



CHANGES OF THE ZONA PELLUCIDA PATTERNS DURING OOCYTE MATURATION, FERTILIZATION AND EMBRYO DEVELOPMENT IN MAMMALS: MINI-REVIEW

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Abstract

The mammalian zona pellucida (ZP) is an extracellular matrix that surrounds immature and mature oocytes and early embryos until the stage of a blastocyst and its implantation. This mini-review summarizes basic information on the ZP and its morphologic and functional changes during *in vitro* oocyte maturation and fertilization and *in vivo* pre-implantation embryo development.

Running title: ZP during maturation, fertilization and embryo development

Keywords: mammals, zona pellucida morphology, ZP glycoproteins, *in vitro* maturation and fertilization, *in vivo* pre-implantation development

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Introduction

The zona pellucida (ZP) is secreted during the growth of mammalian oocytes in secondary ovarian follicles by both the oocytes and the granulosa cells in the form of extracellular patches that further coalesce into a uniform layer [1-3]. Immature and mature oocytes can be collected from the tertiary follicles of animals and humans and used subsequently for *in vitro* maturation and fertilization. During normal fertilization, spermatozoa attack cumulus cells but only one of them penetrates the ZP barrier and interacts with the oolemma of a mature oocyte, leading to the formation of female and male pronuclei, their syngamy, zygote formation and embryonic cleavage [4].

The ZP mediates several events such as sperm activation and induction of the acrosome reaction, including relative species-specificity [5-7], prevents the dispersion of blastomeres during development, facilitates passage of the embryo through the oviduct, and protects it during early stages of development [8].

Some of the aspects from which the ZP has so far been studied are presented in **table 1**.

This mini-review presents the basic knowledge of zona pellucida morphology and function and their changes during *in vitro* oocyte maturation and fertilization and *in vivo* pre-implantation embryo development.

ZP morphology

There are differences in ZP thickness among mammalian oocytes [9], varying from ~16 µm in cows to ~18 µm in pigs to ~12 µm in humans and ~8 µm in mice [10-12]. Also the ZP structure varies. In the cow, pig, mouse and hamster, two structures are distinguished. One is a net-like structure formed by different layers of string-like material perforated by

numerous pores. The other has a totally different appearance with a smooth and compact structure without any pores [13]. When the ZP porous surfaces were compared, the smallest pores were found in the cow, the largest pores occurred in the mouse, rabbit and cat [14]. While in cow oocytes, the diameter of pores was 182 nm, in pig and rat oocytes, the diameters were only about 50–100 nm [9,15]. In general, the shape of ZP pores varies both among and within mammalian species, with many having an elliptical rather than a circular appearance.

Ultrastructural studies of mammalian oocytes have shown that cumulus cells are anchored to the ZP surface by a mesh-like network of fibres and granules [16].

ZP glycoproteins

Depending on the mammalian species, the ZP consists of three or four kinds of glycoproteins. While the ZPs of mice and cows are made of only three glycoproteins (ZPA/ZP2, ZPC/ZP3, ZP1 and ZPA/ZP2, ZPB/ZP4 and ZPC/ZP3, respectively), human and rat ZPs are composed of four proteins (ZPA/ZP2, ZPB/ZP4, ZPC/ZP3 and ZP1), as shown in **figure 1** [17].

Pig, rabbit, and dog ZPs contain three glycoproteins (ZPA/ZP2, ZPB/ZP4, and ZPC/ZP3; [17,18]. Horse, cat, monkey and hamster ZPs consist of four glycoproteins, (ZPA/ZP2, ZPB/ZP4, ZPC/ZP3, and ZP1 [19,20].

In the mouse, it is generally believed that ZP3 is the primary sperm receptor that binds to the anterior head of capacitated spermatozoa and induces the acrosome reaction onset [21,22]. After the acrosome reaction downset, only the ZP2 glycoprotein binds to acrosome-reacted spermatozoa and acts as a secondary sperm receptor [21-23].

TABLE 1 Characteristics of zona pellucida in terms of surface, glycoproteins, sperm-oocyte binding proteins and pre-implantation embryo development

ASPECTS	METHODS	REFERENCES
Ultrastructure of the surface	ICSI, confocal laser scanning microscopy, SEM, TEM	1, 2, 3, 8, 9, 11, 12, 13, 14, 16, 17, 24, 30, 31, 32, 33, 35, 40, 41, 43, 50, 51, 52, 53, 67
Glycoproteins	X-ray crystallography, mass spectrometry, spectroscopy, 2 D-electrophoresis, SDSPAGE, SEM, TEM	5, 18, 19, 20, 25, 26, 27, 28, 29, 42
Sperm-oocyte binding proteins	phylogenetic analysis, gene targeting, autoradiographic analysis, Western blotting, mass spectrometry, stereochemistry, 3D-structural analysis, SEM, TEM	4, 6, 7, 10, 21, 22, 23, 34, 36, 37, 38, 39, 44, 45, 46, 47, 48, 49
Pre-implantation embryo development	inverted microscope, stereomicroscope, immunofluorescence, TEM, time-lapse videomicrography and computer imaging	15, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 68, 69, 70

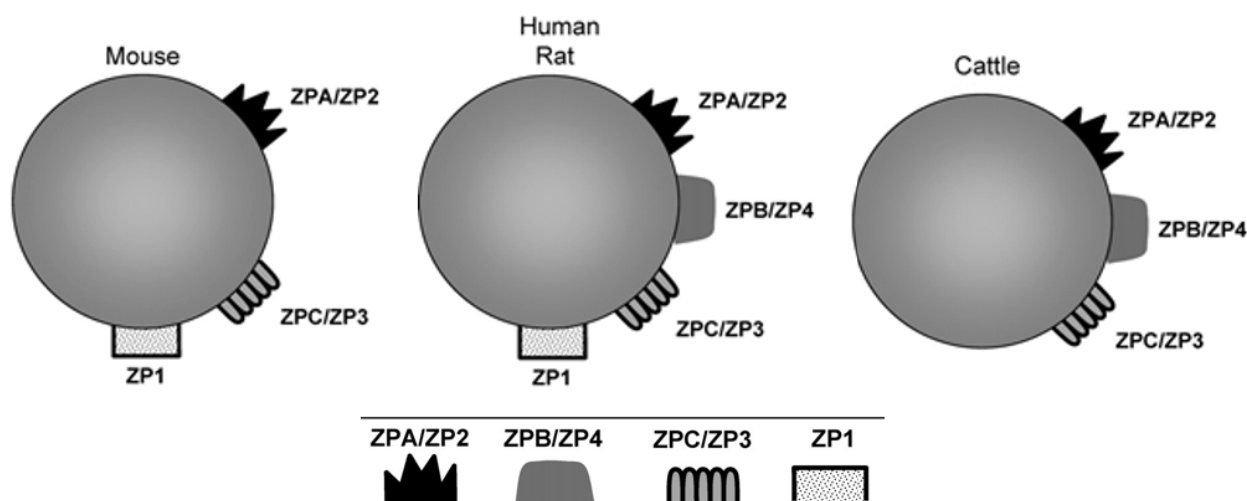


FIGURE 1 Composition of ZP glycoproteins in mouse, human, rat and cow oocytes [17]

Mouse ZP1 is postulated to cross-link the filaments formed by ZP2-ZP3 heterodimers and to provide structural integrity and stability to the ZP matrix [21,24].

Ultrastructure of ZP glycoproteins

In all mammalian oocytes, the ZP consists of three or four glycosylated proteins (ZP1 to ZP4), each with a unique polypeptide chain which includes a ZP domain consisting of a linker region and two subdomains (ZP-N and ZP-C) with an Ig-like three-dimensional structure [25,26]. ZP1 to ZP4 proteins have approximately 270 amino acid positions in the ZP domain and eight conserved cysteine residues present as four intramolecular disulfides [26,27]. The ZP-N subdomains of ZP2 and ZP3 proteins are required for an assembly of fibrils during oocyte growth as well as for the fibril cross-linker. In addition, ZP1 protein has a proline-rich N-terminal region that may be responsible for ZP flexibility or elasticity [28,29].

ZP patterns during maturation

The *in vitro* maturation (IVM) is a process during which mammalian oocytes manifest their intrinsic ability to resume meiosis and support the embryo development. This process involves a complex of distinctly linked nuclear and cytoplasmic events.

During IVM, the ZP ultrastructure of mammalian oocytes is changing in terms of its morphology, i.e., in the number, shape and distribution of pores.

Before maturation, the ZP of cow oocytes shows pores of irregular shape and uneven distribution, crevices and projections. After maturation, the ZP network becomes thinner, the number of pores increases and holes seem to be shallower [30].

The ZP surface in cow oocytes has also been described by Suzuki et al. (1994) [31]. These authors have reported that, before maturation, the ZP is characterized by a fine net-like structure with nu-

merous wide meshes and deep holes while, after maturation, it appears to be finer and meshes and holes seem not to be so deep.

During maturation, the ZP surface is rougher, probably due to remnants of cumulus and corona radiata cells the residuals of which produce cytoplasmic processes. The latter cells pass through the zona pellucida and terminate with gap junctions on the vitelline membrane of the oocyte [32]. It is known that, after maturation, most of these processes retract or disintegrate, and channels left by them are closed by ZP matrix [15]. Pores on the outer ZP surface of mature cow oocytes, which probably mark entries of these cell processes, have also been detected by Vanroose et al. (2000) [9].

In pig oocytes, the ZP has a more net-like and porous structure before maturation than after it; the ZP surface of mature oocytes is more compact and has a distinct mesh-like structure [13,33-35].

In the horse, the ZP has a compact mesh-like structure with small pores in both immature and mature oocytes; differences in its structure between horse and pig oocytes are evident [35]. In humans, immature oocytes have compact ZP surfaces with no pores and mature oocytes have net-like, porous surfaces [33,34].

ZP patterns during fertilization

The fertilization process, one of the most carefully regulated interactions between female and male gametes, is an intricate cascade of events. It starts with sperm capacitation and gamete recognition [36]. The fundamental mechanism of gamete recognition seems to be conserved throughout evolution, from marine vertebrates to mammals, in the way that sperm surface molecules interact with oligosaccharide ligands of oocyte envelope glycoproteins [10]. These events are, to a large extent, determined by compatibility between ligand and receptor molecules on the gamete surfaces [37].

Primary binding of spermatozoa to the ZPs of oocytes is one of the many steps necessary for successful fertilization [38]. In most mammals, gamete binding starts with contact of the equatorial segment of a spermatozoon with the outer surface of the ZP. Besides this flat sperm position, many other types of sperm-ZP binding position have been found including that with the tip of the head first [13].

Primary and secondary receptors for an acrosome-intact sperm cell on the ZP are associated with ZP3 and ZP2 glycoproteins, respectively [37]. These authors hypothesize that proacrosin/acrosin is a multifunctional protein of acrosome-reacted spermatozoa. It binds non-enzymically to oocyte ZP glycoproteins and retains a sperm cell on the ZP surface long enough to allow it to penetrate the oocyte [37]. Recognition is followed by sperm-oolemma adhesion, gamete fusion, oocyte penetration and activation, zygote formation and embryo cleavage [36].

The penetration of oocytes by acrosome-reacted spermatozoa is relatively easy due to the ZP structure and its elasticity [7]. It has been described that the lytic action of sperm enzymes after the acrosome reaction is responsible for structural changes in the ZP network and is accompanied by modification of glycoproteins and ZP permeability known as „ZP hardening.” Following fertilization and cortical granule exocytosis, the cleavage of ZP2 modifies the three-dimensional structure of the zona pellucida matrix to prevent penetration by additional sperm [39,31,40,9,41].

Differences in ZP morphology between mature cow oocytes and those fertilized *in vitro* (IVF) have been reported by Suzuki et al. (1994) [31]. These authors have found that, after IVF, oocytes show compact ZP surfaces without pores, hollows or meshes, probably due to fusion of several layers of the network; no meshes have been found on the ZP surface in fertilized cow oocytes [9,31].

It has been verified that changes in ZP morphology during IVM and IVF can be used to assess the efficiency of maturation and fertilization in cow oocytes [30].

The ZP has a vital role in blocking polyspermy during IVF of oocytes [3,42]. A ZP block to polyspermy occurs as a result of the increased calcium level induced in oocytes by spermatozoa and due to exocytosis of cortical granules [43]. This, in turn, leads to a proteolytic cleavage of the ZP2 to ZP2f by metalloprotease ovastacin from cortical granules [44-48].

In cow oocytes, the enzymes located in the ZP, i.e. ZP2 and ZP3 glycoproteins, are converted by sperm receptors to ZP3- α and ZP2, thus inducing oocyte modifications essential to prevent polyspermy [49,50]. Therefore, the number and diameter of pores may become a key component in the mechanism of sperm-ZP binding and oocyte penetration.

The rate of polyspermy after IVF is relatively low for developmentally competent cow oocytes. How-

ever, polyspermy increases in less competent oocytes due to a low number of ZP pores with large diameters [8].

In comparison with cow and horse oocytes, the polyspermy rate after IVF is generally high in pig oocytes. The number of pores on the ZP is lower and their diameter is larger in pig than in horse oocytes. Some differences in sperm-binding and oocyte penetration have also been observed between pig and horse oocytes matured either *in vivo* or *in vitro* [35]. It can be speculated that the high incidence of polyspermic penetration in pig oocytes after an IVF procedure is related to the insufficient maturity of their ZPs due to conditions not optimal for oocyte maturation [35].

The extracellular matrix of cumulus cells is required for mouse spermatozoa. During *in vivo* fertilization, corona radiate cells lose contact with the oocytes, the cytoplasmic processes crossing the ZP retract and the synthesized hyaluronic acid matrix is entangled in pores of the internal oocyte envelope [16,51,52]. The synthesis of a provisional hyaluronic acid matrix provides favourable conditions for the fertilization of mammalian oocytes with high embryo developmental competence [53].

ZP patterns during embryo pre-implantation

The hatching of an embryo from the ZP is a prerequisite for implantation of a blastocyst to the endometrium and for embryo placentation. This process is important for maintaining pregnancy, as documented by an improved pregnancy rate after assisted hatching in cattle and humans [54]. Three mechanisms are known to be involved in blastocyst hatching: 1) mechanical forces exerted on the ZP by blastocyst expansion [55-57]; 2) weakening of the ZP by enzymatic degradation [58-60]; 3) penetration of the ZP by projections of trophectodermal cells [61,62].

There are variations among mammals in the nature of proteinases implicated in dissolution of the ZP, including a trypsin-like enzyme in the mouse [63,64] and a urokinase-type plasminogen activator in the cow [59,65]. Whereas cow blastocysts preferentially hatch from the embryonic pole, in mice [63], humans [66] and hamsters [67], blastocysts preferentially hatch from the pole opposite to the inner cell mass.

It has also been reported that, in the cow, hatching takes place in the uterus [68] and the blastocyst resides free-floating in the uterus for 10 or more days before attaching to the endometrium [69]. In other mammals, blastocysts undergo implantation in the endometrium shortly after hatching [70].

It seems that the orientation of hatching is less critical in species in which the embryo spends a prolonged period free of permanent attachment to the endometrium [69].

Conclusions

The present mini-review deals with the basic characteristics of the mammalian zona pellucida which are related to oocyte-sperm interaction. Morphological and functional changes of the zona pellucida during *in vitro* maturation and fertilization of oocytes and *in vivo* pre-implantation development of embryos in mammals are reported.

Ethical approval

The conducted research is not related to either human or animal use.

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Conflict of interest

The authors declare they have no conflict of interest.

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