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The Oviduct: Functional Genomic and Proteomic Approach

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Contents

The mammalian oviduct is an anatomical part of the female reproductive tract, which plays several important roles in the events related to fertilization and embryo development. This review examines and compares several studies related to the proteomic and transcriptomic profile of the oviduct in different domestic animals. This information could be important for clarifying the role of oviductal factors in different events regulating fertilization and early embryo development, as well as for improving synthetic media for *in vitro* maturation/*in vitro* fertilization/embryo culture techniques (IVM/IVF/EC).

Introduction

The concept of the oviduct as a passive structure involved in the transport of gametes has been substituted by that of a dynamic structure actively involved in several functions. The oviduct, also called the Fallopian tube in primates, is the organ in which fertilization takes place. Moreover, numerous studies have indicated that the oviduct and, especially, oviductal secretions play a key role in aspects related to gamete maturation, sperm capacitation and the development of the preimplantation embryo (Hunter 1998; Avilés et al. 2010).

Anatomically, the oviduct consists of four regions designated infundibulum, ampulla, isthmus and uterine-tubal junction. The mucosa of the oviduct shows primary and secondary folds of different height and orientation with a typical tree branch–like structure of varying degrees of complexity. Interspersed among these mucosal projections is a complex system of crypts, pockets and grooves (Hunter et al. 1991; Yániz et al. 2000). This complex anatomical structure contributes to sperm selection and probably participates in the regulation of the number of sperm that reach the site of fertilization, thus controlling polyspermy and providing different oviduct microenvironments (Hunter 2012). The epithelium is mainly formed by two different cell types: ciliated cells and non-ciliated cells or secretory cells (Fig. 1). The distribution and morphology of both cell types changes during the oestrous cycle, the anatomical region of the oviduct and even the specific region of the mucosa fold (apical or basal regions). Thus, it has been reported that ciliated cells are more abundant along lateral walls and in the apical region of longitudinal folds than in the basal regions among the mucosa folds (Abe 1996; Yániz et al. 2000; Yániz et al. 2006). In pig, a morphometric analysis even showed differences in the epithelial cells between two breeds (Abe and Hoshi 2008). In addition to these anatomical and histological differences, there are species-specific differences in the physiology of the oviduct. For example, in some species, ovulation is restricted to one of the two oviducts. It was previously reported that the concentration of different hormones (e.g. progesterone, prostaglandins) and the gene expression pattern in the ipsilateral oviduct differs from that observed in the contralateral oviduct (Wijayagunawardane et al. 1998; Bauersachs et al. 2003).

The Oviduct Provides the Most Efficient Environment for the Success of Fertilization and Early Embryo Development

For most domestic animals, the fertility rate is generally higher than 50%. If a uniparous female animal is served during the oestrous period by a male with good sperm quality, the pregnancy rate may reach 60–70%. Among animals that deliver more than one offspring, in many cases the pregnancy rate can even exceed 90% (De Kruif 2003). However, in some cases, it is necessary to use assisted reproductive techniques (ARTs) to solve infertility and subfertility problems. For example, ARTs are linked to the protection and saving of species threatened by extinction, research and genetic improvement (Cseh and Solti 2000).

Nowadays, the production and development of embryos until the blastocyst stage in most of mammalian species can be achieved under *in vitro* conditions, with limitations that depend on the species (Table 1 and Data S1). In pig, for example, the developmental competence of *in vitro*-produced embryos is low compared with their *in vivo* counterparts (Kikuchi et al. 1999). An insufficient cytoplasmic ability for the development and polyspermy of *in vitro* matured oocytes and improper culture conditions for IVP embryos are thought to be responsible for this low efficacy (reviewed in Nagai et al. 2006). In cattle, although polyspermy is not a real problem in *in vitro* embryo production, the process is considered inefficient; while maturation and fertilization may appear to proceed normally, the proportion of embryos reaching the transferable stage is rarely over 40% and those that do reach this stage are often compromised in quality and competence. In equine species, IVF and development rates remain low (Hinrichs et al. 2002; Goudet 2011). The technology of *in vitro* oocyte maturation followed by the application of ICSI has been established to achieve fertilization *in vitro*. In this way, the rates of embryo development ranged from 5% to 10% in early studies but can reach 40% in later ones.

As mentioned above, fertilization and early embryonic development occurs in the oviduct. The quality of

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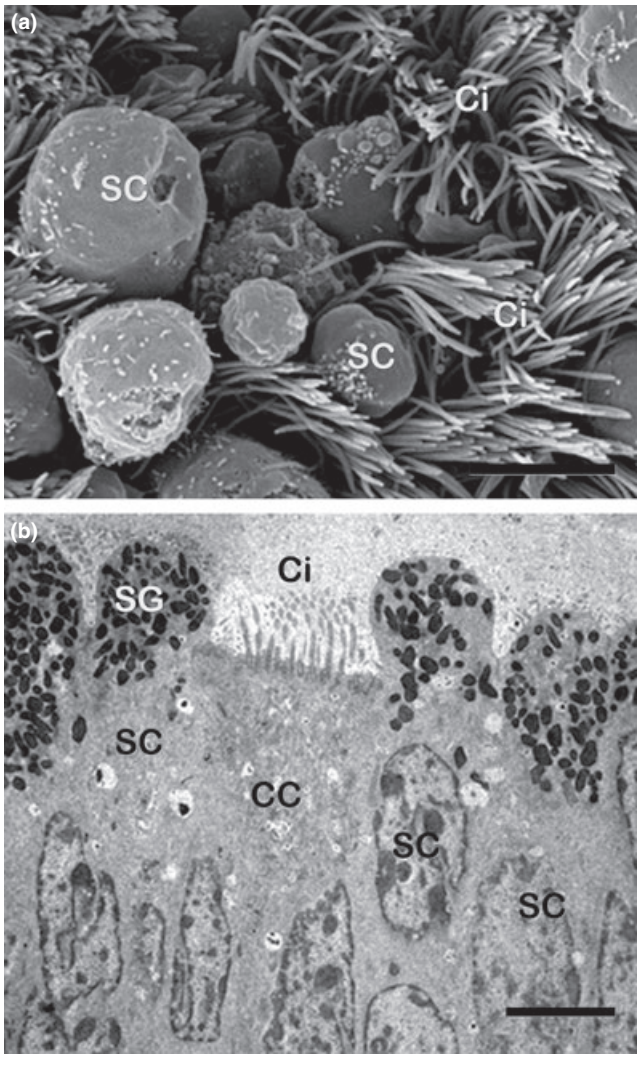

Fig. 1. Epithelial cells of the ampullary-isthmic junction (AIJ) of bovine oviduct. (a) Scanning electron micrographs of the epithelial surface of the ampulla of bovine oviduct in late follicular phase. (b) Electron micrograph of the epon-embedded AIJ. Ciliated cells (CC), secretory cells (SC), secretory granules (SG) and cilia (Ci). Bar: 5 μm

Table 1. Rate of *in vitro* fertilization (IVF), polyspermy, cleavage, morula and blastocyst formation in different domestic animals

Species[a]	*In vitro* fertilization (IVF) (%)	Polyspermy (%)	Cleavage (%)	Morula (%)	Blastocyst (%)
Cat[1]	46–80		30–80	70–97	0–46
Cow[2]	60–92	34–45.6	64–95	60–80	32–65
Dog[3]	10–78	41.2–68	10	0–4	0
Goat[4]	32–37		42–54		10–20.8
Horse[5]	0–84	0–2.4	9–80[b]		4–40[b]
Pig[6]	30–100	32–92.5	20–80		2–40
Rabbit[7]	77–85		64–92		0–85
Sheep[8]		3–21	30–80	50	3.9–57

[a]References used to prepare this table are included in the Supporting Information.
[b]Results obtained after ICSI.

the *in vitro*-produced embryos does not reflect the quality of their *in vivo* counterparts. Results of many studies suggest that culture conditions during *in vitro* embryo production may influence the developmental potential of the early embryo and its quality (Lonergan et al. 2007). Strategies developed to improve embryo development include the use of coculture media with epithelial cells, culture media supplemented with different proteins or growth factors and embryo culture in a foreign oviduct. The trans-species transfer of embryo to oviducts has been used to optimize early embryo development in different species, with the oviduct proving to be the best environment (Gandolfi and Moor 1987; Gutiérrez-Adán et al. 2004).

The Oviduct is Involved in Regulation of Sperm, Oocyte and Embryo Physiology

The efficiency of fertilization is lower *in vitro* than *in vivo* for most species. However, it is unknown whether this is the result of (i) failures in final gamete maturation, (ii) deficient sperm–oocyte interaction or (iii) the lower ability of the recently formed zygote to develop. All these steps, which probably jointly affect the final outcome of *in vitro* procedures, take place in the oviduct under physiological conditions and, consequently, a study of the factors affecting them is of great importance for further advances in the reproductive biology field.

Sperm

The role of the oviduct in male gamete capacitation mediated by binding of the spermatozoa to oviductal epithelial cells has been described in several species (Suarez 1998; Hunter 2012; Goudet 2011). In most cases, bound spermatozoa in the isthmus have been shown to decrease their movement and to prolong their survival, delaying the capacitation process (Suarez 2008; Fazeli et al. 2003). Partial identification of different proteins and carbohydrates involved in sperm binding and release from the oviduct (Suarez 2001; Talevi and Gualtieri 2010; Gualtieri et al. 2010; Talevi et al. 2010), as well as of the relationship between ovulation and the release of capacitated spermatozoa, has also been made (Gualtieri et al. 2005; Suarez 2007). However, a complete description of all the molecular pathways involved in these processes remains under research (Hunter 2012), and better understanding of these pathways will offer new tools for improving *in vitro* reproduction in domestic animals and also in humans.

Other possible roles of the oviduct as regards the male gamete have been related to the selection and guidance of spermatozoa towards the egg (Holt and Fazeli 2010). Moreover, the arrival of spermatozoa within the oviduct regulates gene expression in oviductal epithelial cells (Thomas et al. 1995; Fazeli et al. 2004; Georgiou et al. 2005, 2007). The oviduct may also be involved in a sperm selection process (Rodríguez-Martínez et al. 2005). After mating, where a large number of sperm are deposited in the vagina or uterus, very few are able to reach the site of fertilization. Severely deformed sperm cannot enter the oviduct (Styrna et al. 2002); however, sperm with a normal morphology or with few anomalies and a progressive linear movement can penetrate the uterotubal junction and enter the isthmus (Shalgi et al. 1992; Holt and Van Look 2004; Nakanishi

et al. 2004). Then, sperm with appropriate receptors on their surface may bind to the epithelial cells for a period up to 30 h and form a preovulatory sperm reservoir (Rodríguez-Martínez et al. 2005; Hunter 2012). A seminal plasma protein called, BSP1 (or PDC-109), and annexin present in the apical membrane of the epithelial cells play a key role in this process (reviewed in Hung and Suarez 2010).

It has been reported that the female genital tract has a positive effect on the fertilization potential of spermatozoa that have been genetically altered (Kawano et al. 2010; Turunen et al. 2012). In mouse, Turunen et al. pointed to an 80% decrease in *in vitro* fertilization with sperm that lack CRISP4 compared with the wild type. These data indicated that, even if the physiology of the sperm is seriously compromised, the genetically modified mice were fertile, as wild-type animals, in normal mating. In our opinion, these results provide a new view of the uterine/oviductal contribution to sperm maturation in the genital tract.

The above studies could contribute to the development of sperm treatments with uterine and/or oviductal secretions (or uterine/oviductal tissue explants) to improve the sperm quality of animals with a low seminal quality, or in the case of damaged sperm after cryopreservation. We consider that this finding in mouse is worth investigating in other domestic animals and also in humans.

Oocyte

With regard to the oocyte, the role of the oviduct on its final maturation, especially at the zona pellucida (ZP) level, has not been deeply studied, although it was suggested more than 20 years ago that oviductal glycoproteins may act to enhance the various functions of the ZP (Yang and Yanagimachi 1989). Recently, this role has been partially clarified when OVGP1 and heparin-like glycosaminoglycans from the oviductal fluid were seen to bind to the ZP and make it resilient to enzymatic digestion and to sperm binding and penetration (Coy et al. 2008). This mechanism represents a novel view of the so-called 'ZP hardening', which had been considered until now as a post-fertilization event associated with cortical granule exocytosis (cortical reaction). Now, it is known that the ZP undergoes those maturational changes in the oviduct before the arrival of spermatozoa and that these modifications may be crucial for any further oocyte response to sperm entry. As an example, polyspermy levels in the pig and cow are significantly affected by the contact of the ZP with oviductal secretions and, as a consequence, the final rate of fertilization is modified (Coy et al. 2008). A similar effect of the oviductal fluid on ZP maturation has recently been shown in the sheep and goat (Mondéjar 2011).

Embryo

Finally, it cannot be forgotten that the recently formed zygote remains in the oviduct for a variable period of time, depending on the species but never < 48 h. During this time, oviductal secretions are subjected to important changes derived from hormonal transition from an oestrogen-dependent to a progesterone-dependent environment. As we reported previously, the oviductal fluid protects the embryo against adverse impacts on mtDNA transcription/replication and apoptosis (Lloyd et al. 2009a). Moreover, a number of embryotrophic factors from the oviduct have been described (Lee and Yeung 2006; revised by Avilés et al. 2010), although functional experiments to clarify the specific role of each one and their potential use for improving *in vitro* culture systems remain to be performed. In the following paragraphs the identification and role of some of these factors will be discussed.

Functional Genomic and Proteomic Analysis of the Oviductal Cells and Secretions

Oviductal fluid is a complex fluid formed by different metabolic and macromolecular components from blood plasma and epithelial cell secretions (reviewed in Buhi et al. 2000; Aguilar and Reyley 2005; Georgiou et al. 2005; Leese et al. 2008; Avilés et al. 2010). Most studies of the oviductal fluid have identified one, or a low number of, protein(s) in the oviduct by means of conventional analytical methods (Avilés et al. 2010). Other studies have tried to identify more components using complex technologies that include two dimensional electrophoresis (Gandolfi et al. 1989; Buhi et al. 2000). However, until now, it has been possible to identify only some of the proteins detected in the 2D gel by preparing specific antibodies. Fortunately, thanks to the development of the mass spectrometry instruments and the deciphering of the genome of different species, it is nowadays possible to identify a large number of proteins contained in complex body fluids and to study gene expression patterns in different tissues. Here, we describe the results previously reported in the literature and by our group obtained by using transcriptomic and proteomic analysis.

Transcriptomic Analysis

A transcriptomic analysis of the oviduct has been performed in bovine, human and mouse species (Bauersachs et al. 2003; Fazeli et al. 2004; Bauersachs et al. 2004; Tone et al. 2008; George et al. 2011).

In cattle, the epithelial cells were obtained by scraping the mucosal epithelial layer of the complete oviduct using a glass slide from heifers in oestrous and dioestrous phases (Bauersachs et al. 2004) and in the postovulatory period (Bauersachs et al. 2003). During the postovulatory period, authors found differences for 35 genes when comparing gene expression in the ipsilateral and contralateral oviduct. Twenty-seven genes were up-regulated in the ipsilateral oviduct, and eight were down-regulated (Bauersachs et al. 2003). The comparative analysis of the gene expression between oestrous and dioestrous phase showed that 77 genes were differentially expressed; 37 and 40 genes were up-regulated in the oestrous and dioestrous phases, respectively (Bauersachs et al. 2004). These genes have been related to the immune response, protein secretion and

modification, endocytosis, signalling and the regulation of transcription.

In women, a recent study compared the gene expression profile of epithelial cells of the Fallopian tube between the follicular and luteal phases (George et al. 2011). The authors identified five genes up-regulated and 15 down-regulated in the luteal phase (supplementary file in George et al. 2011). Some of these genes are of potential interest for different aspects related to fertilization and embryo development. For example, mRNA for heparanase was detected in the human oviductal mucosa. In a previous study, our group provide strong evidence to support a role for OVGP1 and heparin in blocking polyspermy (Coy et al. 2008). It was observed that heparin contributes to the stabilization of the OVGP1 effect. Our proteomic analysis also identified the existence of heparanase in the porcine oviductal fluid, agreeing with the results mentioned previously for the human oviduct. Heparin molecules have also been related to the release of bovine sperm bound to the oviductal epithelia (Gualtieri et al. 2010). Therefore, it seems possible that the heparanase present in the oviduct contributes to the regulation of these processes. Future experiments are necessary to confirm this hypothesis.

GPX3 mRNA, a glutathione peroxidase, is another gene differentially expressed in the human oviduct. This enzyme is involved in the redox balance and could make an important contribution to the control of DNA damage that affects gametes and the embryo (Aitken and De Iuliis 2010) and also to the sperm binding to the oviduct through the reduction of SS to SH (Gualtieri et al. 2009).

We have performed a detailed analysis of human specimens from the microarray experiment stored in the Gene Expression Omnibus (GEO) accessible through GEO Series accession number GSE10971 (Tone et al. 2008; Data S2). A total of 5703 genes of the original 54 675 probes present in the microarray were detected. This number of expressed genes is in accordance with a previous study performed in other human tissues using microarray analysis (Su et al. 2004). The list of genes was analysed and classified using the DAVID Bioinformatics Resource 6.7 (DAVID). Genes were classified according to the cellular localization, and genes encoding secreted proteins were also included (Fig. 2). It can be observed that 394 (8%) correspond to genes that codify for plasma membrane proteins and 245 (5%) of the expressed genes correspond to secreted proteins that can be classified into different groups (Fig. 3).

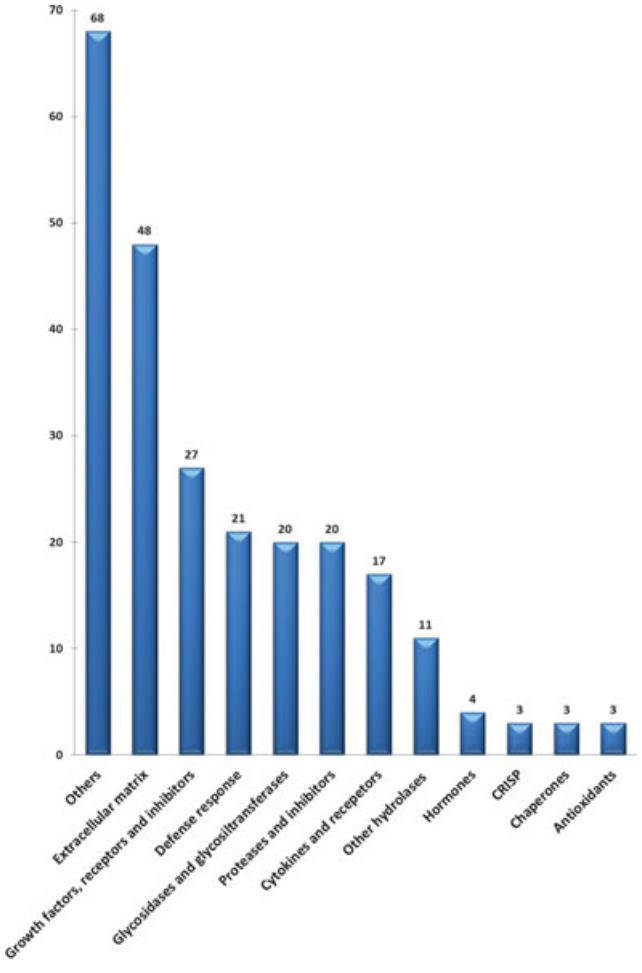

Fig. 3. Functional clustering of genes classified as 'secreted' using the DAVID bioinformatic tool using data from normal Fallopian tube reported in Tone et al. (2008)

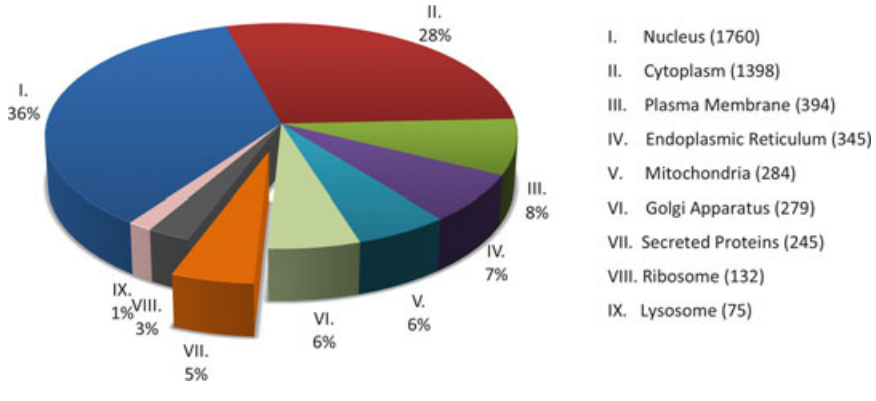

Fig. 2. Gene expression analysis of normal Fallopian tube (GEO: GSE10971). Gene clustering was performed according to the DAVID. The values represent the number and percentage of genes identified

Some of the mRNA detected in this study has previously been identified in the oviductal epithelium in other species (Avilés et al. 2010); however, future studies are needed to confirm these data. The study by Tone et al. (2008) of gene expression analysis was performed using the oviductal mucosa and laser microdissection. Therefore, other cells (fibroblast, endothelial cells, lymphocyte, mast cells, etc.) present in the laminae propia are included in this analysis, and, consequently, some of the genes detected may not correspond to the epithelial cells covering the oviductal lumen. Other techniques like immunocytochemical and *in situ* hybridization analysis can be used to demonstrate the direct relationship between the expressed genes and the oviductal epithelium.

A gene expression study of the porcine oviduct in different phases of the oestrous cycle is currently in progress in our laboratories. In this review, we will provide unpublished information about the gene expression in the oviduct in the preovulatory phase of the cycle. For the analysis, the ampullary-isthmic junction region of the oviduct was selected because fertilization takes place in this region. The hybridization was performed using a microarray of 43 803 probes (Porcine (V2) 4x44K) from Agilent (Agilent Technologies, Madrid, Spain). Further details of the methodology employed is described in Data S2. Then, a total of 2968 genes were detected; this number is low compared with the human samples owing to incomplete annotation of the porcine genome. More than 480 genes are shared between the human and the porcine oviduct. Similarities between the porcine and bovine gene expression are even lower owing to the low number of gene available for comparative purposes (Bauersachs et al. 2007) (Fig. 4). The known genes expressed in the bovine oviduct epithelial cells are reduced owing to the fact that only differentially expressed genes were analysed using a subtracted library. In mice, a comparison of the only available data (125 genes) (Fazeli et al. 2004) showed that 82 of the genes are shared with the human oviduct, 17 are shared with the pig, and only two genes are expressed in the four species studied. In general, these results indicate that some genes are expressed in all four species, suggesting that basic components play a similar function and are evolutionarily conserved. These results are in agreement with functional events observed in a heterologous situation. Thus, embryo development from bovine species can be produced in the ewe oviduct (Rizos et al. 2007). Moreover, a pre-fertilization hardening of the ZP was observed when oocytes and oviductal fluids from different species are used (Mondéjar 2011); but this process is not always produced on the same scale or in all species. This finding is probably due to the existence of expressed genes that are not shared among the species.

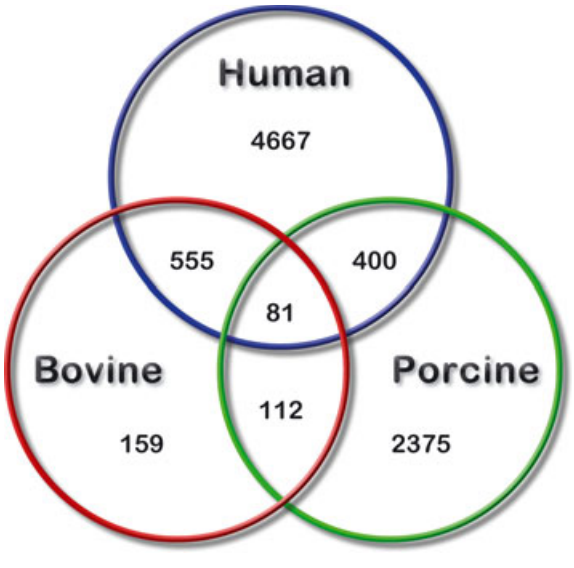

Fig. 4. Venn diagram showing overlapping and non-overlapping gene expression on human, bovine and porcine oviduct

Proteomic Analysis

More than 160 proteins have been seen to be expressed or secreted by the oviduct of different species (Avilés et al. 2010). In pig, numerous proteins have been identified in the epithelial cells (Buhi et al. 2000; Georgiou et al. 2005; Sostaric et al. 2006; Seytanoglu et al. 2008) and also in the oviductal secretions (Georgiou et al. 2005, 2007; Mondéjar et al. 2009; Mondéjar 2011). At least 32 proteins from the oviductal fluid are affected by the presence of gametes, most of which are affected by the presence of the male gamete (Georgiou et al. 2007). In our laboratory, we performed an analysis of the porcine oviductal fluid from preovulatory phase. The oviductal fluid was obtained by luminal aspiration of previously dissected oviducts as previously reported (Carrasco et al. 2008). After centrifugation (5200 × ***g*** for 10 min) to remove the mucus and cellular debris, the samples were analysed by one dimensional SDS-PAGE electrophoresis under reducing conditions. The different bands were visualized by silver staining and were cut and digested with trypsin for subsequent proteomic analysis. The data were analysed by LC/MSD Trap Data Analysis Version 3.2 (Bruker Daltonik, GmbH, Germany), and the search for matches was conducted with the Spectrum Mill MS Proteomics Workbench (Agilent Technologies, Santa Clara, CA, USA) against the most recent version of the NCBInr database.

A total of 291 proteins were identified in the porcine oviductal secretions (Mondéjar 2011); however, only 35 of these proteins were detected in our microarray analysis (Mondéjar 2011), probably due to the incomplete array annotation. The different proteins can be classified into different groups as performed previously (Avilés et al. 2010) (Fig. 5). In this analysis, only 27 secreted proteins (9.3%) were identified; however, most of the proteins correspond to cellular components (90.7%). It is striking that a low number of the proteins identified correspond to typically secreted proteins compared with the total number of proteins. In the oviductal fluid, other proteins that are not typically secreted by the epithelial cells can be detected. These proteins may be divided into two main groups: proteins that come from the transudate of blood (for example, albumin and plasminogen) and other non-secreted cellular proteins. Some of them are proteins present in cellular organelles such as the nucleus, mitochondria,

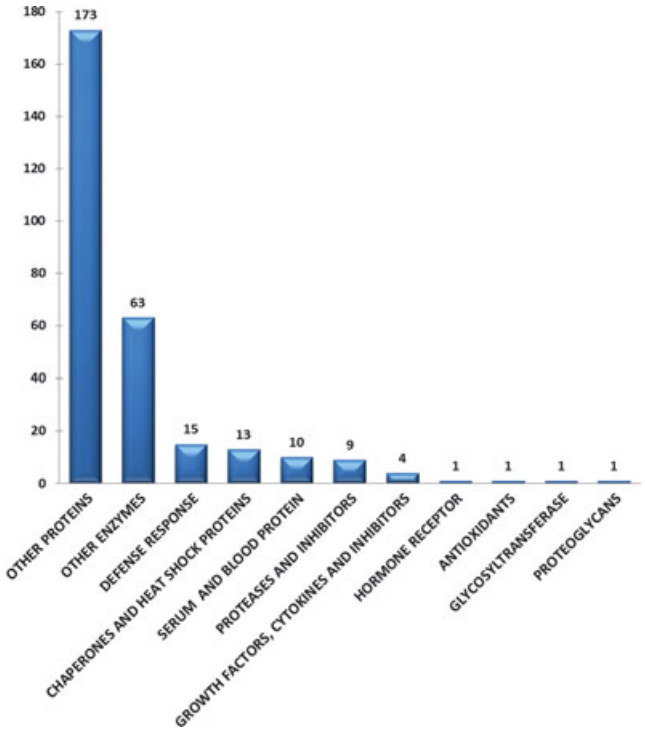

Fig. 5. Classification of the different proteins identified in the porcine oviductal fluid by proteomic analysis

endoplasmic reticulum and lysosomes. The origin of these proteins is probably related to different processes such as epithelial cell renewal or the secretory activity by apocrine and holocrine processes (Crow et al. 1994; Steffl et al. 2008); however, blood or oviductal cells contamination cannot be excluded.

The most abundant peptides identified correspond to albumin and the OVGP1 as expected owing to the fact that these proteins are abundant in oviductal fluid. Owing to space restriction, only three of the identified proteins are discussed in more detail.

Several heat shock proteins (HSP) were detected in our proteomic analysis, confirming their existence, as reported previously (Bauersachs et al. 2004; Georgiou et al. 2005). These proteins are usually considered to be intracellular proteins, although they have also been detected in the human serum and plasma (Molvarec et al. 2010). It was observed that HSP70 can be secreted by a non-classical pathway (Mambula and Calderwood 2006). A similar process, which remains to be confirmed, could exist in the oviduct. It was reported that HSPA8 and HSP60 are able to associate with spermatozoa, thus improving their survival in several species (Elliott et al. 2009; Lloyd et al. 2009b).

Another interesting protein identified in our study is annexin, which is considered a cytosolic protein; however, it was also reported that this protein can be secreted by a non-classical pathway (Christmas et al. 1991). It was previously reported that the bovine sperm interaction with the oviductal epithelium is mediated by BSP1 associated with the sperm membrane and with annexin molecules in the oviductal epithelium (Ignotz et al. 2007). Annexin in the oviductal fluid could regulate this type of interaction.

In our study, we detected, by both gene expression and proteomic analysis, the deleted in malignant brain tumours 1 (DMBT1) protein, confirming its presence at mRNA level in the bovine (Bauersachs et al. 2004) and human (Tone et al. 2008) oviduct. Very recently, it was reported that the DMBT1, previously called SPG, is expressed in the porcine oviduct (Teijeiro et al. 2012). In this study, the authors demonstrated the presence of this protein at the apical surface of the epithelial cells.

Concluding Remarks

A large body of evidence strongly supports the complexity of and the important role played by the oviduct and its secretion in different aspects of gamete maturation, fertilization and embryo development. The oviduct undergoes important changes in several aspects, including its anatomy in different regions, changes in the histology and physiology of the mucosa during the ovarian cycle and a complex gene expression pattern that is modified according to the ovarian cycle status and also to the presence of gametes and embryos. More precise information is needed about the different genes expressed and proteins synthesized and secreted by the oviduct in its different regions, hormonal and other physiological conditions to clarify the role played by the oviduct. However, for this, knowledge of the complete genome annotation of different animals is necessary. The information obtained for different animals will also contribute to understanding the mechanisms conserved in the different species and also others that are responsible for species specificity. This information will contribute to improving different aspects of the methodology used in ARTs in domestic animals, endangered wildlife species and even human beings.

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Conflict of interest

None of the authors have any conflicts of interest to declare.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Data S1. References.

Data S2. Methods.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

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