E, Baba T, Iwamatsu A, Mori T (1993) Purification and characterization of a 38-kDa protein, sp38, with zona pellucida-binding property from porcine epididymal sperm. *Biochem Biophys Res Commun* 196(1):196–202
- Mori E, Kashiwabara S, Baba T, Inagaki Y, Mori T (1995) Amino acid sequences of porcine Sp38 and proacrosin required for binding to the zona pellucida. *Dev Biol* 168(2):575–583
- Mori E, Fukuda H, Imajoh-Ohmi S, Mori T, Takasaki S (2012) Purification of N-acetylglucosamine-binding activity from the porcine sperm membrane: possible involvement of an ADAM complex in the carbohydrate-binding activity of sperm. *J Reprod Dev* 58(1):117–125
- Mtango NR, Sutovsky M, Susor A, Zhong Z, Latham KE, Sutovsky P (2011) Essential role of maternal UCHL1 and UCHL3 in fertilization and preimplantation embryo development. *J Cell Physiol* 227(4):1592–1603
- Muro Y, Hasuwa H, Isotani A, Miyata H, Yamagata K, Ikawa M, Yanagimachi R, Okabe M (2016) Behavior of mouse spermatozoa in the female reproductive tract from soon after mating to the beginning of fertilization. *Biol Reprod* 94(4):80
- Murray SC, Smith TT (1997) Sperm interaction with fallopian tube apical membrane enhances sperm motility and delays capacitation. *Fertil Steril* 68(2):351–357
- Myles DG, Hyatt H, Primakoff P (1987) Binding of both acrosome-intact and acrosome-reacted guinea pig sperm to the zona pellucida during in vitro fertilization. *Dev Biol* 121(2):559–567
- Nakano M, Hatanaka Y, Sawai T, Kobayashi N, Tobita T (1987) Fractionation of glycoproteins from porcine zonae pellucidae into three families by high-performance liquid chromatography. *Biochem Int* 14(3):417–423
- Nozawa K, Satouh Y, Fujimoto T, Oji A, Ikawa M (2018) Sperm-borne phospholipase C zeta-1 ensures monospermic fertilization in mice. *Sci Rep* 8(1):1315
- Okamura N, Tajima Y, Soejima A, Masuda H, Sugita Y (1985) Sodium bicarbonate in seminal plasma stimulates the motility of mammalian spermatozoa through direct activation of adenylate cyclase. *J Biol Chem* 260(17):9699–9705
- Okamura N, Tamba M, Liao HJ, Onoe S, Sugita Y, Dacheux F, Dacheux JL (1995) Cloning of complementary DNA encoding a 135-kilodalton protein secreted from porcine corpus epididymis and its identification as an epididymis-specific alpha-mannosidase. *Mol Reprod Dev* 42(2):141–148
- Oko R, Aarabi M, Mao J, Balakier H, Sutovsky P (2017) Sperm specific WW-domain binding proteins. In: DeJonge C, Barratt C (eds) The sperm cell: production, maturation, fertilization, regeneration. Cambridge University Press, Cambridge, UK
- O’Leary S, Jasper MJ, Warnes GM, Armstrong DT, Robertson SA (2004) Seminal plasma regulates endometrial cytokine expression, leukocyte recruitment and embryo development in the pig. *Reproduction* 128(2):237–247
- Pacey AA, Davies N, Warren MA, Barratt CL, Cooke ID (1995a) Hyperactivation may assist human spermatozoa to detach from intimate association with the endosalpinx. *Hum Reprod* 10(10):2603–2609
- Pacey AA, Hill CJ, Scudamore IW, Warren MA, Barratt CL, Cooke ID (1995b) The interaction in vitro of human spermatozoa with epithelial cells from the human uterine (fallopian) tube. *Hum Reprod* 10(2):360–366
- Peng L, Wen M, Liu Q, Peng J, Tang S, Hong Y, Liu S, Xiao Y (2018) Persistence and transcription of paternal mtDNA dependent on the delivery strategy rather than mitochondrial source in fish embryos. *Cell Physiol Biochem* 47(5):1898–1908
- Perez-Patino C, Barranco I, Parrilla I, Martinez EA, Rodriguez-Martinez H, Roca J (2016a) Extensive dataset of boar seminal plasma proteome displaying putative reproductive functions of identified proteins. *Data Brief* 8:1370–1373
- Perez-Patino C, Barranco I, Parrilla I, Valero ML, Martinez EA, Rodriguez-Martinez H, Roca J (2016b) Characterization of the porcine seminal plasma proteome comparing ejaculate portions. *J Proteome* 142:15–23
- Perreault SD, Wolff RA, Zirkin BR (1984) The role of disulfide bond reduction during mammalian sperm nuclear decondensation in vivo. *Dev Biol* 101(1):160–167
- Peterson RN, Hunt WP (1989) Identification, isolation, and properties of a plasma membrane protein involved in the adhesion of boar sperm to the porcine zona pellucida. *Gamete Res* 23(1):103–118
- Petrunkina AM, Harrison RA, Topfer-Petersen E (2000) Only low levels of spermadhesin AWN are detectable on the surface of live ejaculated boar spermatozoa. *Reprod Fertil Dev* 12(7–8):361–371
- Petrunkina AM, Gehlhaar R, Drommer W, Waberski D, Topfer-Petersen E (2001) Selective sperm binding to pig oviductal epithelium in vitro. *Reproduction* 121(6):889–896
- Petrunkina AM, Lakamp A, Gentzel M, Ekhlasi-Hundrieser M, Topfer-Petersen E (2003) Fate of lactadherin P47 during post-testicular maturation and capacitation of boar spermatozoa. *Reproduction* 125(3):377–387
- Pilch B, Mann M (2006) Large-scale and high-confidence proteomic analysis of human seminal plasma. *Genome Biol* 7(5):R40
- Plante G, Therien I, Lachance C, Leclerc P, Fan J, Manjunath P (2014) Implication of the human Binder of Sperm Homolog 1 (BSPH1) protein in capacitation. *Mol Hum Reprod* 20(5):409–421
- Plante G, Prud’homme B, Fan J, Lafleur M, Manjunath P (2016) Evolution and function of mammalian binder of sperm proteins. *Cell Tissue Res* 363(1):105–127
- Politi Y, Gal L, Kalifa Y, Ravid L, Elazar Z, Arama E (2014) Paternal mitochondrial destruction after fertilization is mediated by a common endocytic and autophagic pathway in *Drosophila*. *Dev Cell* 29(3):305–320

- Porter JC, Hogg N (1998) Integrins take partners: cross-talk between integrins and other membrane receptors. *Trends Cell Biol* 8(10):390–396
- Primakoff P, Myles DG (2000) The ADAM gene family: surface proteins with adhesion and protease activity. *Trends Genet* 16(2):83–87
- Puga Molina L, Luque G, Balestrini P, Marín-Briggiler C, Romarowski A, Romarowski A (2018) Molecular basis of human sperm capacitation. *Front Cell Dev Biol* 6(72)
- Que EL, Duncan FE, Bayer AR, Phillips SJ, Roth EW, Bleher R, Gleber SC, Vogt S, Woodruff TK, O'Halloran TV (2017) Zinc sparks induce physicochemical changes in the egg zona pellucida that prevent polyspermy. *Integr Biol (Camb)* 9(2):135–144
- Rebeiz M, Miller DJ (1999) Porcine sperm surface beta1, 4galactosyltransferase binds to the zona pellucida but is not necessary or sufficient to mediate sperm-zona pellucida binding. *Mol Reprod Dev* 54(4):379–387
- Redgrove KA, Anderson AL, Dun MD, McLaughlin EA, O'Bryan MK, Aitken RJ, Nixon B (2011) Involvement of multimeric protein complexes in mediating the capacitation-dependent binding of human spermatozoa to homologous zonae pellucidae. *Dev Biol* 356(2):460–474
- Reid AT, Redgrove K, Aitken RJ, Nixon B (2011) Cellular mechanisms regulating sperm-zona pellucida interaction. *Asian J Androl* 13(1):88–96
- Rodríguez-Martínez H, Peña Vega FJ (2013) Semen technologies in domestic animal species. *Anim Front* 3(4):8
- Rodríguez-Martínez H, Iborra A, Martínez P, Calvete JJ (1998) Immunoelectronmicroscopic imaging of spermadhesin AWN epitopes on boar spermatozoa bound in vivo to the zona pellucida. *Reprod Fertil Dev* 10(6):491–497
- Rodríguez-Martínez H, Kvist U, Saravia F, Wallgren M, Johannisson A, Sanz L, Pena FJ, Martínez EA, Roca J, Vazquez JM, Calvete JJ (2009) The physiological roles of the boar ejaculate. *Soc Reprod Fertil Suppl* 66:1–21
- Rodríguez-Martínez H, Saravia F, Wallgren M, Martínez EA, Sanz L, Roca J, Vazquez JM, Calvete JJ (2010) Spermadhesin PSP-I/PSP-II heterodimer induces migration of polymorphonuclear neutrophils into the uterine cavity of the sow. *J Reprod Immunol* 84(1):57–65
- Rojansky R, Cha MY, Chan DC (2016) Elimination of paternal mitochondria in mouse embryos occurs through autophagic degradation dependent on PARKIN and MUL1. *eLife* 5:e17896
- Roldan ML, Teijeiro JM, Ruiz Alvarez J, Marini PE (2018) Sperm binding to porcine oviductal cells is mediated by SRCR domains contained in DMBT1. *J Cell Biochem* 119(4):3755–3762
- Rozeboom KJ, Troedsson MH, Molitor TW, Crabo BG (1999) The effect of spermatozoa and seminal plasma on leukocyte migration into the uterus of gilts. *J Anim Sci* 77(8):2201–2206
- Rubinstein E, Ziyat A, Prenant M, Wrobel E, Wolf JP, Levy S, Le Naour F, Boucheix C (2006) Reduced fertility of female mice lacking CD81. *Dev Biol* 290(2):351–358
- Rybar R, Fladikova L, Faldyna M, Machatkova M, Rubes J (2004) Bull and boar sperm DNA integrity evaluated by sperm chromatin structure assay in the Czech Republic. *Vet Med Czech* 49:1–8
- Ryu J, Lee K (2017) CRISPR/Cas9-mediated gene targeting during embryogenesis in swine. *Methods Mol Biol* 1605:231–244
- Santi CM, Orta G, Salkoff L, Visconti PE, Darszon A, Trevino CL (2013) K⁺ and Cl⁻ channels and transporters in sperm function. *Curr Top Dev Biol* 102:385–421
- Sanz L, Calvete JJ, Jonakova V, Topfer-Petersen E (1992) Boar spermadhesins AQN-1 and AWN are sperm-associated acrosin inhibitor acceptor proteins. *FEBS Lett* 300(1):63–66
- Sartini BL, Berger T (2000) Identification of homologous binding proteins in porcine and bovine gametes. *Mol Reprod Dev* 55(4):446–451
- Sato M, Sato K (2011) Degradation of paternal mitochondria by fertilization-triggered autophagy in *C. elegans* embryos. *Science* 334(6059):1141–1144
- Saunders CM, Larman MG, Parrington J, Cox LJ, Royle J, Blayney LM, Swann K, Lai FA (2002) PLC zeta: a sperm-specific trigger of Ca²⁺ oscillations in eggs and embryo development. *Development* 129(15):3533–3544
- Schjenken JE, Robertson SA (2014) Seminal fluid and immune adaptation for pregnancy—comparative biology in mammalian species. *Reprod Domest Anim* 49(Suppl 3):27–36
- Schlondorff J, Blobel CP (1999) Metalloprotease-disintegrins: modular proteins capable of promoting cell-cell interactions and triggering signals by protein-ectodomain shedding. *J Cell Sci* 112(Pt 21):3603–3617
- Schuberth HJ, Taylor U, Zerbe H, Waberski D, Hunter R, Rath D (2008) Immunological responses to semen in the female genital tract. *Theriogenology* 70(8):1174–1181
- Schwartz M, Vissing J (2002) Paternal inheritance of mitochondrial DNA. *N Engl J Med* 347(8):576–580
- Shalgi R, Phillips D (1980) Mechanics of sperm entry in cycling hamsters. *J Ultrastruct Res* 71(2):154–161
- Sharkey DJ, Tremellen KP, Jasper MJ, Gemzell-Danielsson K, Robertson SA (2012) Seminal fluid induces leukocyte recruitment and cytokine and chemokine mRNA expression in the human cervix after coitus. *J Immunol* 188(5):2445–2454
- Shitara H, Kaneda H, Sato A, Inoue K, Ogura A, Yonekawa H, Hayashi JJ (2000) Selective and continuous elimination of mitochondria microinjected into mouse eggs from spermatids, but not from liver cells, occurs throughout embryogenesis. *Genetics* 156(3):1277–1284
- Shitara H, Kaneda H, Sato A, Iwasaki K, Hayashi J, Taya C, Yonekawa H (2001) Non-invasive visualization of sperm mitochondria behavior in transgenic mice with introduced green fluorescent protein (GFP). *FEBS Lett* 500(1–2):7–11
- Silva E, Frost D, Li L, Bovin N, Miller DJ (2017) Lactadherin is a candidate oviduct Lewis X trisaccharide receptor on porcine spermatozoa. *Andrology* 5(3):589–597
- Snell WJ, White JM (1996) The molecules of mammalian fertilization. *Cell* 85(5):629–637
- Soede NM, Helmond FA, Kemp B (1994) Periovarian profiles of oestradiol, LH and progesterone in relation to oestrus and embryo mortality in multiparous sows using transrectal ultrasonography to detect ovulation. *J Reprod Fertil* 101(3):633–641
- Song X, Li F, Cao G, Zhang J, Han Y (2007) Distribution of alpha-D-mannose residues on zona pellucida and their role(s) in fertilization in pigs. *Sci China C Life Sci* 50(2):170–177
- Song WH, Yi YJ, Sutovsky M, Meyers S, Sutovsky P (2016) Autophagy and ubiquitin-proteasome system contribute to sperm mitophagy after mammalian fertilization. *Proc Natl Acad Sci U S A* 113(36):E5261–E5270
- Spargo SC, Hope RM (2003) Evolution and nomenclature of the zona pellucida gene family. *Biol Reprod* 68(2):358–362
- Suarez SS (1987) Sperm transport and motility in the mouse oviduct: observations in situ. *Biol Reprod* 36(1):203–210
- Suarez SS (1996) Hyperactivated motility in sperm. *J Androl* 17(4):331–335
- Suarez SS (1998) The oviductal sperm reservoir in mammals: mechanisms of formation. *Biol Reprod* 58(5):1105–1107
- Suarez SS (2008) Regulation of sperm storage and movement in the mammalian oviduct. *Int J Dev Biol* 52(5–6):455–462
- Suarez SS (2015) Gamete and Zygote Transport. In: Plant TM, Zeleznik AJ (eds) Knobil and Neill's physiology of reproduction, vol. 1. Academic Press, Cambridge, pp 197–232
- Suarez SS (2016) Mammalian sperm interactions with the female reproductive tract. *Cell Tissue Res* 363(1):185–194

- Suarez S, Redfern K, Raynor P, Martin F, Phillips DM (1991) Attachment of boar sperm to mucosal explants of oviduct in vitro: possible role in formation of a sperm reservoir. *Biol Reprod* 44(6):998–1004
- Suarez SS, Dai XB, DeMott RP, Redfern K, Mirando MA (1992) Movement characteristics of boar sperm obtained from the oviduct or hyperactivated in vitro. *J Androl* 13(1):75–80
- Suarez SS, Varosi SM, Dai X (1993) Intracellular calcium increases with hyperactivation in intact, moving hamster sperm and oscillates with the flagellar beat cycle. *Proc Natl Acad Sci U S A* 90(10):4660–4664
- Sun QY, Nagai T (2003) Molecular mechanisms underlying pig oocyte maturation and fertilization. *J Reprod Dev* 49(5):347–359
- Sutovsky P (2009) Sperm-egg adhesion and fusion in mammals. *Expert Rev Mol Med* 11:e11
- Sutovsky P, Schatten G (1997) Depletion of glutathione during bovine oocyte maturation reversibly blocks the decondensation of the male pronucleus and pronuclear apposition during fertilization. *Biol Reprod* 56(6):1503–1512
- Sutovsky P, Navara CS, Schatten G (1996) Fate of the sperm mitochondria, and the incorporation, conversion, and disassembly of the sperm tail structures during bovine fertilization. *Biol Reprod* 55(6):1195–1205
- Sutovsky P, McCauley TC, Sutovsky M, Day BN (2003) Early degradation of paternal mitochondria in domestic pig (*Sus scrofa*) is prevented by selective proteasomal inhibitors lactacystin and MG132. *Biol Reprod* 68(5):1793–1800
- Sutovsky P, Van Leyen K, McCauley T, Day BN, Sutovsky M (2004) Degradation of paternal mitochondria after fertilization: implications for heteroplasmy, assisted reproductive technologies and mtDNA inheritance. *Reprod BioMed Online* 8(1):24–33
- Sutovsky P, Kerns K, Zigo M, Zuidema D (2019) Boar semen improvement through sperm capacitation management, with emphasis on zinc ion homeostasis. *Theriogenology* 137:50–55
- Suzuki T, Fujinoki M, Shibahara H, Suzuki M (2010) Regulation of hyperactivation by PPP2 in hamster spermatozoa. *Reproduction* 139(5):847–856
- Talevi R, Gualtieri R (2010) Molecules involved in sperm-oviduct adhesion and release. *Theriogenology* 73(6):796–801
- Tanihara F, Nakai M, Men NT, Kato N, Kaneko H, Noguchi J, Otoi T, Kikuchi K (2014) Roles of the zona pellucida and functional exposure of the sperm-egg fusion factor 'IZUMO' during in vitro fertilization in pigs. *Anim Sci J* 85(4):395–404
- Tanphaichitr N, Moase C, Taylor T, Surewicz K, Hansen C, Namking M, Berube B, Kamolvarin N, Lingwood CA, Sullivan R, Rattanachaiyanont M, White D (1998) Isolation of antiSLIP1-reactive boar sperm P68/62 and its binding to mammalian zona pellucida. *Mol Reprod Dev* 49(2):203–216
- Tanphaichitr N, Carmona E, Bou Khalil M, Xu H, Berger T, Gerton GL (2007) New insights into sperm-zona pellucida interaction: involvement of sperm lipid rafts. *Front Biosci* 12:1748–1766
- Tanphaichitr N, Kongmanas K, Kruevaisayawan H, Saewu A, Sugeng C, Fernandes J, Souda P, Angel JB, Faull KF, Aitken RJ, Whitelegge J, Hardy D, Berger T, Baker M (2015) Remodeling of the plasma membrane in preparation for sperm-egg recognition: roles of acrosomal proteins. *Asian J Androl* 17(4):574–582
- Tardif S, Cormier N (2011) Role of zonadhesin during sperm-egg interaction: a species-specific acrosomal molecule with multiple functions. *Mol Hum Reprod* 17(11):661–668
- Tardif S, Dube C, Chevalier S, Bailey JL (2001) Capacitation is associated with tyrosine phosphorylation and tyrosine kinase-like activity of pig sperm proteins. *Biol Reprod* 65(3):784–792
- Tardif S, Dube C, Bailey JL (2003) Porcine sperm capacitation and tyrosine kinase activity are dependent on bicarbonate and calcium but protein tyrosine phosphorylation is only associated with calcium. *Biol Reprod* 68(1):207–213
- Tardif S, Wilson MD, Wagner R, Hunt P, Gertsenstein M, Nagy A, Lobe C, Koop BF, Hardy DM (2010) Zonadhesin is essential for species specificity of sperm adhesion to the egg zona pellucida. *J Biol Chem* 285(32):24863–24870
- Tateno H, Krapf D, Hino T, Sanchez-Cardenas C, Darszon A, Yanagimachi R, Visconti PE (2013) Ca²⁺ ionophore A23187 can make mouse spermatozoa capable of fertilizing in vitro without activation of cAMP-dependent phosphorylation pathways. *Proc Natl Acad Sci U S A* 110(46):18543–18548
- Tavalae M, Parivar K, Shahverdi AH, Ghaedi K, Nasr-Esfahani MH (2017) Status of sperm-born oocyte activating factors (PAWP, PLCzeta) and sperm chromatin in uncapacitated, capacitated and acrosome-reacted conditions. *Hum Fertil (Camb)* 20(2):96–103
- Teijeiro JM, Cabada MO, Marini PE (2008) Sperm binding glycoprotein (SBG) produces calcium and bicarbonate dependent alteration of acrosome morphology and protein tyrosine phosphorylation on boar sperm. *J Cell Biochem* 103(5):1413–1423
- Teijeiro JM, Ignatz GG, Marini PE (2009) Annexin A2 is involved in pig (*Sus scrofa*) sperm-oviduct interaction. *Mol Reprod Dev* 76(4):334–341
- Teijeiro JM, Roldan ML, Marini PE (2016) Annexin A2 and S100A10 in the mammalian oviduct. *Cell Tissue Res* 363(2):567–577
- Tesarik J, Drahorad J, Peknicova J (1988) Subcellular immunochemical localization of acrosin in human spermatozoa during the acrosome reaction and zona pellucida penetration. *Fertil Steril* 50(1):133–141
- Topfer-Petersen E (1999) Carbohydrate-based interactions on the route of a spermatozoon to fertilization. *Hum Reprod Update* 5(4):314–329
- Topfer-Petersen E, Calvete JJ (1995) Molecular mechanisms of the interaction between sperm and the zona pellucida in mammals: studies on the pig. *Int J Androl* 18(Suppl 2):20–26
- Topfer-Petersen E, Henschen A (1987) Acrosin shows zona and fucose binding, novel properties for a serine proteinase. *FEBS Lett* 226(1):38–42
- Topfer-Petersen E, Friess AE, Nguyen H, Schill WB (1985) Evidence for a fucose-binding protein in boar spermatozoa. *Histochemistry* 83(2):139–145
- Topfer-Petersen E, Mann K, Calvete JJ (1993) Identification of porcine oocyte 55 kDa alpha and beta proteins within the zona pellucida glycoprotein families indicates that oocyte sperm receptor activity is associated with different zone pellucida proteins in different mammalian species. *Biol Chem Hoppe Seyler* 374(7):411–417
- Topfer-Petersen E, Romero A, Varela PF, Ekhlasi-Hundrieser M, Dostalova Z, Sanz L, Calvete JJ (1998) Spermadhesins: a new protein family. Facts, hypotheses and perspectives. *Andrologia* 30(4–5):217–224
- Topfer-Petersen E, Ekhlasi-Hundrieser M, Tsovalova M (2008) Glycobiology of fertilization in the pig. *Int J Dev Biol* 52(5–6):717–736
- Tsai PS, Garcia-Gil N, van Haften T, Gadella BM (2010) How pig sperm prepares to fertilize: stable acrosome docking to the plasma membrane. *PLoS One* 5(6):e11204
- Tsai PS, Brewis IA, van Maaren J, Gadella BM (2012) Involvement of complexin 2 in docking, locking and unlocking of different SNARE complexes during sperm capacitation and induced acrosomal exocytosis. *PLoS One* 7(3):e32603
- Vadnais ML, Althouse GC (2011) Characterization of capacitation, cryoinjury, and the role of seminal plasma in porcine sperm. *Theriogenology* 76(8):1508–1516
- Vadnais ML, Roberts KP (2007) Effects of seminal plasma on cooling-induced capacitative changes in boar sperm. *J Androl* 28(3):416–422
- Vadnais ML, Kirkwood RN, Tempelman RJ, Sprecher DJ, Chou K (2005) Effect of cooling and seminal plasma on the capacitation status of fresh boar sperm as determined using chlortetracycline assay. *Anim Reprod Sci* 87(1–2):121–132

- Vadnais ML, Foster DN, Roberts KP (2008) Molecular cloning and expression of the CRISP family of proteins in the boar. *Biol Reprod* 79(6):1129–1134
- Veselsky L, Jonakova V, Sanz ML, Topfer-Petersen E, Cechova D (1992) Binding of a 15 kDa glycoprotein from spermatozoa of boars to surface of zona pellucida and cumulus oophorus cells. *J Reprod Fertil* 96(2):593–602
- Veselsky L, Peknicova J, Cechova D, Kraus M, Geussova G, Jonakova V (1999) Characterization of boar spermadhesins by monoclonal and polyclonal antibodies and their role in binding to oocytes. *Am J Reprod Immunol* 42(3):187–197
- Veselsky L, Jonakova V, Dostal J, Holan V, Voburka Z, Drahorad J, Manaskova P, Zelezna B (2000) Inhibition of the antibody responses to rat blood transfusion antigens in mice by boar seminal immunosuppressive fraction. *Am J Reprod Immunol* 44(6):325–335
- Visconti PE, Florman HM (2010) Mechanisms of sperm-egg interactions: between sugars and broken bonds. *Sci Signal* 3(142):e35
- Visconti PE, Moore GD, Bailey JL, Leclerc P, Connors SA, Pan D, Olds-Clarke P, Kopf GS (1995) Capacitation of mouse spermatozoa. II. Protein tyrosine phosphorylation and capacitation are regulated by a cAMP-dependent pathway. *Development* 121(4):1139–1150
- Visconti PE, Krapf D, de la Vega-Beltran JL, Acevedo JJ, Darszon A (2011) Ion channels, phosphorylation and mammalian sperm capacitation. *Asian J Androl* 13(3):395–405
- Waberski D, Helms D, Beyerbach M, Weitze KF, Bollwein H, Bluemig P, Willeke H, Acevedo N, Saacke RG (2002) Sperm chromatin structure in boars used in artificial insemination. *Reprod Domest Anim* 37(4):257
- Wagner A, Ekhlasi-Hundrieser M, Hettel C, Petrunkina A, Waberski D, Nimtz M, Topfer-Petersen E (2002) Carbohydrate-based interactions of oviductal sperm reservoir formation-studies in the pig. *Mol Reprod Dev* 61(2):249–257
- Wardrip NJ, Hedrick JL (1985) Pig zona pellucida 25K and 65K glycoproteins are derived from Hydrolysis and reduction of the 90K family. *J Cell Biol* 101:378a
- Wassarman PM (1999) Mammalian fertilization: molecular aspects of gamete adhesion, exocytosis, and fusion. *Cell* 96(2):175–183
- Wassarman PM (2009) Mammalian fertilization: the strange case of sperm protein 56. *Bioessays* 31(2):153–158
- Whitworth KM, Lee K, Benne JA, Beaton BP, Spate LD, Murphy SL, Samuel MS, Mao J, O'Gorman C, Walters EM, Murphy CN, Driver J, Mileham A, McLaren D, Wells KD, Prather RS (2014) Use of the CRISPR/Cas9 system to produce genetically engineered pigs from in vitro-derived oocytes and embryos. *Biol Reprod* 91(3):78
- Williams TA, Barnes K, Kenny AJ, Turner AJ, Hooper NM (1992) A comparison of the zinc contents and substrate specificities of the endothelial and testicular forms of porcine angiotensin converting enzyme and the preparation of isoenzyme-specific antisera. *Biochem J* 288(Pt 3):875–881
- Williams M, Hill CJ, Scudamore I, Dunphy B, Cooke ID, Barratt CL (1993) Sperm numbers and distribution within the human fallopian tube around ovulation. *Hum Reprod* 8(12):2019–2026
- Wolff JN, Gemmell NJ (2013) Mitochondria, maternal inheritance, and asymmetric fitness: why males die younger. *Bioessays* 35(2):93–99
- Wright SJ, Longo FJ (1988) Sperm nuclear enlargement in fertilized hamster eggs is related to meiotic maturation of the maternal chromatin. *J Exp Zool* 247(2):155–165
- Wu AT, Sutovsky P, Manandhar G, Xu W, Katayama M, Day BN, Park KW, Yi YJ, Xi YW, Prather RS, Oko R (2007) PAWP, a sperm-specific WW domain-binding protein, promotes meiotic resumption and pronuclear development during fertilization. *J Biol Chem* 282(16):12164–12175
- Yamasaki K, Yoshida K, Yoshiike M, Shimada K, Nishiyama H, Takamizawa S, Yanagida K, Iwamoto T (2017) Relationship between Semenogelins bound to human sperm and other semen parameters and pregnancy outcomes. *Basic Clin Androl* 27:15
- Yanagimachi R (1994) Mammalian fertilization. In: Knobil E, Neill JD (eds) *The Physiology of Reproduction*, vol. 1. Raven Press, New York, pp 189–317
- Yefimova M, Bourmeyster N, Becq F, Burel A, Lavault MT, Jouve G, Veau S, Pimentel C, Jegou B, Ravel C (2019) Update on the cellular and molecular aspects of cystic fibrosis transmembrane conductance regulator (CFTR) and male fertility. *Morphologie* 103(341):4–10
- Yi YJ, Manandhar G, Sutovsky M, Li R, Jonakova V, Oko R, Park CS, Prather RS, Sutovsky P (2007) Ubiquitin C-terminal hydrolase-activity is involved in sperm acrosomal function and anti-polyspermy defense during porcine fertilization. *Biol Reprod* 77(5):780–793
- Yi YJ, Manandhar G, Sutovsky M, Jonakova V, Park CS, Sutovsky P (2010a) Inhibition of 19S proteasomal regulatory complex subunit PSMD8 increases polyspermy during porcine fertilization in vitro. *J Reprod Immunol* 84(2):154–163
- Yi YJ, Manandhar G, Sutovsky M, Zimmerman SW, Jonakova V, van Leeuwen FW, Oko R, Park CS, Sutovsky P (2010b) Interference with the 19S proteasomal regulatory complex subunit PSMD4 on the sperm surface inhibits sperm-zona pellucida penetration during porcine fertilization. *Cell Tissue Res* 341(2):325–340
- Yonezawa N, Hatanaka Y, Takeyama H, Nakano M (1995) Binding of pig sperm receptor in the zona pellucida to the boar sperm acrosome. *J Reprod Fertil* 103(1):1–8
- Yonezawa N, Fukui N, Kudo K, Nakano M (1999) Localization of neutral N-linked carbohydrate chains in pig zona pellucida glycoprotein ZPC. *Eur J Biochem* 260(1):57–63
- Yonezawa N, Amari S, Takahashi K, Ikeda K, Imai FL, Kanai S, Kikuchi K, Nakano M (2005) Participation of the nonreducing terminal beta-galactosyl residues of the neutral N-linked carbohydrate chains of porcine zona pellucida glycoproteins in sperm-egg binding. *Mol Reprod Dev* 70(2):222–227
- Yoon S, Chang KT, Cho H, Moon J, Kim JS, Min SH, Koo DB, Lee SR, Kim SH, Park KE, Park YI, Kim E (2014) Characterization of pig sperm hyaluronidase and improvement of the digestibility of cumulus cell mass by recombinant pSPAM1 hyaluronidase in an in vitro fertilization assay. *Anim Reprod Sci* 150(3–4):107–114
- Yu Y, Xu W, Yi YJ, Sutovsky P, Oko R (2006) The extracellular protein coat of the inner acrosomal membrane is involved in zona pellucida binding and penetration during fertilization: characterization of its most prominent polypeptide (IAM38). *Dev Biol* 290(1):32–43
- Yuan Y, Spate LD, Redel BK, Tian Y, Zhou J, Prather RS, Roberts RM (2017) Quadrupling efficiency in production of genetically modified pigs through improved oocyte maturation. *Proc Natl Acad Sci U S A* 114(29):E5796–e5804
- Yurewicz EC, Sacco AG, Subramanian MG (1987) Structural characterization of the Mr = 55,000 antigen (ZP3) of porcine oocyte zona pellucida. Purification and characterization of alpha- and beta-glycoproteins following digestion of lactosaminoglycan with endo-beta-galactosidase. *J Biol Chem* 262(2):564–571
- Yurewicz EC, Pack BA, Sacco AG (1991) Isolation, composition, and biological activity of sugar chains of porcine oocyte zona pellucida 55K glycoproteins. *Mol Reprod Dev* 30(2):126–134
- Zapata-Carmona H, Baron L, Zuniga LM, Diaz ES, Kong M, Drobnis EZ, Sutovsky P, Morales P (2019) The activation of the chymotrypsin-like activity of the proteasome is regulated by soluble adenylyl cyclase / cAMP / protein kinase a pathway and required for human sperm capacitation. *Mol Hum Reprod* 25(10):587–600
- Zayas-Perez H, Casas E, Bonilla E, Betancourt M (2005) Inhibition of sperm-zona pellucida binding by a 55 kDa pig sperm protein in vitro. *Arch Androl* 51(3):195–206
- Zhu J, Massey JB, Mitchell-Leef D, Elsner CW, Kort HI, Roudebush WE (2006) Platelet-activating factor acetylhydrolase activity affects sperm motility and serves as a decapacitation factor. *Fertil Steril* 85(2):391–394
- Zigo M, Jonakova V, Sulc M, Manaskova-Postlerova P (2013) Characterization of sperm surface protein patterns of ejaculated

- and capacitated boar sperm, with the detection of ZP binding candidates. *Int J Biol Macromol* 61:322–328
- Zigo M, Dorosh A, Pohlova A, Jonakova V, Sulc M, Manaskova-Postlerova P (2015) Panel of monoclonal antibodies to sperm surface proteins as a tool for monitoring localization and identification of sperm-zona pellucida receptors. *Cell Tissue Res* 359(3):895–908
- Zigo M, Kerns K, Sutovsky M, Sutovsky P (2018) Modifications of the 26S proteasome during boar sperm capacitation. *Cell Tissue Res* 372(3):591–601
- Zigo M, Jonakova V, Manaskova-Postlerova P, Kerns K, Sutovsky P (2019a) Ubiquitin-proteasome system participates in the deaggregation of spermadhesin and DQH protein during boar sperm capacitation. *Reproduction* 157(3):283–295
- Zigo M, Manaskova-Postlerova P, Jonakova V, Kerns K, Sutovsky P (2019b) Compartmentalization of the proteasome-interacting proteins during sperm capacitation. *Sci Rep* 9(1):12583
- Zimmerman SW, Manandhar G, Yi YJ, Gupta SK, Sutovsky M, Odhiambo JF, Powell MD, Miller DJ, Sutovsky P (2011) Sperm proteasomes degrade sperm receptor on the egg zona pellucida during mammalian fertilization. *PLoS One* 6(2):e17256
- Zuidema D, Sutovsky P (2019) The domestic pig as a model for the study of mitochondrial inheritance. *Cell Tissue Research*. <https://doi.org/10.1007/s00441-019-03100-z>

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