Chapter 8
Boar Spermatozoa Within the Oviductal Environment (III): Fertilisation

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Abstract This chapter is the last of a series of four chapters about the transit of boar spermatozoa within the female reproductive tract and deals with the fertilisation events that take place during and after a capacitated spermatozoon interacts with the ZP of the oocyte. Thus, we will focus on the mechanisms of gamete binding and interaction, including acrosome exocytosis, ZP-penetration and fusion between sperm and oocyte plasma membranes. In addition, subsequent events that take place after sperm-oocyte fusion, such as sperm chromatin remodelling and sperm-mediated oocyte activation will also be dealt with. To conclude, other novel aspects about the role played by sperm in post-fertilisation and early embryo development events will also be discussed.

8.1 Introduction

Fertilisation is the process by which two haploid gametes, namely the spermatozoon and the oocyte, unite to produce a genetically distinct individual (Brewis et al. 2005; Signorelli et al. 2012).

In mammalian species, fertilisation involves a number of sequential steps (Yanagimachi 1994a, b) (Fig. 8.1):

1. Sperm migration through the female genital tract (see Chaps. 5, 6 and 7)
2. Sperm penetration through the cumulus cells
3. Sperm adhesion and binding to the zona pellucida (ZP)
4. Acrosome exocytosis
5. Sperm penetration through the ZP
6. Fusion of sperm plasmalemma to oolemma.

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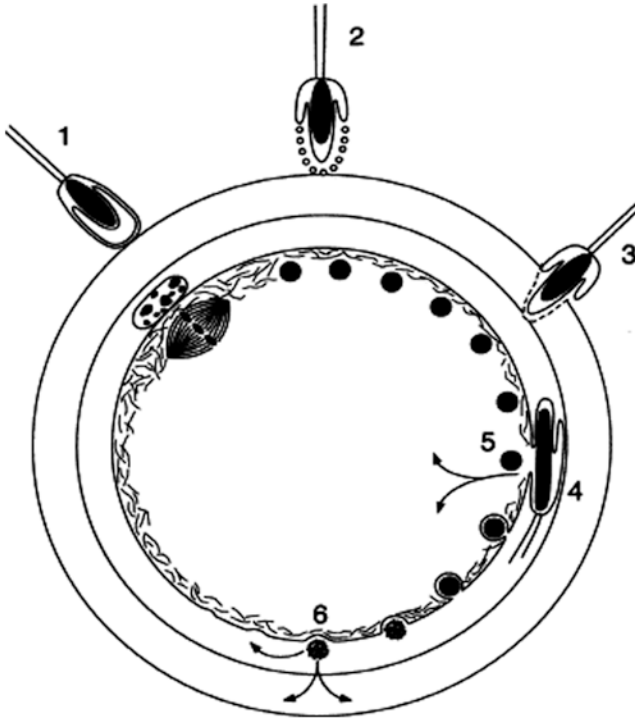e-mail: marc.yeste@uab.cat

Fig. 8.1 Schematic representation of the sequence of interactions between the male and female gametes leading to fertilisation: (*1*) sperm binding to the ZP (restricted to the apical ridge subdomain area), (*2*) the acrosome reaction (fusions restricted to the apical ridge and pre-equatorial subdomain areas), (*3*) the penetration of the zona pellucida (equatorial membranes remain intact and that the mixed vesicles resulting from the acrosome reaction are shed of the penetrating sperm, see also Chap. 9), (*4*) binding and fusion with the oolemma (restricted to the equatorial subdomain area), (*5*) activation of the fertilised oocyte by a soluble sperm factors, and (*6*) polyspermy block by the cortical reaction (Boerke et al. 2008) Reproduced with permission

In short, at fertilisation, the capacitated mammalian spermatozoon binds in a specific manner to the ZP (outer coating of the egg), and in response to this contact exocytosis its acrosome (Töpfer-Petersen et al. 2008). It then penetrates the ZP by means of a combination of flexured ('hyperactive') motility and the activity of released or unmasked acrosomal hydrolases. Finally, the spermatozoon binds to the oolemma and enters the ooplasm (Barroso et al. 2009). Once fertilised, the oocyte is called a zygote.

Recently, Mugnier et al. (2009) identified four key elements of the fertilisation process from in vitro studies:

(a) During sperm-ZP binding, the composition and structure of the ZP play a critical role.

(b) The capacity of spermatozoa to undergo the acrosome reaction is essential in this step of fertilisation.

(c) The plasma membrane of the oocyte is crucial in the mechanism of gamete fusion.

(d) The competence of the ooplasm is a major element in pronuclei formation.

8.2 Ovaries and Ova Transport

8.2.1 The Ovaries

Ovaries are the primary structures of the female reproductive tract that perform two main functions: to produce the female germ cells (i.e. the oocytes) and to synthesise and release oestrogens (oestradiol, oestrone and oestriol) and progesterone. They are suspended from the abdominal roof by long mesovaria and each ovary is surrounded by a thin membrane called the infundibulum, which acts as a funnel to collect ova and divert them to the oviduct (Hafez 1993).

With regard to the hormonal control of the ovaries, the hypothalamus located at the basis of the brain secretes the gonadotropin-releasing hormone (GnRH) which regulates the pituitary gland to secrete follicle stimulating hormone (FSH) and luteinising hormone (LH) into the blood. These two gonadotropins stimulate the production of the ovarian hormones, oestrogens and progesterone, which in turn regulate the reproductive process (Espey and Lipner 1994; Madej et al. 2005).

In the pig, ovaries are approximately 5 cm long and irregular in shape, due to numerous follicles and/or corpora lutea protruding from the surface, as occur in cyclic animals. The oocytes are contained within follicles in different stages of development—primary, secondary and antral follicles. Antral follicles may undergo further development into mature follicles that ovulate (Espey and Lipner 1994).

8.2.2 Ovulation and Transport of Ova

Ovulation is the rupture of a mature follicle at the surface of the ovary, thereby releasing an oocyte surrounded by cumulus cells (COCs) into the oviduct, and is initiated by an increase in gonadotropic hormones (Richards et al. 2002). Then, a set of structural changes in the follicular wall and vascular microcirculation takes place, due to the action of proteolytic enzymes and prostaglandins (Espey and Lipner 1994; Brüssow et al. 2008). After this, the oocytes are transported by the cilia-covered fimbria towards the ampulla, in a process that takes about 30–45 min (Brüssow et al. 2008), and the ova spend about 2 days in the oviducts (Mwanza et al. 2002a, b). Finally, the cumulus cells disperse within 6 h after ovulation (Hunter 1984; Brüssow et al. 2008). This process is influenced by the presence of spermatozoa in the oviduct (Rodríguez-Martínez et al. 2001).

The mechanism of ova transport and its regulation still remains unknown, but it has been hypothesised that myosalpingeal peristalsis (Rodríguez-Martínez

et al. 1982; Mwanza et al. 2002b), cumulus cells and extracellular matrix, which increase the size of the egg plug making ciliary beating, are involved (Talbot et al. 1999).

8.3 An overview of Fertilisation and Early Embryonic Development

8.3.1 A General View of Fertilisation

As occurs for all sexually reproducing species, the fertilisation of an oocyte is the completion of the role of the sperm and involves the creation of a new individual (Töpfer-Petersen et al. 2008). In mammalian species, fertilisation is an internal process that takes place in the upper third of the oviduct, specifically in the ampullary-isthmic junction. In swine, the success rate of this event is about 100 % when a fertile boar mounts the female at the correct time relative to ovulation (Hafez 1993).

As widely explained in Chap. 6, the oviduct plays a crucial role in the events leading to fertilisation, since it first stores the spermatozoa until an ovulated oocyte is released forming the sperm reservoir, and then capacitates the spermatozoa before they bind the ZP-glycoproteins (Darson et al. 2007; Salicioni et al. 2007). In Chap. 7, the importance of sperm capacitation before binding to ZP has been extensively discussed.

Binding and interaction of a capacitated spermatozoon with an ovulated oocyte start as a coordinated series of events that lead to the formation of the diploid zygote and initiates embryonic development. This process begins when a capacitated spermatozoon interacts with the ZP of the oocyte, which is a surrounding extracellular matrix that contains glycoproteins. This event, like sperm binding to oviductal epithelial cells, is mediated by carbohydrate interactions (see also Sect. 6.7). In this case, two different binding phases can be distinguished, and both involve carbohydrate mediation (Töpfer-Petersen 1999; Wassarman 2005; Wassarman et al. 2005; Töpfer-Petersen et al. 2008) (Figs. 8.1 and 8.2):

(a) Primary binding that consists of recognition between the capacitated spermatozoon and the ZP-oocyte and triggers the acrosome reaction.
(b) Secondary binding, when an acrosome-reacted spermatozoon interacts with the oolemma. After this secondary binding, the cortical reaction occurs and the ZP is hardened to impede polyspermia (Funahashi et al. 2000; Funahashi and Nagai 2001).

After the acrosome reaction, the equatorial region of the sperm membrane is exposed, adheres to and fuses with the oolemma, facilitating the introduction of the sperm nucleus into the oocyte. Once in the ooplasm, the sperm nucleus undergoes several structural changes that involve chromatin remodelling (Ajduk et al. 2006). This step leads the oocyte, stopped at Metaphase II, to complete the
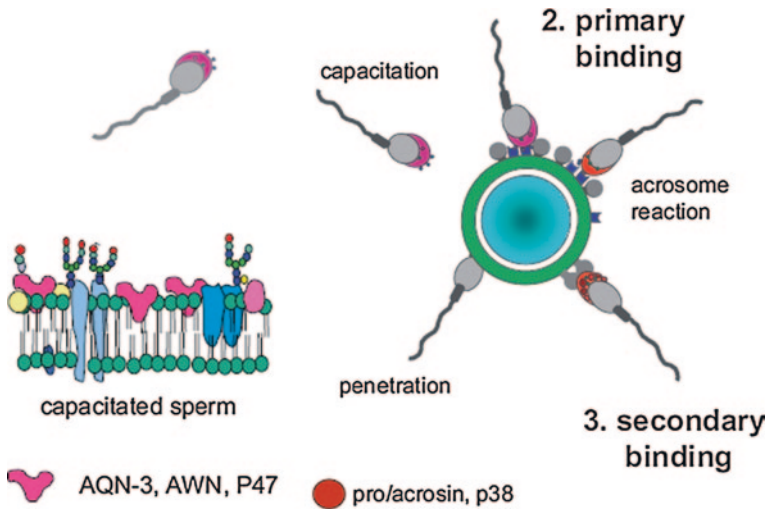
Fig. 8.2 Sperm-ZP binding. Capacitated spermatozoa binds to ZP by recognising a set of neutral complex N-glycans (primary binding). Upon binding, the acrosome reaction is induced and the acrosome reacted spermatozoa binds to polysulfated glycan structures of the zona pellucida via pro/acrosin, p38 and others (secondary binding) (Töpfer-Petersen et al. (2008) Reproduced with permission)

second meiotic division. The next step is the migration of pronuclei and chromosome alignment when the two haploid nuclei (called pronuclei) come together and combine their chromosomes into a single diploid nucleus (Yanagimachi 1994b; Töpfer-Petersen et al. 2008). In the case of mammals, the two pronuclei do not fuse directly as they do in many other species like *Xenopus laevis* (Edwards and Beard 1997). Instead, they approach each other (pronuclear apposition), but remain distinct until after the membrane of each pronucleus has broken down, preparing for the first mitotic division of the zygote (Alberio et al. 2001; Alberts et al. 2008; Snook et al. 2011).

8.3.2 Some General Concepts Related to Early Embryonic Development

Embryos remain for a long period of time (up to 26 h) at the one-cell stage, but only between 6 and 8 h in the 2- and 4-cell embryo stage (Hunter 1984; Brüssow 1985). The early cleavage of embryos takes place in the isthmus and embryos usually leave the oviduct about 50–60 h post-ovulation (Brüssow et al. 2008).

About 2 days after fertilisation, embryos enter the uterus. They are at the 4–8 cell stage and begin to space themselves at equal distances in the uterine horns. It is worth noting that blastocysts can migrate from one horn to the other during this stage (Hafez 1993).

One of the most critical periods of pregnancy is from approximately 11–16 days after mating. At this time, the blastocysts elongate into long, stringy masses and they begin to attach to the uterine wall. During this stage of development, the greatest potential loss in litter size may occur because of attachment failures. In fact, some factors have been suggested to limit the number of blastocysts that can attach in a given uterus. In this regard, embryonic survival might be related to certain uterine secretions which have yet to be elucidated. On the other hand, stressing factors, such as high temperatures or animal regrouping, can also negatively influence both implantation and embryo survival. In addition, a minimum number (four) of the implanted blastocysts in the uterus is required for maintaining pregnancy (Hafez 1993; Brüssow et al. 2008).

By days 25–35, the length of the embryo is between 2.5 and 3.8 cm, and the major body systems are well formed. Each embryo is surrounded by a separate series of fluid-filled membranes, the amnion and chorion, which comprise the placenta. These membranes help to protect and nourish the growing embryo. Nutrients, waste, gases and certain antibodies cross the membranes between the dam and embryo blood systems (Pusateri et al. 1990; Bazer et al. 2012).

The foetal period begins at approximately day 36. Gender may be easily determined by external examination and the main systems of the body are still better defined. Foetal orientation is random; some are head to head, some are tail to tail and some are head to tail. At farrowing, about half are born tail first and half are born head first. Embryos which die before day 35–40 are usually reabsorbed by the dam. However, progressive calcification of the skeleton begins to occur from day 36 onwards and deaths taking place after this point result in mummification (Hafez 1993; Knobil and Neil 1994; Bazer et al. 2012).

At day 109, the weight of foetuses is between 0.6 and 0.8 kg and at farrowing this weight reaches 1.2–1.6 kg. On the other hand, the uterus gradually enlarges over gestation from about 0.8–1.2 kg at mating to up to 24 kg, including foetal contents, during the last week of pregnancy. In fact, some females lose up to 10–11 % of their body weight at parturition.

8.3.3 Effect of 'Stressful' Events After Ovulation

The effects of stressful conditions on post-ovulatory events (fertilisation, ova transport, ova cleavage rate and number of spermatozoa attached to the ZP) have also been investigated by fasting or ACTH administration (Mburu et al. 1998; Mwanza et al. 2000a, b; Razdan et al. 2001, 2002; Einarsson et al. 2008).

Food deprivation for 2 days immediately after ovulation reduces the numbers of viable spermatozoa in the sperm reservoir, and delays the cleavage rate of fertilised ova (Mburu et al. 1998). Fasting for 2 days after ovulation elevates the plasma levels of cortisol and $PGF_{2\alpha}$, decreases oestradiol-17β levels and raises the isthmic intraluminal pressure and the frequencies of pressure fluctuations (Mwanza et al. 2000a). Fasting also delays ova transport through the isthmic part

of the oviduct, perhaps owing to a prolonged α-adrenergic response in the smooth circular musculature of the isthmus (Mwanza et al. 2000a, b; Razdan et al. 2001).

As far as induction of stress by ACTH-stimulation after ovulation is concerned, this does not affect the activity of the isthmus and frequency distribution of ova within the isthmus, but it does reduce the number of sperm cells attached to ZP and the number of embryos retrieved from the sow reproductive tract (Mwanza et al. 2000b; Razdan et al. 2002; Einarsson et al. 2008). In contrast, ACTH also increases the velocity of embryo transport along the uterus (Brandt et al. 2007).

8.4 Gamete Binding and Interaction: Ovum Surface

The mechanism of gamete recognition is conserved throughout the biological evolution from marine vertebrates to eutherian mammals. As previously mentioned, the oocyte is coated by the ZP in the case of mammals. This ZP plays a protective role, which modulates sperm function during fertilisation and prevents polyspermia (Töpfer-Petersen et al. 2008).

Since recognition and interaction between spermatozoa and oocyte involves sperm-surface associated proteins that recognise oligosaccharide ligands of the ZP-glycoproteins, this section and the next will focus on these molecules.

8.4.1 ZP-Glycoproteins: ZPA, ZPB and ZPC

In mammals, ZP is formed by more than one glycoprotein (Harris et al. 1994). All ZP-glycoproteins share a common domain of about 260 residues that play a critical role in the polymerisation of ZP-glycoproteins into filaments (Litscher and Wassarman 2007).

Glycoproteins from the ZP are encoded by seven gene families that have originated from a duplication of an ancestral gene during vertebrate evolution (Spargo and Hope 2003; Hughes 2007). Related to this, Goudet et al. (2008) used phylogenetic analysis to classify ZP-genes in six subfamilies: *ZP1*, *ZPA/ZP2*, *ZPB/ZP4*, *ZPC/ZP3*, *ZPAX* and *ZPD*. However, the mammalian-ZP is not always formed by the same number of ZP-glycoproteins, but differences exist among species. Thus, in pigs, cattle, dogs and cats, ZP is constituted by three glycoproteins: ZPA/ZP2, ZPB/ZP4 and ZPC/ZP3 (Noguchi et al. 1994; Goudet et al. 2008; Mugnier et al. 2009), while in humans (Lefièvre et al. 2004; Conner et al. 2005; Barroso et al. 2009), rats (Hoodbhoy et al. 2005), hamsters (Izquierdo-Rico et al. 2009), macaques, chimpanzees (Goudet et al. 2008) and horses, it is formed by four glycoproteins (ZPA/ZP2, ZPB/ZP4, ZPC/ZP3 and ZP1). Differences between the species presenting three or four ZP-glycoproteins have been attributed either to the loss of the gene in the case of pigs or to the 'death' of the ZP1 gene, as in bovine and canine species (Goudet et al. 2008). In addition, it is worth noting that ZPAX and ZPD proteins are not present in any of the mentioned species.

As stated, porcine-ZP is formed by three different glycoproteins that are encoded by the gene subfamilies *ZPA/ZP2*, *ZPB/ZP4* and *ZPC/ZP3*. From these three ZP-glycoproteins, two (ZPB and ZPC) are synthesised by the developing oocyte during the early phases of follicle development (primordial and primary follicles), while the other (ZPA) is later released and is not observed in immature oocytes. As follicles grow, the contribution of the oocyte in the synthesis of ZPB and ZPC decreases, while that of the follicular cells increases (Sinowatz et al. 2001). In tertiary follicles (also known as Graafian follicles), ZPA is present in the oocyte cytoplasm and ZPB and ZPC are found in ZP (Niemann and Rath 2001). This finding suggests spatio-temporal secretion of ZPA and ZPB, and ZPC is differently regulated in pigs during folliculogenesis.

ZPA has been reported to harden ZP, thereby preventing polyspermia. This occurs after fertilisation and is related with the formation of intra-/inter-molecular disulphide bonds within/between monomers of this protein, as has been observed in bovine (Iwamoto et al. 1999) and porcine species (Töpfer-Petersen et al. 2008). In mice, ZPA/ZP2 also mediates secondary binding of spermatozoa, and cleavage of ZPA/ZP2 by proteases released through cortical granule reaction causing ZP-'hardening' and thus preventing polyspermy (Barroso et al. 2009).

Both ZPB/ZP4 and ZPC/ZP3 are post-translationally modified. The carbohydrate chains in ZPB are involved in the recognition of spermatozoa (Kudo et al. 1998), and maximal sperm binding is observed when ZPB/ZP4 forms heteromultimeric complexes with ZPC/ZP3 (Yurewicz et al. 1998). In fact, it seems that it is the interaction of these two ZP-glycoproteins that induce the conformational changes in ZPB/ZP4 and allows its sperm-binding domains to be exposed (Töpfer-Petersen et al. 2008).

8.4.2 *Morphological Structure and Organisation of ZP*

The morphological structure of ZP in the pig consists of a meshwork with pores in a similar fashion to other species (Funahashi and Nagai 2001; Fléchon et al. 2004; Mugnier et al. 2009). In this case, ZPB/ZP4 and ZPC/ZP3 form heteromultimeric complexes that may be interconnected by ZPA/ZP2 (Yurewicz et al. 1998) and are organised forming a filamentous and homogenous ring around the oocyte (Mugnier et al. 2009).

In mice, early work by Greve and Wassarman (1985) showed that ZP consists of a matrix constituted by repeating units of ZPA/ZP2 and ZPC/ZP3 heterodimers that are cross-linked by ZP1 homodimers and form a fibrous pattern (East and Dean 1984). Related to this, a hypothetical model for those ZPs formed by four ZP glycoproteins has been proposed; its organisation consisting of ZPA/ZP2 and ZPC/ZP3 heterodimers arranged in filaments and connected by ZP1 and ZPB/ZP4. This organisation would appear to be conserved through species (Familiari et al. 2008).

In humans, ZP is formed by four different glycoproteins known as ZP1, ZP2, ZP3 and ZP4 (Lefièvre et al. 2004; Conner et al. 2005); ZPC/ZP3 forms an homogenous ring around the oocyte in a similar fashion to pigs (Hinsch et al. 1994).

In the case of equine species, Mugnier et al. (2009) have proposed that ZPB/ZP4 and ZPC/ZP3 form heteromultimeric complexes interconnected by equine ZPA/ZP2, so that equine-ZP might contain different types of filament cross-linkers such as ZP1 homodimers, ZPA/ZP2 homodimers and/or ZP1 and ZPA/ZP2 heterodimers. Thus, while in porcine species ZPB/ZP4 and ZPC/ZP3 are organised forming a filamentous meshwork, in equine species they form homogenous patches (Mugnier et al. 2009).

When comparing the size and the number of pores, Mugnier et al. (2009) have observed that porcine-ZP presents fewer pores than equine ZP, but their diameter is larger. This observation is related to differences in ZP-composition in both species and may explain why the penetration rate is lower in equine than in pigs after in vitro fertilisation (IVF) procedures. Taking into account this observation, a general model has been proposed on the basis of porcine and equine species assuming that polyspermy rates are higher when the ZP presents fewer and larger pores (Santos et al. 2008; Mugnier et al. 2009).

8.4.3 Glycosylation and Glycans in ZP-Glycoproteins

Among the most critical domains of ZP-glycoproteins are those formed by carbohydrate chains, since they are involved in the recognition of binding to the sperm surface.

In the case of ZP-glycoproteins, glycosylation consists of neutral and acidic O- and N-linked oligosaccharides that are arranged in N-acetyl-lactosamine residues (Töpfer-Petersen et al. 2008). Regarding O-linked glycans, these are mainly core-1 type (Galβ1-3GalNAc) but they can also be, to a minor extent, core-3 type glycans (GlcNAcβ1-3GalNAc) (Hokke et al. 1994).

As far as N-linked chains are concerned, they mainly belong to the complex type containing bi, tri and tetra antennae, which are all α(1,6)-fucosylated at the innermost N-acetyl-glucosamine residues (Noguchi and Nakano 1992; Noguchi et al. 1992; Von Witzendorff et al. 2005). Amounts of acidic N-glycans in ZP-glycoproteins are approximately two times higher than neutral ones.

On the other hand, it is worth noting that acidic N-glycans containing a single sulphate group (SO_3^-) is abundant. Accordingly, sulphation mainly occurs at the C-6 position of non-repeated GlcNAc residues, of N-acetyl-lactosamine repeating units, and of non-repeated residues of N-glycans (Noguchi and Nakano 1992; Noguchi et al. 1992; Mori et al. 1998). In addition, sulphation is also possible at the C-3 position of the innermost N-acetylglucosamine residues (Mori et al. 1998), but it is less frequent. In contrast, there is no sulphation of repeated N-acetylglucosamine residues (Töpfer-Petersen et al. 2008).

When the glycan profiles of ZP-glycoproteins are compared, high-mannose type glycans appear to be exclusively linked to ZPA/ZP2 (Töpfer-Petersen et al. 2008), while in ZPB/ZP4 and ZPC/ZP3, acidic N-glycans are elongated by up to six sulfated N-acetyl-lactosamine repeats carrying N-acetyl-or N-glycolylneuraminic acid residues at the non-reducing ends (Noguchi and Nakano 1992).

ZP-glycoproteins carry different numbers of potential N-glycosylation sites. In the case of ZPB/ZP4 and ZPC/ZP3, Töpfer-Petersen et al. (2008) have predicted that all N-glycosylation sites are located within the ZP-domain (ZPB/ZP4: N_{202}, N_{220}, N_{333}; ZPC/ZP3: N_{124}, N_{146}, N_{271}). However, only the asparagine residues located at position 220 (N_{220}) of ZPB/ZP4 and at position 271 (N_{271}) in ZPC/ZP3 carry tri-, and tetra-antennary complex N-glycans (Kudo et al. 1998; Yonezawa et al. 1999). In ZPA/ZP2, there are five glycosylation sites: N_{84}, which carry tri- and tetra-antennary glycans and are located at the N-terminal domain, and N_{268}, N_{316}, N_{323} and N_{530}, which are located in the ZP-domain and carry bi-antennary chains (Töpfer-Petersen et al. 2008). According to Von Witzendorff et al. (2005), glycosylation site N_{268} in ZPA/ZP2 also contains mannosyl residues (Man_5GlcNA_2).

Within the glycans linked to the ZP-glycoproteins, sperm-binding activity has been located at the neutral tri- and tetra-antennary complex N-glycans of ZPB/ZP4 expressing non-reducing terminal β-galactosyl residues (Kudo et al. 1998; Yonezawa et al. 2005). These carbohydrate ligands are found in the surface of the ZP and are recognised by the carbohydrate-binding proteins attached to the sperm surface (see also Sect. 8.5.2).

On the other hand, glycosylation is highly important because it regulates the function of ZP-glycoproteins. In mice, for example, sugars have been reported to regulate the ligand abilities of ZPC/ZP3, since O-glycosylation, and particularly terminal galactose residues of O-linked oligosaccharides, and the amino sugar N-acetylglucosamine play a critical role in the maintenance of gamete interaction. In contrast, the acrosome exocytosis triggering activity of ZPC/ZP3 does not depend on sugars but on the integrity of the protein backbone (Chapman and Barratt 1996). In humans, Chiu et al. (2008) observed that the activity of ZPC/ZP3 and ZPB/ZP4 in terms of sperm-ZP binding and of acrosome exocytosis-induced ability also depended on their glycosylation. These authors also reported that N-linked glycosylation appeared to be more significant than O-linked glycosylation.

Finally, it is worth noting that ZP-glycoproteins present considerable heterogeneity in carbohydrate-linked chains. This is related to different degrees of sialylation and sulphation, and depends on oocyte maturation, the age of the gilts/sows and even on breed and diet (Noguchi et al. 1992; Noguchi and Nakano 1992; Takasaki et al. 1999; Töpfer-Petersen et al. 2008). Specifically, differences in ZP-glycoproteins related to oocyte maturation are discussed in the next section (Sect. 8.4.3).

8.4.4 Maturation of ZP

Apart from the maturation of nucleus and cytoplasm in the pig oocyte, the ZP also undergoes a maturation process prior to achieving full fertilisation competence as observed in pigs, humans, mice or cats (Calafell et al. 1992; Familiari et al. 1992; Hermansson et al. 2007). As previously stated, the ZP of an immature and in vitro-matured oocyte differs from that of a mature one, since the former has a spongy

appearance with numerous large pores while the latter presents small pores and a more compact surface (Rath et al. 2005; Michelmann et al. 2007; Mugnier et al. 2009). In addition, kinetics of the acrosome reaction are faster in the ZP of mature oocytes than in the ZP of immature oocytes (Rath et al. 2006).

In fact, the relevance of ZP-maturation during oocyte development is seen in IVF procedures. Specifically for the case of porcine species, there is a low IVF success rate due to high polyspermic rates (Coy et al. 2008) that appear to be related to the defective in vitro maturation (IVM) of collected oocytes. Indeed, both immature oocytes from pre-pubertal animals and IVM-oocytes show a delayed secretion of ZPA/ZP2 from the oocyte. This incomplete secretion of ZPA/ZP2 may influence the three-dimensional architecture of the ZP, thereby accounting for the aforementioned high polyspermic rates after IVF (Niemann and Rath 2001; Töpfer-Petersen et al. 2008).

Maturation of ZP involves a set of changes, including modification in the carbohydrate antennae chains (Rath et al. 2005). For example, ZP-glycoproteins from the oocytes of adult animals or IVM are slightly more acid than those from pre-pubertal or immature animals (pI pre-pubertal: 3–7 vs. pI adult: 3–5.5) (Rath et al. 2005). In addition, ZP-glycans of the oocytes from adult animals are more N-sulphated than those from pre-pubertal gilts (Noguchi and Nakano 1992). Indeed, neutral N-glycans are dominant in immature oocytes, containing high-mannose type glycans with five mannosyl residues and a core made up of bi-antennary N-glycans with one and two terminal galactose residues (Töpfer-Petersen et al. 2008). Therefore, ZP-maturation implies an increase in sulphated N-acetyl-lactosamine repeated units in ZP-glycoproteins. The high occurrence of polysulphate structures in mature oocytes affects the sperm penetration of oocyte through ZP (Töpfer-Petersen et al. 2008).

8.5 Gamete Binding and Interaction (II): Sperm Surface

8.5.1 A General Overview of Sperm Receptors for ZP-Proteins in Mammals

Research on carbohydrate-binding mechanisms between spermatozoa and oviductal cells (see Chap. 6) and between sperm and ZP started more than 25 years ago (Töpfer-Petersen et al. 1985). In this regard, one of the most important efforts has been to identify whether an individual protein or a carbohydrate side chain working as 'sperm receptor' existed (Barroso et al. 2009). Accordingly, different studies have been conducted and have proposed the following primary sperm receptors for ZP:

1. A 95 kDa protein-kinase in mice (Leyton et al. 1992).
2. SP56 (Bookbinder et al. 1995) in mice.
3. Trypsin-like protein (Boettger-Tong et al. 1993) in humans.

4. β1,4-galactosyltransferase (GalTase) in mice (Shur 1994).
5. Zona-adhesins/Spermadhesins in mice (Gao and Garbers 1998) and ungulates
   (Töpfer-Petersen et al. 2008), such as pigs.

At present, none of these molecules have been unequivocally established as an
active receptor, but growing evidence indicates that spermadhesins play a relevant
role in ungulates, as will be further discussed. In addition, recent progress in mam-
malian spermatozoa has shown that two proteins that interact with ZP3 are zona-
adhesin and PKDREJ (Polycystic Kidney Disease Polycystin) and REJ (sperm
receptor for egg jelly homolog, sea urchin homolog). Evolutionary studies made
within and between primate species have identified promising regions of the proteins
that seem to interact and co-evolve with ZP3 (Bi et al. 2003; Sutton et al. 2006).

8.5.2 The Case of Porcine Species: Primary and Secondary Binding of Spermatozoa to ZP

In the case of sperm-ZP interaction in pigs, boar spermatozoa were first described as
having fucoidan-binding proteins, especially at the apical region of the sperm head
(Töpfer-Petersen et al. 1985; Friess et al. 1987). Within these proteins, identified
as the major ZP-binding proteins of boar spermatozoa, we can distinguish between
sperm-surface associated proteins (mainly spermadhesins and P47/SED1) that have a
molecular mass of 12–16 kDa (Töpfer-Petersen et al. 1998; Petrunkina et al. 2003)
(see also Sect. 6.7.3) and pro/acrosin, which is an intra-acrosomal serine protease that
has a molecular mass of 53–55 kDa (Töpfer-Petersen and Henschen 1987) (Fig. 8.2).
The different localisation of sperm-surface attached proteins and proacro-
sin (the former are peripheral membrane-associated proteins while the latter is
an intra-acrosomal protein) seems to be related to the sequence of carbohydrate-
mediated events occurring within the oviduct, i.e. the formation of sperm reservoir
and binding to the ZP (Töpfer-Petersen et al. 2008). Thus, sperm-surface associ-
ated proteins not only participate in the formation of sperm reservoir but also in
the primary binding with ZP-proteins of the oocyte. After primary binding, proacr-
osin is activated to acrosin, leading to acrosomal exocytosis and subsequently
allows secondary binding. In addition, sperm-surface attached proteins are thought
to stabilise the plasma membrane over the acrosomal vesicle between ejaculation
and fertilisation (see also Chaps. 1 and 6).

8.5.3 Sperm-Surface Attached Proteins Involved in Primary Binding to ZP

Different sperm-binding ZP proteins have been identified, which represent the
contribution of multiple sperm proteins in recognition and binding events. These
proteins are sperm-surface attached proteins (spermadhesins AWN and AQN-3,

and protein DQH; see Sect. 6.7.3), lactadherin (also known as P47 and SED-1), fertilin β (also known as ADAM-2) and peroxiredoxin 5 (PRDX5), which belong to a novel family of peroxidases and is usually intracellular (Blobel 2000; Brewis et al. 2005; Primakoff and Myles 2000; Van Gestel et al. 2007) (Figs. 6.4 and 8.2).

As extensively discussed in Chap. 6, there are different sperm-surface attached proteins, but the major ones are spermadhesins. The first interaction of spermadhesins to spermatozoa is mediated by non-aggregated AWN and AQN-3 proteins that directly bind the phosphorylethanolamine molecules (Calvete et al. 1996; Dostàlovà et al. 1995a; Ensslin et al. 1995). Then, there is another spermadhesin, AQN-1, which is located over AWN and AQN-3 and indirectly attaches to the sperm surface via binding to DQH. This DQH interacts, in turn, with the sperm membrane through phosphorylcholine-binding sites (Calvete et al. 1997; Ekhlasi-Hundrieser et al. 2007).

Three spermadhesins (AQN-1, AQN-3 and AWN) play a crucial role in the carbohydrate-mediated events that take place in the oviduct (i.e. sperm reservoir and fertilisation) (Töpfer-Petersen et al. 2008). These three proteins differ, however, in the carbohydrates they recognise, since AWN only interacts with α- and β-linked galactose residues, while AQN-1 also recognises Man α1-3(Manα1-6)Man structures (Ekhlasi-Hundrieser et al. 2005). This fact has been related to the role of these sperm proteins within the oviduct, since AQN-1 participates in the formation of the sperm reservoir by interacting with the glycoconjugates of the oviductal epithelium, while AQN-3 and AWN take part in primary binding of the spermatozoon to ZP-glycoproteins during fertilisation. In this latter case, it must be mentioned that AQN-3, AWN and P47/SED1 are masked by AQN-1 before capacitation. During capacitation, AQN-1 is removed from the sperm surface (Sanz et al. 1993; Calvete et al. 1997), so that AQN-3, AWN and P47/SED1 become unmasked, accessible and able to interact with ZP-glycans (Rodríguez-Martínez et al. 1998; Wagner et al. 2002; Petrunkina et al. 2003; Ekhlasi-Hundrieser et al. 2005). Related to this, and despite AWN and AQN-3 presenting a similar binding affinity for ZP-glycoproteins that contain Galβ(1-3)GlcNAc and Galβ(1-4)GlcNAc sequences either in N- or O-linked oligosaccharides, they differ regarding the recognition of tri-/tetra-antennary N-glycans (Dostàlovà et al. 1995b; Töpfer-Petersen et al. 2008).

Finally, there is another protein named lactadherin (the porcine homolog of SED1, also known as sperm-surface protein P47/SED1, MFGM or Milk fat globule-EGF factor 8 protein), which is expressed in the testis and in the epididymis of mammals (Ensslin et al. 1998), binds the sperm surface, and also seems to participate in the interaction between the spermatozoon and oocyte, as has been suggested in mouse experimental models (Shur et al. 2004). However, it still remains to be elucidated whether P47/SED 1 interacts with ZP by carbohydrate protein or protein–protein interactions.

8.5.4 Proacrosin and Secondary Binding

When the sperm-surface attached proteins primarily interact with the ZP-glycoproteins, a signal cascade is triggered leading to the exocytosis of the acrosome, which contains a large amount of hydrolytic enzymes like acrosin

(Jungnickel et al. 2001; see also Chap. 1). The acrosome-reacted spermatozoon is then able to penetrate the ZP by alternating cycles of sperm-ZP binding, limited proteolysis of the matrix and release from the ZP (Fig. 8.1). In these cycles, the force generated by the forward motility of hyperactivated spermatozoa also plays a crucial role (O'Rand et al. 1986; Green 1987; Suarez 2008).

Proacrosin has two forms, i.e. the inactive (zymogen) named proacrosin and the active (serine proteinase) called acrosin, and exerts a key function in the interaction between spermatozoon and oocyte. Genes encoding the proacrosin have been reported to be highly conserved in mammals during their biological evolution (Klemm et al. 1991).

Proacrosin, the inactive form, is stored in the acrosomal vesicle until the acrosome reaction is triggered and has a single chain molecule of 53/55 kDa that presents the catalytic triad (formed by residues of Histidine (His) at position 70, Aspartate (Asp) at position 124 and Serine (Ser) at position 222) like other serine proteinases, and a sequence of 125 amino acidic residues at the C-terminus that contain 23 consecutive prolines. Zelezna et al. (1989) suggested that this proline-rich domain could be responsible for the strong binding of proacrosin to biological membranes and possibly to the sperm inner acrosomal membrane.

Proacrosin presents a high affinity for sulphated oligosaccharide chains such as ZP-glycoproteins that, together with fucoidan and heparin, have been reported to accelerate the activation process from proacrosin to acrosin (Töpfer-Petersen and Henschen 1987; Töpfer-Petersen and Henschen 1988; Töpfer-Petersen et al. 1990a). Accordingly, this activation process is mediated by a basic peptide of 18 residues, between Isoleucine at position 43 (Ile_{43}) and Leucine at position 60 (Leu_{60}) that contains one polysulphate-binding site (Moreno and Barros 2000). In fact, activation from proacrosin to acrosin as well as control of enzyme activity depends on the spatial proximity of the proacrosin-active site and the polysulphate-binding sites, which are recognised by ZP-components and trigger the acrosome reaction and subsequent downstream events (Tranter et al. 2000).

After the activation mediated by Ile_{43}-Leu_{60} peptide, conversion of proacrosin to acrosin occurs via the autocatalytic-proteolytic cleavage between arginine and valine residues located at positions 23 and 24, respectively (Arg_{23} and Val_{24}). This leads to the formation of an active two-chain molecule of the same molecular mass in which the light chain (23 amino acids) remains linked to the heavy chain by two disulphide bonds (Cechova et al. 1988). Each chain presents one N-glycosylation site in asparagine residues, which are respectively located at position 3 (Asn_3) in the light chain and at position 10 (Asn_{10}) in the heavy chain (Cechova et al. 1988; Töpfer-Petersen et al. 1990b).

Apart from the activation-proteolytic site located between Arg_{23} and Val_{24} residues, proacrosin presents another processing site at C-terminal, which releases the last 77 amino acids originating β-acrosin, an mature acrosin form of 38-kDa (Baba et al. 1989; Puigmulé et al. 2011).

Although proacrosin may take part in all three stages of the mentioned cycles (i.e. binding, digestion and release and proacrosin reactions), its exact role is not clear, since proacrosin null mouse spermatozoa are able to penetrate the ZP and

to fertilise the oocyte (Honda et al. 2002) but at a lower velocity than the wild type (Adham et al. 1997; Nayernia et al. 2002). In addition, Jin et al. (2011) have recently observed that mice spermatozoa that undergo acrosome reaction before interacting with ZP are also able to fertilise the oocyte. We mention here data available in mice, as no similar studies have been conducted in pigs. Care must be taken, however, when extrapolating these findings in mice to pigs, since the thickness of ZP in pig oocytes is greater than in mice, so that acrosin may play a more critical role in pigs in the secondary binding and sperm penetration through ZP.

Finally and still related to the actual role of proacrosin, secondary binding also seems to involve other enzymes and binding proteins apart from proacrosin, which could act synergistically to achieve maximal fertilisation. In this regard, two other serine proteases have been identified in mouse sperm (Honda et al. 2002), and another ZP-binding protein (SP38, also known as ZPBP) has been found in boar spermatozoa (Mori et al. 1995; see also Chap. 1).

8.6 Gamete Binding and Interaction (III): Sperm-ZP Recognition and Acrosome Exocytosis

8.6.1 Introduction

Several studies have previously shown that sperm-ZP interaction plays a critical role for successful fertilisation (Yanagimachi 1994b). This interaction takes place through two sequential steps. The first one occurs when ZPC/ZP3 interacts with spermatozoa. Then, a sperm receptor that is coupled to an inhibitory regulative G-protein (G_i) activates phospholipase Cβ1 and regulates adenylyl cyclase leading to an increase in cAMP levels. Cyclic AMP activates, in turn, protein kinase A (PKA) to open a calcium channel in the outer acrosomal membrane, resulting in a small increase of cytosolic Ca^{2+}. Then, Ca^{2+} activates phospholipase C$_\gamma$, which is coupled to the second tyrosine kinase receptor. The two products of phospholipase C (PLC) activity, diacylglycerol (DAG) and inositol triphosphate (IP_3), activate protein kinase C (PKC) and IP_3 receptor. Upon activation, PKC opens a Ca^{2+}-channel in the membrane, and IP_3 activates the Ca^{2+}-channel in the outer acrosomal membrane that leads to a higher increase in cytosol calcium. This set of intracellular changes results in membrane fusion and completion of the acrosome reaction (Breitbart and Naor 1999; Breitbart 2002; Florman et al. 1989, 1992) (Fig. 8.3).

A second binding then follows which involves ZPA/ZP2 and the inner acrosomal-sperm membrane. This second binding finally leads to ZP-penetration (Wassarman 1995, 1999).

Cleavage of ZPA/ZP2 by proteases released through the cortical granule reaction causes the hardening of ZP and thus prevents polyspermy (Barroso et al. 2009). Related to this, uncleaved ZPA/ZP2 seems to be related with post-fertilisation persistence of mouse sperm binding to 'humanised' ZP (Castle and Dean 1999).
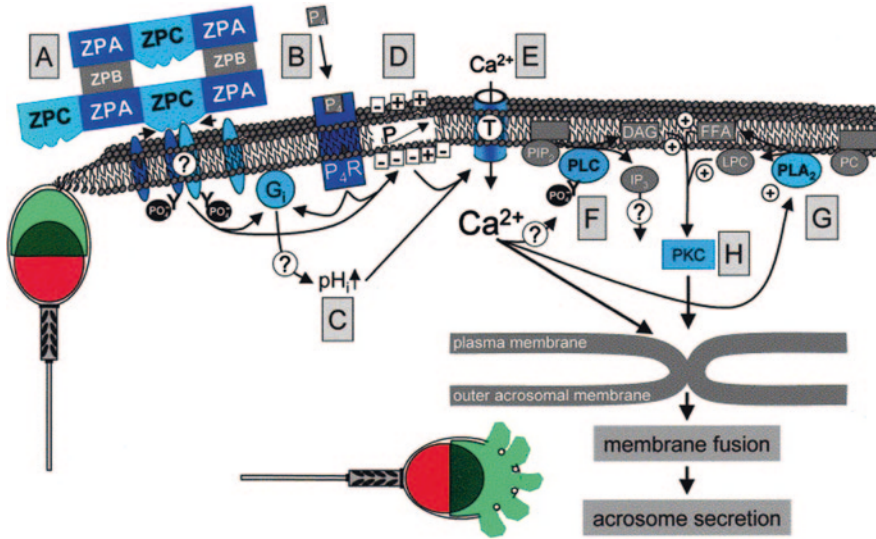
Fig. 8.3 Proposed sequence of the ZP and progesterone induced acrosome exocytosis. (*A*) ZP proteins (most likely ZPC) bind to sperm ZP receptors, leading to aggregation and tyrosine (Y) phosphorylation. (*B*) The direct environment of the ZP contains high levels of progesterone that can bind to its non-genomic receptor (P_4R) on the sperm surface. Both ZP and progesterone have a dual effect on sperm cells. (*C*) The intracellular pH (pH_i) is increased via (G_i) and (*D*) the plasma membrane potential depolarises. (*E*) Both the increased pH_i and depolarisation induce the entry of calcium via a T-type voltage dependent Ca^{2+} channel. (*F*) The higher intracellular Ca^{2+} levels activate PLC that has been translocated to the plasma membrane during capacitation. PLC converts PIP_2 to DAG and IP_3. (*G*) Increased Ca^{2+} levels activate PLA_2, which degrades PC to LPC and free fatty acids (FFA). (*H*) The role of IP_3 is unclear, but DAG, FFA and LPC activate PKC. Both the increased intracellular Ca^{2+} levels and PKC activation are necessary for the fusion of the plasma membrane with the underlying acrosomal membrane, which leads to the subsequent secretion of acrosomal enzymes (Flesch and Gadella 2000) Reproduced with permission

For this reason, it has been hypothesised that the supramolecular structure of ZP necessary for sperm binding is modulated by the cleavage status of ZPA/ZP2 (Rankin et al. 1998, 2003; Castle and Dean 1999; Hoodbhoy and Dean 2004).

8.6.2 Species Specificity of Sperm-ZP Recognition: Barriers to Penetration

Specificity of sperm-oocyte interaction exists that varies between vertebrate species and represents a barrier for fertilisation between some species. Some of these barriers are located at the ZP level, so that ZP often works as a barrier for heterologous crosses in vitro (Wassarman et al. 2005). Indeed, for example, complex N-glycans with terminal fucose and GlcNAc residues are involved in the sperm-ZP binding in *Xenopus laevis* (Vo and Hedrick 2000), O-linked oligosaccharides

of ZP-glycoproteins take part in sperm-oocyte interaction in mice (Wassarman et al. 2005), and the ZP of human oocyte presents mannose-binding sites that play a crucial role in the recognition mechanisms of gametes (Rosano et al. 2007).

However, there are also exceptions to this rule in those species presenting both four and three ZP-glycoproteins. Thus, for example, rat spermatozoa are able to bind mouse-ZP, but mouse spermatozoa are less able to adhere to rat-ZP (Hoodbhoy et al. 2005). Mouse spermatozoa are, in turn, able to bind human-ZP. In contrast, human spermatozoa cannot bind mouse-ZP (Hoodbhoy et al. 2005), but they are able to interact with porcine-ZP (Cánovas et al. 2007).

In cattle, sperm-oocyte binding involves the polyvalent presentation of terminal α-mannosyl residues of high-mannose N-glycans (Amari et al. 2001), while this interaction is mediated by terminal β-galactosyl residues of complex N-glycans (Yonezawa et al. 2005) in pigs. In both species, sugars mediating this interaction are linked to the ZPB/ZP4 glycoprotein, and despite receptor-ligand systems being different, porcine and equine spermatozoa can bind bovine-ZP in vitro, and equine spermatozoa are even able to penetrate the bovine-ZP and to enter the bovine oocyte (Sinowatz et al. 2003). When comparing equine and porcine species, Mugnier et al. (2009) observed that although the number of porcine spermatozoa bound to equine ZP is similar to that of equine spermatozoa, equine spermatozoa are less able to bind porcine ZP. Thus, it seems that that equine-ZP is less selective than porcine ZP.

All these data suggest that there are some ZPs that are not able to support heterologous sperm binding, whereas others are more permissive (Mugnier et al. 2009). This selective ability, apart from emphasising the key role of the composition and structure of ZP in sperm-ZP binding, seems to be related with the phylogenetic distance between species. In this regard, it is worth noting that tiger spermatozoa appear to be able to fertilise cat oocytes (Donoghue et al. 1992), while goat and ram spermatozoa are able to fertilise bovine oocytes (Cox et al. 1994; Kouba et al. 2001; García-Álvarez et al. 2008) and deer sperm can fertilise sheep oocytes (Soler et al. 2008).

This heterologous in vitro recognition and interaction may be explained by the existence of glycans with a similar structure that can be recognised by different ZP-binding proteins.

In addition, the heterologous interaction observed in vitro suggests that there are other mechanisms in vivo, apart from the specific recognition between spermatozoon and oocyte, which also act as inter-species barriers. These mechanisms are related to the anatomy and behaviour of males and females, species specificity of the sperm oviductal reservoir (see Chap. 6), fusion between sperm and oocyte membranes and post-fertilisation events (Töpfer-Petersen et al. 2008).

8.6.3 Lessons from In Vitro Studies: Porcine versus Equine Spermatozoa

As stated, penetration and polyspermy rates after IVF are higher in equine (Dell'Aquila et al. 1996; Alm et al. 2001; Hinrichs et al. 2002) than in porcine

species (Day 2000; Nagai et al. 2006; Mugnier et al. 2009). This fact can be related to two findings recently reported by Mugnier et al. (2009):

1. During sperm-ZP binding, equine-ZP is less selective than porcine-ZP.
2. During sperm penetration, equine ZP represents a barrier for both homologous (equine) and heterologous (porcine) spermatozoa, whereas porcine ZP represents a barrier for heterologous but not for homologous spermatozoa.

Since equine and porcine ZPs differ in the presence/absence of ZP1, Mugnier et al. (2009) have suggested that this protein might be involved in the different sperm attachment and penetrating ability in equine and porcine species, the former presenting a higher sperm binding but a lower penetration rate than the latter. Despite the fact that ZP1 does not seem to play a direct role in sperm-ZP interaction, the ability of ZP1 to form intermolecular disulphide bonds may allow it to be involved in the three-dimensional structure of the ZP. Since Hoodbhoy and Dean (2004) have proposed the supramolecular structure as a key component in sperm-oocyte recognition, ZP1 could take part in sperm-ZP binding via a role in this supramolecular structure (Mugnier et al. 2009).

On the other hand, ZP-glycoproteins are differently located in equine and porcine species, and this may be related to differences in ZP-composition in both species. Thus, in pigs, ZPB/ZP4 and ZPC/ZP3 are organised forming a filamentous meshwork and in equine species they form homogenous patches (Mugnier et al. 2009). Therefore, a different molecular organisation of ZP-glycoproteins exists in these porcine and equine species, since ZP1 is present in equine but not in pigs, and ZPA/ZP2 is differently located in equine and porcine species.

When comparing the size and the number of pores, Mugnier et al. (2009) have observed that porcine-ZP presents a lower number of pores than equine-ZP, but their diameter is greater. This observation may explain why the penetration rate is low in equine IVF, whereas the penetration and polyspermy rates are high in porcine IVF. Therefore, a general model has been proposed on the basis of porcine and bovine species assuming that polyspermy rates are higher when the ZP presents fewer and larger pores (Santos et al. 2008; Mugnier et al. 2009).

In short, the main differences between equine- and porcine-ZP are the number of ZP-glycoproteins, three in porcine, four in equine species, their localisation and the molecular organisation of ZP, including the number and the size of pores (Mugnier et al. 2009). These differences may underlie the different penetration and polyspermy rates in equine and porcine species.

8.6.4 ZP-Modifications Mediated by the Oviductal-Specific Glycoprotein to Prevent Polyspermia

Since polyspermy is an important anomaly of fertilisation in placental mammals, it is important to mention that there must exist mechanisms avoiding this polyspermy (Snook et al. 2011; Iwao 2012).

Although most studies have used rodents as models, ungulates may differ in their mechanisms to prevent polyspermy. Related to this, Coy et al. (2008), working with pigs, observed that periovulatory oviductal fluid from sows increased ZP resistance to digestion with pronase (a parameter commonly used to measure the block to polyspermy). These authors also reported an increase in monospermy and a decrease in sperm-ZP binding when the oocytes were previously exposed to oviductal fluid. In addition, Coy et al. (2008) also observed that an heparin-depleted medium abolished the resistance of oviductal fluid-exposed oocytes to pronase, but a medium with heparin did not alter this resistance. When these authors analysed the content released in the heparin-depleted medium after removing the oocytes, they isolated and identified the oviduct-specific glycoprotein (OVGP1, also known as OSP; see Sect. 6.5). Taking into account all these data, Coy et al. (2008) hypothesised that ZP-changes during the transit of oocyte along the oviductal ampulla modulated the interaction with spermatozoa. In this context, oviduct-specific glycoprotein-heparin protein complex would be the responsible for these ZP changes, thereby affecting sperm binding and contributing to the regulation of polyspermy.

We can thus conclude that, according to Coy and Avilés (2010), there are three groups of mechanisms that contribute to block the polyspermy in mammals:

1. Oviduct-based mechanisms, avoiding a massive arrival of spermatozoa in the proximity of the oocyte.
2. Egg-based mechanisms, including changes in the membrane and ZP in reaction to the fertilising sperm.
3. Modifications of the ZP in the oviduct before the oocyte interact with spermatozoa, known as 'pre-fertilisation ZP hardening'. As stated, this mechanism is mediated by the OVGP1 secreted by the oviductal epithelial cells around the time of ovulation, and is reinforced by heparin-like glycosaminoglycans (S-GAGs) present in the oviductal fluid (Coy and Avilés 2010).

8.6.5 Role of ZP in Triggering Acrosome Exocytosis

Solubilised ZP triggers the acrosome reaction of spermatozoa, not only in pigs, but also in humans (Cross et al. 1988) and other mammalian species. However, when human spermatozoa are treated with pertussis toxin, an inactivator of inhibitory G-like proteins, there is a decrease in the percentage of acrosome-reacted spermatozoa after incubation with solubilised ZP. In contrast, calcium ionophore A-23187, also used to induce acrosome reaction, is able to trigger the acrosome reaction of human spermatozoa, despite the latter having previously been treated with pertussis toxin (Lee et al. 1992). This acrosome reaction triggering effect mediated by ZP is dose-dependent and involves inhibitory G_i (Franken et al. 1996). Related to this, Schuffner et al. (2002) demonstrated that the AR mediated by homologous ZP depends on the activation of inhibitory G_is (toxin-sensitive)
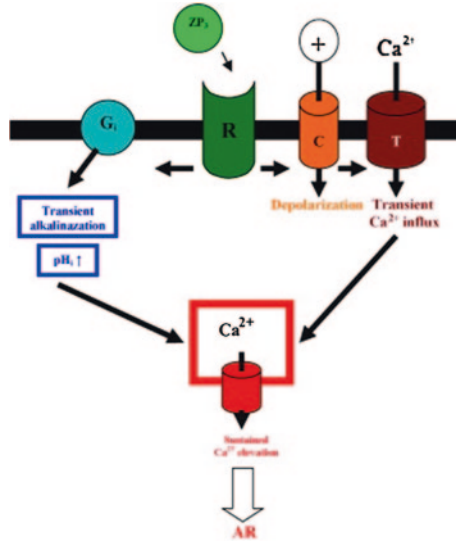
Fig. 8.4 Schematic representation of the proposed mechanism of sperm ion channel activation by the zona pellucida (ZP3/ZPC). One pathway consists of the activation of a cation channel (*C*), which leads to a depolarisation of the sperm plasma membrane; the result of membrane depolarisation is the activation of another, low-voltage-activated T-type channel (*T*). The other signalling pathway results from a transient alkalinisation of the internal pH (pH$_i$). Both pathways lead to a sustained Ca^{2+} elevation, necessary for acrosome exocytosis (Witte and Schäfer-Somi 2007) Reproduced with permission

and on the presence of extracellular Ca^{2+} (Fig. 8.4). In addition, the oviductal environment, particularly progesterone and follicular fluid, exert a priming role in the ZP-mediated induction of acrosome (Schuffner et al. 2002).

Although as is widely known, sperm binding to ZP triggers the acrosome reaction (Yanagimachi 1994b), Mugnier et al. (2009) have shown that ZP-mediated induction is not species specific. Thus, porcine ZP is able to induce acrosome exocytosis of horse spermatozoa and equine ZP is also able to induce the acrosome reaction of boar spermatozoa, thereby indicating that the ZP-ligand inducing acrosome reaction may be conserved between different species.

On the other hand, there are other interesting reports that have studied how acrosome exocytosis occurs when ZP is not present (i.e. in the case of zona-free oocytes). These reports have shown that despite porcine- and equine-ZP presenting a similar ability to induce acrosome exocytosis in boar and horse spermatozoa, equine spermatozoa are less able than boar sperm to undergo acrosome reaction regardless of the ZP-origin (Mugnier et al. 2009). This suggests that acrosome exocytosis depends on the capacity of the spermatozoa to undergo it, rather than the structure and composition of the ZP. Thus, the composition/conformation of ZP appears to be a critical element for sperm-oocyte binding and recognition but not for triggering acrosome reaction. The mentioned differences between boar and stallion spermatozoa in terms of acrosome reaction induced by ZP might be

related to differences in sperm capacitation, to the second binding of the spermatozoa, and/or to the penetration of the spermatozoa through the ZP.

8.6.6 SNARE Proteins and Acrosome Exocytosis

Yunes et al. (2000) were the first to hypothesise that the essential components involved in the membrane fusion of intracellular compartments in somatic cells, such as Rab3A, GTPase and SNAREs, are also present in mammalian sperm and can be involved in membrane fusion during the acrosome reaction. We must remember that conformational changes of SNARE proteins occur during sperm capacitation as has been described in Chap. 7 (Fig. 7.5), in a prior step to fertilisation (Tsai et al. 2010).

In the exocytosis of somatic cells mediated by SNARE proteins, the Q-SNARE proteins located in the cell membrane interact with the R-SNARE proteins located in the vesicle membrane and form a 7S or a 20S trans-SNARE protein complex (Chernomordik and Kozlov 2008). This complex then undergoes a conformational change depending on calcium levels, and this leads to membrane fusion (Martens and McMahon 2008; Sudhof and Rothman 2009). Therefore, it is worth noting that membrane fusion does not take place directly after the assemblage of SNARE proteins, but needs the appropriate stimulus (i.e. extracellular calcium influx) as well as other factors, such as RAS-associated protein RAB3A, N-ethylmaleimide-sensitive factor (NSF) or complexin, that mediate the fusion of the two membranes (Michaut et al. 2000; Maximov et al. 2009; Tsai et al. 2010; Vrljic et al. 2010). Furthermore, the formation of the trans-SNARE complex involves multiple regulatory pathways in a complicated process (Verhage and Toonen 2007).

Taking into account all the aforementioned, Verhage (2009) distinguishes three stages in the fusion between cell and vesicle membranes:

1. Membrane docking, when Q- and R-SNARE proteins interact to form the trans-SNARE complex (Jahn and Scheller 2006).
2. Priming or preparation of the fusion event when trans-SNARE complex pulls the interacting membranes into a close and tight apposition (Jahn and Scheller 2006; Verhage and Toonen 2007).
3. Membrane fusion.

Specifically for acrosome exocytosis, Tsai et al. (2007, 2010) have investigated how SNARE proteins are involved in the regulation of acrosome docking, priming and fusion in the male gametes. Accordingly, upon sperm capacitation, the redistribution of both Q- and R-SNAREs at the apical head region appears to be a bicarbonate- and albumin-dependent event (Tsai et al. 2007; see also Chap. 7). This redistribution step is required for further membrane fusion. In the case of acrosome reaction, the fusion points are located in the sperm head plasma (Q-SNARE) and in the outer acrosome membranes (R-SNARE) and form a trans-SNARE protein complex when interacting with ZP-glycoproteins (Tsai et al. 2010).

8.6.7 The Role of Glycodelins

As previously mentioned, carbohydrate-binding proteins on the sperm surface mediate gamete recognition by binding with high affinity and specificity to complex glycoconjugates of the ZP (Miller et al. 1992; Clark et al. 1996, 1997; Oehninger 2001). In the case of humans, the sperm-binding proteins and ZP-glycans involved in this recognition system have been reported to be linked to natural killer cell inhibition (Clark et al. 1996; Oehninger 2001, 2003; Ozgur et al. 1998; Patankar et al. 1997), i.e. to immune response.

This hypothesis has been reinforced by the discovery of Glycodelin-A, a protein that is not present either on the sperm surface or in the ZP but is involved in the recognition and binding between gametes. Indeed, Glycodelin-A is an endometrial epithelial protein that in vitro inhibits sperm-ZP binding in a dose-dependent manner (Oehninger 2001; Oehninger et al. 1995; Seppälä et al. 2002). In addition, oligosaccharides associated with Glycodelin-A also block selectin-mediated adhesions. Therefore, carbohydrate binding specificity of the receptors mediating sperm-oocyte recognition seems to converge with that of lymphocyte/leukocyte adhesion, since Glycodelin-A exerts a contraceptive and immunosuppressive role, which depends on carbohydrates (Clark et al. 1996; Dell et al. 1995, 2003).

Glycodelin-A binds to N-glycans, specifically to high mannose bi-antennary bisecting type, and to biantennary, triantennary and tetra-antennary oligosaccharides, that terminate with Lewis-x (Lex) and Lewis-y (Ley) sequences (Dell et al. 1995, 2003). It is worth noting that, at least in humans, the major N-glycans of spermatozoa, which are Lex/Ley-terminated (Pang et al. 2007), are related to the inhibition of both innate and adaptive immune responses (Barroso et al. 2009). Following this, Clark et al. (1996, 1997) linked the expression of carbohydrate functional groups with the protection of gametes and the developing human embryo in the uterus, giving rise to the 'fetoembryonic defense system hypothesis' for eutherian mammals.

More recently, Chiu et al. (2007) have observed that a sperm protein identified as fucosyltransferase-5 (FUT5) is able to bind both to Glycodelin-A and to intact and solubilised ZP. For this reason, these authors have suggested that Glycodelin-A inhibits sperm-ZP binding, because it blocks the binding of sperm-FUT5 to ZP (Chiu et al. 2007).

8.7 Gamete Binding and Interaction (IV): Fusion of Sperm and Oocyte Membranes

8.7.1 Introduction

Fusion between a spermatozoon and an oocyte is a cell–cell membrane fusion event (Barroso et al. 2009; Sutovsky 2009) that is only possible when acrosome exocytosis has occurred (Yanagimachi 1994b). Thus, binding and fusion of spermatozoa

Fig. 8.5 Binding and fusion between both gametes. **a** Sperm initially binds with the apical tip of the inner acrosomal membrane to the oolemma. **b** After proper positioning, the apical binding is diminished and has facilitated a lateral binding of the sperm cell to the oolemma. The equatorial region of the sperm head plasma membrane is involved in this process. **c** The spermatozoon is now capable of fusing its equatorial plasma membrane with the oolemma. **d** The multidomain organisation of fertilin has been proposed to be directly involved in processes (**b**) and (**c**) (Flesch and Gadella 2000) Reproduced with permission
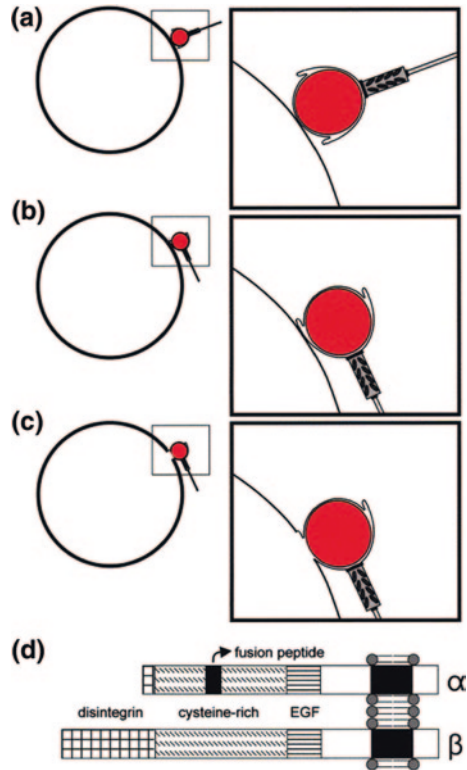
with the oolemma occurs at the post-acrosomal/equatorial region, since after the completion of acrosome reaction and ZP-penetration, plasma and inner and outer acrosomal membranes of this region remain intact (Stein et al. 2004) (Fig. 8.5).

As early studies conducted in mice showed, an acrosome-reacted spermatozoon fuses with numerous microvilli of the oolemma during fertilisation, and it is the whole spermatozoon (with the exception of part of the sperm plasmalemma, most of the outer acrosomal membrane and the acrosomal components) that is incorporated into the oocyte (Yanagimachi 1998).

8.7.2 Protein Candidates Involved in Sperm-Oocyte Fusion

As far as molecules involved in binding/fusion between sperm and oocyte membranes are concerned, different studies have suggested a list of candidate proteins that may mediate this mechanism (Snell and White 1996). However, the specific roles of these molecules still need to be validated (Vjugina and Evans 2008). These proteins are:

- Two cysteine-rich secretory proteins (CRISP), also known as epididymal protein CRISP1, and testicular protein TPX1 (CRISP2) that interact with egg-binding sites (Busso et al. 2005; Da Ros et al. 2007; Vadnais et al. 2008).

- A member of the ADAM family of trans-membrane proteins on sperm (ADAM-1 and ADAM-2, Fàbrega et al. 2011).
- IZUMO1, a family member of immunoglobulin proteins, in spermatozoa (Inoue et al. 2005, 2008).
- Equatorin, which takes its name from its localisation at the equatorial segment of the acrosome (Toshimori et al. 1998).
- Integrin $\alpha v \beta_1$ on oocytes (Linfor and Berger 2000).
- CD9, known to complex with integrins, on oocytes (Kaji et al. 2000; Miyado et al. 2000).

From all these candidates, we must mention that sperm binding to oocyte-integrinβ_1 is a necessary step prior to fusion between spermatozoon and oocyte (Blobel 2000; Cowan et al. 2001). Oocyte integrinβ_1 recognises fertilin-β (ADAM-2), a sperm-surface protein also known as PH-30 (Fàbrega et al. 2011) (Fig. 8.5).

Another protein candidate that has been reported to be involved in sperm plasmalemma- and oolemma-binding is P-selectin. This protein has been detected in the oolemma of human and hamster oocytes following sperm adhesion on the equatorial region of human spermatozoa (Fusi et al. 1996).

Finally, Inoue et al. (2005) identified a gene known as *IZUMO1* that seems to mediate sperm-oocytes fusion at least in humans and hamsters. However, the function of the protein product encoded by this gene does not seem to be dependent on glycosylation.

8.7.3 Membrane Fusion Between Gametes as a Barrier to Penetration

Apart from the role that ZP can play as a barrier to penetration between species, fusion between sperm plasmalemma and oolemma after secondary binding and acrosome reaction can act, in some species, as an intra/inter-specific barrier (Mugnier et al. 2009).

In several studies evaluating heterologous IVF and using ZP-free oocytes, horse and boar spermatozoa have been seen to fuse with equine and porcine oolemma (Mugnier et al. 2009). In addition, horse spermatozoa are able to fuse with bovine and hamster oolemma (Landim-Alvarenga et al. 2001; Matsukawa et al. 2002; Choi et al. 2003), and rat sperm have also been seen to fuse with porcine oolemma (Zhao et al. 2002). All these findings suggest that the ligand-receptor mediating sperm-oolemma binding may be conserved through some species (Sartini and Berger 2000; Mugnier et al. 2009). However, despite horse and boar spermatozoa being able to fuse with equine and porcine oolemma, both are less able to penetrate ZP-free equine oocytes than ZP-free porcine oocytes. This suggests that the oolemma may represent a barrier of equine oocytes to be penetrated by homologous or heterologous spermatozoa (see also Sect. 8.6; Mugnier et al. 2009).

On the other hand, it is worth noting that zona-free hamster eggs bind sperm from almost any species, including human (Tyler et al. 1981; Matson et al. 1987) and, even, birds (Samour et al. 1986), and allow sperm penetration (Maleszewski et al. 1995). However, while guinea-pig and human spermatozoa can activate hamster oocytes as efficiently as hamster spermatozoa, mouse spermatozoa cannot. This means that sperm-oocyte membrane fusion *per se* is not enough to trigger oocyte activation, but there is another species-specific barrier related to sperm-derived oocyte activating factors (Maleszewski et al. 1995).

8.8 Remodelling of Sperm Chromatin and Pronuclei Formation After Fertilisation

8.8.1 Organisation of Chromatin in Mature Spermatozoa

During spermatogenesis, DNA is progressively compacted by the replacement of nuclear histones for sperm-specific proteins known as protamines (see Chap. 3). These protamines contain a large amount of cysteine residues that establish inter- and intra-molecular disulphide bonds formed after the oxidation of the sulphydryl groups that these cysteine-residues contain. This forms a toroid shaped 'doughnut' of coiled chromatin loops that are much tighter than the normal solenoid loop typical of somatic cell nuclei (Calvin and Bedford 1971; Ward and Coffey 1991; Ward 1993).

Despite the aforementioned substitution, sperm chromatin also contains histones. Accordingly, studies conducted in humans have demonstrated that about 15–20 % of sperm DNA, which in contrast to porcine sperm (that only present protamine-1), contains two different protamine-types (i.e. protamine-1 and protamine-2), is structured around histones (O'Brien and Zini 2005). This finding is highly relevant, since the structure associated with histone-linked DNA domains is much less compact and rigid than the one related to protamine-linked DNA domains (Wykes and Krawetz 2003; O'Brien and Zini 2005). In addition, these histone-linked domains have been reported to be located at the telomeric sequences, so that they present a given function (O'Brien and Zini 2005), that may be related to fertilisation and/or early embryo development (Barroso et al. 2009).

Chromatin in spermatozoa does not only contain protamines but also histones, as has been reported in humans (Gatewood et al. 1990) and pigs (Flores et al. 2011), amongst other mammalian species. The presence of histone has been associated with telomeres (Gineitis et al. 2000; Zalenskaya et al. 2000). In addition, chromatin packaging in the nucleus is not homogeneous, since some zones are more condensed than others (Flores et al. 2011) and sperm chromosomes are arranged in territories (Foster et al. 2005). This might be related with the hypothesis that sperm chromatin retains some features of active chromatin, mainly acetylated histones, and the arrangement of certain chromatin domains in the nucleosomes.

On the other hand, Nazarov et al. (2008) have proposed a model for the organisation of human sperm chromatin, in which sperm chromosomes are organised at different structural levels in a non-random way. Similarly, Flores et al. (2011) have also proposed a model for the nuclear architecture of boar spermatozoa, of areas with higher or fewer chromatin condensation levels.

Finally, we must also mention that protamine-1 contains more cysteine residues in pigs than in other mammalian species (Gosálvez et al. 2011). As studies conducted in boar sperm cryopreservation have shown (Flores et al. 2011; Yeste et al. 2012), these elevated number of cysteine residues appear to be related with their contribution to the stability of nucleoprotein structure, through the disulphide bonds that such residues establish between them.

8.8.2 *Remodelling of Sperm Chromatin*

After fusion of sperm and oocyte membranes, a global remodelling of sperm chromatin takes place. This process entails the participation of different elements/events, such as reduced glutathione (GSH), histone-assembling, chromatin maturation, nucleosome positioning or addition of other DNA-binding proteins (McLay and Clarke 2003; Ajduk et al. 2006).

Early studies conducted in mice showed that sperm chromatin remodelling in ooplasm consisted of three phases: decondensation, recondensation and male pronuclei formation (Adenot et al. 1991).

Decondensation overlaps with the anaphase of the second meiotic division in the oocyte. During this stage, GSH present in the oocyte cytoplasm decondenses sperm DNA by reducing the protamine-disulphide bonds (Calvin and Bedford 1971; Kvist 1980; Perreault et al. 1984, 1988; Sutovsky and Schatten 1997). As the links are cleaved, the 'doughnut' loops unfold, the sperm chromatin is uncoiled, and protamines can be removed from this paternal chromatin (Perreault 1992). Interestingly, using intracytoplasmic injection (ICSI), it has been reported that equine and porcine cytoplasm decondenses equine and porcine sperm chromatin (Mugnier et al. 2009). In addition, bovine oocyte-cytoplasm decondenses sperm chromatin from human (Nakamura et al. 2001) and equine species (Li et al. 2003), and porcine ooplasm is able to decondense bovine, mouse and human sperm chromatin (Kim et al. 1999; Cánovas et al. 2007). Thus, the molecules involved in decondensing sperm chromatin again appear to be partially conserved throughout mammalian species.

After decondensation, sperm chromatin recondensation takes place that consists first of a protamine–histone exchange, in a process that is mediated by nucleoplasmin in *Xenopus* (Philpott and Leno 1992), and seems to be mediated by nucleoplasmin-3 (NPM3) and nucleosome assembly proteins in the case of mammals (McLay and Clarke 2003). To date, the exact time during which protamines are exchanged with histones still remains to be elucidated, since the literature has provided inconsistent data (Nonchev and Tsanev 1990; Shimada et al. 2000; van der

Heijden et al. 2005). The end of chromatin recondensation overlaps with the telophase of the second meiotic division in the oocyte and at that time sperm DNA is bound to histones and already presents a nucleosome-based structure (McLay and Clarke 2003; van der Heijden et al. 2005).

The last stage of sperm chromatin remodelling is the formation of the male pronucleus after decondensation of the tightly histone-recondensed sperm chromatin. During this phase, sperm chromatin interacts with proteins involved in kinetochore formation (Schatten et al. 1988) and/or proteins involved in DNA replication (McLay and Clarke 2003).

8.8.3 Centrosome and Pronuclei Migration

The process of pronuclear migration takes longer in mammals (about 12 h) than in other species, such as sea urchin (one hour). During this process, the mammalian male pronucleus enlarges while the oocyte nucleus completes its second meiotic division. This male pronucleus is associated with the centrosome, which acts as a microtubule nucleation centre to form the sperm aster. The sperm aster grows and drives the centrosome and the associated male pronucleus from the cell cortex towards the centre of the oocyte (Barroso et al. 2009; Snook et al. 2011). Thus, the role of the male pronucleus in pronuclear formation and chromosome alignment is crucial. This is supported by other observations made in humans that have related infertility with structural abnormalities and incomplete junctioning of the centrosome (Hewitson et al. 2002).

In contrast, the female pronucleus presents neither an associated centrosome nor microtubule activity (Barroso et al. 2009). As the female pronucleus is able to migrate along microtubules from the cell cortex towards the centrosome located in the centre of sperm aster, it is quite reasonable to suggest that this movement along the microtubule lattice involves a dynein motor, similar to organelle motility (Schatten 1994; Allan 1996). In fact, dynein and dynactin are involved in the union of both pronuclei. However, while dynein is exclusively located around the female pronucleus, dynactin has been observed around both pronuclei in association with nucleoporins, vimentin and dynein (Helfand et al. 2002; Barroso et al. 2009). Thus, a sperm aster is required for dynein to localise the female pronucleus and the microtubules are necessary to retain dynein, but not dynactin, at their surface. This emphasises the relevance of the integrity of the sperm nucleus beyond fertilisation and suggest that nucleoporins, vimentin and dynactin associate upon pronuclear formation (Payne et al. 2003).

Finally, the sperm aster contacts the female pronucleus allowing dynein to interact with nucleoporins, vimentin and dynactin (Helfand et al. 2002; Sheeman et al. 2003). Then, each pronucleus migrates towards the other and DNA is replicated as it travels. Upon meeting, the two nuclear envelopes break down. However in mammals, instead of producing a common syngamy (as happens in sea urchin fertilisation), the chromatin condenses into chromosomes that are disposed on a

common mitotic spindle. Therefore, a true diploid nucleus in mammals is first seen not in the zygote, but at the two-cell stage (Alberts et al. 2008).

To conclude with this section, we must mention that there are differences between species in terms of oocyte competence to form pronuclei. Accordingly, Mugnier et al. (2009) have reported that despite penetration rates being higher in porcine than in equine oolemma, the fertilisation rates after ICSI are higher in equine oocyte cytoplasm than in the porcine ooplasm. This finding indicates that, at least under ICSI conditions, the equine ooplasm is more competent for the formation of pronuclei than the porcine ooplasm.

8.8.4 The Relevance of Sperm Chromatin Integrity in Embryo Viability

In mammals, several reports have shown that sperm chromatin integrity is related to fertility and in vitro embryo development up to the blastocyst stage (Silva and Gadella 2006; Didion et al. 2009). Chromatin packaging has also been reported to be the main cause of ICSI failure (Nikolettos et al. 1999).

Esterhuizen et al. (2002); Zalensky and Zalenskaya (2007) have emphasised the relevance of sperm chromatin organisation during fertilisation, since such genomic architecture appears to govern the orchestrated chromatin decondensation and the ordered activation of the male genome. These authors have thereby suggested the paternal contribution to the epigenetic level of embryos. Related to this, the presence of the histone human testis/sperm-specific histone H2B (hTSH2B), which is expressed in spermatogenic germline cells and in some mature spermatozoa, has been related to chromatin decondensation, pronuclei formation and the activation of paternal genes after fertilisation and during early embryonic development (Singleton et al. 2007). On the other hand, Avendaño et al. (2009) have shown that infertile men can present DNA fragmentation in morphologically normal spermatozoa. The presence of these DNA-fragmented spermatozoa is negatively correlated with embryo quality and pregnancy outcome. Again, this finding highlights the relevance of sperm chromatin integrity in embryo viability.

Therefore, it is important to evaluate the integrity of sperm DNA due to its robust power to predict fertilisation in vitro (Duran et al. 1998; Larson et al. 2000), through tests such as TUNEL, Comet assay, SCSA or SCDt (see also Chap. 9). The origin of DNA damage in boar spermatozoa can be explained by different causes, such as defective packaging during the transition of histone to protamine complex during spermiogenesis, the result of oxidative damage or even apoptosis, although the actual existence of apoptotic mechanisms in mature spermatozoa is quite challenged (Tamburrino et al. 2012). In this regard, Koppers et al. (Koppers et al. 2011) have suggested that spermatozoa have a special kind of apoptosis that only involves the early stages of apoptosis but not DNA fragmentation. Indeed, these authors have identified the involvement of PI3 K (phosphoinositide 3-kinase)/AKT pathway in sperm apoptosis and they have observed that

the apoptotic cascade in spermatozoa is characterised by loss of rapid motility, generation of mitochondrial ROS, activation of caspases in the cytosol, annexin V binding to the cell surface, cytoplasmic vacuolisation and oxidative DNA damage. However, DNA fragmentation does not occur as a direct result of apoptosis in spermatozoa as it does in somatic cells.

Taking into account these findings, it has been suggested that this truncated apoptotic cascade prepares spermatozoa for silent phagocytosis within the female tract and prevents DNA-damaged spermatozoa from participating in fertilisation (Koppers et al. 2011). Intriguingly, phosphatidylserine externalisation is a late event in sperm apoptosis that may facilitate the silent phagocytosis of moribund cells in the female reproductive tract, i.e. the phagocytosis of senescent spermatozoa without generating an inflammatory response (Aitken et al. 2012). In this context, this process will culminate in a regulated cell death that will prepare the spermatozoa for phagocytosis by leucocytes in the female reproductive tract (see Sect. 5.8 about Reproductive Immunology in Swine).

In addition, some of these apoptotic changes have also been associated with capacitation, so that Aitken (2011) has hypothesised that capacitation and apoptosis might be part of a continuous process driven by peroxynitrite and oxysterol formation. According to this hypothesis, if the spermatozoon fails to find an egg, then the continued generation of peroxynitrite might promote oxysterol formation in the mitochondrial membranes, leading to activation of the intrinsic apoptotic cascade that ultimately results in cell death. (Aitken 2011).

8.9 The Ubiquitin–Proteasome System During Fertilisation

8.9.1 Introduction

One of the most important post-translational changes for protein is ubiquitination. Ubiquitination consists of a stable and covalent modification that marks defective or outlived intracellular proteins and alters their activity by proteolytic degradation through 26S proteasomes or lysosomes (Hochstrasser 1996; Sutovsky et al. 1999; see also Chap. 7). This route is essential for those events that require degradation of substrate-specific proteins, such as progression within the cell cycle, endocytosis of membrane receptors, presentation of antigens in the humoral response and notwithstanding fertilisation (Sawada et al. 2002; Sutovsky 2011; Yi et al. 2012). However, despite the relevance of sperm proteasome during fertilisation being currently better known, there are also indices that suggest the proteasome pathway could be involved in other regulation processes of sperm physiology. In this regard, the studies dealing with this issue to date have been scarce, and more evidence is still needed to learn about the actual role of ubiquitin proteasome in regulating sperm function.

The presence of proteasome has been reported in spermatozoa of different species, including invertebrates (Sakai et al. 2004; Yokota and Sawada 2007) and vertebrates (Zimmerman and Sutovsky 2009). In mammals, the components of

the 26S proteasome have been found in different species, such as pigs (Sutovsky et al. 2004a; Yi et al. 2012; Zimmerman et al. 2011), humans (Baker et al. 2007; Morales et al. 2003; Tipler et al. 1997; Wojcik et al. 2000), rats (Baker et al. 2008; Haraguchi et al. 2007; Pizarro et al. 2004) and mice (Baker et al. 2008; Pizarro et al. 2004; Tipler et al. 2007). Indeed, the presence of multiple ubiquitin-specific proteases (Baker et al. 2008) and ubiquitin-conjugating enzymes E2 (Fischer et al. 2005; Sutovsky et al. 2000; Tipler et al. 1997) and E3 (Rivkin et al. 2009; Rodríguez and Stewart 2007; Wong et al. 2002) have been found identified in the proteomes of mammalian spermatozoa.

Other findings that support the view of the ubiquitin–proteasome system being involved in sperm function are related to the presence of ubiquitinated proteins in the sperm nucleus (Haraguchi et al. 2007) and with the nature of proteasome α6 subunit which has been found as a substrate for Tyr phosphorylation during capacitation (Arcelay et al. 2008; Ficarro et al. 2003).

Ubiquitination–proteasome pathway is not restricted to capacitation and post-fertilisation events (see also Chap. 7), but it also plays a critical role during spermatogenesis, when ubiquitination allows the replacement of the spermatid's nuclear histones by transition nuclear proteins (TNP) and then by permanent substitution with protamines (Baarends et al. 1999a, b). Ubiquitination also participates in the dramatic reduction of sperm centrosome during spermatid elongation. In addition, and although protein ubiquitination typically occurs in the cell cytosol or nucleus, defective mammalian spermatozoa are ubiquitinated on their surface during sperm maturation in the epididymis (Sutovsky et al. 2001a). Related to this, morphologically abnormal ubiquitinated spermatozoa have been found in human ejaculates (Rawe et al. 2008; Sutovsky et al. 2001a), thereby indicating that they escaped from epididymal degradation. Baska et al. (2008) found these ubiquitinated spermatozoa mainly presented defects in head and/or axoneme. Finally, some reports have found a relationship between sperm ubiquitination and DNA fragmentation (Sutovsky et al. 2002, b) and have associated this degradation pathway with poor quality sperm parameters (Sutovsky et al. 2001b). However, this finding has been challenged by Marchiani et al. (2007) who found apoptotic bodies in human semen, so that it still remains to be firmly elucidated whether ubiquination and/or apoptosis pathways take place in mature spermatozoa.

8.9.2 The Sperm Proteasome During In Vitro Fertilisation and Acrosome Exocytosis

To date, the relevance of sperm proteasome during IVF and acrosome exocytosis has been documented in different species (Pizarro et al. 2004; Sutovsky 2011), and as mentioned before the presence of an extracellular pool of proteasomes has been observed in the plasma membrane of the head of pig (Yi et al. 2007), human (Morales et al. 2004), cattle (Sánchez et al. 2011), mouse (Pasten et al. 2005) and ascidian (Sawada et al. 2002) spermatozoa. Related to this, Zimmerman et al.

([2011](#)) have demonstrated that the sperm acrosome-borne 26S proteasomes recognise and degrade ubiquitinated ZP proteins during pig fertilisation (Zimmerman et al. [2011](#)).

One aspect that is well-known is the involvement of the sperm proteasome in the sustained phase of the Ca^{2+} influx that precedes acrosome exocytosis (Morales et al. [2003](#)). Proteasome inhibitors block sperm capacitation (see also Chap. 7) and sperm-penetration through ZP but they do not affect sperm motility and ZP-binding (Morales et al. [2007](#); Kong et al. [2009](#)). In addition, proteasome phosphorylation and activity are regulated by the extracellular matrix proteins, fibronectin and laminin (Díaz et al. [2007](#); Tapia et al. [2011](#)). This supports the idea that COCs and their matrix play an important role in mammalian fertilisation (Jin et al. [2011](#)).

We must finally mention other previous studies conducted on human sperm that have reported a relationship between proteasome and male fertility. Indeed, human spermatozoa of poor quality and/or with defective centriolar/pericentriolar structures present lower proteasomal activity. This deficiency in intrinsic proteasome activity seems to be related to a lower fertilising ability (Rawe et al. [2008](#); Rosales et al. [2011](#)).

8.9.3 Mechanism of Ubiquitination During Acrosome Exocytosis and Sperm-ZP Interaction: UBA1

In the ubiquitination mechanism, ubiquitin plays a significant role. This protein is a small and highly conserved chaperone protein presents in all eukaryotic cells that binds covalently to substrate proteins via an isopeptide bond between the C-terminal glycine of ubiquitin and the E-amino group of a lysine in substrate proteins.

Ubiquitination follows with a multistep enzymatic pathway, catalysed by the enzymatic activities of ubiquitin-activating enzyme E1 (UBA1), ubiquitin-conjugating enzyme E2 and a substrate-specific ubiquitin ligase E3. During the initial step of ubiquitin-protein binding, mediated by the E1-type ubiquitin-activating enzyme (UBA1), one molecule of ubiquitin interacts with an internal lysine residue of the substrate protein (Fig. [8.6](#)). Following this, additional molecules of ubiquitin are ligated in tandem forming a multi-ubiquitin isopeptide (Hershko and Ciechanover [1998](#); Glickman and Ciechanover [2002](#)). The polyubiquitinated substrate proteins are then recognised and degraded by the 26S proteasome, which is composed of a proteolytic complex (20S) and one/two regulatory complexes (19S) (Glickman et al. [1998](#); Voges et al. [1999](#)). Substrate proteins are degraded and the multi-ubiquitin isopeptide is finally released from the substrate protein and disassembled by deubiquitinating enzymes (Wilkinson [1997](#)).

The presence and role of UBA1 in reproductive cells has also been investigated. In this regard, it has been reported to be necessary for mouse (Odorisio et al. [1996](#)) and human spermatogenesis (Zhu et al. [2004](#)), and highly expressed in rat spermatogonia (Du et al. [2008](#)). Furthermore, an active form of UBA1 enzyme

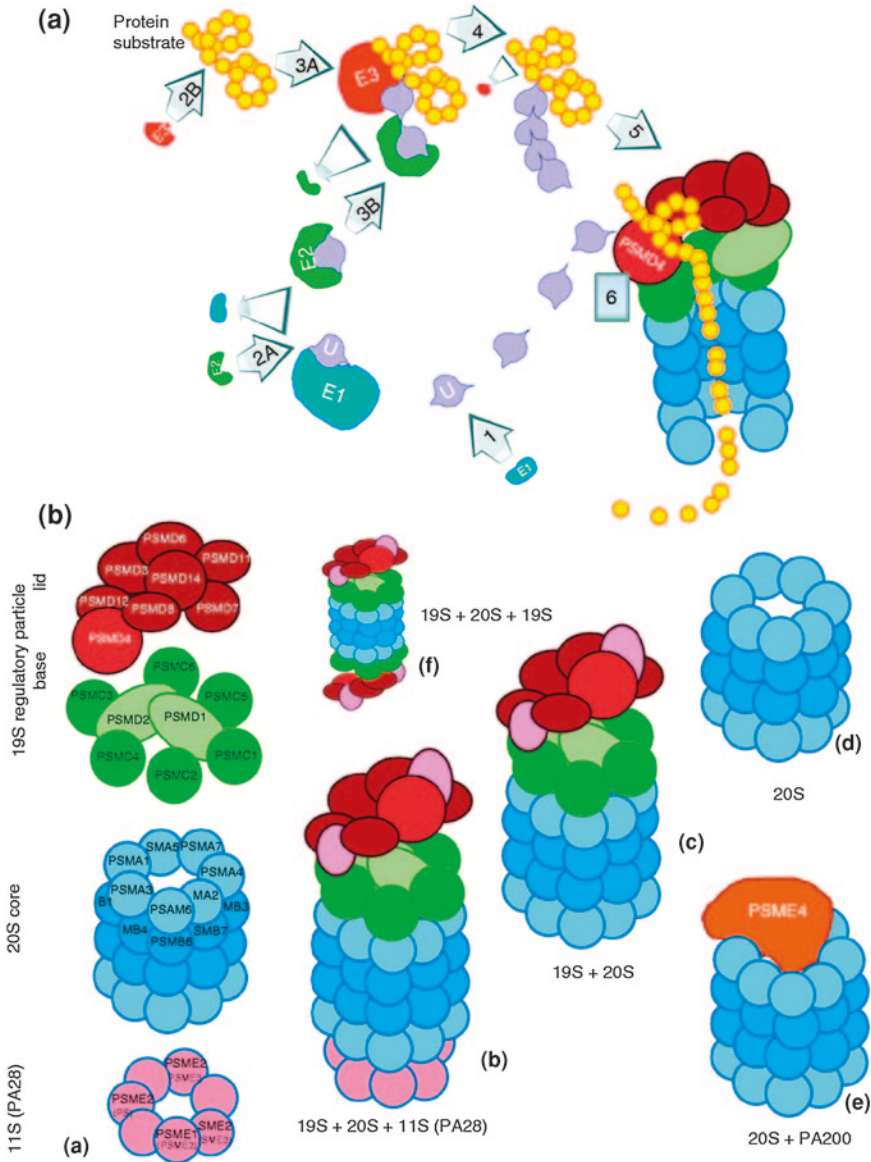**Fig. 8.6 a** Diagram of protein ubiquitination and degradation by the 26S proteasome. **b** Variations on the subunit composition of the 26S proteasome (Sutovsky 2011) Reproduced with permission

has been found in bovine epididymal fluid, thus suggesting that it could also play some role in epididymal sperm maturation (Baska et al. 2008).

In mature rat spermatozoa, UBA1 is also present and it is phosphorylated during sperm capacitation (Baker et al. 2010). This protein also plays a key role
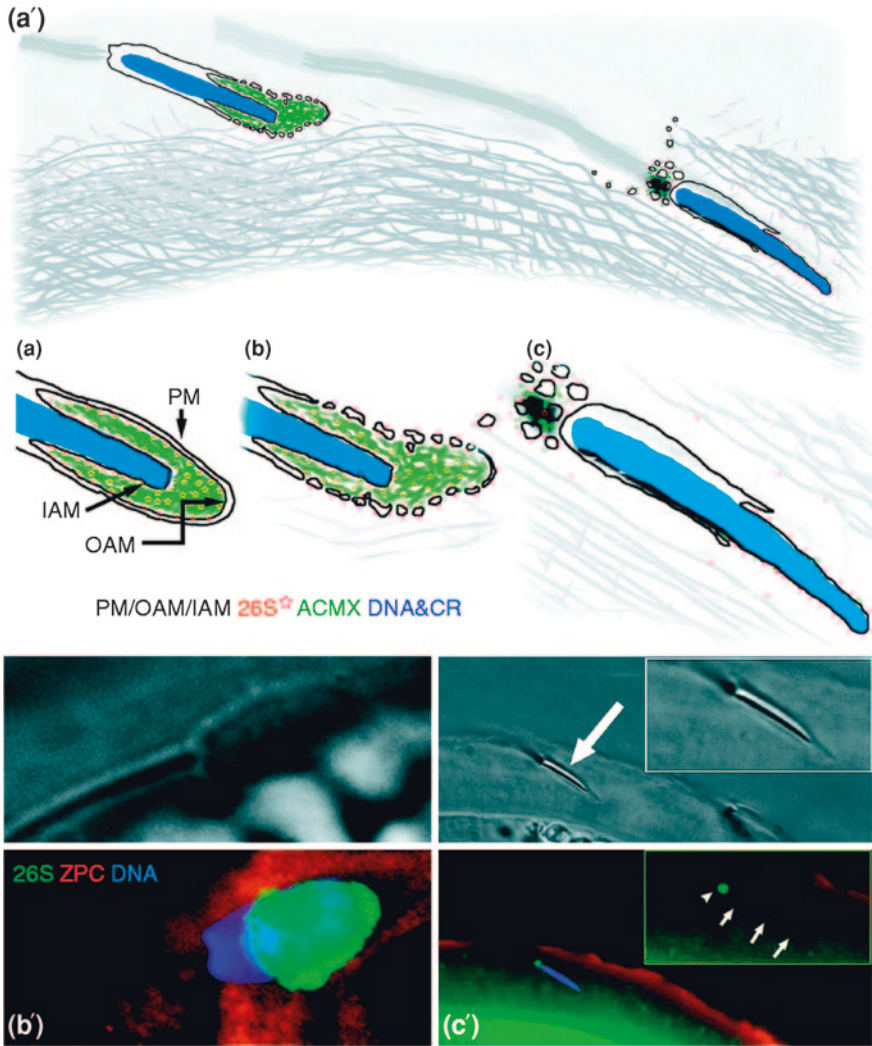
Fig. 8.7 Acrosome remodeling and proteasome localisation during the zona pellucida-induced acrosome reaction in the pig IVF system. *a′* Diagram based on immunofluorescence and transmission electron microscopy images of boar sperm acrosomal exocytosis. In an intact acrosome (**a**), proteasomes (*red star*) are bound to inner (*IAM*) and outer (*OAM*) acrosomal membranes and also dispersed throughout acrosomal matrix (ACMX; *green*). On sperm–zona binding, the *IAM* and *OAM* give rise to acrosomal membrane vesicles coated with proteasomes (**b**), which together with loosened ACMX form a zona-bound acrosomal shroud, a transient but structurally stable structure. During sperm–zona penetration, (**c**) remnants of the acrosomal shroud envelope the sperm tail connecting piece, while a subpopulation of proteasomes remain associated with *IAM*. (*b′*) Immunodetection of proteasomes (*green*) in the acrosomal shroud of a ZP-bound, acrosome-reacted boar spermatozoon. Sperm receptor protein ZPC is labelled red; DNA is counterstained blue with DAPI (*c′*) Proteasomes (*green*) are detected on the *IAM* (*arrows*) and in the cluster of acrosomal membrane vesicles associated with the sperm tail connecting piece (*arrowhead*) in a spermatozoon passing through ZP (*red*; anti-*ZPC* antibody) (Sutovsky 2011) Reproduced with permission

during fertilisation in porcine and other mammalian species, since different reports have shown that the ubiquitin–proteasome pathway participates in sperm–oocyte interaction (Sutovsky et al. 2004a; Yi et al. 2007a, b, 2012; Zimmerman and Sutovsky 2009, 2011) (Fig. 8.7). Intriguingly, proteasomal proteolysis of proteins from spermatozoa and oocyte takes place when sperm acrosome interacts with the ZP of the oocyte during fertilisation (Fig. 8.7). Related to this, Sutovsky et al. (2004a) observed that proteasome inhibitors impeded sperm penetration of ZP. On the other hand, sperm-acrosomal ubiquitin C-terminal hydrolases have also been reported to be involved in antipolyspermy defense (Yi et al. 2007).

Yi et al. (2012) have recently studied the requirement of UBA1 activity for sperm capacitation, acrosome exocytosis and spermatozoa–ZP penetration during porcine IVF. These authors sought to elucidate whether the substrates were solely ubiquitinated during gametogenesis or *de novo* ubiquitination also took place during acrosome exocytosis and fertilisation. In fact, during spermatogenesis in boars, UBA1 is accumulated in the spermatogonia, spermatocytes and spermatids, and in the acrosome regions of round and elongating spermatids, so that an active form of UBA1 is present in the acrosomal extracts of boar spermatozoa.

Against this background, Yi et al. (2012) assessed the effect of an UBA1 inhibitor (PYR-41) on sperm capacitation and oocyte fertilisation. The incubation of this inhibitor altered the reorganisation of the outer acrosomal membrane during sperm capacitation, and reduced the fertilising ability of spermatozoa. These authors also showed that PYR-41 altered the capacitation-induced modification of two acrosomal proteins: the spermadhesin AQN1 and the acrosin inhibitor SPINK2. In the presence of PYR-41, spermatozoa showed normal motility and sperm–ZP binding, but they were unable to penetrate the oocyte, thereby suggesting that the course of acrosomal exocytosis had been altered. These data have led them to conclude that *de novo* protein ubiquitination involving UBA1 contributes to sperm capacitation and acrosomal function during fertilisation.

8.10 Sperm Contributions Beyond Fertilisation

8.10.1 Introduction

The spermatozoon does not only act as a simple vector that delivers paternal DNA to the oocyte after fertilisation, but it also contributes to other events during the first stages of pre-implantation embryo development. Thus, the oocyte needs the presence of the spermatozoon for its activation, and at the same time the spermatozoon needs to fertilise the oocyte to decondensate its chromatin.

The spermatozoon has, therefore, a very dynamic and critical participation in normal embryogenesis that clearly extends beyond the fertilisation process (Barroso et al. 2009). Although the molecules involved in fertilisation and postfertilisation developmental steps remain largely unknown, defective fertilising spermatozoa may cause arrest of development at multiple levels during embryo

pre-implantation development. In addition, evidence suggests sub-lethal effects can be carried over after implantation, resulting in untoward embryonic/foetal defects (Barroso et al. 2009).

To the authors' knowledge, there is no specific report on pigs that reviews sperm contribution beyond fertilisation. For this reason, this section essentially mentions the role of various human sperm components/molecules on early embryo development, since they are the most abundant so far.

The contribution of sperm tail to embryonic development remains largely unknown. Despite microinjection of isolated sperm fractions (heads or tails) allowing oocyte activation and pronuclei formation, these oocytes are not able to undergo normal mitotic division (Moomjy et al. 1999; Alberio et al. 2001). This finding points to the fact that the structural integrity of fertilising spermatozoon contributes to normal embryogenesis, since there are abnormalities of the mitotic apparatus when sperm integrity is damaged (Colombero et al. 1999).

8.10.2 Oocyte Activation as a Sperm-Mediated Step

As mentioned above, the contribution of a given fertilising spermatozoon in the formation of a new zygote after fertilisation is not only related to the paternal haploid genome, but is also involved in signalisation processes that lead to oocyte activation and to the microtubule assembly leading to the formation of mitotic spindles during the initial zygote development.

It is well-known that only fertilised oocytes are able to continue meiosis from metaphase II, where they are arrested at the time of ovulation (Alberio et al. 2001). This phenomenon, also known as oocyte activation, is related to a signalling mechanism related to spermatozoa. According to Barroso et al. (2009), three theories have been proposed to explain this event:

1. *The fusion theory* that proposes the presence of active calcium-releasing components in the sperm head (Dale et al. 1985; Fissore et al. 1999).
2. *The receptor theory* that suggests a signal transduction pathway mediated by a receptor located on the oolemma (Kline et al. 1988).
3. *The calcium pump theory* that hypothesises that Ca^{2+} enters the oocyte upon fertilisation, either from stores in the sperm itself or through channels of the sperm plasma membrane (Jaffe 1983).

Although a sperm factor located at the equatorial segment and known as oscillin was initially identified by Parrington et al. (1996) as being responsible for calcium oscillation in spermatozoa that leads to oocyte fertilisation in mammals, Tesarik (1998) later reported that oscillin was not responsible for these calcium oscillations in mammalian sperm cells.

Another interesting finding in sperm contribution to oocyte activation is related to sperm PLC-zeta (PLCζ) (Saunders et al. 2002, 2007). PLCζ is a novel form of PLC specific for mammalian spermatozoa that appears to trigger calcium

oscillations leading to oocyte activation (Kouchi et al. 2004). This observation was confirmed when recombinant PLCζ was injected into mouse oocytes and this was seen to lead to egg activation (Kouchi et al. 2004). Following this, it has been proposed that after fusion of sperm and oocyte plasma membranes, sperm-derived PLCζ diffuses into the oocyte cytosol that results in hydrolysis of phosphatidylinositol 4, 5-bisphosphate (PIP_2) from an unknown source to inositol 1,4,5-trisphosphate (IP_3) (Swann et al. 2004; Saunders et al. 2007).

Two of the earliest indications of oocyte activation are the extrusion of cortical granules by exocytosis and the resumption of meiosis. For the time being, we know that both events are triggered by Ca^{2+} transient oscillations, but it remains to be elucidated which pathways leading to the intracellular Ca^{2+}-release are involved. Indeed, Ca2$^+$ oscillations allow the resumption of meiosis as the activity decreases of both a M-phase-promoting factor and a cytostatic factor, as seen in *Xenopus* oocytes (Dupont 1998). The extrusion of cortical granules seems to be mediated either by Ca^{2+} transients and/or by PKC (Eliyahu and Shalgi 2002). Although the exact mechanism leading to the extrusion of cortical granules has not been completely understood, it is worth noting that kinases from Src-family have been suggested to be involved in calcium release and oocyte activation (Talmor-Cohen et al. 2004).

Finally, another interesting contribution about the role of sperm presence in oocyte activation concerns two different sperm-borne proteins that induce pronuclei formation in oocytes. These sperm proteins are:

1. The truncated c-Kit tyrosine kinase that activates the dormant/quiescent oocyte by eliciting intracellular Ca^{2+} oscillations. In this context, Ca^{2+} as a secondary messenger for downstream effectors/events of zygotic development (Sette et al. 1997, 2002).
2. The post-acrosomal sheath WWP domain-binding protein (PAWP), which is exclusively located in the post-acrosomal sheath of the sperm perinuclear theca (Wu et al. 2007). The WWP domain (also known as WW) is a protein domain with two highly conserved tryptophans that binds proline-rich peptide motifs.

Interestingly, Wu et al. (2007) observed that when recombinant PAWP or an extract of perinuclear theca is injected into metaphase-II-arrested porcine, bovine, macaque or xenopus oocytes, a high rate of pronuclear formation takes place. In contrast, these authors also observed that fertilisation was arrested and pronuclei formation prevented when ICSI of porcine oocytes was performed together with injection of PAWP-competitive peptide or of an anti-recombinant-PAWP (Wu et al. 2007). This finding thus points out the relevant role of sperm's PAWP in oocyte activation.

8.10.3 The Role of Paternal Mitochondrial DNA in the New Zygote

Contribution of sperm DNA in post-fertilisation events has been previously discussed in the specific section about the integrity of sperm nuclear chromatin and

its relevance in early embryo development (see Sect. 8.8.4). However, sperm mitochondrial DNA (mtDNA) also plays a relevant role in the development of the new zygote. This issue will be discussed in this Section.

To the best of our knowledge, paternal mtDNA inheritance has most assuredly not been reported so far. However, some studies have hinted at this possibility (Schwartz and Vissing 2002). Notwithstanding, Brenner et al. (2004) reported heteroplasmic or mixed mtDNA inheritance after ooplasmic transfusion (ooplasmic transfusion consists of injecting ooplasm from young oocytes to older oocytes from older women).

Most somatic cells of the body contain between 103 and 104 copies of mtDNA. Oocytes contain slightly higher copies of mtDNA (mtDNA), which can be related to the preparation for energetic demands of the new individual during embryogenesis (Shadel and Clayton 1997). As explained in Chap. 2, spermatozoa from porcine and other species switch between aerobic and anaerobic metabolism, depending on the oxygen tensions of the surrounding environment, from near anoxia in the testis and epididymis to ambient tension in the vagina and in vitro.

Sperm mtDNA, like somatic cells, is highly vulnerable to mutation (Cummins 1998) and in humans it has been reported that a high number of mtDNA deletions are found in at least 50 % of normospermic men (Cummins et al. 1998). This high vulnerability of sperm mtDNA to mutation is related to the length of the spermatogenic wave, which consists of spermatogenesis (34–36 days) and epididymal maturation (12–15 days) and lasts between 46 and 51 days (See also Chap. 3). During these processes, spermatozoa are exposed to mutagenic agents that can damage DNA. This is of particular importance, especially if we bear in mind that spermatozoa present few DNA-repair mechanisms.

In this context, defective sperm mtDNA needs to be eliminated to prevent this detrimental paternal inheritance contributing to the embryo. Therefore, mtDNA from mammalian embryo is mainly inherited from the maternal rather than paternal line. In this regard, Short (1998) proposed that this asymmetric inheritance could be the fundamental driving force behind amphimixis and anisogamy because of the need to conserve a healthy stock of mtDNA for embryo development by means of a long period of quiescence in meiosis. Indeed, the strictly maternal inheritance of mtDNA in mammals represents a paradox, especially when we take into consideration that a fertilising spermatozoon introduces up to 100 functional mitochondria into the oocyte cytoplasm at the time of fertilisation (Ingman et al. 2000).

However, it is quite likely that destruction of paternal mitochondria after fertilisation results from an evolutionary and developmental advantage (Ankel-Simons and Cummins 1996), since sperm mtDNA can be compromised by deleterious action of ROS that spermatozoa encounter in their surrounding environment during spermatogenesis, storage, transport along the female tract and fertilisation, as Aitken (1995) pointed out. Kaneda et al. (1995) found that membrane proteins from spermatozoa, rather than mtDNA, determined whether sperm mitochondria and mtDNA die or are degraded. Related to this, we must indicate that one of the most significant processes related to the ubiquitin–proteasome pathway in

fertilisation (see also Sect. 8.9) is the destruction of sperm mitochondria (Sutovsky et al. 1999, 2000). Thompson et al. (2003) found that a mitochondrial membrane protein known as prohibitin, was one of the ubiquitinated substrates that made the sperm mitochondria responsible for the egg's ubiquitin–proteasome dependent, proteolytic machinery after fertilisation. Notwithstanding, abnormalities of this recognition system seem to be related to deregulations of mitochondrial inheritance and leads them to escape from the 'sperm quality control' that oocyte manages.

8.10.4 'Early' and 'Late' Paternal Effects

Assisted reproductive technologies have been widely implemented in human and other mammalian species during the last 20 years, and some lessons about early and late paternal contributions can be obtained from these technologies.

Apart from the relationship between successful fertilisation and oocyte quality/characteristics in human (Van Blerkom 2000; Swain and Pool 2008) and other species, some evidence has supported that contribution of sperm to faulty fertilisation and abnormal embryogenes (Silva and Gadella 2006). Indeed, abnormal sperm morphology and/or function, which is related to oxidative damage, DNA fragmentation or chromatin defective packaging, can result in failed or delayed fertilisation and/or aberrant embryo development. This has been reported in human but also in bovine and other species (Oehninger et al. 1989, 1996; Ron-el et al. 1991; Parinaud et al. 1993; Grow et al. 1994; Gorczyca et al. 1993).

Early cleavage divisions of the new zygote depend on the endogenous machinery of the oocyte, and transcription of the embryo genome occurs between the four-cell and eight-cell stage of preimplantation development (Braude et al. 1998). For this reason, alterations of sperm nucleus (like DNA fragmentation) are not detected up to the eight-cell stage when a major expression of sperm-derived genes has begun, while deficiencies in sperm cytoplasm are detected at the 1-cell stage and during preimplantation embryo development (Tesarik et al. 2004).

From these observations, one can talk about 'early' and 'late' paternal effects depending on which pathologies are related to sperm cytoplasm and nucleus (Tesarik 2005):

– 'Early' paternal effects are related to abnormal embryo morphology and low cleavage speed, and are not associated with DNA fragmentation of spermatozoa. Early paternal effects may also be due to alterations of the oocyte-activating factor, which can be caused by no sperm delivery of this factor or by a dysfunctional oocyte-activating factor, and to deficiencies of the centrosome-cytoskeletal apparatus.
– 'Late' paternal effects are related to poor development competence and implantation failure and appear to be associated with the sperm chromatin integrity of spermatozoa (Barroso et al. 2009). In humans, ICSI with testicular spermatozoa has been regarded as an efficient treatment to avoid the 'late paternal effect, as ejaculated spermatozoa can contain fragmented DNA due to the ROS-mediated

effects of DNA damage during epididymal maturation (Greco et al. 2005). As more recent studies have reported, late paternal effects may also be related to sperm mitochondrial dysfunctions or abnormal sperm mRNA delivery.

Finally, alterations due to genomic imprinting anomalies may result in both early and late paternal effects.

8.10.5   Disorders Beyond Fertilisation due to Defective Spermatozoa

8.10.5.1   Disorders of Oocyte Activation

As mentioned above, early paternal effects can be provoked by a defective/absent oocyte-activation factor. Dysfunction of the oocyte-activation factor occurs when a fertilising-spermatozoon enters the oocyte but there is no stimulation for oocyte activation. This leads to failure to resume meiosis or to undergo extrusion of cortical granules. In humans, 40 % of failed fertilisation cases after ICSI are related to the absence or dysfunction of the sperm's oocyte-activating factor (Rawe et al. 2000). Yoon et al. (2008) have related this defective oocyte-activating factor with undetectable levels of PLCζ in sperm, which results in the inability of fertilising spermatozoon to induce calcium oscillations in the oocyte.

Apart from early paternal effects, there are other cases of non-response of the oocyte to the sperm's oocyte-activating factor that are due to multiple elements of the oocyte responsive system (PIP$_2$, IP$_3$ receptor, PKC, etc.), rather than the oocyte-activating factor itself.

8.10.5.2   Disorders Related to Microtubule Organisation

Another dysfunction that may result in fertilisation arrest is related to defective organisation of microtubules/centrosome (Asch et al. 1995; see also Sect. 8.8), as correlation between sperm aster formation and embryonic cleavage rate has shown (Terada et al. 2004). In a study conducted in humans, fertilisation arrest was observed to occur at various levels:

1.   At metaphase II
2.   After successful fertilisation
3.   After formation of the sperm aster
4.   During meiotic cell cycle progression
5.   During mitotic cell cycle progression.

From the failed fertilisation procedures after human IVF, Rawe et al. (2000) reported that 55.5 % were due to no sperm penetration. In the case of ICSI, the same authors observed that failed fertilisation was due to incomplete oocyte activation in 39.9 % of cases. However, care must be taken when talking about

unsuccessful fertilisation and its underlying causes in ICSI procedures. Indeed, the presence of the sperm acrosome and apical perinuclear theca, structures normally removed at the oolemma during in vivo and IVF, may result in abnormal nuclear remodelling during sperm decondensation and a delay in DNA synthesis (Hewitson et al. 2000, 2003). In fact, as described in Chap. 1, sperm acrosome contains a variety of hydrolytic enzymes that are released into the ooplasm in ICSI, and may thus generate some kind of damage and physical disturbance when decondensing sperm chromatin (Tesarik and Mendoza 1999). Also related to this, Katayama et al. (2002) working in pigs, observed differences between ICSI and IVF in the RNA-binding properties of sperm head components introduced into the ooplasm.

8.10.6 The Role of Sperm mRNAs

Mature and ejaculated mammalian spermatozoa contain mRNA transcripts, as detected in human (Ostermeier et al. 2004, 2005; Zhao et al. 2006), boars (Yang et al. 2009), and other species. In addition, the amounts of mRNA in human spermatozoa are 600 times lower than somatic cells (Zhao et al. 2006), and Pessot et al. (1989) found RNA in sperm nucleus and residual DNA, and RNA-polymerases activity has also been early reported (Hecht 1974). After these findings, the main question is to determine the role of these mRNAs, and when they have been synthesised. There are different possibilities:

1. One possibility is that these mRNAs are residual and non-functional material.
2. Another possibility is that these mRNAs have a function during late spermiogenesis. As explained in Chap. 3, spermiogenesis is a complex process in which the round spermatids undergo deep changes, such as condensation of the nuclear chromatin, formation of the flagellum and development of the acrosomal cap (Wykes et al. 1997). Spermatids lose most of their cytoplasm when they have been transformed into spermatozoa. It is logical, therefore, to consider that mature spermatozoa are unlikely to transcribe novel RNAs and translate protein-coding RNAs (Grunewald et al. 2005). Many of the late post-meiotically transcribed genes are most likely synthesised during the final burst of transcription prior to histone-protamine exchange in haploid spermatids, and then later actively translated in elongated spermatids (Kramer and Krawetz 1997).

    In this second possibility, mRNAs would equilibrate imbalances in sperm phenotypes brought about by meiotic recombination and segregation. In addition, these mRNAs have also been hypothesised to be involved in early embryo development. Specifically, they could establish imprints during the transition from maternal to embryonic genes (Barroso et al. 2009).
3. Another explanation for the presence of mRNA in ejaculated spermatozoa could be related to the existence of translational activities that Naz (1998) reported during capacitation and acrosome reaction of human spermatozoa.

4. Another possibility is that mRNAs accumulated in the sperm nucleus play some role in early embryogenesis (Krawetz 2005). This more recent approach is backed by other observations made on ejaculated sperm in mice (Hayashi et al. 2003), humans (Ostermeier et al. 2004) and pigs (Yang et al. 2009) that emphasises the role of sperm-mRNA delivery to oocytes. In fact, sperm transcripts encoding proteins that participate in fertilisation and embryo development have been found in early embryos after IVF, but not in the non-fertilised oocyte (Signorelli et al. 2012). In heterologous experiments conducted with human spermatozoa and hamster oocytes, Avendaño et al. (2008) have observed that two human sperm-specific transcripts (PSG1 and HLA-E) involved in implantation are found in hamster-fertilised oocytes, fertilised after 24 h post-fertilisation. This finding clearly indicates that mature spermatozoa deliver mRNA into the oocyte, and provides specific transcripts that are needed for embryo development before the activation of its own genome, thereby contributing in embryo survival and development.

On the other hand, some of the transcripts present in ejaculated spermatozoa have been determined and although this list is, of course, incomplete, it provides us with some idea about the nature of the information about these mRNAs encode (Dadoune et al. 2005; Kumar et al. 1993; Miller et al. 1994; Ostermeier et al. 2002):

– Protamine 1 (PRM1) and protamine 2 (PRM2)
– Transition nuclear protein 1 (TNP1, also known as TP1 and STP1), which protein products replace histones and are subsequently replaced by protamines in mature spermatozoa
– Heat shock protein 70 (HSP1A1)
– Heat shock protein 90 (HSP90AA1)
– c-MYC (a protooncogen involved in several carcinogenic processes)
– HLA class 1
– β-integrins
– β-actin
– Variants of phosphodiesterase
– Sperm progesterone receptor
– Aromatase
– Transcript encoding factors (NFkB, HOX2A, ICSBP, JNK2, HBEGF, RXRb, and ErbB3).

In one of the scarce studies about RNAs in mature boar sperm, Yang et al. (2009) have evaluated the presence/absence of different rRNAs and mRNAs. These mRNAs are related to nucleic acid binding, structural modifications and transcriptional regulation, and can therefore be related to spermiogenic processes and/or later fertilisation events. In addition, these authors found that the two major rRNAs essential for 80S cytoplasmic ribosome assembly within eukaryotic cells, were absent in boar spermatozoa, in agreement with other studies conducted with other species, like human (Ostermeier et al. 2002; Lambard et al. 2004), and bovine species (Gilbert et al. 2007).

Yang et al. (2009) also found remnant spermatozoal mRNA transcripts within mature boar ejaculated spermatozoa. It is quite evident that these transcripts play relevant functions mostly of genes in various types of male germ cells (Kramer and Krawetz, 1997; Gilbert et al. 2007). However, the role and the mechanism of action for mRNAs within the mature spermatozoon during fertilisation and embryonic development have yet to be determined.

Despite Hayashi et al. (2003) reporting that spermatozoal mRNA transcripts in mice did not have any effect on embryogenesis and were degraded rapidly in embryos at late 1-cell stage, Yang et al. (2009) suggested that these spermatozoal mRNA transcripts were found to survive up to the 4-cell period. Thus, spermatozoa deliver not only genetic materials but also spermatozoal RNAs into the oocyte during the process of fertilisation. These authors suggested that mRNAs from sperm-specific antigen 2 (SSFA2), and Sestrin 1 (SESN1) could be involved in early embryo development.

Other mRNAs found in mature ejaculated spermatozoa, but less clearly involved in early embryo development, are protamine P1 (PRM1)-mRNAs. According to Yang et al. (2009), the importance of *PRM1* might be related to male infertility, but this should not rule out the possibility that these *PRM1* mRNA residues in spermatozoa may influence early embryo development indirectly. Related to this, Hwang et al. (2012), also working with pigs, have recently reported that the abundance of the following mRNA transcripts: *MYC, CYP19, ADAM-2, PRM1* and *PRM2* is related to early embryo development. In addition, these authors have also determined that *MYC*-mRNA is less abundant in capacitated than in uncapacitated spermatozoa, thereby suggesting that sperm-RNAs also regulate capacitation.

8.10.7 The Role of Sperm MicroRNAs

MicroRNAs (miRNAs) are endogenous or artificial single-stranded siRNAs capable of interfering with mRNA (RNAi) via complete or partial complementarities (Lagos-Quintana et al. 2001). Initially, miRNAs were named small temporal RNAs after being discovered in *Caenorhabditis elegans* and in *Drosophila melanogaster* as regulators of gene expression (Lee et al. 1993; Olsen and Ambros 1999; Reinhart et al. 2000).

Different types of miRNAs have been observed in diverse species. One class of these miRNAs are intron-derived miRNAs, which are derived from intron sequences, and differ from the other intergenic miRNAs in that they require RNA II polymerases and spliceosomal components for their biogenesis (Lin et al. 2007). Intron-derived miRNAs are firstly transcribed from introns as hairpin structures (primary miRNAs) and then matured as single-stranded RNAs by Dicer enzyme (mature miRNAs) in several organisms (Hutvagner et al. 2001; Ketting et al. 2001; Lau et al. 2001; Mourelatos et al. 2002; Tabara et al. 2002). Then, the mature miRNA binds to the three prime untranslated region (3'-UTR) of its corresponding mRNAs (Provost et al. 2002). As a general mechanism of RNAi, RISC-like is also involved in the miRNA pathway (Hutvagner and Zamore 2002).

In zebrafish, RNAi mediated by miRNAs regulates early development. In contrast, in the case of mammals, the exact role of miRNAs in mammalian fertilisation still remains unknown. To date, studies conducted in mice have demonstrated that although sperm-borne prototypical miRNAs are present in mouse spermatozoa and enter the oocyte at fertilisation, they play either a marginal role or no role in mammalian fertilisation and early preimplantation development (Amanai et al. 2006).

8.11 Conclusions

When spermatozoa become completely capacitated, they are able to recognise and bind to the ZP of the egg by recognising properly arranged neutral complex N-glycans of ZP glycoproteins. This interaction triggers the acrosome reaction and the acrosomal content is exocytosed. Then, proacrosin binds to the polysulfated glycans of the ZP and mediates secondary binding of the acrosome-reacted sperm to the matrix. The next steps are the fusion of sperm membrane with oolemma, sperm chromatin remodelling and pronuclei migration. Finally, chromosome alignment takes place and the zygote immediately starts the first and subsequent mitotic divisions. After fertilisation, some spermatozoal components, such as some specific sperm mRNAs can also influence early embryonic development.

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 28:23–34

Adham IM, Nayernia K, Engel W (1997) Spermatozoa lacking acrosin protein show delayed fertilization. Mol Reprod Dev 46:370–376

Aitken RJ (1995) Free radicals, lipid peroxidation and sperm function. Reprod Fertil Dev 7:659–668

Aitken RJ (2011) The capacitation-apoptosis highway: oxysterols and mammalian sperm function. Biol Reprod 85:9–12

Aitken RJ, De Iuliis GN, Gibb Z, Baker MA (2012) The Simmet lecture: new horizons on an old landscape—oxidative stress, DNA damage and apoptosis in the male germ line. Reprod Domest Anim 47(Suppl. 4):7–14

Ajduk A, Yamauchi Y, Ward MA (2006) Sperm chromatin remodeling after intra-cytoplasmic sperm injection differs from that of in vitro fertilization. Biol Reprod 75:442–451

Alberio R, Zakhartchenko V, Motlik J, Wolf E (2001) Mammalian oocyte activation: lessons from the sperm and implications for nuclear transfer. Int J Dev Biol 45:797–809

Alberts B, Johnson A, Walter P, Lewis J, Raff M, Roberts K (2008) Molecular biology of the cell, 5th edn. Garland Science, New York

Allan V (1996) Role of motor proteins in organizing the endoplasmic reticulum and Golgi apparatus. Semi Cell Dev Biol 7:335–342

Alm H, Torner H, Blottner S, Nurnberg G, Kanitz W (2001) Effect of sperm cryopreservation and treatment with calcium ionophore or heparin on in vitro fertilization of horse oocytes. Theriogenology 56:817–829

Amanai M, Brahmajosyula M, Perry ACF (2006) A restricted role for sperm-borne microRNAs in mammalian fertilization. Biol Reprod 75:877–884

Amari S, Yonezawa N, Mitsui S, Katsmata T, Hamano S, Kuwyama M, Hasimoto Y, Suzuki A, Takeda Y, Nakano M (2001) Essential role of the nonreducing terminal α-mannosyl residues of the N-linked carbohydrate chain of bovine zona pellucida glycoproteins in sperm-egg binding. Mol Reprod Dev 59:221–226

Ankel-Simons F, Cummins JM (1996) Misconceptions about mitochondria and mammalian fertilization: implications for theories on human evolution. Proc Natl Acad Sci USA 93:13859–13863

Arcelay E, Salicioni AM, Wertheimer E, Visconti PE (2008) Identification of proteins undergoing tyrosine phosphorylation during mouse sperm capacitation. Int J Dev Biol 52:463–472

Asch R, Simerly C, Ord T, Ord VA (1995) Schatten G (the stages at which human fertilization arrests: microtubule and chromosome configurations in inseminated oocytes which failed to complete fertilization and development in humans. Hum Reprod 10:1897–1906

Avendaño C, Franchi A, Jones E, Oehninger S (2008) Pregnancy-specific β-1-glycoprotein 1 and human leukocyte antigen-E mRNA in human sperm: differential expression in fertile and infertile men and evidence of a possible functional role during early development. Hum Reprod 24:270–277

Avendaño C, Franchi A, Taylor S, Morshedi M, Bocca S, Oehninger S (2009) Fragmentation of DNA in morphologically normal human spermatozoa. Fertil Steril 91:1077–1084

Baarends WM, Hoogerbrugge JW, Roest HP, Ooms M, Vreeburg J, Hoeijmakers JH, Grootegoed JA (1999a) Histone ubiquitination and chromatin remodeling in mouse spermatogenesis. Dev Biol 207:322–333

Baarends M, Roest P, Grootegoed A (1999b) The ubiquitin system in gametogenesis. Mol Cell Endocrinol 151:5–16

Baba T, Kashiwabara S, Watanabe K, Itoh H, Michikawa Y, Kimura K, Takada M, Fukamizu A, Arai Y (1989) Activation and maturation mechanisms of boar acrosin zymogen based on the deduced primary structure. J Biol Chem 264:11920–11927

Baker MA, Reeves G, Hetherington L, Muller J, Baur I, Aitken RJ (2007) Identification of gene products present in triton X-100 soluble and insoluble fractions of human spermatozoa lysates using LC-MS/MS analysis. Proteomics Clin Appl 1:524–532

Baker MA, Hetherington L, Reeves G, Muller J, Aitken RJ (2008) The rat sperm proteome characterized via IPG strip prefractionation and LC-MS/MS identification. Proteomics 8:2312–2321

Baker MA, Smith ND, Hetherington L, Taubman K, Graham ME, Robinson PJ, Aitken RJ (2010) Label-free quantitation of phosphopeptide changes during rat sperm capacitation. J Proteom Res 9:718–729

Barroso G, Valdespin C, Vega E, Kershenovich R, Avila R, Avendaño C, Oehninger S (2009) Developmental sperm contributions: fertilization and beyond. Fertil Steril 92:835–848

Baska KM, Manandhar G, Feng D, Agca Y, Tengowski MW, Sutovsky M, Yi YJ, Sutovsky P (2008) Mechanism of extracellular ubiquitination in the mammalian epididymis. J Cell Physiol 215:684–696

Bazer FW, Song G, Kim J, Dunlap KA, Satterfield MC, Johnson GA, Burghardt RC, Wu G (2012) Uterine biology in pigs and sheep. J Anim Sci Biotechnol 3:23

Bi M, Hickox R, Winfrey P, Olson E, Hardy M (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 375:477–488

Blobel CP (2000) Functional processing of fertilin: evidence for a critical role of proteolysis in sperm maturation and activation. Rev Reprod 5:75–83

Boerke A, Tsai PS, García-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 70:1188–1196

Boettger-Tong L, Aarons J, Biegler E, George B, Poirier R (1993) Binding of a murine proteinase inhibitor to the acrosome region of the human sperm head. Mol Reprod Dev 36:346–353

Bookbinder H, Cheng A, Bleil D (1995) Tissue- and species-specific expression of SP56, a mouse sperm fertilization protein. Science 269:86–89

Braude P, Bolton V, Moore S (1998) Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature 332:459–461

Brandt Y, Madej A, Rodríguez-Martínez H, Einarsson S (2007) Effects of exogenous ACTH during oestrus on early embryo development and oviductal transport in the sow. Reprod Domest Anim 42:118–125

Breitbart H (2002) Role and regulation of intracellular calcium in acrosomal exocytosis. J Reprod Immunol 53:151–159

Breitbart H, Naor Z (1999) Protein kinases in mammalian sperm capacitation and the acrosome reaction. Rev Reprod 4:151–159

Brenner A, Kubisch M, Pierce E (2004) Role of the mitochondrial genome in assisted reproductive technologies and embryonic stem cell-based therapeutic cloning. Reprod Fertil Dev 16:743–751

Brewis IA, Van Gestel RA, Gadella BM, Jones R, Publicover SJ, Roldan ER, Frayne J, Barratt CL (2005) The spermatozoon at fertilisation: current understanding and future research directions. Hum Fertil (Camb) 8:241–251

Brüssow KP (1985) Distribution of oocytes in oviducts of gilts, following synchronized ovulation. Mh Vet Med 40:264–268

Brüssow KP, Rátky J, Rodríguez-Martínez H (2008) Fertilization and early embryonic development in the porcine fallopian tube. Reprod Domest Anim 43(Suppl 2):245–251

Busso D, Cohen DJ, Hayashi M, Kasahara M, Cuasnicú PS (2005) Human testicular protein TPX1/CRISP-2: localization in spermatozoa, fate after capacitation and relevance for gamete interaction. Mol Hum Reprod 11:299–305

Calafell JM, Nogués C, Ponsà M, Santaló J, Egozcue J (1992) Zona pellucida surface of immature and in vitro matured mouse oocytes: analysis by scanning electron microscopy. J Assist Reprod Genet 9:365–372

Calvete JJ, Dostàlovà Z, Sanz L, Adermann K, Thole HH, Töpfer-Petersen E (1996) Mapping the heparin-binding domain of boar spermadhesins. FEBS Lett 379:207–211

Calvete JJ, Raida M, Gentzel M, Urbanke C, Sanz L, Töpfer-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 407:201–206

Calvin HI, Bedford JM (1971) Formation of disulphide bonds in the nucleus and accessory structures of mammalian spermatozoa during maturation in the epididymis. J Reprod Fertil Suppl 13:65–75

Cánovas S, Coy P, Gómez E (2007) First steps in the development of a functional assay for human sperm using pig oocytes. J Androl 28:273–281

Castle E, Dean J (1999) Manipulating the genome to study reproduction. Mice with ''humanized'' zonae pellucidae. Hum Reprod 14:1927–1939

Cechova D, Töpfer-Petersen E, Henschen E (1988) Boar proacrosin is a single chain molecule which ahs the N-terminus of the acrosin-A chain (light chain). FEBS Lett 241:136–140

Chapman R, Barratt L (1996) The role of carbohydrate in sperm-ZP3 adhesion. Mol Hum Reprod 2:767–774

Chernomordik LV, Kozlov MM (2008) Mechanics of membrane fusion. Nat Struct Mol Biol 15:675–683

Chiu PC, Chung MK, Koistinen R, Koistinen H, Seppala M, Ho PC, Ng EH, Lee KF, Yeung WS (2007) Glycodelin-A interacts with fucosyltransferase on human sperm plasma membrane to inhibit spermatozoa-zona pellucida binding. J Cell Sci 120:33–44

Chiu PC, Wong BS, Chung MK, Lam KK, Pang RT, Lee KF, Sumitro SB, Gupta SK, Yeung WS (2008) Effects of native human zona pellucida glycoproteins 3 and 4 on acrosome reaction and zona pellucida binding of human spermatozoa. Biol Reprod 79:869–877

Choi YH, Landim-Alvarenga FC, Seidel GE, Squires EL (2003) Effect of capacitation of stallion sperm with polyvinylalcohol or bovine serum albumin on penetration of bovine zona-free or partially zona-removed equine oocytes. J Anim Sci 81:2080–2087

Clark F, Oehninger S, Patankar S, Koistinen R, Dell A, Morris HR, Koistinen H, Seppälä M (1996) A role for glycoconjugates in human development: the human feto-embryonic defence system hypothesis. Hum Reprod 11:467–473

Clark F, Dell A, Morris R, Patankar M, Oehninger S, Seppälä M (1997) Viewing AIDS from a glycobiological perspective: potential linkages to the human fetoembryonic defence system hypothesis. Mol Hum Reprod 3:5–13

Colombero T, Moomjy M, Silis S, Rosenwaks Z, Palermo D (1999) The role of structural integrity of the fertilizing spermatozoon in early human embryogenesis. Zygote 7:157–163

Conner J, Lefievre L, Hughes C, Barratt L (2005) Cracking the egg: increased complexity in the zona pellucida. Hum Reprod 20:1148–1152

Cowan E, Koppel E, Vargas A, Hunnicutt R (2001) Guinea pig fertilin exhibits restricted lateral mobility in epididymal sperm and becomes freely diffusing during capacitation. Dev Biol 15:502–509

Cox JF, Avila J, Saravia F, Santa Maria A (1994) Assessment of fertilizing ability of goat spermatozoa by in vitro fertilization of cattle and sheep intact oocytes. Theriogenology 41:1621–1629

Coy P, Cánovas S, Mondéjar I, Saavedra MD, Romar R, Grullón L, Matás C, Avilés M (2008) Oviduct-specific glycoprotein and heparin modulate sperm-zona pellucida interaction during fertilization and contribute to the control of polyspermy. Proc Natl Acad Sci USA 105:15809–15814

Coy P, Avilés M (2010) What controls polyspermy in mammals, the oviduct or the oocyte? Biol Rev Camb Philos Soc 85:593–605

Cross L, Morales P, Overstreet W, Hanson W (1988) Induction of acrosome reactions by the human zona pellucida. Biol Reprod 38:235–244

Cummins J (1998) Mitochondrial DNA in mammalian reproduction. Rev Reprod 3:172–182

Cummins J, Wakayama T, Yanagimachi R (1998) Fate of microinjected spermatid mitochondria in the mouse oocyte and embryo. Zygote 6:213–222

Da Ros V, Busso D, Cohen DJ, Maldera J, Goldweic N, Cuasnicu PS (2007) Molecular mechanisms involved in gamete interaction: evidence for the participation of cysteine-rich secretory proteins (CRISP) in sperm-egg fusion. Soc Reprod Fertil Suppl 65:353–356

Dadoune JP, Pawlak A, Alfonso MF, Siffro JP, Beebe S (2005) Identification of transcripts by macroarrays, RT-PCR and in situ hybridization in human ejaculate spermatozoa. Mol Hum Reprod 11:133–140

Dale B, DeFelice LJ, Ehrenstein G (1985) Injection of a soluble sperm extract into sea urchin eggs triggers the cortical reaction. Experientia 41:1068–1070

Darson A, Treviño CL, Wood C, Galindo B, Rodríguez-Miranda E, Acevedo JJ, Hernández-González EO, Beltrán C, Martínez-López P, Nishigaki T (2007) Ion channels in sperm motility and capacitation. Soc Reprod Fertil Suppl 65:229–244

Day BN (2000) Reproductive biotechnologies: current status in porcine reproduction. Anim Reprod Sci 60:161–172

Dell A, Morris HR, Easton RL, Panico M, Patankar M, Oehniger S, Koistinen R, Koistinen H, Seppala M, Clark GF (1995) Structural analysis of the oligosaccharides derived from glycodelin, a human glycoprotein with potent immunosuppressive and contraceptive activities. J Biol Chem 270:24116–24126

Dell A, Chalabi S, Easton RL, Haslam SM, Sutton-Smith M, Patankar MS, Lattanzio F, Panico M, Morris HR, Clark GF (2003) Murine and human zona pellucida 3 derived from mouse eggs express identical O-glycans. Proc Natl Acad Sci USA 100:15631–15636

Dell'Aquila ME, Fusco S, Lacalandra GM, Maritato F (1996) In vitro maturation and fertilization of equine oocytes recovered during the breeding season. Theriogenology 45:547–560

Díaz ES, Kong M, Morales P (2007) Effect of fibronectin on proteasome activity, acrosome reaction, tyrosine phosphorylation and intracellular calcium concentrations of human sperm. Hum Reprod 22:1420–1430

Didion BA, Kasperson KM, Wixon RL, Evenson DP (2009) Boar fertility and sperm chromatin structure status: a retrospective report. J Androl 30:655–660

Donoghue AM, Johnston LA, Seal US, Armstrong DL, Simmons LG, Gross T, Tilson RL, Wildt DE (1992) Ability of thawed tiger (*Panthera tigris*) spermatozoa to fertilize conspecific eggs and bind and penetrate domestic cat eggs in vitro. J Reprod Fertil 96:555–564

Dostàlovà Z, Calvete JJ, Töpfer-Petersen E (1995a) 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 376:237–242

Dostàlovà Z, Calvete JJ, Sanz L, Töpfer-Petersen E (1995b) Boar spermadhesin AWN-1: oligo-saccharide and zona pellucida binding characteristics. Eur J Biochem 230:329–336

Du Y, Liu ML, Jia MC (2008) Identification and characterization of a spermatogenesis-related gene Ube1 in rat testis. Sheng Li Xue Bao 60:382–390

Dupont G (1998) Theoretical insights into the mechanism of spiral Ca^{2+} wave initiation in xeno-pus oocytes. Am J Physiol 275:C317–C322

Duran EH, Gürgan T, Günalp S, Enginsu ME, Yarali H, Ayhan A (1998) A logistic regression model including DNA status and morphology of spermatozoa for prediction of fertilization in vitro. Hum Reprod 13:1235–1239

East IJ, Dean J (1984) Monoclonal antibodies as probes of the distribution of ZP2, the major sul-fated glycoprotein of the murine zona pellucida. J Cell Biol 98:795–800

Edwards RG, Beard HK (1997) Oocyte polarity and cell determination in early mammalian embryos. Mol Hum Reprod 3:863–905

Einarsson S, Brandt Y, Rodríguez-Martínez H, Madej A (2008) Conference lecture: influence of stress on estrus, gametes and early embryo development in the sow. Theriogenology 70:1197–1201

Ekhlasi-Hundrieser M, Gohr K, Wagner A, Tsolova M, Petrunkina AM, Töpfer-Petersen E (2005) Spermadhesin AQN1 is a candidate receptor molecule involved in the formation of the oviductal sperm reservoir in the pig. Biol Reprod 73:536–545

Ekhlasi-Hundrieser M, Schäfer B, Philipp U, Kuiper H, Leeb T, Mehta M, Kirchhoff C, Töpfer-Petersen E (2007) Sperm-binding fibronectin type II-module proteins are genetically linked and functionally related. Gene 392:253–265

Eliyahu E, Shalgi R (2002) A role for protein kinase C during rat egg activation. Biol Reprod 67:189–195

Ensslin M, Calvete JJ, Thole HH, Sierralta WD, Adermann K, Sanz L, Töpfer-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 376:733–738

Ensslin M, Vogel T, Calvete JJ, Thole HH, Schmidtke J, Matsuda T, Töpfer-Petersen E (1998) Molecular cloning an characterization of P47: a novel boar sperm-associated zona pelluci-cida-bninding protein homologous to a family of mammalian secretory proteins. Biol Reprod 58:1057–1064

Espey LL, Lipner H (1994) Ovulation. In: Knobil E, Neill JD (eds) The physiology of reproduction. Raven Press, New York, pp 725–780

Esterhuizen D, Franken R, Becker J, Lourens G, Muller I, van Rooyen H (2002) Defective sperm decondensation: a cause for fertilization failure. Andrologia 34:1–7

Fàbrega A, Guyonnet B, Dacheux JL, Gatti JL, Puigmulé 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

Familiari G, Heyn R, Relucenti M, Sathananthan H (2008) Structural changes of the zona pellu-cida during fertilization and embryo development. Front Biosci 13:6730–6751

Familiari G, Nottola SA, Macchiarelli G, Micara G, Aragona C, Motta PM (1992) Human zona pellucida during in vitro fertilization: an ultrastructural study using saponin, ruthenium red and osmium-thiocarbohydrazide. Mol Reprod Dev 32:51–61

Ficarro S, Chertihin O, Westbrook VA, White F, Jayes F, Kalab P, Marto JA, Shabanowitz J, Herr JC, Hunt DF, Visconti PE (2003) Phosphoproteome analysis of capacitated human sperm. Evidence of tyrosine phosphorylation of a kinase-anchoring protein 3 and valosin-contain-ing protein/p97 during capacitation. J Biol Chem 278:11579–11589

Fischer KA, van Leyen K, Lovercamp KW, Manandhar G, Sutovsky M, Feng D, Safranski T, Sutovsky P (2005) 15-lipoxygenase is a component of the mammalian sperm cytoplasmic droplet. Reproduction 130:213–222

Fissore A, Reis M, Palermo D (1999) Isolation of the Ca^{2+} releasing component(s) of mammalian sperm extracts: the search continues. Mol Hum Reprod 5:189–192

Fléchon JE, Kopecny V, Pivko J, Pavlok A, Motlik J (2004) Texture of the zona pellucida of the mature pig oocyte: the mammalian egg envelope revisited. Reprod Nutr Dev 44:207–218

Flesch FM, Gadella BM (2000) Dynamics of the mammalian sperm plasma membrane in the process of fertilization. Biochim Biophys Acta 1469:197–235

Flores E, Ramió-Lluch L, Bucci D, Fernández-Novell JM, Peña A, Rodríguez-Gil JE (2011) Freezing-thawing induces alterations in histone H1-DNA binding and the breaking of protein-DNA disulfide bonds in boar sperm. Theriogenology 76:1450–1464

Florman M, Corron E, Kim D, Babcock F (1992) Activation of voltage-dependent calcium channels of mammalian sperm is required for zona pellucida-induced acrosomal exocytosis. Dev Biol 152:304–314

Florman M, Tombes M, First L, Babcock F (1989) An adhesion-associated agonist from the zona pellucida activates G protein-promoted elevations of internal Ca^{2+} and pH that mediate mammalian sperm acrosomal exocytosis. Dev Biol 135:133–146

Foster HA, Abeydeera LR, Griffin DK, Bridger JM (2005) Non-random chromosome positioning in mammalian sperm nuclei, with migration of the sex chromosomes during late spermatogenesis. J Cell Sci 118:1811–1820

Franken DR, Morales J, Habenicht F (1996) Inhibition of G protein in human sperm and its influence on acrosome reaction and zona pellucida binding. Fertil Steril 66:1009–1011

Friess AE, Töpfer-Petersen E, Nguyen H, Schill WB (1987) Electron micorscopic localization of a fucose-binding protein in acrosomereacted boar spermatozoa by the fucosyl-peroxidase-gold method. Histochemistry 86:297–303

Funahashi H, Ekwall H, Rodríguez-Martínez H (2000) The zona reaction in porcine oocytes fertilized in vivo and in vitro as seen with scanning electron microscopy. Biol Reprod 63:1437–1442

Funahashi H, Nagai T (2001) Regulation of in vitro penetration of frozen-thawed boar spermatozoa by caffeine and adenosine. Mol Reprod Dev 58:424–431

Fusi F, Montesano M, Bernocchi N, Panzeri C, Ferrara F, Villa A, Bronson R (1996) P-selectin is expressed on the oolemma of human and hamster oocytes following sperm adhesion and is also detected on the equatorial region of acrosome-reacted human spermatozoa. Mol Hum Reprod 2:341–347

Gao Z, Garbers DL (1998) Species diversity in the structure of zonadhesin, a sperm-specific membrane protein containing multiple cell adhesion molecule-like domains. J Biol Chem 273:3415–3421

García-Álvarez O, Maroto-Morales A, Martínez-Pastor F, Fernández-Santos MR, Esteso MC, Pérez-Guzman MD, Soler AJ (2008) Heterologous in vitro fertilization is a good procedure to assess the fertility of thawed ram spermatozoa. Theriogenology 71:643–650

Gatewood M, Cook R, Balhorn R, Schmid W, Bradbury M (1990) Isolation of four core histones from human sperm chromatin representing a minor subset of somatic histones. J Biol Chem 265:20662–20666

Gilbert I, Bissonnette N, Boissonneault G, Vallée M, Robert C (2007) A molecular analysis of the population of mRNA in bovine spermatozoa. Reproduction 133:1073–1086

Gineitis A, Zalenskaya A, Yau M, Bradbury M, Zalensky O (2000) Human sperm telomere-binding complex involves histone H2B and secures telomere membrane attachment. J Cell Biol 151:1591–1598

Glickman MH, Rubin DM, Coux O, Wefes I, Pfeifer G, Cjeka Z, Baumeister W, Fried VA, Finley D (1998) A subcomplex of the proteasome regulatory particle required for ubiquitin-conjugate degradation and related to the COP9-signalosome and eIF3. Cell 94:615–623

Glickman MH, Ciechanover A (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 82:373–428

Gorczyca W, Traganos F, Jesionowska H, Darzynkiewicz Z (1993) Presence of DNA strand breaks and increased sensitivity of DNA in situ to denaturation in abnormal human sperm cells: analogy to apoptosis of somatic cells. Exp Cell Res 207:202–205

Gosálvez J, López-Fernández C, Fernández JL, Gouraud A, Holt WV (2011) Relationships between the dynamics of iatrogenic DNA damage and genomic design in mammalian spermatozoa from eleven species. Mol Reprod Dev 78:951–961

Goudet G, Mugnier S, Callebaut I, Monget P (2008) Phylogenetic analysis and identification of pseudogenes reveal a progressive loss of zona pellucida genes during evolution of vertebrates. Biol Reprod 78:796–806

Greco E, Scarselli F, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Franco G, Anniballo N, Mendoza C, Tesarik J (2005) Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa. Hum Reprod 20:226–230

Green DPL (1987) Mammalian sperm can not penetrate the zona pellcuida soley by force. Exp Cell Res 169:31–38

Greve JM, Wassarman PM (1985) Mouse egg extracellular coat is a matrix of interconnected filaments possessing a structural repeat. J Mol Biol 181:253–264

Grow DR, Oehninger S, Seltman HJ, Toner JP, Swanson RJ, Kruger TF, Muasher SJ (1994) Sperm morphology as diagnosed by strict criteria: probing the impact of teratozoospermia on fertilization rate and pregnancy outcome in a large in vitro fertilization population. Fertil Steril 62:559–567

Grunewald S, Paasch U, Glander HJ, Anderegg U (2005) Mature human spermatozoa do not transcribe novel RNA. Andrologia 37:69–71

Hafez ESE (1993) Anatomy of female reproductive tract. In: Hafez ESE (ed) Reproduction in farm animals, 6th edn. Lea and Febiger, Philadelphia

Haraguchi CM, Mabuchi T, Hirata S, Shoda T, Tokumoto T, Hoshi K, Yokota S (2007) Possible function of caudal nuclear pocket: degradation of nucleoproteins by ubiquitin-proteasome system in rat spermatids and human sperm. J Histochem Cytochem 55:585–595

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 4:361–393

Hayashi S, Yang J, Christenson L, Yanagimachi R, Hecht NB (2003) Mouse preimplantation embryos developed from oocytes injected with round spermatids or spermatozoa have similar but distinct patterns of early messenger RNA expression. Biol Reprod 69:1170–1176

Hecht NB (1974) A DNA polymerase isolated from bovine spermatozoa. J Reprod Fertil 41:345–354

Helfand T, Mikami A, Vallee B, Goldman D (2002) A requirement for cytoplasmic dynein and dynactin in intermediate filament network assembly and organization. J Cell Biol 157:795–806

Hermansson U, Axnér E, Holst SB (2007) Application of a zona pellucida binding assay (ZBA) in the domestic cat benefits from the use of in vitro matured oocytes. Acta Vet Scand 49:28

Hershko A, Ciechanover A (1998) The ubiquitin system. Annu Rev Biochem 67:425–479

Hewitson L, Simerly C, Schatten G (2000) Cytoskeletal aspects of assisted fertilization. Semin Reprod Med 18:151–159

Hewitson L, Simerly CR, Schatten G (2002) Fate of sperm components during assisted reproduction: implications for infertility. Hum Fertil (Camb) 5:110–116

Hewitson L, Simerly CR, Schatten G (2003) ICSI, male pronuclear remodeling and cell cycle checkpoints. Adv Exp Med Biol 518:199–210

Hinrichs K, Love CC, Brinsko SP, Choi YH, Varner DD (2002) In vitro fertilization of in vitro-matured equine oocytes: effect of maturation medium, duration of maturation, and sperm calcium ionophore treatment, and comparison with rates of fertilization in vitro after oviductal transfer. Biol Reprod 67:256–262

Hinsch KD, Hinsch E, Meinecke B, Töpfer-Petersen E, Pfisterer S, Schill WB (1994) Identification of mouse ZP3 protein in mammalian oocytes with antisera against synthetic ZP3 peptides. Biol Reprod 51:193–204

Hochstrasser M (1996) Ubiquitin-dependent protein degradation. Annu Rev Genet 30:405–439

Hokke CH, Damm JB, Penninkhof B, Aitken RJ, Kamerling JP, Vliegenthart JF (1994) Structure of the O-linked carbohydrate chains of porcine zona pellucida glycoproteins. Eur J Biochem 221:491–512

Honda A, Siruntawinwti J, Baba T (2002) Role of acrosomal matrix proteinases in sperm-zona pellucida interactiosn. Hum Reprod Update 5:405–412

Hoodbhoy T, Dean J (2004) Insights into the molecular basis of sperm-egg recognition in mammals. Reproduction 127:417–422

Hoodbhoy T, Joshi S, Boja ES, Williams SA, Stanley P, Dean J (2005) Human sperm do not bind to rat zonae pellucidae despite the presence of four homologous glycoproteins. J Biol Chem 280:12721–12731

Hughes DC (2007) ZP genes in the avian species illustrates the dynamic evolution of the vertebrate egg envelope. Cytogenet Genome Res 117:86–91

Hunter RH (1984) Pre-ovulatory arrest and peri-ovulatory redistribution of competent spermatozoa in the isthmus of the pig oviduct. J Reprod Fertil 72:203–211

Hutvagner G, McLachlan J, Pasquinelli AE, Balint E, Tuschl T, Zamore PD (2001) A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. Science 293:834–838

Hutvagner G, Zamore PD (2002) A microRNA in a multiple-turnover RNAi enzyme complex. Science 297:2056–2060

Hwang JY, Mulligan BP, Kim, HM, Y BC, Lee CK (2012) Quantitative analysis of sperm mRNA in the pig: relationship with early embryo development and capacitation. Reprod Fertil Dev, http://dx.doi.org/10.1071/RD12160 (in press)

Ingman M, Kaessmann H, Pääbo S, Gyllensten U (2000) Mitochondrial genome variation and the origin of modern humans. Nature 408:708–713

Inoue N, Ikawa M, Isotani A, Okabe M (2005) The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. Nature 434:234–238

Inoue N, Ikawa M, Okabe M (2008) Putative sperm fusion protein IZUMO and the role of N-glycosylation. Biochem Biophys Res Comm 377:910–914

Iwamoto K, Ikeda K, Yonezawa N, Noguchi S, Kudo K, Hamano S, Kuwayama M, Nakano M (1999) Disulfide formation in bovine zona pellucida glycoproteins during fertilization: evidence for the involvement of cystine cross-linkages in hardening of the zona pellucida. J Reprod Fertil 117:395–402

Iwao Y (2012) Egg activation in physiological polyspermy. Reproduction 144:11–12

Izquierdo-Rico MJ, Jiménez-Movilla M, Llop E, Pérez-Oliva AB, Ballesta J, Gutiérrez-Gallego R, Jiménez-Cervantes C, Avilés M (2009) Hamster zona pellucida is formed by four glycoproteins: ZP1, ZP2, ZP3, and ZP4. J Proteome Res 8:926–941

Jaffe F (1983) Sources of calcium in egg activation: a review and hypothesis. Dev Biol 99:265–276

Jahn R, Scheller RH (2006) SNAREs-engines for membrane fusion. Nat Rev Mol Cell Biol 7:631–643

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 USA 108:4892–4896

Jungnickel MK, Marrero H, Birnbaumer L, Lemos JR, Florman HM (2001) Trp2 regulates entry of Ca^{2+} into mouse sperm triggered by egg ZP3. Nat Cell Biol 3:499–502

Kaji K, Oda S, Shikano T, Ohnuki T, Uematsu Y, Sakagami J, Tada N, Miyazaki S, Kudo A (2000) The gamete fusion process is defective in eggs of CD9-deficient mice. Nat Genet 24:279–282

Kaneda H, Hayashi J, Takahama S, Taya C, Lindahl KF, Yonekawa H (1995) Elimination of paternal mitochondrial DNA in intraspecific crosses during early mouse embryogenesis. Proc Natl Acad Sci USA 92:4542–4546

Katayama M, Koshida M, Miyake M (2002) Fate of the acrosome in ooplasm in pigs after IVF and ICSI. Hum Reprod 17:2657–2664

Ketting RF, Fischer SE, Bernstein E, Sijen T, Hannon GJ, Plasterk RH (2001) Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in *C. elegans*. Genes Dev 15:2654–2659

Kim NH, Jun SH, Do JT, Uhm SJ, Lee HT, Chung KS (1999) Intracytoplasmic injection of porcine, bovine, mouse, or human spermatozoon into porcine oocytes. Mol Reprod Dev 53:84–91

Klemm U, Müller-Esterl W, Engle W (1991) Acrosin, the peculiar sperm-specific serine protease. Hum Genet 87:635–641

Kline D, Simoncini L, Mandel G, Maue RA, Kado RT, Jaffe LA (1988) Fertilization events induced by neurotransmitters after injection of mRNA in xenopus eggs. Science 241:464–467

Knobil E, Neill JD (1994) The physiology of reproduction, 2nd edn. Raven Press, New York

Kong M, Días ES, Morales P (2009) Participation of the human sperm proteasome in the capacitation process and its regulation by protein kinase A and tyrosine kinase. Biol Reprod 80:1026–1035

Koppers AJ, Mitchell LA, Wang P, Lin M, Aitken RJ (2011) Phosphoinositide 3-kinase signalling pathway involvement in a truncated apoptotic cascade associated with motility loss and oxidative DNA damage in human spermatozoa. Biochem J 436:687–698

Kouba AJ, Atkinson MW, Gandolf AR, Roth TL (2001) Species-specific sperm-egg interaction affects the utility of a heterologous bovine in vitro fertilization system for evaluating antelope sperm. Biol Reprod 65:1246–1251

Kouchi Z, Fukami K, Shikano T, Oda S, Nakamura Y, Takenawa T, Miyazaki S (2004) Recombinant phospholipase Cζ has high Ca^{2+} sensitivity and induces Ca^{2+} oscillations in mouse eggs. J Biol Chem 279:10408–10412

Kramer JA, Krawetz SA (1997) RNA in spermatozoa: implications for the alternative haploid genome. Mol Hum Reprod 3:473–478

Krawetz A (2005) Paternal contribution: new insights and future challenges. Nat Rev Genet 6:633–642

Kudo K, Yonezawy 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 Biol 252:492–499

Kumar G, Patel D, Naz RK (1993) c-MYC mRNA is present in human sperm cells. Cell Mol Biol Res 39:111–117

Kvist U (1980) Sperm nuclear chromatin decondensation ability. An in vitro study on ejaculated human spermatozoa. Acta Physiol Scand Suppl 486:1–24

Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T (2001) Identification of novel genes coding for small expressed RNAs. Science 294:853–858

Lambard S, Galeraud-Denis I, Martin G, Levy R, Chocat A, Carreau S (2004) Analysis and significance of mRNA in human ejaculated sperm from normozoospermic donors: relationship to sperm motility and capacitation. Mol Hum Reprod 10:535–541

Landim-Alvarenga FC, Alvarenga MA, Seidel GE Jr, Squires EL, Graham JK (2001) Penetration of zona-free hamster, ovine and equine oocytes by stallion and bull spermatozoa pretreated with equine follicular fluid, dilauroylphosphatidylcholine or calcium ionophore A23187. Theriogenology 56:937–953

Larson L, DeJonge J, Barnes M, Jost K, Evenson P (2000) Sperm chromatin structure assay parameters as predictors of failed pregnancy following assisted reproductive techniques. Hum Reprod 15:1717–1722

Lau NC, Lim LP, Weinstein EG, Bartel DP (2001) An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. Science 294:858–862

Lee A, Check H, Kopf A (1992) Guanine nucleotide-binding regulatory protein in human sperm mediates acrosomal exocytosis induced by the human zona pellucida. Mol Reprod Dev 31:78–86

Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843–854

Lefièvre L, Conner SJ, Salpekar A, Olufowobi O, Ashton P, Pavlovic B, Lenton W, Afnan M, Brewis IA, Monk M, Hughes DC, Barratt CL (2004) Four zona pellucida glycoproteins are expressed in the human. Hum Reprod 19:1580–1586

Leyton L, LeGuen P, Bunch D, Saling PM (1992) Regulation of mouse gamete interaction by a sperm tyrosine kinase. Proc Natl Acad Sci USA 89:11692–11695

Li GP, Seidel GE, Squires EL (2003) Intracytoplasmic sperm injection of bovine oocytes with stallion spermatozoa. Theriogenology 59:1143–1155

Lin SL, Kim H, Ying SY (2007) Intron-mediated RNA interference and microRNA (miRNA). Front Biosci 13:2216–2230

Linfor J, Berger T (2000) Potential role of αv and β1 integrins as oocyte adhesion molecules during fertilization in pigs. J Reprod Fertil 120:65–72

Litscher ES, Wassarman PM (2007) Egg extracellular coat proteins: from fish to mammals. Histol Histopathol 22:337–347

Madej A, Lang A, Brandt Y, Kindahl H, Madsen MT, Einarsson S (2005) Factors regulating ovarian function in pigs. Domestic Anim Endocrinol 29:347–361

Maleszewski M, Kline D, Yanagimachi R (1995) Activation of hamster zona-free oocytes by homologous and heterologous spermatozoa. J Reprod Fertil 105:99–107

Marchiani S, Tamburrino L, Maoggi A, Vannelli GB, Forti G, Baldi E, Muratori M (2007) Characterization of M540 bodies in human semen: evidence that they are apoptotic bodies. Mol Hum Reprod 13:621–631

Matson PL, Vaid P, Parsons JH, Goswamy R, Collins WP, Pryor JP (1987) Use of the heterologous ovum penetration test to predict the fertilising capacity of human spermatozoa. Clin Reprod Fertil 5:5–13

Martens S, McMahon HT (2008) Mechanisms of membrane fusion: disparate players and common principles. Nat Rev Mol Cell Biol 9:543–556

Matsukawa K, Takahashi M, Hanada A (2002) Assay for penetrability of frozen-thawed stallion spermatozoa into zona-free hamster oocytes. J Reprod Dev 48:539–544

Maximov A, Tang J, Yang X, Pang ZP, Sudhof TC (2009) Complexin controls the force transfer from SNARE complexes to membranes in fusion. Science 323:516–521

Mburu JN, Einarsson S, Kindahl H, Madej A, Rodríguez-Martínez H (1998) Effects of post-ovulatory food deprivation on oviductal sperm concentration, embryo development and hormonal profiles in the pig. Anim Reprod Sci 52:221–234

McLay DW, Clarke HJ (2003) Remodelling the paternal chromatin at fertilization in mammals. Reproduction 125:625–633

Michaut M, Tomes CN, De BG, Yunes R, Mayorga LS (2000) Calcium triggered acrosomal exocytosis in human spermatozoa requires the coordinated activation of Rab3A and N-ethylmaleimide-sensitive factor. Proc Natl Acad Sci USA 97:9996–10001

Michelmann HW, Rath D, Töpfer-Petersen E, Schwartz P (2007) Structural and functional events on the porcine zona pellucida during maturation, fertilization and embryonic development: a scanning electron microscopy analysis. Reprod Domest Anim 42:594–602

Miller D, Tang PZ, Skinner C, Lilford R (1994) Differential RNA finger printing as a tool in the analysis of spermatozoal gene expression. Hum Reprod 9:864–869

Miller J, Macek B, Shur D (1992) Complementarity between sperm surface beta -1,4-galactosyltransferase and egg-coat ZP3 mediates sperm-egg binding. Nature 357:589–593

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 287:321–324

Moomjy M, Colombero LT, Veeck L, Rosenwaks Z, Palermo D (1999) Sperm integrity is critical for normal mitotic division and early embryonic development. Mol Hum Reprod 5:836–844

Morales P, Kong M, Pizarro E, Pasten C (2003) Participation of the sperm proteasome in human fertilization. Hum Reprod 18:1010–1017

Morales P, Pizarro E, Kong M, Jara M (2004) Extracellular localization of proteasomes in human sperm. Mol Reprod Dev 68:115–124

Morales P, Díaz 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

Moreno RD, Barros C (2000) A basic 18-amino acid peptide contains the polysulfate-binding domain responsible for activation of the boar proacrosin/acrosin system. Biol Reprod 62:1536–1542

Mori E, Kashiwabara S, Baba T, Inagaki AY, Mori T (1995) Amino acid sequences of porcine Sp38 and proacrosin required for binding to the zona pellucida. Dev Biol 168:575–593

Mori E, Hedrick JL, Wardrip NJ, Mori T, Takasaki S (1998) Occurrence of reducing terminal N-acetylglucosamine 3-sulfate and fucosylated outer chains in acidic N-glycans of porcine zona pellucida glycoproteins. Glycoconj J 15:447–456

Mourelatos Z, Dostie J, Paushkin S, Sharma A, Charroux B, Abel L, Rappsilber J, Mann M, Dreyfuss G (2002) miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs. Genes Dev 16:720–728

Mugnier S, Dell'Aquila ME, Pelaez J, Douet C, Ambruosi B, De Santis T, Lacalandra GM, Lebos C, Sizaret PY, Delaleu B, Monget P, Mermillod P, Magistrini M, Meyers SA, Goudet G (2009) New insights into the mechanisms of fertilization: comparison of the fertilization steps, composition, and structure of the zona pellucida between horses and pigs. Biol Reprod 81:856–870

Mwanza AM, Englund P, Kindahl H, Lundeheim N, Einarsson S (2000a) Effects of post-ovulatory food deprivation on the hormonal profiles, activity of the oviduct and ova transport in sows. Anim Reprod Sci 59:185–199

Mwanza AM, Madej A, Kindahl H, Lundeheim N, Einarsson S (2000b) Postovulatory effect of repeated administration of ACTH on the contractile activity of the oviduct, ova transport and endocrine status of recently ovulated and unrestrained sows. Theriogenology 54:1305–1316

Mwanza AM, Razdan P, Hultén F, Einarsson S (2002a) Transport of fertilised and unfertilised ova in sows. Anim Reprod Sci 74:69–74

Mwanza AM, Einarsson S, Madej A, Lundeheim N, Rodríguez-Martínez H, Kindahl H (2002b) Postovulatory effect of repeated administration of prostaglandin $F_{2\alpha}$ on the endocrine status, ova transport, binding of accessory spermatozoa to the zona pellucida and embryo development of recently ovulated sows. Theriogenology 58:1111–1124

Nagai T, Funahashi H, Yoshioka K, Kikuchi K (2006) Update of in vitro production of porcine embryos. Front Biosci 11:2565–2573

Nakamura S, Terada Y, Horiuchi T, Emuta C, Murakami T, Yaegashi N, Okamura K (2001) Human sperm aster formation and pronuclear decondensation in bovine eggs following intracytoplasmic sperm injection using a piezo-driven pipette: a novel assay for human sperm centrosomol function. Biol Reprod 65:1359–1363

Nayernia K, Adham IM, Shamsadin R, Müller C, Sancken U, Engel W (2002) Proacrosin-deficient mice and zona pellucida modifications in an experimental model of multifactorial infertility. Mol Hum Reprod 8:434–440

Naz RK (1998) Effect of actinomycin D and cycloheximide on human sperm function. Arch Androl 41:135–142

Nazarov IB, Shlyakhtenko LS, Lyubchenko YL, Zalenskaya IA, Zalensky AO (2008) Sperm chromatin released by nucleases. Syst Biol Reprod Med 54:37–46

Niemann H, Rath D (2001) Progress in reproductive biotechnology in swine. Theriogenology 56:1291–1304

Nikolettos N, Küpker W, Demirel C, Schöpper B, Blasig C, Sturm R, Felberbaum R, Bauer O, Diedrich K, Al-Hasani S (1999) Fertilization potential of spermatozoa with abnormal morphology. Hum Reprod 14(Suppl 1):47–70

Noguchi S, Hatanaka Y, Tobita T, Nakano M (1992) Structural analysis of the N-linked carbohydrate chains of the 55-kDa glycoprotein family (PZP3) from porcine zona pellucida. Eur J Biochem 207:1130

Noguchi S, Nakano M (1992) Structure of the acidic N-linked carbohydrate chains of the 55-kDa glycoprotein family (PZP3) from porcine zona pellucida. Eur J Biochem 209:883–894

Noguchi S, Yonezawa N, Katsumata T, Hashizume K, Kuwayama M, Hamano S, Watanabe S, Nakano M (1994) Characterization of the zona pellucida glycoproteins from bovine ovarian and fertilized eggs. Biochim Biophys Acta 1201:7–14

Nonchev S, Tsanev R (1990) Protamine-histone replacement and DNA replication in the male mouse pronucleus. Mol Reprod Dev 25:72–76

O'Brien J, Zini A (2005) Sperm DNA integrity and male infertility. Urology 65:16–22

O'Rand M, Welch JE, Fisher SJ (1986) Sperm membrane and zona pellucida interaction during fertilization. In: Dhinsa DS, Bahl O (eds) Molecular and cellular aspects of reproduction. Plenum Press, New York, pp 131–144

Odorisio T, Mahadevaiah SK, McCarrey JR, Burgoyne PS (1996) Transcriptional analysis of the candidate spermatogenesis gene Ube1y and of the closely related Ube1x shows that they are coexpressed in spermatogonia and spermatids but are repressed in pachytene spermatocytes. Dev Biol 180:336–343

Oehninger S (2001) Molecular basis of human sperm-zona pellucida interaction. Cells Tissues Organs 168:58–64

Oehninger S (2003) Biochemical and functional characterization of the human zona pellucida. Reprod Biomed Online 7:641–648

Oehninger S, Acosta A, Veeck L, Simonetti S, Muasher J (1989) Delayed fertilization during in vitro fertilization and embryo transfer cycles: analysis of causes and impact on overall results. Fertil Steril 52:991–997

Oehninger S, Veeck L, Lanzendorf S, Maloney M, Toner J, Muasher S (1995) Intracytoplasmic sperm injection: achievement of high pregnancy rates in couples with severe male factor infertility is dependent primarily upon female and not male factors. Fertil Steril 64:977–981

Oehninger S, Kruger TF, Simon T, Jones D, Mayer J, Lanzendorf S, Toner JP, Muasher SJ (1996) A comparative analysis of embryo implantation potential in patients with severe teratozoospermia undergoing in vitro fertilization with a high insemination concentration or intracytoplasmic sperm injection. Hum Reprod 11:1086–1089

Olsen PH, Ambros V (1999) The lin-4 regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation. Dev Biol 216:671–680

Ostermeier C, Dix J, Millar D, Khatri P, Krawetz A (2002) Spermatozoal RNA profiles of normal fertile men. Lancet 360:772–777

Ostermeier C, Miller D, Huntriss D, Diamond P, Krawetz A (2004) Reproductive biology: delivering spermatozoan RNA to the oocyte. Nature 429:154

Ostermeier C, Goodrich J, Moldenhauer S, Diamond P, Krawetz A (2005) A suite of novel human spermatozoal RNAs. J Androl 26:70–74

Ozgur K, Patankar MS, Oehninger S, Clark GF (1998) Direct evidence for the involvement of carbohydrate sequences in human sperm-zona pellucida binding. Mol Hum Reprod 4:318–324

Pang PC, Tissot B, Drobnis EZ, Sutovsky P, Morris HR, Clark GF, Dell A (2007) Expression of bisecting type and Lewisx/Lewisy terminated N-glycans on human sperm. J Biol Chem 282:36593–36602

Parinaud J, Mieusset R, Vieitez G, Labal B, Richoilley G (1993) Influence of sperm parameters on embryo quality. Fertil Steril 60:888–892

Parrington J, Swann K, Shevchenko VI, Sesay AK, Lai FA (1996) Calcium oscillations in mammalian eggs triggered by a soluble sperm protein. Nature 379:364–368

Pasten C, Morales P, Kong M (2005) Role of the sperm proteasome during fertilization and gamete interaction in the mouse. Mol Reprod Dev 71:209–219

Patankar MS, Ozgur K, Oehninger S, Dell A, Morris H, Seppala M, Clark GF (1997) Expression of glycans linked to natural killer cell inhibition on the human zona pellucida. Mol Hum Reprod 3:501–505

Payne C, Rawe V, Ramalho-Santos J, Simerly C, Schatten G (2003) Preferentially localized dynein and perinuclear dynactin associate with nuclear pore complex proteins to mediate genomic union during mammalian fertilization. J Cell Sci 116:4727–4738

Perreault SD (1992) Chromatin remodeling in mammalian zygotes. Mutat Res 296:43–55

Perreault SD, Wolff RA, Zirkin BR (1984) The role of disulfide bond reduction during mammalian sperm nuclear decondensation in vivo. Dev Biol 101:160–167

Perreault SD, Barbee RR, Slott VL (1988) Importance of glutathione in the acquisition and maintenance of sperm nuclear decondensing activity in maturing hamster oocytes. Dev Biol 125:181–186

Pessot A, Brito M, Figueroa J, Concha I, Yanez A, Burzio O (1989) Presence of RNA in the sperm nucleus. Biochem Biophys Res Comm 158:272–278

Petrunkina AM, Läkamp A, Gentzel M, Eklhasi-Hundrieser M, Töpfer-Petersen E (2003) Fate of lactadherin P47 during post-testicular maturation and capacitation of boar spermatozoa. Reproduction 125:377–387

Philpott A, Leno GH (1992) Nucleoplasmin remodels sperm chromatin in Xenopus egg extracts. Cell 69:759–767

Pizarro E, Pasten C, Kong M, Morales P (2004) Proteasomal activity in mammalian spermatozoa. Mol Reprod Dev 69:87–93

Primakoff P, Myles DG (2000) The ADAM gene family: surface proteins with adhesion and protease activity. Trends Genet 16:83–87

Provost P, Dishart D, Doucet J, Frendewey D, Samuelsson B, Radmark O (2002) Ribonuclease activity and RNA binding of recombinant human Dicer. EMBO J 21:5864–5874

Puigmulé M, Fàbrega A, Yeste M, Bonet S, Pinart E (2011) Study of the proacrosin-acrosin system in epididymal, ejaculated and in vitro capacitated boar spermatozoa. Reprod Fertil Dev 23:837–845

Pusateri AE, Rothschild MF, Warner CM, Ford SP (1990) Changes in morphology, cell number, cell size and cellular estrogen content of individual littermate pig conceptuses on days 9 to 13 of gestation. J Anim Sci 68:3727–3735

Rankin TL, Coleman JS, Epifano O, Hoodbhoy T, Turner SG, Castle PE, Lee E, Gore-Langton R, Dean J (2003) Fertility and taxon-specific sperm binding persist after replacement of mouse "sperm receptors" with human homologs. Dev Cell 5:33–43

Rankin TL, Tong ZB, Castle PE, Lee E, Gore-Langton R, Nelson LM, Dean J (1998) Human ZP3 restores fertility in Zp3 null mice without affecting order-specific sperm binding. Development 125:2415–2424

Rath D, Töpfer-Petersen E, Michelmann HW, Schwartz P, Ebeling S (2005) Zona pellucida characteristics and sperm binding patterns of in vivo and in vitro produced porcine oocytes inseminated with differently prepared spermatozoa. Theriogenology 63:352–362

Rath D, Töpfer-Petersen E, Michelmann HW, Schwartz P, Von Witzendorff D, Ebeling S, Ekhlasi-Hundrieser M, Piehler E, Petrunkina A, Romar R (2006) Structural, biochemical and functional aspects of sperm-oocyte interactions in pigs. Soc Reprod Fertil Suppl 62:317–330

Rawe Y, Olmedo B, Nodar N, Doncel D, Acosta A, Vitullo D (2000) Cytoskeletal organization defects and abortive activation in human oocytes after IVF and ICSI failure. Mol Hum Reprod 6:510–516

Rawe VY, Días ES, Abdelmassih R, Wojcik C, Morales P, Sutovsky P, Chemes HE (2008) The role of sperm proteasomes during sperm aster formation and early zygote development: implications for fertilization failure in humans. Hum Reprod 23:573–580

Razdan P, Mwanza A, Kindahl H, Hultén F, Einarsson S (2001) Effects of post-ovulatory food deprivation on ova transport, hormonal profiles and metabolic changes in sows. Acta Vet Scand 42:45–55

Razdan P, Mwanza A, Kindahl H, Rodríguez-Martínez H, Hultén F, Einarsson S (2002) Effect of repeated ACTH-stimulation on early embryonic development and hormonal profiles in sows. Anim Reprod Sci 70:127–137

Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G (2000) The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature 403:901–906

Richards JS, Russell DL, Ochsner S, Espey LL (2002) Ovulation: new dimensions and new regulators of the inflammatory-like response. Annu Rev Physiol 64:69–92

Rivkin E, Kierszenbaum AL, Gil M, Tres LL (2009) Rnf19a, a ubiquitin protein ligase, and Psmc3, a component of the 26S proteasome, tether to the acrosome membranes and the head-tail coupling apparatus during rat spermatid development. Dev Dyn 238:1851–1861

Rosano G, Caille AM, Gallardo-Ríos M, Munuce MJ (2007) D-Mannose-binding sites are putative sperm determinants of human oocyte recognition and fertilization. Reprod Biomed Online 15:182–190

Rodríguez CI, Stewart CL (2007) Disruption of the ubiquitin ligase HERC4 causes defects in spermatozoon maturation and impaired fertility. Dev Biol 312:501–508

Rodríguez-Martínez H, Einarsson S, Larsson B (1982) Spontaneous motility of the oviduct in the anaesthetized pig. J Reprod Fertil 66:615–624

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 10:491–497

Rodríguez-Martínez H, Tienthai P, Suzuki K, Funahashi H, Ekwall H, Johannisson A (2001) Involvement of oviduct in sperm capacitation and oocyte development in pigs. Reproduction Suppl 58:129–145

Ron-el R, Nachum H, Herman A, Golan A, Caspi E, Soifer Y (1991) Delayed fertilization and poor embryonic development associated with impaired semen quality. Fertil Steril 55:338–344

Rosales O, Opazo C, Días ES, Villegas JV, Sanchez R, Morales P (2011) Proteasome activity and proteasome subunit transcripts in human spermatozoa separated by a discontinuous percoll gradient. Andrologia 43:106–113

Sakai N, Sawada MT, Sawada H (2004) Non-traditional roles of ubiquitin-proteasome system in fertilization and gametogenesis. Int J Biochem Cell Biol 36:776–784

Salicioni AM, Platt MD, Wertheimer EV, Arcelay E, Allaire A, Sosnik J, Visconti PE (2007) Signalling pathways involved in sperm capacitation. Soc Reprod Fertil Suppl 65:245–259

Samour J, Moore HD, Smith CA (1986) Avian spermatozoa penetrate zona-free hamster oocytes in vitro. J Exp Zool 239:295–298

Sánchez R, Deppe M, Schulz M, Bravo P, Villegas J, Morales P, Risopatron J (2011) Participation of the sperm proteasome during in vitro fertilisation and the acrosome reaction in cattle. Andrologia 43:114–120

Santos P, Chaveiro A, Simoes N, Moreira da Silva F (2008) Bovine oocyte quality in relation to ultrastructural characteristics of zona pellucida, polyspermic penetration and developmental competence. Reprod Domest Anim 43:685–689

Sanz L, Calvete JJ, Mann K, Gabius HJ, Töpfer-Petersen E (1993) Isolation and biochemical of heparin-binding proteins from boar seminal plasma: a dual role for spermadhesins in fertilization. Mol Reprod Dev 35:37–43

Sartini BL, Berger T (2000) Identification of homologous binding proteins in porcine and bovine gametes. Mol Reprod Dev 55:446–451

Saunders CM, Larman MG, Parrington J, Cox LJ, Royse J, Blayney LM, Swann K, Lai FA (2002) PLCζ: a sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. Development 129:3533–3544

Saunders CM, Swann K, Lai FA (2007) PLCzeta, a sperm-specific PLC and its potential role in fertilization. Biochem Soc Symp 74:23–36

Sawada H, Sakai N, Abe Y, Tanaka E, Takahashi Y, Fujino J, Kodama E, Takizawa S, Yokosawa H (2002) Extracellular ubiquitination and proteasome-mediated degradation of the ascidian sperm receptor. Proc Natl Acad Sci USA 99:1223–1228

Schatten G (1994) The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. Dev Biol 165:299–335

Schatten G, Simerly C, Palmer DK, Margolis RL, Maul G, Andrews BS, Schatten H (1988) Kinetochore appearance during meiosis, fertilization and mitosis in mouse oocytes and zygotes. Chromosoma 96:341–352

Schuffner AA, Bastiaan HS, Duran HE, Lin ZY, Morshedi M, Franken DR, Oehninger S (2002) Zona pellucida-induced acrosome reaction in human sperm: dependency on activation of pertussis toxin-sensitive G_i protein and extracellular calcium, and priming effect of progesterone and follicular fluid. Mol Hum Reprod 8:722–727

Schwartz M, Vissing J (2002) Paternal inheritance of mitochondrial DNA. N Engl J Med 347:576–580

Seppälä M, Taylor N, Koistinen H, Koistinen R, Milgrom E (2002) Glycodelin: a major lipocalin protein of the reproductive axis with diverse actions in cell recognition and differentiation. Endocr Rev 23:401–430

Sette C, Bevilacqua A, Bianchini A, Mangia F, Geremia R, Rossi P (1997) Parthenogenetic activation of mouse eggs by microinjection of a truncated c-kit tyrosine kinase present in spermatozoa. Development 124:2267–2274

Sette C, Paronetto MP, Barchi M, Bevilacqua A, Geremia R, Rossi P (2002) Tr-kit-induced resumption of the cell cycle in mouse eggs requires activation of a Src-like kinase. EMBO J 21:5386–5395

Shadel S, Clayton A (1997) Mitochondrial DNA maintenance in vertebrates. Annu Rev Biochem 66:409–435

Sheeman B, Carvalho P, Sagot I, Geiser J, Kho D, Hoyt MA, Pellman D (2003) Determinants of S. cerevisiae dynein localization and activation: implications for the mechanism of spindle positioning. Curr Biol 13:364–372

Shimada A, Kikuchi K, Noguchi J, Akama K, Nakano M, Kaneko H (2000) Protamine dissociation before decondensation of sperm nuclei during in vitro fertilization of pig oocytes. J Reprod Fertil 120:247–256

Shur BD (1994) Glycobiology. The beginning of a sweet tale. Curr Biol 4:996–999

Shur BD, Ensslin MA, Rodeheffer C. (2004) SED1 function during mammalian sperm-egg adhesion. Curr Opin Cell Biol 16: 477-485

Short RV (1998) Difference between a testis and an ovary. J Exp Zool 281:359–361

Signorelli J, Días ES, Morales P (2012) Kinases, phosphatases and proteases during sperm capacitation. Cell Tissue Res 349:765–782

Silva PF, Gadella BM (2006) Detection of damage in mammalian sperm cells. Theriogenology 65:958–978

Singleton S, Zalensky A, Doncel GF, Morshedi M, Zalenskaya IA (2007) Testis/sperm-specific histone 2B in the sperm of donors and subfertile patients: variability and relation to chromatin packaging. Hum Reprod 22:743–750

Sinowatz F, Kölle S, Töpfer-Petersen E (2001) Biosynthesis and expression of zona pellcucida glycoproteins in mammals. Cell Tissues Organs 168:24–35

Sinowatz F, Wessa E, Neumüller C, Palma G (2003) On the species specificity of sperm binding and sperm penetration of the zona pellucida. Reprod Domest Anim 38:141–146

Snell J, White M (1996) The molecules of mammalian fertilization. Cell 85:629–637

Snook RR, Hosken DJ, Karr TL (2011) The biology and evolution of polyspermy: insights from cellular and functional studies of sperm and centrosomal behavior in the fertilized egg. Reproduction 142:779–792

Soler AJ, Poulin N, Fernández-Santos MR, Cognie Y, Esteso MC, Garde JJ, Mermillod P (2008) Heterologous in vitro fertility evaluation of cryopreserved Iberian red deer epididymal spermatozoa with zona-intact sheep oocytes and its relationship with the characteristics of thawed spermatozoa. Reprod Domest Anim 43:293–298

Spargo SC, Hope RM (2003) Evolution and nomenclature of the zona pellucida gene family. Biol Reprod 68:358–362

Stein KK, Primafoff P, Myles D (2004) Sperm-egg fusion: events at the plasma membrane. J Cell Sci 117:6269–6274

Suarez SS (2008) Control of hyperactivation in sperm. Hum Reprod Update 14:647–657

Sudhof TC, Rothman JE (2009) Membrane fusion: grappling with SNARE and SM proteins. Science 323:474–477

Sutovsky P (2009) Sperm-egg adhesion and fusion in mammals. Expert Rev Mol Med 11:e11

Sutovsky P (2011) Sperm proteasome and fertilization. Reproduction 142:1–14

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 56:1503–1512

Sutovsky P, Moreno R, Ramalho-Santos J, Dominko T, Simerly C, Schatten G (1999) Ubiquitin tag for sperm mitochondria. Nature 402:371–372

Sutovsky P, Moreno RD, Ramalho-Santos J, Dominko T, Simerly C, Schatten G (2000) Ubiquitinated sperm mitochondria, selective proteolysis, and the regulation of mitochondrial inheritance in mammalian embryos. Biol Reprod 63:582–590

Sutovsky P, Moreno R, Ramalho-Santos J, Dominko T, Thompson WE, Schatten G (2001a) A putative, ubiquitin-dependent mechanism for the recognition and elimination of defective spermatozoa in the mammalian epididymis. J Cell Sci 114:1665–1675

Sutovsky P, Terada Y, Schatten G (2001b) Ubiquitin-based sperm assay for the diagnosis of male factor infertility. Hum Reprod 16:250–258

Sutovsky P, Neuber E, Schatten G (2002) Ubiquitin-dependent sperm quality control mechanism recognizes spermatozoa with DNA defects as revealed by dual ubiquitin-TUNEL assay. Mol Reprod Dev 61:406–413

Sutovsky P, Manandhar G, McCauley TC, Caamano JN, Sutovsky M, Thompson WE, Day BN (2004a) Proteasomal interference prevents zona pellucida penetration and fertilization in mammals. Biol Reprod 71:1625–1637

Sutovsky P, Hauser R, Sutovsky M (2004b) Increased levels of sperm ubiquitin correlate with semen quality in men from an andrology laboratory clinic population. Hum Reprod 19:628–638

Sutton A, Jungnickel K, Ward J, Harris C, Florman M (2006) Functional characterization of PKDREJ, a male germ cell-restricted polycystin. J Cell Physiol 209:493–500

Swain JE, Pool TB (2008) ART failure: oocyte contributions to unsuccessful fertilization. Hum Reprod Update 14:431–446

Swann K, Larman G, Saunders M, Lai A (2004) The cytosolic sperm factor that triggers Ca^{2+} oscillations and egg activation in mammals is a novel phospholipase C: PLCzeta. Reproduction 127:431–439

Tabara H, Yigit E, Siomi H, Mello CC (2002) The dsRNA binding protein RDE-4 interacts with RDE-1, DCR-1, and a DExH-box helicase to direct RNAi in *C. elegans*. Cell 109:861–871

Takasaki S, Mori E, Mori T (1999) Structures of sugar chains included in mammalian zona pellucida glycoproteins and their potential roles in sperm-egg interaction. Biochim Biophys Acta 1473:206–215

Talbot P, Geiske C, Knoll M (1999) Oocyte pickup by the mammalian oviduct. Mol Biol Cell 10:5–8

Talmor-Cohen A, Tomashov-Matar R, Eliyahu E, Shapiro R, Shalgi R (2004) Are Src family kinases involved in cell cycle resumption in rat eggs? Reproduction 127:455–463

Tamburrino L, Marchiani S, Montoya M, Elia Marino F, Natali I, Cambi M, Forti G, Baldi E, Muratori M (2012) Mechanisms and clinical correlates of sperm DNA damage. Asian J Androl 14:24–31

Tapia S, Rojas M, Morales P, Ramírez MA, Díaz ES (2011) The laminin-induced acrosome reaction in human sperm is mediated by Src kinases and the proteasome. Biol Reprod 85:357–366

Terada Y, Nakamura S, Simerly C, Hewitson L, Murakami T, Yaegashi N, Okamura K, Schatten G (2004) Centrosomal function assessment in human sperm using heterologous ICSI with rabbit eggs: a new male factor infertility assay. Mol Reprod Dev 67:360–365

Tesarik J (1998) Sperm-induced calcium oscillations. Oscillin-reopening the hunting season. Mol Hum Reprod 4:1007–1012

Tesarik J (2005) Paternal effects on cell division in the human preimplantation embryo. Reprod Biomed Online 10:370–375

Tesarik J, Mendoza C (1999) In vitro fertilization by intracytoplasmic sperm injection. BioEssays 21:791–801

Tesarik J, Greco E, Mendoza C (2004) Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum Reprod 19:611–615

Thompson WE, Ramalho-Santos J, Sutovsky P (2003) Ubiquitination of prohibitin in mammalian sperm mitochondria: possible roles in the regulation of mitochondrial inheritance and sperm quality control. Biol Reprod 69:254–260

Tipler CP, Hutchon SP, Hendil K, Tanaka K, Fishel S, Mayer RJ (1997) Purification and characterization of 26S proteasomes from human and mouse spermatozoa. Mol Hum Reprod 3:1053–1060

Töpfer-Petersen E (1999) Carbohydrate-based interactions on the route of a spermatozoon to fertilization. Hum Reprod Update 5:314–329

Töpfer-Petersen E, Henschen A (1987) Acrosin shows zona and fucose- binding, novel properties for a serine proteinase. FEBS Lett 226:38–42

Töpfer-Petersen E, Henschen A (1988) Zona pellucida-binding and fucose-binding of boar sperm acrosin is not correlated to proteolytic activity. Biol Chem Hoppe Seyler 369:69–76

Töpfer-Petersen E, Friess AE, Nguyen H, Schill WB (1985) Evidence for a fucose-bindng protein in boar spermatozoa. Histochemistry 83:139–145

Töpfer-Petersen E, Steinberger M, Von Eschenbach CE, Zucker A (1990a) Zona pellucida-binding of boar sperm acrosin is associated with the N-terminal peptide of the acrosin B-chain (heavy chain). FEBS Lett 265:51–54

Töpfer-Petersen E, Calvete JJ, Schäfer W, Henschen A (1990b) Complete localization of disulfide bridges and glycosylation sites in boar sperm acrosin. FEBS Lett 275:139–142

Töpfer-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 30:217–224

Töpfer-Petersen E, Ekhlasi-Hundrieser M, Tsolova M (2008) Glycobiology of fertilization in the pig. Int J Dev Biol 52:717–736

Toshimori K, Saxena DK, Tanii I, Yoshinaga K (1998) An MN9 antigenic molecule, equatorin, is required for successful sperm-oocyte fusion in mice. Biol Reprod 59:22–29

Tranter R, Read JA, Jones R, Brady RL (2000) Effector sites in the three-dimensional structure of mammalian sperm β-acrosin. Structure 8:1179–1188

Tsai PS, De Vries KJ, De Boer-Brouwer M, Garcia-Gil N, Van Gestel RA, Colenbrander B, Gadella BM, Van Haeften T (2007) Syntaxin and VAMP association with lipid rafts depends on cholesterol depletion in capacitating sperm cells. Mol Membr Biol 24:313–324

Tsai PS, Garcia-Gil N, van Haeften T, Gadella BM (2010) How pig sperm prepares to fertilize: stable acrosome docking to the plasma membrane. PLoS ONE 5:e11204

Tyler JP, Pryor JP, Collins WP (1981) Heterologous ovum penetration by human spermatozoa. J Reprod Fertil 63:499–508

Vadnais ML, Foster DN, Roberts KP (2008) Molecular cloning and expression of the CRISP family of proteins in the boar. Biol Reprod 79:1129–1134

Van Blerkom J (2000) Intrafollicular influences on human oocyte developmental competence: perifollicular vascularity, oocyte metabolism and mitochondrial function. Hum Reprod 15(Suppl 2):173–188

Van der Heijden GW, Dieker JW, Derijck AA, Muller S, Berden JH, Braat DD, van der Vlag J, de Boer P (2005) Asymmetry in histone H3 variants and lysine methylation between paternal and maternal chromatin of the early mouse zygote. Mech Dev 122:1008–1022

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 13:445–454

Verhage M (2009) Organelle docking: R-SNAREs are late. Proc Natl Acad Sci USA 106:19745–19746

Verhage M, Toonen RF (2007) Regulated exocytosis: merging ideas on fusing membranes. Curr Opin Cell Biol 19:402–408

Vjugina U, Evans JP (2008) New insights into the molecular basis of mammalian sperm-egg membrane interactions. Front Biosci 13:462–476

Vo LH, Hedrick JL (2000) Independent and hetero-oligomeric-dependent sperm binding to egg envelope glycoprotein ZPC of *Xenopus laevis*. Biol Reprod 62:766–774

Voges D, Zwickl P, Baumeister W (1999) The 26S proteasome: a molecular machine designed for controlled proteolysis. Annu Rev Biochem 68:1015–1068

Von Witzendorff D, Ekhlasi-Hundrieser M, Dostàlovà Z, Resch M, Rath D, Michelmann HW, Töpfer-Petersen E (2005) Analysis of N-linked glycans of porcine zona pellucida glycoprotein, ZPA by MALDITOF MS: a contribution to understanding zona pellucida structure. Glycobiology 15:475–488

Vrljic M, Strop P, Ernst JA, Sutton RB, Chu S, Brunger AT (2010) Molecular mechanism of the synaptotagmin-SNARE interaction in Ca2 + -triggered vesicle fusion. Nat Struct Mol Biol 17:325–331

Wagner A, Ekhlasi-Hundrieser M, Hettel C, Petrunkina A, Waberski D, Nimtz M, Töpfer-Petersen E (2002) Carbohydrate-based interactions of oviductal sperm reservoir formation-studies in the pig. Mol Reprod Dev 61:249–257

Ward WS (1993) Deoxyribonucleic acid loop domain tertiary structure in mammalian spermatozoa. Biol Reprod 48:1193–1201

Ward WS, Coffey DS (1991) DNA packaging and organization in mammalian spermatozoa: comparison with somatic cells. Biol Reprod 44:569–574

Wassarman PM (1995) Mammalian fertilization: egg and sperm (glyco)proteins that support gamete adhesion. Am J Reprod Immunol 33:253–258

Wassarman PM (1999) Mammalian fertilization: molecular aspect of gamete adhesion exocytosis, and fusion. Cell 96:175–183

Wassarman PM (2005) Contribution of mouse egg zona pellucida glycoproteins to gamete recognition during fertilization. J Cell Physiol 204:388–391

Wassarman PM, Jovine L, Qi H, Williams Z, Darie C, Litscher ES (2005) Recent aspects of mammalian fertilization research. Mol Cell Endocrinol 234:95–103

Wilkinson KD (1997) Regulation of ubiquitin-dependent processes by deubiquitinating enzymes. FASEB J 11:1245–1256

Witte TS, Schäfer-Somi S (2007) Involvement of cholesterol, calcium and progesterone in the induction of capacitation and acrosome reaction of mammalian spermatozoa. Anim Reprod Sci 102:181–193

Wojcik C, Benchaib M, Lornage J, Czyba JC, Guerin JF (2000) Proteasomes in human spermatozoa. Int J Androl 23:169–177

Wong EY, Tse JY, Yao KM, Tam PC, Yeung WS (2002) VCY2 protein interacts with the HECT domain of ubiquitin-protein ligase E3A. Biochem Biophys Res Commun 296:1104–1111

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 282:12164–12175

Wykes SM, Krawetz SA (2003) The structural organization of sperm chromatin. J Biol Chem 278:29471–29477

Wykes SM, Visscher DW, Krawetz SA (1997) Haploid transcripts persist in mature human spermatozoa. Mol Hum Reprod 3:15–19

Yanagimachi R (1994a) Fertility of mammalian spermatozoa: its development and relativity. Zygote 2:371–372

Yanagimachi R (1994b) Mammalian fertilization. In: Knobil E, Neill JD (eds) The physiology of reproduction, 2nd edn. Raven Press, New York, pp 189–317

Yanagimachi R (1998) Intracytoplasmic sperm injection experiments using the mouse as a model. Hum Reprod 13:87–98

Yang CC, Lin YS, Hsu CC, Wu SC, Lin EC, Cheng WT (2009) Identification and sequencing of remnant messenger RNAs found in domestic swine (*Sus scrofa*) fresh ejaculated spermatozoa. Anim Reprod Sci 113:143–155

Yeste M, Flores E, Estrada E, Bonet S, Rigau T, Rodríguez-Gil JE (2012) Reduced glutathione and procaine hydrochloride protect the nucleoprotein structure of boar spermatozoa during

freezing/thawing through stabilisation of disulphide bonds. Reprod Fertil Dev, http://dx.doi.org/10.1071/RD12230 (in press)

Yi YJ, Manandhar G, Sutovsky M, Li R, Jonáková 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 77:780–793

Yi YJ, Manandhar G, Sutovsky M, Jonáková V, Park CS, Sutovsky P (2010a) Inhibition of 19S proteasomal regulatory complex subunit PSMD8 increases polyspermy during porcine fertilization in vitro. J Reprod Immunol 84:154–163

Yi YJ, Manandhar G, Sutovsky M, Zimmerman SW, Jonáková 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 341:325–340

Yi YJ, Zimmerman SW, Manandhar G, Odhiambo JF, Kennedy C, Jonáková V, Maňásková-Postlerová P, Sutovsky M, Park CS, Sutovsky P (2012) Ubiquitin-activating enzyme (UBA1) is required for sperm capacitation, acrosomal exocytosis and sperm–egg coat penetration during porcine fertilization. Int J Androl 35:196–210

Yokota N, Sawada H (2007) Sperm proteasomes are responsible for the acrosome reaction and sperm penetration of the vitelline envelope during fertilization of the sea urchin Pseudocentrotus depressus. Dev Biol 308:222–231

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 260:57–63

Yonezawa N, Amari S, Takahashi K, Ikeda K, Imai FL, Kanai S, Kikuchi K, Nakano M (2005) Participation of the non-reducing terminal β-galactosyl residues of the neutral N-linked carbohydrate chains of porcine zona pellucida glycoproteins in sperm-egg binding. Mol Reprod Dev 70:222–227

Yoon SY, Jellerette T, Salicioni AM, Lee HC, Yoo MS, Coward K, Parrington J, Grow D, Cibelli JB, Visconti PE, Mager J, Fissore RA (2008) Human sperm devoid of PLC, zeta-1 fail to induce Ca^{2+} release and are unable to initiate the first step of embryo development. J Clin Invest 118:3671–3681

Yunes R, Michaut M, Tomes C, Mayorga LS (2000) Rab3A triggers the acrosome reaction in permeabilized human spermatozoa. Biol Reprod 62:1084–1089

Yurewicz EC, Sacco AG, Ghupta SK, Xu N, Gage DA (1998) Heterooligomerisation dependent binding of pig oocyte zona pellucida glycoproteins ZPB and ZPC to boar sperm membrane vesicles. J Biol Chem 273:7488–7494

Zalenskaya A, Bradbury M, Zalensky O (2000) Chromatin structure of telomere domain in human sperm. Biochem Biophys Res Commun 279:213–218

Zalensky A, Zalenskaya I (2007) Organization of chromosomes in spermatozoa: an additional layer of epigenetic information? Biochem Soc Trans 35:609–611

Zelezna B, Cechova D, Henschen A (1989) Isolation of boar sperm acrosin peptide released during conversion of α-form to β-form. Biol Chem Hoppe Seyler 370:323–327

Zhao XM, Song XX, Kawai Y, Niwa K (2002) Penetration in vitro of zona-free pig oocytes by homologous and heterologous spermatozoa. Theriogenology 58:995–1006

Zhao Y, Li Q, Yao C, Wang Z, Zhou Y, Wang Y, Liu L, Wang Y, Wang L, Qiao Z (2006) Characterization and quantification of mRNAtranscripts in ejaculated spermatozoa of fertilemenby serial analysis of gene expression. Hum Reprod 21:1583–1590

Zhu H, Zhou ZM, Huo R, Huang XY, Lu L, Lin M, Wang LR, Zhou YD, Li JM, Sha JH (2004) Identification and characteristics of a novel E1 like gene nUBE1L in human testis. Acta Biochim Biophys Sin (Shanghai) 36:227–234

Zimmerman SW, Sutovsky P (2009) The sperm proteasome during sperm capacitation and fertilization. J Reprod Immunol 83:19–25

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:e17256