Mammalian Zona Pellucida Glycoproteins

Subjects: Biochemistry & Molecular Biology

Contributor: Pavla Postlerova

Zona pellucida (ZP) plays an important role in the oocyte lifespan providing mechanical protection and defense against polyspermic fertilization by directly modulating sperm function.

Keywords: zona pellucida ; glycoproteins ; mammals ; gamete interaction

1. Introduction

Mammalian fertilization is a species-specific event that involves a series of interactions between sperm protein molecules and zona pellucida (ZP) glycoproteins of the oocyte. The initial gamete interaction, also known as the primary binding of the spermatozoa to the ZP of the oocytes, is facilitated by the complementary sperm and zona surface molecules.

To gain the ability to bind to the ZP of an oocyte, spermatozoa undergo a sequence of post-testicular maturation events resulting in changes in the sperm protein composition, especially those localized to the sperm plasma membrane. Ejaculated spermatozoa have a fully differentiated morphology with a myriad of different protein molecules present on their surface [1][2][3]. During sperm transit through the female reproductive tract, the protein composition of the sperm plasma membrane changes dramatically, adapting spermatozoa to survival in the uterine environment [4] with the final step of capacitation leading to exposure of the receptors on the sperm surface responsible for ZP binding [5][6]. The sperm surface proteins are complementary to the oligosaccharide chains that decorate the ZP of the oocyte. Spermatozoa bind the ZP carbohydrate moieties via their membrane protein receptors resulting in, for most, part species-specific gamete recognition (reviewed by Clark [7]).

The differences in ZP carbohydrate moieties and sperm surface proteins are considered the main factor in the species specificity of sperm-ZP recognition and binding. While the concept of strict species-specificity applies to mice [8] and humans [9], this does not hold true for domestic animals such as pigs and cattle [10][11][12].

The initial interaction between the spermatozoa and oocyte takes place at the level of ZP. Therefore, receptors on the surface of capacitated spermatozoa are key to the fertilization process. The species-specificity of the sperm-ZP interaction can be ensured on the one hand by the presence of a certain receptor and, on the other hand, by a particular glycosylation pattern of the ZP.

2. Zona Pellucida Glycoproteins

Zona pellucida (ZP) plays an important role in the oocyte lifespan providing mechanical protection [13] and defense against polyspermic fertilization by directly modulating sperm function [14][15]. The mammalian ZP is composed of three to four glycoproteins most commonly designated ZP1, ZP2, ZP3, and ZP4, with inter-species differences addressed below (Table 1). Four mammalian ZP glycoproteins are the products of three genes: *ZPA*, *ZPB*, and *ZPC* [16]. Phylogenic studies revealed that ZP2, encoded by *ZPA* and ZP3, coded *ZPC* is common in all the mammalian species so far investigated; meanwhile, ZP1 and ZP4 are products of the common progenitor *ZPB* gene, a duplication event that occurred during the evolution of the amniotes [17][18], see Table 1. Some authors differentiate *ZPB* paralogues into (*ZP1*/*ZPB1*) coding ZP1 and (*ZPB*/*ZPB2*) coding ZP4 [19]. In newer literature, genes encoding four ZP glycoproteins are termed *ZP1-4* to avoid nomenclature confusion [20], which is in accordance with HUGO nomenclature. From here on, we will use the HUGO nomenclature of ZP glycoproteins. Depending on species, either ZP1 or ZP4, or both are present. Synthesis of ZP glycoproteins was attributed to the growing oocyte in mice [13] whereas, in humans and other species (e.g., domestic pig, cattle, rabbit, and dog), granulosa/cumulus oophorus cells contribute to the synthesis and deposition of ZP as well [20]. ZP glycoproteins are conserved throughout the mammalian species sharing a high amino acid sequence identity between individual ZP1-4 homologs.

Table 1. Summary of zona pellucida (ZP) glycoproteins in different mammalian species. ZP protein AA sequences were taken from the UniProtKB database, uniport.org and the sequence alignment was performed using BLAST® software blast.ncbi.nlm.nih.gov/BlastAlign.cgi.

Mammalian Species	ZP Gene	ZP Protein	Molecular Weight (kDa)	Homology with				References
				Mouse	Human	Porcine	Bovine	
Mouse	*ZP1 (ZPB1)*	ZP1	200 (dimer)	-	68%	-	-	[21][22][23][24][25]
	ZP2 (ZPA)	ZP2	120	-	58%	55%	57%	
	ZP3 (ZPC)	ZP3	83	-	68%	66%	64%	
	ZP4 (ZPB/ZPB2)	not expressed	-	-	-	-	-	
Human	*ZP1 (ZPB1)*	ZP1	65	68%	-	-	-	[26][27][28][29]
	ZP2 (ZPA)	ZP2	120	58%	-	64%	67%	
	ZP3 (ZPC)	ZP3	58	68%	-	74%	72%	
	ZP4 (ZPB/ZPB2)	ZP4	65	-	-	68%	69%	
Porcine	*ZP1 (ZPB1)*	not expressed	-	-	-	-	-	[30][31][32][33][34][35][36][37][38]
	ZP2 (ZPA)	ZP2/PZPL	90	55%	64%	-	78%	
	ZP3 (ZPC)	ZP3/ZP3-β	55	66%	74%	-	84%	
	ZP4 (ZPB/ZPB2)	ZP4/ZP-α	55	-	68%	-	76%	
Bovine	*ZP1 (ZPB1)*	not expressed	-	-		-	-	[39][40][41]
	ZP2 (ZPA)	ZP2	76	57%	67%	78%	-	
	ZP3 (ZPC)	ZP3	47	64%	72%	84%	-	
	ZP4 (ZPB/ZPB2)	ZP4	68	-	69%	76%	-	

2.1. ZP Glycoproteins in the Mouse Model

In the best-studied animal model, a mouse, ZP is composed of three glycoproteins: mZP1 (200 kDa, dimer), mZP2 (120 kDa, monomer), and mZP3 (83 kDa, monomer) [23]. mZP1 shares the domain architecture with ZP4 that is expressed in other mammals such as human, pig, bovine, and dog (see relevant references in Fahrenkamp et al. [15]), and their genes are considered paralogous [22][24]. *ZP4* (*ZPB*/*ZPB1*) is a pseudogene in mice and therefore not expressed. The basic structural elements of murine ZP are repeating fibers formed by a pair of glycoproteins mZP2 and mZP3 (heterodimers) linked together by a dimer of mZP1 glycoprotein [23][25]. The estimated molar ratio of ZP1/ZP2/ZP3 is 1:4:4 [41]. Functional ZP glycoproteins consist of domains, including the signal peptide, ZP “domain” modules responsible for ZP polymerization, the consensus protease cleavage site, and a GPI-anchor [21]. ZP1 and ZP4, on top of the aforementioned domains, also contain the trefoil domain.

2.2. ZP Glycoproteins in the Humans

Contrary to the mouse, humans express all four ZP genes resulting in four ZP glycoproteins termed hZP1, hZP2, hZP3, and hZP4 [28]. hZP1 and hZP4 are paralogs, and their amino acids sequences share 47% identity. Human hZP1, hZP2, hZP3 amino acid sequences show 68%, 58%, and 68% homology with mouse mZP1, mZP2, and mZP3 glycoproteins, respectively (https://blast.ncbi.nlm.nih.gov/). Comparing the amino acid sequences between human ZP2, ZP3, and ZP4 and porcine glycoprotein homologs, there is 64%, 74%, and 68% sequence identity [27]. SDS-PAGE analysis revealed hZP2 as a 120 kDa band, hZP3 as a 58 kDa band, and the 65 kDa band contained both hZP4 and hZP1 [26]. The assembly of ZP glycoproteins into a matrix has been studied in a mouse model and was discussed above. It was reported recently that a frameshift mutation in the human *ZP1* gene caused primary female infertility as a result of the absence of the ZP2-ZP3 filament crosslinking and the inability to form a stable ZP matrix [29].

2.3. ZP Glycoproteins in the Pig Model

Porcine ZP is composed of three ZP glycoproteins, pZP2-4. *ZP1* is a pseudogene in the pig, and therefore ZP1 is not expressed. SDS-PAGE analysis revealed pZP2 (*ZPA*/PZPL) as a 90 kDa band that splits under reducing conditions into two smaller bands of 65 kDa and 25 kDa [31][32][33][36]. Both pZP3 (*ZPC*/ZP3-β) and pZP4 (*ZPB*/ZP3-α) migrated as 55 kDa protein bands [38]. pZP3 and pZP4 make about 80% of total porcine ZP glycoproteins [30][32]. The pZP2 and mouse mZP2 homologs share a 55% amino acid sequence identity, while pZP3 and mouse mZP3 share a 66% amino acid sequence identity (https://blast.ncbi.nlm.nih.gov/). The pZP4 was implied to have the same function as the mZP1 paralogue [35][37]. It was later predicted that similar to mice, pig ZP filaments are formed by pZP3 and pZP4 heterodimers, crosslinked with pZP2 based on their estimated molar ratio of 1:6:6 (pZP2:pZP3:pZP4) [34].

2.4. ZP Glycoproteins in the Bovine Model

Similarly, as in the pig, three glycoproteins were identified in bovine ZP, termed bZP2 (*ZPA*), bZP3 (*ZPC*), and bZP4 (*ZPB*) [39], and the *ZP1* is a pseudogene. Furthermore, SDS-PAGE analysis of deglycosylated ZP glycoproteins showed that bZP2 migrated at 76 kDa, bZP3 at 47 kDa, and bZP4 at 68 kDa. Similar to the domestic pig, bZP2, under reducing conditions, split into two smaller bands of 63 kDa and 21 kDa [39]. Amino acid sequences of bovine ZP glycoproteins show high similarity to their pig counterparts, i.e., 78%, 84%, and 76% for ZP2, ZP3, and ZP4, respectively (https://blast.ncbi.nlm.nih.gov/). bZP4 was found to have the strongest sperm-binding activity among the components, while bZP3 had about one-sixth that of bZP4 [40]. The estimated molar ratio of bZP2/bZP3/bZP4 in bovine is 1:2:1 [41].

3. Carbohydrate Structure and Glycosylation of ZP Glycoproteins

All ZP glycoproteins are highly heterogeneous due to post-translational modification by glycosylation of serine/threonine (O-linked glycosylation) and asparagine (N-linked glycosylation) residues, which are mostly sulfated and sialylated. Structures of the glycan portion of ZP proteins have been characterized by in-depth and reviewed in-detail [7][42][43][44]. The carbohydrate content of ZP is estimated at 15–54% *(w/w)*, and its heterogeneity is reflected as sets of trailing spots on 2-DE electrophoretograms. The glycosylation sites of individual oligosaccharides and cognate carbohydrate-binding proteins are involved in the sperm-ZP binding in many species in a species-specific manner [45][46][47].

In the 1990s, the sugar structures of ZP have deducted from lectin-binding studies. Some conserved carbohydrate structures were found in almost all species investigated, such as mannose and N-acetylglucosamine that are common components of the core of N-linked oligosaccharides [48][49][50]. On the other hand, β-galactose was found in mouse and bovine but not in porcine ZP [51]. Terminal N-acetylgalactosamine and α-galactose residues constitute minor components in murine and bovine ZP, whereas porcine N-glycans are lacking these N-acetylgalactosamine and α-galactose residues [45]. Human ZP also contains mannosyl, N-acetylglucosaminyl, and β-galactosyl residues and βGal-(1–3)GalNAc sugar sequences that are exposed only after removing terminal sialic acid residues [49]. Sialyl-Lewis^x structures are uniquely present in human ZP [52].

3.1. Glycosylation in the Mouse Model

The basic structure of N-linked oligosaccharides (complex-type) in mice is similar to porcine ZP [53][54]. Also, bovine N-linked glycans show practically the same structure as their murine and porcine homologs [55]. Species-specific differences are most obvious in the structure of neutral N-linked carbohydrates [56]. In the pig and cattle, neutral oligosaccharides represent about 25% of the total carbohydrate portion, whereas in the mouse they are present at less than 5%. Variations in other species are in di-, tri-, tetra-antennary chains, sulfation, and sialylation. The number of sulfated lactosamine repeats and degree of sialylation in both N- and O-glycans are the causes of enormous heterogeneity of the ZP glycoproteins in all species [45][57].

Mouse ZP contains N-linked oligosaccharides with high-mannose and complex-type structures (such as di-, tri-, and tetra-antennary branched N-glycans) as well as O-linked oligosaccharides [58]. The mZP oligosaccharides are complexes containing fucose residues [51] and form mainly acidic tri- and tetra-antennary chains containing lower amounts of sulfates and sialic acids in the N-linked chains [51][58][59]. N-glycans are fucosylated and elongated by non-branched N-acetyllactosamine chains. Acidic glycans contain sialic acids at the nonreducing end or sulfates in the C-6 position of the N-acetylglucosamine residues of the lactosamine repeats [45][55]. N-acetyl-D-lactosamine (LacNAc), sialized LacNAc, and terminal N-acetylglucosamine (GlcNAc) were found as terminal units of N-linked oligosaccharides. In O-linked oligosaccharides, the majority were core-2 type O-N-acetylgalactosamine [58], with mainly sialic acid found as a terminal unit [60]. Mouse ZP glycoproteins are composed of 16 potential N-glycosylation sites, with 15 of them being actually occupied [61]. The mZP1 contains four, mZP2 six and mZP3 six N-glycosylation sites. Mouse ZP has many additional potential O-glycosylation sites that are less utilized. There are as many as 82 potential O-linkage sites in mZP1, 84 in mZP2 and 58 in mZP3 [61]. mZP1 is more O-glycosylated than N-glycosylated, whereas mZP2 is predominantly N-glycosylated, with low or no O-glycosylation, and mZP3 is more N-glycosylated with relatively low O-glycosylation [61].

3.2. Glycosylation in the Humans

The glycan profile of human ZP is unique compared to other mammalian species [62]. Even though the lectin studies initially indicated a high content of D-mannose in human ZP [49], ultrasensitive mass spectrometric analyses revealed the absence of the high-mannose type chain [63]. Human N-linked ZP glycans have bi-, tri, and tetra- antennary fucosylated complex-type structures, and are terminated with sialyl-Lewis^x (SLEX) and sialyl-Lewis^x-Lewis^x. O-linked glycans in human ZP are core-1, and -2 type O-N-acetylgalactosamine, but only core-2 type possess terminal SLEX [63]. Sialyl-Lewis^x sequences on O- and N-glycans are important for sperm-oocyte binding. Human sperm-egg binding depends primarily on the recognition of terminal SLEX that is expressed on about 85% of all N-glycans [52][63]. SLEX was found to be expressed more densely in the outer region of ZP than in the inner layer [52]. In human hZP2, hZP3 and hZP4 glycoproteins, the N-linked glycosylation is predominant. Although N-linked glycosylation occupies 37%, 27% and 18% of the molecular mass of hZP2, hZP3, and hZP4, respectively, the percentages of O-linked glycosylation are only 8% for hZP2, 9% for hZP3 and hZP4 seems to be without O-linked glycosylation [26].

3.3. Glycosylation in the Pig Model

As in the other species previously discussed, porcine ZP glycoproteins are highly heterogeneous due to varied amounts of sialylated and/or sulfated poly-N-acetyllactosamine [64]. N-linked chains are composed of neutral and acidic chains at a molar ratio of about 1:3 that constitute di-, tri- and tetra-antennary N-glycans complex with α-fucosyl residue in the innermost N-acetylglucosamine [65]. The main neutral N-glycans of porcine ZP glycoproteins belong to the di-antennary fucosylated glycans containing N-acetyllactosamine chains [45] and are implicated in sperm-oocyte recognition [34]. Highly sulfated acidic N-glycans consist of poly-N-acetyllactosamine sequences of different lengths, sulfated at the C-6 position of GlcNAc [54]. In contrast to the N-glycans of ZP in cyclic sows, a lower degree of glycan sulfation in the prepuberal zona pellucida has been reported [66]. N-linked glycans contain fucose residues but no high mannose chains [51]. The largest ZP glycoprotein in the pig, pZP2 has six, pZP3 three, and pZP4 five potential N-glycosylation sites. In addition, pZP4 contains three and pZP3 six potential O-glycosylation sites [37]. Sugar-mapping of pZP4 glycopeptides has revealed that all three potential N-glycosylation sites Asn203, Asn220, and Asn333 of the mature pZP4 carry neutral bi-antennary N-glycans, whereas only Asn220 is also glycosylated with neutral tri- and tetra-antennary chains. At least one disulfide bond between the neighboring cysteine residues Cys224 and Cys243 has been localized in the N-terminal part of pZP4 [45][57]. O-linked glycans comprise 9 neutral and 26 acidic unbranched chains of core-1 O-N-acetylgalactosamine type [67]. Similar to N-linked glycans, the O-linked glycans are sulfated at the C-6 position of GlcNAc and/or sialylated. The N-glycosylation of porcine ZP glycoproteins, which occurs during meiotic maturation is crucial in sperm-ZP interactions, including sperm binding to ZP and induction of AE in ZP-bound sperm [68]. Nevertheless, the binding and induction of AE in boar spermatozoa do not require the participation of terminal Galα1-3Gal sequences [69].

3.4. Glycosylation in the Bovine Model

Thus far, only N-linked glycans have been reported in bovine ZP [51]. Bovine ZP glycoproteins are contained with 23% of neutral carbohydrate chains, of which the main constituent is high-mannose-type oligosaccharide structure, and 77% of acidic chains with a high content of sialic acid as opposed to the high content of sulfation that is typical for the pig [59]. Bovine ZP glycans are therefore more similar to those of the mouse than the pig and human. The acidic N-linked glycans of bovine ZP contain di-, tri- and tetra-antennary sialylated complex-type structures with a fucose residue at their reducing ends [51]. Molecular cloning of bovine ZP revealed five potential N-glycosylation sites in bZP4 (*ZPB)*, three potential glycosylation sites in bZP3 (*ZPC*), and four potential N-glycosylation sites in bZP2 (*ZPA*) [40][70]. Further studies confirmed bZP2 being N-glycosylated at Asn83, Asn191, and Asn527 [71], and bZP2 being N-glycosylated at Asn124, and Asn146 [70].

References

1. Brewis, I.A.; Gadella, B.M. Sperm surface proteomics: From protein lists to biological function. Mol. Hum. Reprod. 201 0, 16, 68–79.
2. Byrne, K.; Leahy, T.; McCulloch, R.; Colgrave, M.L.; Holland, M.K. Comprehensive mapping of the bull sperm surface p roteome. Proteomics 2012, 12, 3559–3579.
3. Kasvandik, S.; Sillaste, G.; Velthut-Meikas, A.; Mikelsaar, A.V.; Hallap, T.; Padrik, P.; Tenson, T.; Jaakma, U.; Koks, S.; Salumets, A. Bovine sperm plasma membrane proteomics through biotinylation and subcellular enrichment. Proteomics 2015, 15, 1906–1920.
4. Rickard, J.P.; de Graaf, S.P. Sperm surface changes and their consequences for sperm transit through the female repr oductive tract. Theriogenology 2020.
5. Yanagimachi, R. Mammalian fertilization. In The Physiology of Reproduction; Knobil, E., Neill, J.D., Eds.; Raven Press: New York, NY, USA, 1994; Volume 1, pp. 189–317.
6. Gadella, B.M.; Boerke, A. An update on post-ejaculatory remodeling of the sperm surface before mammalian fertilizatio n. Theriogenology 2016, 85, 113–124.
7. Clark, G.F. A role for carbohydrate recognition in mammalian sperm-egg binding. Biochem. Biophys. Res. Commun. 20 14, 450, 1195–1203.
8. Wassarman, P.M. Contribution of mouse egg zona pellucida glycoproteins to gamete recognition during fertilization. J. Cell. Physiol. 2005, 204, 388–391.
9. Rosano, G.; Caille, A.M.; Gallardo-Rios, M.; Munuce, M.J. D-Mannose-binding sites are putative sperm determinants of human oocyte recognition and fertilization. Reprod. Biomed. Online 2007, 15, 182–190.
10. Sinowatz, F.; Wessa, E.; Neumüller, C.; Palma, G. On the species specificity of sperm binding and sperm penetration of the zona pellucida. Reprod. Domest. Anim. 2003, 38, 141–146.
11. Topfer-Petersen, E.; Ekhlasi-Hundrieser, M.; Tsolova, M. Glycobiology of fertilization in the pig. Int. J. Dev. Biol. 2008, 5 2, 717–736.
12. Takahashi, K.; Kikuchi, K.; Uchida, Y.; Kanai-Kitayama, S.; Suzuki, R.; Sato, R.; Toma, K.; Geshi, M.; Akagi, S.; Nakan o, M.; et al. Binding of Sperm to the Zona Pellucida Mediated by Sperm Carbohydrate-Binding Proteins is not Species-Specific in Vitro between Pigs and Cattle. Biomolecules 2013, 3, 85–107.
13. Wassarman, P.M.; Litscher, E.S. The mouse egg’s zona pellucida. In Current Topics in Developmental Biology; Litscher, E.S., Wassarman, P.M., Eds.; Academic Press: Cambridge, MA, USA, 2018; Volume 130, pp. 331–356.
14. Evans, J.P. Preventing polyspermy in mammalian eggs-Contributions of the membrane block and other mechanisms. Mol. Reprod. Dev. 2020, 87, 341–349.
15. Fahrenkamp, E.; Algarra, B.; Jovine, L. Mammalian egg coat modifications and the block to polyspermy. Mol. Reprod. Dev. 2020, 87, 326–340.
16. Harris, J.D.; Hibler, D.W.; Fontenot, G.K.; Hsu, K.T.; Yurewicz, E.C.; Sacco, A.G. Cloning and characterization of zona p ellucida genes and cDNAs from a variety of mammalian species: The ZPA, ZPB and ZPC gene families. DNA Seq. 199 4, 4, 361–393.
17. Spargo, S.C.; Hope, R.M. Evolution and nomenclature of the zona pellucida gene family. Biol. Reprod. 2003, 68, 358–3 62.
18. Goudet, G.; Mugnier, S.; Callebaut, I.; Monget, P. Phylogenetic analysis and identification of pseudogenes reveal a pro gressive loss of zona pellucida genes during evolution of vertebrates. Biol. Reprod. 2008, 78, 796–806.
19. Smith, J.; Paton, I.R.; Hughes, D.C.; Burt, D.W. Isolation and mapping the chicken zona pellucida genes: An insight into the evolution of orthologous genes in different species. Mol. Reprod. Dev. 2005, 70, 133–145.
20. Gupta, S.K. The human egg’s zona pellucida. In Current Topics in Developmental Biology; Litscher, E.S., Wassarman, P.M., Eds.; Academic Press: Cambridge, MA, USA, 2018; Volume 130, pp. 379–411.
21. Bokhove, M.; Jovine, L. Structure of Zona Pellucida Module Proteins. Curr. Top. Dev. Biol. 2018, 130, 413–442.
22. Conner, S.J.; Lefievre, L.; Hughes, D.C.; Barratt, C.L. Cracking the egg: Increased complexity in the zona pellucida. Hu m. Reprod. 2005, 20, 1148–1152.
23. Greve, J.M.; Wassarman, P.M. Mouse egg extracellular coat is a matrix of interconnected filaments possessing a struct ural repeat. J. Mol. Biol. 1985, 181, 253–264.
24. Hughes, D.C.; Barratt, C.L. Identification of the true human orthologue of the mouse Zp1 gene: Evidence for greater co mplexity in the mammalian zona pellucida? Biochim. Biophys. Acta (BBA) Gene Struct. Expr. 1999, 1447, 303–306.

25. Wassarman, P.M. Zona pellucida glycoproteins. Annu. Rev. Biochem. 1988, 57, 415–442.

26. Chiu, P.C.; Wong, B.S.; Lee, C.L.; Pang, R.T.; Lee, K.F.; Sumitro, S.B.; Gupta, S.K.; Yeung, W.S. Native human zona p ellucida glycoproteins: Purification and binding properties. Hum. Reprod. 2008, 23, 1385–1393.

27. Gupta, S.K.; Bhandari, B.; Shrestha, A.; Biswal, B.K.; Palaniappan, C.; Malhotra, S.S.; Gupta, N. Mammalian zona pell ucida glycoproteins: Structure and function during fertilization. Cell Tissue Res. 2012, 349, 665–678.

28. Lefièvre, L.; Conner, S.J.; Salpekar, A.; Olufowobi, O.; Ashton, P.; Pavlovic, B.; Lenton, W.; Afnan, M.; Brewis, I.A.; Mon k, M.; et al. Four zona pellucida glycoproteins are expressed in the human. Hum. Reprod. 2004, 19, 1580–1586.

29. Nishimura, K.; Dioguardi, E.; Nishio, S.; Villa, A.; Han, L.; Matsuda, T.; Jovine, L. Molecular basis of egg coat cross-linki ng sheds light on ZP1-associated female infertility. Nat. Commun. 2019, 10, 3086.

30. Hasegawa, A.; Koyama, K.; Okazaki, Y.; Sugimoto, M.; Isojima, S. Amino acid sequence of a porcine zona pellucida gly coprotein ZP4 determined by peptide mapping and cDNA cloning. J. Reprod. Fertil. 1994, 100, 245–255.

31. Hedrick, J.L.; Wardrip, N.J. Isolation of the zona pellucida and purification of its glycoprotein families from pig oocytes. Anal. Biochem. 1986, 157, 63–70.

32. Hedrick, J.L.; Wardrip, N.J. On the macromolecular composition of the zona pellucida from porcine oocytes. Dev. Biol. 1987, 121, 478–488.

33. Nakano, M.; Hatanaka, Y.; Sawai, T.; Kobayashi, N.; Tobita, T. Fractionation of glycoproteins from porcine zonae pelluci dae into three families by high-performance liquid chromatography. Biochem. Int. 1987, 14, 417–423.

34. Nakano, M.; Yonezawa, N.; Hatanaka, Y.; Noguchi, S. Structure and function of the N-linked carbohydrate chains of pig zona pellucida glycoproteins. J. Reprod. Fertil. Suppl. 1996, 50, 25–34.

35. Topfer-Petersen, E.; Mann, K.; Calvete, J.J. Identification of porcine oocyte 55 kDa alpha and beta proteins within the z ona pellucida glycoprotein families indicates that oocyte sperm receptor activity is associated with different zone pelluci da proteins in different mammalian species. Biol. Chem. 1993, 374, 411–417.

36. Wardrip, N.J.; Hedrick, J.L. Pig zona pellucida 25K and 65K glycoproteins are derived from Hydrolysis and reduction of the 90K family. J. Cell Biol. 1985, 101, 378a.

37. Yurewicz, E.C.; Pack, B.A.; Sacco, A.G. Isolation, composition, and biological activity of sugar chains of porcine oocyte zona pellucida 55K glycoproteins. Mol. Reprod. Dev. 1991, 30, 126–134.

38. Yurewicz, E.C.; Sacco, A.G.; Subramanian, M.G. Structural characterization of the Mr = 55,000 antigen (ZP3) of porcin e oocyte zona pellucida. Purification and characterization of alpha- and beta-glycoproteins following digestion of lactos aminoglycan with endo-beta-galactosidase. J. Biol. Chem. 1987, 262, 564–571.

39. Noguchi, S.; Yonezawa, N.; Katsumata, T.; Hashizume, K.; Kuwayama, M.; Hamano, S.; Watanabe, S.; Nakano, M. Ch aracterization of the zona pellucida glycoproteins from bovine ovarian and fertilized eggs. Biochim. Biophys. Acta (BBA) Gene Struct. Expr. 1994, 1201, 7–14.

40. Yonezawa, N.; Fukui, N.; Kuno, M.; Shinoda, M.; Goko, S.; Mitsui, S.; Nakano, M. Molecular cloning of bovine zona pell ucida glycoproteins ZPA and ZPB and analysis for sperm-binding component of the zona. Eur. J. Biochem. 2001, 268, 3587–3594.

41. Yonezawa, N.; Kanai, S.; Nakano, M. Structural significance of N-glycans of the zona pellucida on species-selective rec ognition of spermatozoa between pig and cattle. Soc. Reprod. Fertil. Suppl. 2007, 63, 217–228.

42. Abou-Haila, A.; Bendahmane, M.; Tulsiani, D.R. Significance of egg's zona pellucida glycoproteins in sperm-egg interac tion and fertilization. Minerva Ginecol. 2014, 66, 409–419.

43. Yonezawa, N. Posttranslational modifications of zona pellucida proteins. Adv. Exp. Med. Biol. 2014, 759, 111–140.

44. Yonezawa, N. Involvement of Carbohydrate Residues of the Zona Pellucida in In Vitro Sperm Recognition in Pigs and Cattle. In Sexual Reproduction in Animals and Plants; Sawada, H., Inoue, N., Iwano, M., Eds.; Springer: Tokyo, Japan, 2014; pp. 409–415.

45. Topfer-Petersen, E. Carbohydrate-based interactions on the route of a spermatozoon to fertilization. Hum. Reprod. Upd ate 1999, 5, 314–329.

46. Wassarman, P.M.; Litscher, E.S. Towards the molecular basis of sperm and egg interaction during mammalian fertilizati on. Cells Tissues Organs 2001, 168, 36–45.

47. Hoodbhoy, T.; Dean, J. Insights into the molecular basis of sperm-egg recognition in mammals. Reproduction 2004, 12 7, 417–422.

48. Shalgi, R.; Maymon, R.; Bar-Shira, B.; Amihai, D.; Skutelsky, E. Distribution of lectin receptors sites in the zona pellucid a of follicular and ovulated rat oocytes. Mol. Reprod. Dev. 1991, 29, 365–372.

49. Maymon, B.B.; Maymon, R.; Ben-Nun, I.; Ghetler, Y.; Shalgi, R.; Skutelsky, E. Distribution of carbohydrates in the zona pellucida of human oocytes. J. Reprod. Fertil. 1994, 102, 81–86.
50. Parillo, F.; Stradaioli, G.; Dall'Aglio, C.; Verini-Supplizi, A. Characterization of the complex carbohydrates in the zona pellucida of mammalian oocytes using lectin histochemistry. Vet. Res. Commun. 1996, 20, 225–236.
51. Katsumata, T.; Noguchi, S.; Yonezawa, N.; Tanokura, M.; Nakano, M. Structural characterization of the N-linked carbohydrate chains of the zona pellucida glycoproteins from bovine ovarian and fertilized eggs. Eur. J. Biochem. 1996, 240, 448–453.
52. Lucas, H.; Bercegeay, S.; Le Pendu, J.; Jean, M.; Mirallie, S.; Barriere, P. A fucose-containing epitope potentially involved in gamete interaction on the human zona pellucida. Hum. Reprod. 1994, 9, 1532–1538.
53. Noguchi, S.; Hatanaka, Y.; Tobita, T.; Nakano, M. Structural analysis of the N-linked carbohydrate chains of the 55-kDa glycoprotein family (PZP3) from porcine zona pellucida. Eur. J. Biochem. 1992, 204, 1089–1100.
54. Noguchi, S.; Nakano, M. Structure of the acidic N-linked carbohydrate chains of the 55-kDa glycoprotein family (PZP3) from porcine zona pellucida. Eur. J. Biochem. 1992, 209, 883–894.
55. Mori, E.; Hedrick, J.L.; Wardrip, N.J.; Mori, T.; Takasaki, S. Occurrence of reducing terminal N-acetylglucosamine 3-sulfate and fucosylated outer chains in acidic N-glycans of porcine zona pellucida glycoproteins. Glycoconj. J. 1998, 15, 447–456.
56. Töpfer-Petersen, E.; Petrounkina, A.M.; Ekhlasi-Hundrieser, M. Oocyte-sperm interactions. Anim. Reprod. Sci. 2000, 60–61.
57. Kudo, K.; Yonezawa, N.; Katsumata, T.; Aoki, H.; Nakano, M. Localization of carbohydrate chains of pig sperm ligand in the glycoprotein ZPB of egg zona pellucida. Eur. J. Biochem. 1998, 252, 492–499.
58. Easton, R.L.; Patankar, M.S.; Lattanzio, F.A.; Leaven, T.H.; Morris, H.R.; Clark, G.F.; Dell, A. Structural analysis of murine zona pellucida glycans. Evidence for the expression of core 2-type O-glycans and the Sd(a) antigen. J. Biol. Chem. 2000, 275, 7731–7742.
59. Noguchi, S.; Nakano, M. Structural characterization of the N-linked carbohydrate chains from mouse zona pellucida glycoproteins ZP2 and ZP3. Biochim. Biophys. Acta (BBA) Gene Struct. Expr. 1993, 1158, 217–226.
60. Dell, A.; Chalabi, S.; Easton, R.L.; Haslam, S.M.; Sutton-Smith, M.; Patankar, M.S.; Lattanzio, F.; Panico, M.; Morris, H.R.; Clark, G.F. Murine and human zona pellucida 3 derived from mouse eggs express identical O-glycans. Proc. Natl. Acad. Sci. USA 2003, 100, 15631–15636.
61. Boja, E.S.; Hoodbhoy, T.; Fales, H.M.; Dean, J. Structural characterization of native mouse zona pellucida proteins using mass spectrometry. J. Biol. Chem. 2003, 278, 34189–34202.
62. Jimenez-Movilla, M.; Aviles, M.; Gomez-Torres, M.J.; Fernandez-Colom, P.J.; Castells, M.T.; de Juan, J.; Romeu, A.; Ballesta, J. Carbohydrate analysis of the zona pellucida and cortical granules of human oocytes by means of ultrastructural cytochemistry. Hum. Reprod. 2004, 19, 1842–1855.
63. Pang, P.C.; Chiu, P.C.; Lee, C.L.; Chang, L.Y.; Panico, M.; Morris, H.R.; Haslam, S.M.; Khoo, K.H.; Clark, G.F.; Yeung, W.S.; et al. Human sperm binding is mediated by the sialyl-Lewis(x) oligosaccharide on the zona pellucida. Science 2011, 333, 1761–1764.
64. Yonezawa, N.; Mitsui, S.; Kudo, K.; Nakano, M. Identification of an N-glycosylated region of pig zona pellucida glycoprotein ZPB that is involved in sperm binding. Eur. J. Biochem. 1997, 248, 86–92.
65. Nakano, M.; Yonezawa, N. Localization of sperm ligand carbohydrate chains in pig zona pellucida glycoproteins. Cells Tissues Organs 2001, 168, 65–75.
66. Von Witzendorff, D.; Maass, K.; Pich, A.; Ebeling, S.; Kölle, S.; Kochel, C.; Ekhlasi-Hundrieser, M.; Geyer, H.; Geyer, R.; Töpfer-Petersen, E. Characterization of the acidic N-linked glycans of the zona pellucida of prepuberal pigs by a mass spectrometric approach. Carbohydr. Res. 2009, 344, 1541–1549.
67. Hokke, C.H.; Damm, J.B.; Penninkhof, B.; Aitken, R.J.; Kamerling, J.P.; Vliegenthart, J.F. Structure of the O-linked carbohydrate chains of porcine zona pellucida glycoproteins. Eur. J. Biochem. 1994, 221, 491–512.
68. Lay, K.M.; Nakada, T.; Tatemoto, H. Involvement of N-glycosylation of zona glycoproteins during meiotic maturation in sperm-zona pellucida interactions of porcine denuded oocytes. Anim. Sci. J. 2013, 84, 8–14.
69. Clark, G.F.; Zimmerman, S.; Lafrenz, D.E.; Yi, Y.J.; Sutovsky, P. Carbohydrate-mediated binding and induction of acrosomal exocytosis in a boar sperm-somatic cell adhesion model. Biol. Reprod. 2010, 83, 623–634.
70. Suzuki, K.; Tatebe, N.; Kojima, S.; Hamano, A.; Orita, M.; Yonezawa, N. The Hinge Region of Bovine Zona Pellucida Glycoprotein ZP3 Is Involved in the Formation of the Sperm-Binding Active ZP3/ZP4 Complex. Biomolecules 2015, 5, 3339–3353.

71. Ikeda, K.; Yonezawa, N.; Naoi, K.; Katsumata, T.; Hamano, S.; Nakano, M. Localization of N-linked carbohydrate chains in glycoprotein ZPA of the bovine egg zona pellucida. Eur. J. Biochem. 2002, 269, 4257–4266.