Extra-oviductal expression of oviductal glycoprotein 1 in mouse: Detection in testis, epididymis and ovary

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Oviductal glycoprotein 1 (OVGP1), also called oviductin, is an oviduct-specific protein and is suggested to play a role in fertilization. Traditionally, *Ovgp1* has been shown to be exclusively expressed by the oviduct; however, recent studies have demonstrated its expression in some cancers. This observation led us to hypothesize that *Ovgp1* might have some extra-oviductal expression. In the current study, we evaluated the mRNA and protein expression of *Ovgp1* in normal reproductive tissues of male and female mice. For the first time, we demonstrate that beyond the oviduct, *Ovgp1* mRNA is expressed in the testis, epididymis and ovary, but not in the uterus, cervix, vagina, breast, seminal vesicles and prostate gland. In the testis, *Ovgp1* mRNA was localized in the cells at the base of seminiferous tubules (most likely, Sertoli cells), while the protein was detected in the round and elongating spermatids. In the epididymis, *Ovgp1* transcripts were localized in epididymal epithelium of the caput but not the corpus and cauda; OVGP1 protein was, however, not detected in any of the segments but was present in the epididymal sperm. In the ovary, *Ovgp1* transcripts and protein were detected in the surface epithelium, granulosa cells of the preantral and the antral follicles and corpus luteum. In both, the ovary and oviduct, the expression of *Ovgp1* was found to be higher at estrus stage than at diestrus stage. To the best of our knowledge, this is the first study demonstrating the extra-oviductal expression of *Ovgp1*. Our data suggests that, beyond fertilization, *Ovgp1* might have specific roles in gonadal physiology.

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1. Introduction

Oviductal secretions provide a microenvironment that supports final maturation of gametes, fertilization and early embryonic development. The non-ciliated secretory cells of the oviductal epithelium secrete a high-molecular-weight, oestrogen-dependent protein, oviduct-specific glycoprotein 1 (*Ovgp1*) also called as oviductin (Buhi 2002). Since its first discovery in mouse (Kapur and Johnson 1988) *Ovgp1* has been extensively characterized in a large number of animal species (Yang *et al.* 2015). Most *in vitro* studies implicate the involvement of *Ovgp1* in fertilization and early embryonic development (Buhi 2002). It is shown that OVGP1 enhances sperm capacitation, viability (King and Killian 1994) and sperm-oocytes binding, and improves fertilization and early embryonic development (Buhi 2002), and decreases polyspermy (Coy *et al.* 2008).

Previous studies have implicated that OVGP1 is an oviduct-specific protein. In mouse using Northern blotting, transcripts of *Ovgp1* were exclusively detected in RNA from the oviduct but not in other tissues like uterus, ovary, spleen and brain (Sendai *et al.* 1995). Since this discovery, a number of studies, using

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tools like RT-PCR and immunolocalization, in humans, mice and other species have demonstrated expression of *Ovgp1* in the oviduct (Rapisarda *et al.* 1993; Bhatt *et al.* 2004). However, in most studies, other tissues have not been investigated, except for one report where Hendrix and co-workers detected *Ovgp1* mRNA in rabbit endocervix (Hendrix *et al.* 2001). Recent reports have demonstrated that beyond the oviduct, OVGP1 is detected in ovarian cancers (Woo *et al.* 2004a); elevated OVGP1 levels have been reported in the serum of women with mucinous, clear cell, low-grade/low-serous ovarian carcinomas (Maines-Bandiera *et al.* 2010). Beyond the ovary, OVGP1 expression is also reported in non-atypical and atypical hyperplastic endometrial tissue (Woo *et al.* 2004b) and in invasive endometriod carcinoma (Wang *et al.* 2009). These observations imply that OVGP1 may be expressed in tissues apart from oviduct. However, it is unknown if the expression of *Ovgp1* is exclusive to pathologic tissues or even normal tissues express *Ovgp1*. We suspected that the failure to detect *Ovgp1* expression in tissues beyond the oviduct in earlier studies could be due to low sensitivity of the methods used. Therefore, the objective of our study was to determine the expression of *Ovgp1* beyond the oviduct in other reproductive tissues.

2. Materials and methods

2.1 *Ethics*

All experimental procedures involving animals were conducted in accordance with animal ethical guidelines of SPP-SPTM NMIMS (deemed-to-be) University.

2.2 *Animals/tissue collection*

Male and female Swiss albino mice (8–10 weeks of age) were housed under controlled temperature and humidity. Female Swiss albino mice were sacrificed at the estrus and diestrus stages and the tissues collected were oviduct, ovary, uterus, cervix and vagina. The estrous cycle of the female mice was monitored by examining the vaginal smears fixed with alcohol and stained using giemsa stain. Male mice were sacrificed and the tissues collected were testis, epididymis, prostate gland and seminal vesicle. All the tissues were either collected in TRI-reagent (Sigma- Aldrich) for RNA extraction or fixed in 4% paraformaldehyde for immunohistochemistry. For *in situ* hybridization, the tissues were fixed in 10% buffered formalin (pH 7.2).

2.3 *Total RNA extraction, cDNA synthesis and RT-PCR*

Total RNA was extracted from the tissues using TRI-reagent and RNA concentrations were determined from absorbance at 260 and 280 nm. After initial treatment with DNase I (Thermo Scientific), RNA (1μg) was reverse-transcribed with a random hexamer (TAKARA BIO INC) and M-MLV reverse transcriptase (TAKARA BIO INC). For negative controls, the reverse transcriptase was omitted from the reaction mixture.

The pair of primers of *Ovgp1* and *Gapdh* (housekeeping gene) were designed using NCBI primer blast, and the sequences are shown in table 1. Care was taken to ensure that the primers span at least one intron to avoid amplification from contaminating genomic DNA. For PCR, each cycle included the following parameters: 94°C for 30 s, specific annealing temperature (table 1) for 30 s, and 72°C for 30 s for 35 cycles, with a final extension step at 72°C for 5 min. Amplified products were resolved on a 2% agarose gel with ethidium bromide and visualized in a UV Transilluminator. The PCR product of *Ovgp1* was sequenced commercially by Sanger's method (Applied Biosystems).

2.4 *Real-time PCR*

Real-time PCR was performed as detailed elsewhere (Godbole and Modi 2010). The relative levels of *Ovgp1* mRNA in relation to *Gapdh* (housekeeping) were estimated by Step One Plus Real Time PCR (Applied Biosystems) using SYBR Green chemistry (TAKARA BIO Inc.). The amplification conditions for *Ovgp1* and *Gapdh* were as mentioned in section 2.3, and the fluorescence emitted was collected for 30 s during the extension step of each cycle. The homogeneity of the PCR amplicons was verified using the melt curve method. All PCR amplifications were carried out in duplicate for at least three biological replicates and data was statistically analysed using Student's *t*-test for statistical significance.

2.5 *Immunohistochemistry*

The detailed protocol for immunohistochemistry has been described previously (Godbole *et al.* 2007). Briefly, paraformaldehyde fixed tissues were embedded in paraffin and 5 μm thick sections were prepared on poly-L-lysine-coated slides (Blue Star), deparaffinized in xylene, and rehydrated in decreasing concentrations of alcohol before rinsing in distilled water. The endogenous peroxidase activity was blocked by incubating the sections for 10 min in 2% H_2O_2 in methanol. The antigenic sites were unmasked by incubation of sections at 90°C in Tris EDTA buffer (10 mM, pH 9.0) for 10 min and rinsed in PBS. This was followed by blocking with 2% bovine serum albumin (BSA) for 60 min followed by overnight incubation at 4°C with the anti-*Ovgp1* antibody (Abcam), at a dilution of 1:500 in PBS. Next day, the sections were rinsed with 0.2% Tween PBS and incubated with HRP conjugated goat anti-rabbit polyclonal (1:100) (Abcam) for 60 min. For peroxidase detection, all sections were incubated with 0.1% 3, 3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma-Aldrich) in PBS and 1%

Table 1. Primer sequences of *Gapdh* and *Ovgp1*

Gene	Accession No.	Primer sequence	Annealing temperature (T_a)	Product size
Gapdh	NM_001289726.1	FP: 5′GGCCGGGGCCCACTTGAAG3′ RP: 5′TGGATGACCTTGGCCAGGGGG3′	68°C	174 bp
Ovgp1	NM_007696.2	FP: 5′ACGTCTTATGATGCGCTCCTT3′ RP: 5′TTATCTGCGGGTGTCCCAAG3′	58°C	185 bp

H_2O_2 for 10 min. The reaction was stopped by washing the slides in distilled water. The sections were counterstained with haematoxylin and rehydrated with increasing concentrations of alcohol and mounted with DPX (HIMEDIA). The slides were observed in the microscope (Olympus BX51). At least three animals were analysed in each case.

2.6 *In situ hybridization*

Localization of *Ovgp1* transcripts was studied in the ovaries, testis and epididymis using non-radioactive *in situ* hybridization as described elsewhere (Modi *et al.* 2006, 2009). All glass and plastic wares used were autoclaved and baked, all solutions and water were treated with diethylpyrocarbonate (DEPC) to inactivate RNase prior to use. The probe used for *in situ* hybridization was an oligoprobe labelled with Digoxygenein (Dig) using the third-generation tailing kit (Roche). Briefly, the sections were deparaffinized in xylene, hydrated and incubated in 2X SSC (Sodium Saline Citrate) for 15 min at room temperature. Pre-hybridization was carried in a pre-hybridization cocktail containing 50% formamide, 4X SSC, 5% Denhardts solution, 0.25% yeast tRNA, 0.5% sheared Salmon sperm DNA and 10% dextran sulphate (all from Sigma Aldrich). The sections were hybridized overnight at 37°C with the labelled antisense probe diluted in the same cocktail at a concentration of 100 µM. As negative controls, sections were incubated using a Dig-labelled sense probe. Next day, the sections were stringently washed in varying concentration of SSC followed by an overnight incubation in alkaline phosphatase-conjugated anti-Dig antibody diluted (1:500). The slides were then extensively washed in 0.1 M Tris–HCl (pH 7.5) and equilibrated in 0.1 M Tris–HCl (pH 9.5) for 20 min. Detection was carried out at pH 9.5 for 1 h at room temperature in a solution of nitro blue tetrazolium (Roche) and 5-bromo-4-chloro-2-indoyl phosphate (Roche). The reaction was stopped in 0.1 M EDTA and the sections mounted in Vectashield (Vector laboratories).

3. Results

3.1 *Ovgp1 mRNA is expressed in multiple male and female reproductive tissues*

RT-PCR was performed on cDNA of various mouse reproductive tissues. In the positive control (oviduct) a single band of expected size (185 bp) was detected. No bands were detected in the reaction without reverse transcriptase, indicating specificity of the amplicons. Of the tissues analysed, beyond the oviduct, transcripts of *Ovgp1* were detected in the ovary, testis and epididymis in all the three biological replicates (figure 1). No bands were observed in any other tissues. The quality of the cDNA was verified by amplifying the housekeeping gene *Gapdh*, which was detected in all the cDNA preparations. The sequence of the PCR product from the ovary had 100% homology to the known mouse *Ovgp1*, verifying the specificity.

3.2 *Localization of Ovgp1 transcripts in ovary, testis and epididymis*

Ovgp1 transcripts were localized in ovary, testis and epididymis by non-radioactive *in situ* hybridization. In the ovary, *Ovgp1* transcripts were localized to the cells of the surface epithelium, granulosa cells of the growing follicles and the corpus luteum (figure 2; supplementary figure 1).

In the testis the *Ovgp1* mRNA was localized in cells juxtaposed on basal membrane at the periphery of the seminiferous tubule, most probably the Sertoli cells (figure 2). The *Ovgp1* mRNA was also localized in epithelium of the caput region of epididymis (figure 2). No transcripts were detected in corpus or caudal epithelium of epididymis (supplementary figure 2).

No staining was detected in any of the sections hybridized with sense probe, indicative of the specificity of reaction.

3.3 *Localization of OVGP1 protein in ovary, testis and epididymis*

The protein expression of OVGP1 was tested by immunohistochemistry in the tissues which expressed *Ovgp1* transcripts. OVGP1 was detected in the ovarian follicles (figure 3) and in the cells within the seminiferous tubules (figure 3). No OVGP1 was detected in the epithelial layer of epididymis; the staining was detected only in the lumen (figure 3). The negative controls incubated without primary antibody and excess of BSA did not show any staining, indicative of the specificity of the reaction.

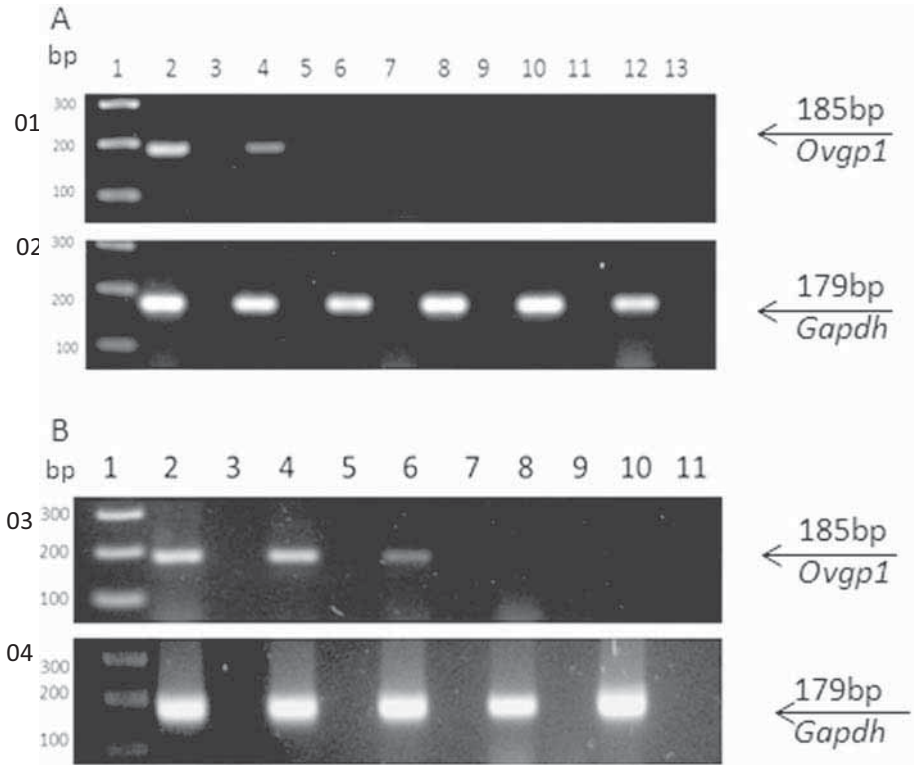

Figure 1. Distribution of *Ovgp1* mRNA in female and male reproductive tissues. RT-PCR for *Ovgp1* was done on RNA isolated from selected male and female reproductive tissues, and representative data is shown. (**A**) Lane 2 is oviduct, lane 4 is ovary, lane 6 is uterus, lane 8 is cervix, lane 10 is vagina, and lane 12 is breast cDNA. The respective RT negatives are in lanes 3, 5, 7, 9, 11 and 13. (**B**) Lane 2 is oviduct, lane 4 is testis, lane 6 is epididymis, lane 8 is seminal vesicles, and lane 10 is prostate gland. The respective RT negatives are in lanes 3, 5, 7, 9 and 11. The bands and the expected product sizes for *Ovgp1* and *Gapdh* are shown by arrow marks. In both the panels lane 1 is 100 bp ladder.

3.4 *Cellular distribution of OVGP1 protein in ovary, testis and epididymis*

In the ovary, OVGP1 protein was localized in cells of the surface epithelium (figure 4). In the growing follicles, the protein was detected in the granulosa cells (figure 4) and the corpus luteum (figure 4). Expression was not detected in the theca cells and large part of the ovarian stroma. In some cases weak staining of OVGP1 was detected in the oocytes. However, this staining was detected (albeit less frequently) in the negative controls.

In the testis, OVGP1 was localized exclusively in the round, elongated spermatids and the residual body of the elongated sperm (figure 4). No staining for OVGP1 was observed in the spermatogonia, spermatocytes and the Sertoli cells. The myoid cells and Leydig cells were devoid of OVGP1 (figure 4).

Irrespective of the segment of the epididymis, OVGP1 protein was not detected in epididymal epithelium. Instead, OVGP1 protein expression was detected in sperm cells of the lumen of caput, corpus and cauda (figure 4). The negative controls in all the cases did not show any staining, indicative of the specificity of the reaction.

3.5 *Expression of OVGP1 is not altered in testis during different stages of spermatogenesis*

In mouse, there are 12 stages of spermatogenesis that can be morphologically classified. The immunostained sections of OVGP1 were classified on the basis of the stages of spermatogenesis. As evident from figure 5 irrespective of the stage of spermatogenesis, OVGP1 was only expressed in the round and elongated spermatids and the residual bodies. No expression was seen in the Sertoli cells, spermatogonia and spermatocytes at any stages of spermatogenesis. The intensity of the OVGP1 staining also remained consistent across all the stages.

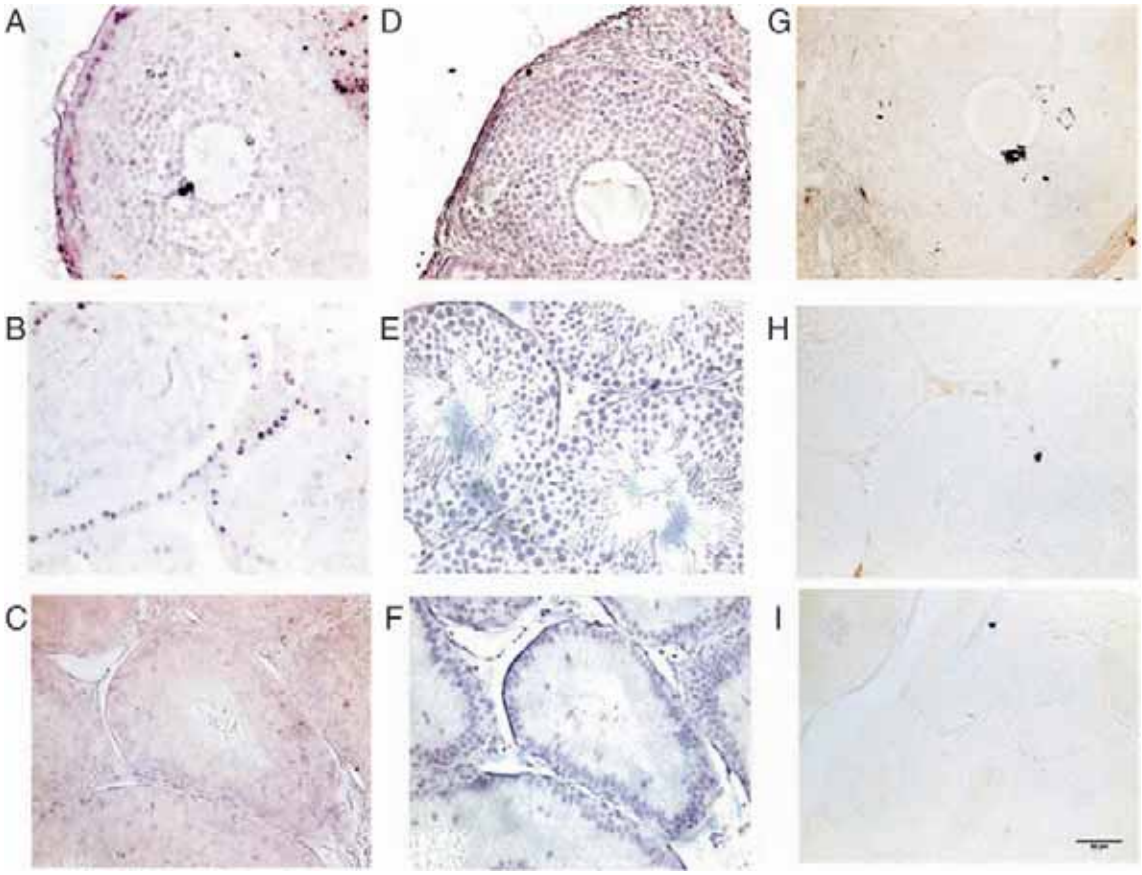

Figure 2. Localization of *Ovgp1* mRNA in mouse ovary, testis and epididymis by non-radioactive *in situ* hybridization. Paraffin sections of the ovary (**A**), testis (**B**) and epididymis (C) hybridized with antisense probe are shown. Purple staining indicates a positive reaction. The images in (**D**), (**E**) and (**F**) are the same sections stained with and haematoxylin re-imaged to show tissue architecture. The negative controls hybridized with sense probe are shown in (**G**), (**H**) and (**I**) for ovary, testis and epididymis respectively. Scale bar in (**I**) is 50 μM.

3.6 *Expression of OVGP1 in the oviduct is estrous-cycle-dependent*

In the oviduct, the immunoperoxidase staining was cytoplasmic and restricted to the luminal epithelia. The immunoreactivity was highest in the epithelium of the infundibulum irrespective of the stage of the estrous cycle. In comparison, the ampullary epithelium showed strong staining of OVGP1 in the estrus stage but was weaker in the diestrus. The immunoreactive OVGP1 was weak in the isthmus at the estrus stage; the expression was not detected at the diestrus stage. No immunostaining was observed in the negative control (figure 6).

To determine if the differential expression of OVGP1 protein in the estrus and diestrus oviduct is transcriptionally regulated, the relative expression of *Ovgp1* mRNA was estimated by quantitative reverse-transcriptase PCR (qPCR) (figure 6). The homogeneity of the products was validated by melt curve analysis and a single melt peak was detected in all the cases (supplementary figure 2). Relative quantification revealed *Ovgp1* mRNA was ~5 times higher in the estrus as compared to the diestrus stage. This difference was statistically significant ($p<0.05$).

3.7 *Expression of OVGP1 in the ovary is estrous-cycle-dependent*

OVGP1 was consistently detected in the surface epithelium, granulosa cells and corpus luteum. Irrespective of the stage of estrous cycle, immunoreactive OVGP1 was not observed in granulosa cells of primary and secondary follicles. Moderate to high immunoreactivity was observed in granulosa cells of the preantral and the antral follicles at estrus stage; the expression was weaker in the diestrus stage. Staining for OVGP1 was detected in the corpus luteum at both the stages in estrous cycle, but the immunoreactivity was higher at estrus than at diestrus stage. The ovarian surface epithelium

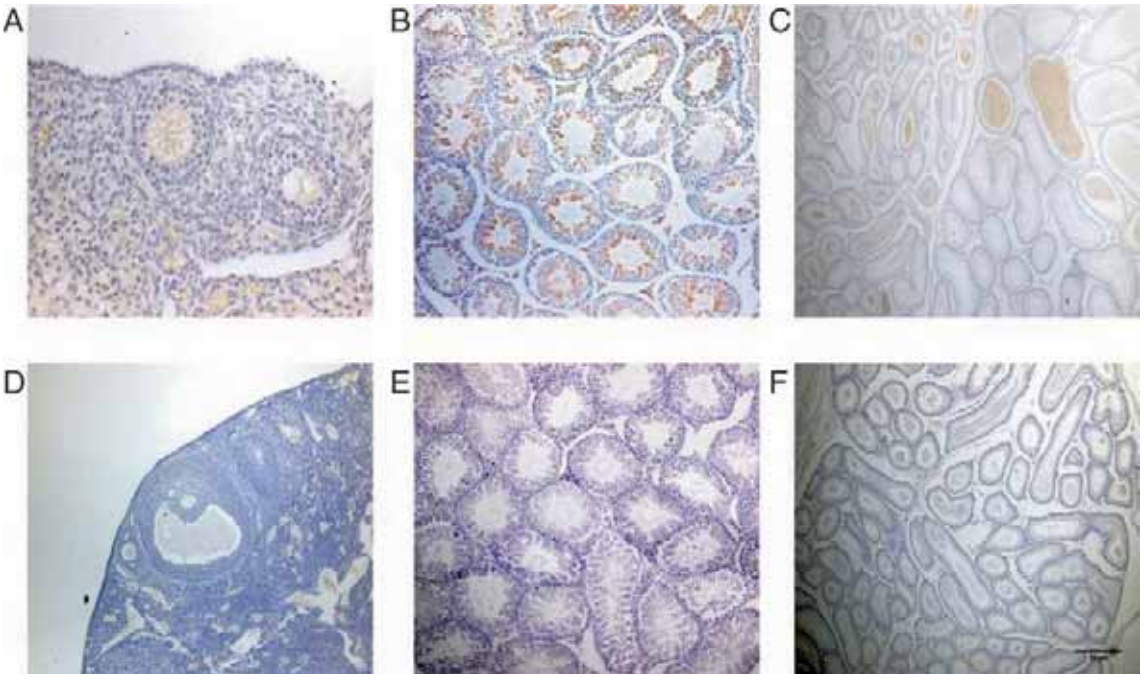

Figure 3. Immunohistochemical localization of OVGP1 in mouse reproductive tissues. Paraffin sections probed with OVGP1 antibody in case of ovary (**A**), testis (**B**) and epididymis (**C**). The corresponding negative controls (without primary antibody) are shown in (**D**), (**E**) and (**F**) respectively. Brown staining is indicative of positive reaction. Blue is counterstain of the nuclei with haematoxylin. Scale bar in (**F**) is 50 μM.

showed strong staining at estrus stage, while the staining was weaker at diestrus stage. The negative controls incubated without primary antibody and excess of BSA did not show any staining, indicative of the specificity of the reaction (figure 7).

The relative expression of *Ovgp1* mRNA was estimated by qPCR. The results revealed that the levels of *Ovgp1* were ~10 times higher in the estrus stage as compared to disetrus stage (figure 7). The differences in the levels of *Ovgp1* was significantly different ($p<0.05$).

4. Discussion

The results of the present study demonstrate that *Ovgp1* is expressed by the ovary, testis and epididymis as well as the oviduct. We further demonstrate that *Ovgp1* is transcriptionally regulated during the mouse estrous cycle.

Ovgp1 gene is transcribed as 2.16 kb mRNA, which encodes for highly glycosylated protein of 120 kDa. Secreted by the oviductal epithelial cells, *Ovgp1* has been considered to be exclusively expressed by the oviduct. *In vitro* experiments have suggested the involvement of oviductal protein OVGP1 in fertilization and sperm–egg interaction (Bhatt *et al.* 2004). OVGP1 has been shown to bind the gametes (Martus *et al.* 1998; Kadam *et al.* 2007) and improve fertilization rates (McCauley *et al.* 2003). However, OVGP1 does not seem to be essential for fertilization as mice lacking this gene does not have any reproductive phenotypes (Araki *et al.* 2003).

Recent studies have demonstrated increase of *Ovgp1* expression in endometrial and ovarian cancers (Woo *et al.* 2004b; Maines-Bandiera *et al.* 2010), suggesting that *Ovgp1* might have some extra oviductal expression and functions. In the present study we investigated if *Ovgp1* is expressed by any other tissues beyond the oviduct in mouse. The results revealed that beyond the oviduct, *Ovgp1* is expressed by the testis, epididymis and ovaries. To the best of our knowledge this is the first report demonstrating extra-oviductal expression of *Ovgp1* in normal tissues.

Our studies demonstrated that *Ovgp1* mRNA was expressed by the adult mouse testis, the gene was seen to be transcribed by the cells juxtaposed to the basement membrane (mostly likely Sertoli cells), and the protein expression is, however, detected in the round and elongated spermatids and the elongated sperm. This implies that *Ovgp1* may be synthesized and secreted by the Sertoli cells and possibly acquired by the germ cells during spermeation. It has been previously shown that spermatozoa from multiple species contain receptors for OVGP1 (Zhao *et al.* 2016). Since we

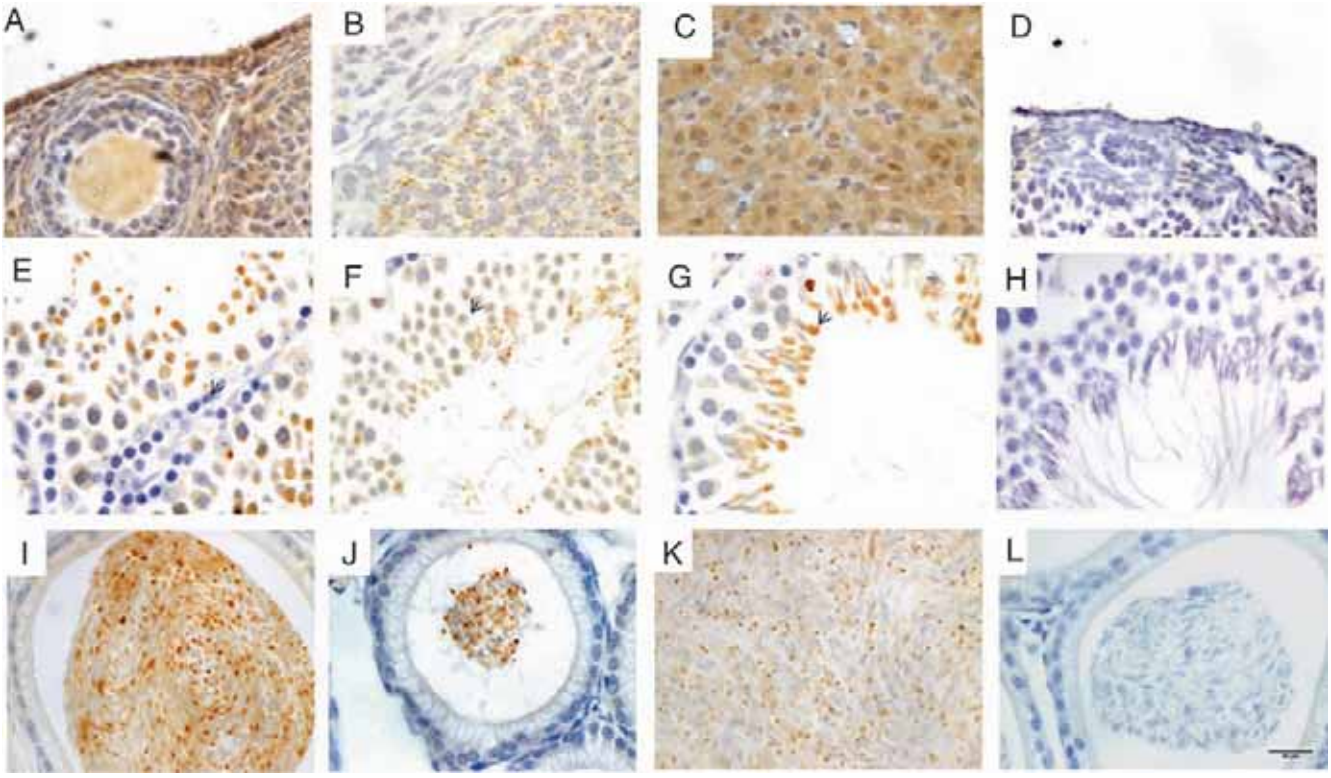

Figure 4. Cellular distribution of OVGP1 in reproductive tissues. Immunohistochemical localization in the surface epithelium and follicle of the ovary (**A**), granulosa cells (**B**) and corpus luteum (**C**). Localization for OVGP1 in the spermatogonia (**E**), round spermatids (**F**), elongated spermatids (**G**). Immunostaning for OVGP1 in the epididymal segments caput (**I**), corpus (**J**) and cauda (**K**). The corresponding negative controls (without primary antibody) are shown in (**D**), (**H**) and (**L**) respectively. Brown staining is indicative of positive reaction and counterstain with haematoxylin stained with blue. Scale bar in (**L**) is 50 μM.

detect the OVGP1 protein only on elongating spermatids, it is likely that OVGP1 receptors are gained on the spermatozoa during spermeogenesis.

Spermatogenesis is a hormonally controlled active process, involving tight communication between germ cells and somatic cells. Spermatozoa are formed from the differentiation of spermatogonial stem cells in a step-wise process whereby the meiotic spermatocytes develop into round and elongating spermatids (Hess 1999). In the mouse, the process of spermatogenesis can be classified into 12 morphologically distinguishable stages (Hess and de Franca 2008). Several proteins that are expressed by the spermatogenic cells are differentially expressed during different stages of spermatogenesis (Unni *et al.* 2009; Murta *et al.* 2013). Such differential distribution of proteins during spermatogenesis implies its differential requirements at each stage. However, unlike these proteins, OVGP1 protein was found to be constitutively expressed in the round and elongating spermatids at all the stages of spermatogenesis. What could be the need of *Ovgp1* expression and its protein binding on the round and elongating spermatids is difficult to speculate. Male knockout mice for *Ovgp1* are fertile, there are no reports describing the testicular phenotypes and sperm counts of such mice. Considering that *Ovgp1* is expressed by testis and acquired in maturing sperm, it will be imperative to study spermeogenesis and sperm counts of mice lacking *Ovgp1* to understand the significance of *Ovgp1* expression in these cells.

Post spermeation, the spermatozoa transits through the epididymis to gain fertilizing potential. Selected proteins secreted by the epididymal epithelium are taken up by the sperm cells which are essential for sperm maturation. By RT-PCR, we readily detected *Ovgp1* mRNA in the epididymis. Interestingly, we could detect transcripts of *Ovgp1* only in the in epithelial cells of the caput of the epididymis, whereas the corpus and cauda were devoid of *Ovgp1* transcripts. These observations imply that epididymis, which is the male developmental counterpart of the oviduct, also transcribe *Ovgp1*. However, by immunohistochemistry, OVGP1 protein was only detected in sperm within the epididymal lumen; no staining was detected in the cells of the caput, corpus and cauda. It is possible that the transcripts

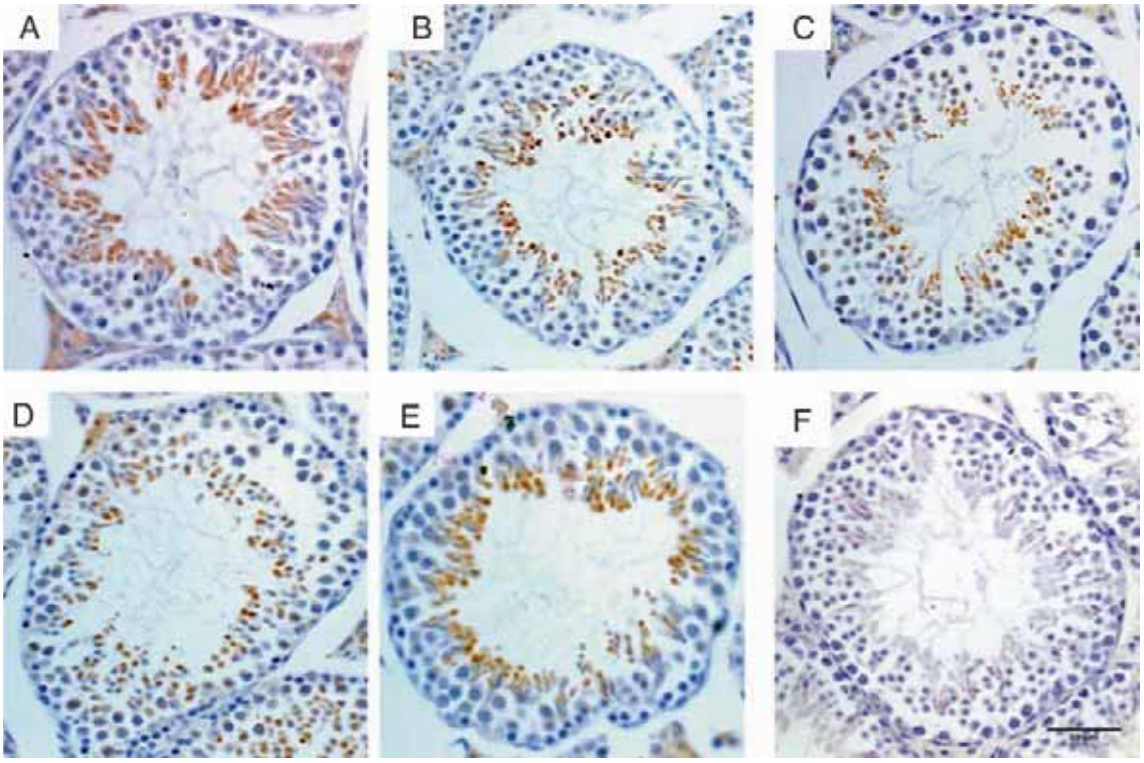

Figure 5. Localization of OVGP1 in different stages of spermatogenesis. stage 1–4 (**A**), stage 5–6 (**B**), stage 7–8 (**C**), stage 9–10 (**D**), stage 11–12 (**E**) and negative control (**F**).Positive staining is indicated by brown colour and counterstained with haematoxylin stained by blue colour. Scale bar in (**F**) is 50 μM.

detected for *Ovgp1* in RT-PCR reactions could be either untranslated or the protein rapidly synthesized and secreted within the epididymis lumen.

Among the female tissues tested, as expected, *Ovgp1* mRNA and protein were detected in the oviduct. Interestingly, we also detected *Ovgp1* mRNA in the RNA extracted from the ovary. This transcript does not arise from the possible contamination of oviductal cells as the OVGP1 protein could be detected in the ovarian follicles by immunohistochemistry. No *Ovgp1* mRNA was detected in the other female reproductive tissues. In the ovarian follicles, OVGP1 was mainly detected in the granulosa cells of the preantral and the antral follicles and also in the corpus luteum. OVGP1 was not detected in the thecal cells of the ovarian stroma. Beyond the granulosa cells, OVGP1 was detected in the oocytes, although inconsistently. In some sections, oocytes in a few large antral follicles stained positive for OVGP1, no expression was detected in other follicles at the same stage. Unfortunately, as the negative control sections also showed some weak brown staining in the oocytes, we cannot confidently determine if OVGP1 is secreted by the oocytes. It is possible that the oocytes in some follicles may acquire the OVGP1 secreted by the granulosa cells like that observed *in vitro* (Bhatt *et al.* 2004). Nevertheless, to the best of our knowledge this is the first report demonstrating the expression of *Ovgp1* by the granulosa cells of the ovarian follicles. Although female mice knockout for *Ovgp1* are fertile, the ovulation kinetics and the ovarian profiles of these mice have not been thoroughly investigated (Araki *et al.* 2003). In light of the observation that *Ovgp1* is expressed by ovarian follicles, it will be interesting to study the ovarian phenotypes of mice lacking *Ovgp1*, to decipher its possible roles in ovary.

In the oviduct, the expression of *Ovgp1* is known to be hormonally regulated. It has been reported in almost all the species that expression of *Ovgp1* is induced by oestrogen (Buhi 2002). Not surprisingly, expression of oviductal *Ovgp1* was higher in the follicular or the estrus phase which is characterized by oestrogen dominance (Abe *et al.* 1993; Sendai *et al.* 1994; Abe *et al.* 1995). Extending these evidence we found that in both the ovary and oviduct, expression of *Ovgp1* was higher in the estrus phase as compared to the diestrus phase. Regional difference in OVGP1 distribution has been reported in golden hamster (Abe and Oikawa 1991), sheep (Gandolfi and Moor 1987), cow (Abe *et al.* 1993) and canine (Saint-Dizier *et al.* 2014). Interestingly, we

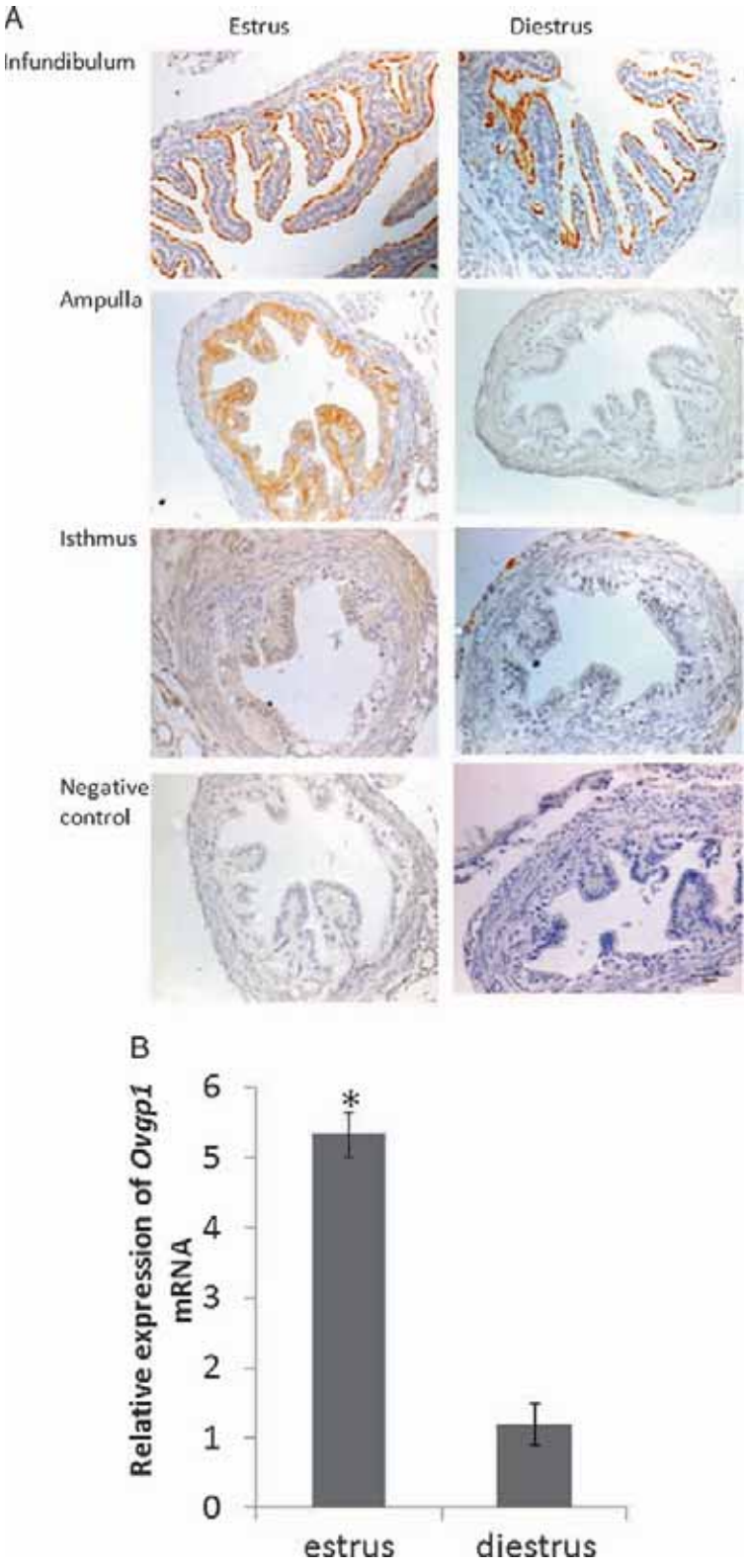

Figure 6. Cyclic regulation of OVGP1 in oviduct. (**A**) Immunohistochemistry for OVGP1 in different segments (infundibulum, ampulla and isthmus) of the mouse oviduct in estrus and diestrus stages. Blue staining is due to counterstaining by haematoxylin and brown staining is indicative of positive reaction. (**B**) Relative expression of *Ovgp1* mRNA in estrus and diestrus phase in oviduct (n=3). Data was analysed using one-tailed Student's *t*-test. *$p<0.05$.

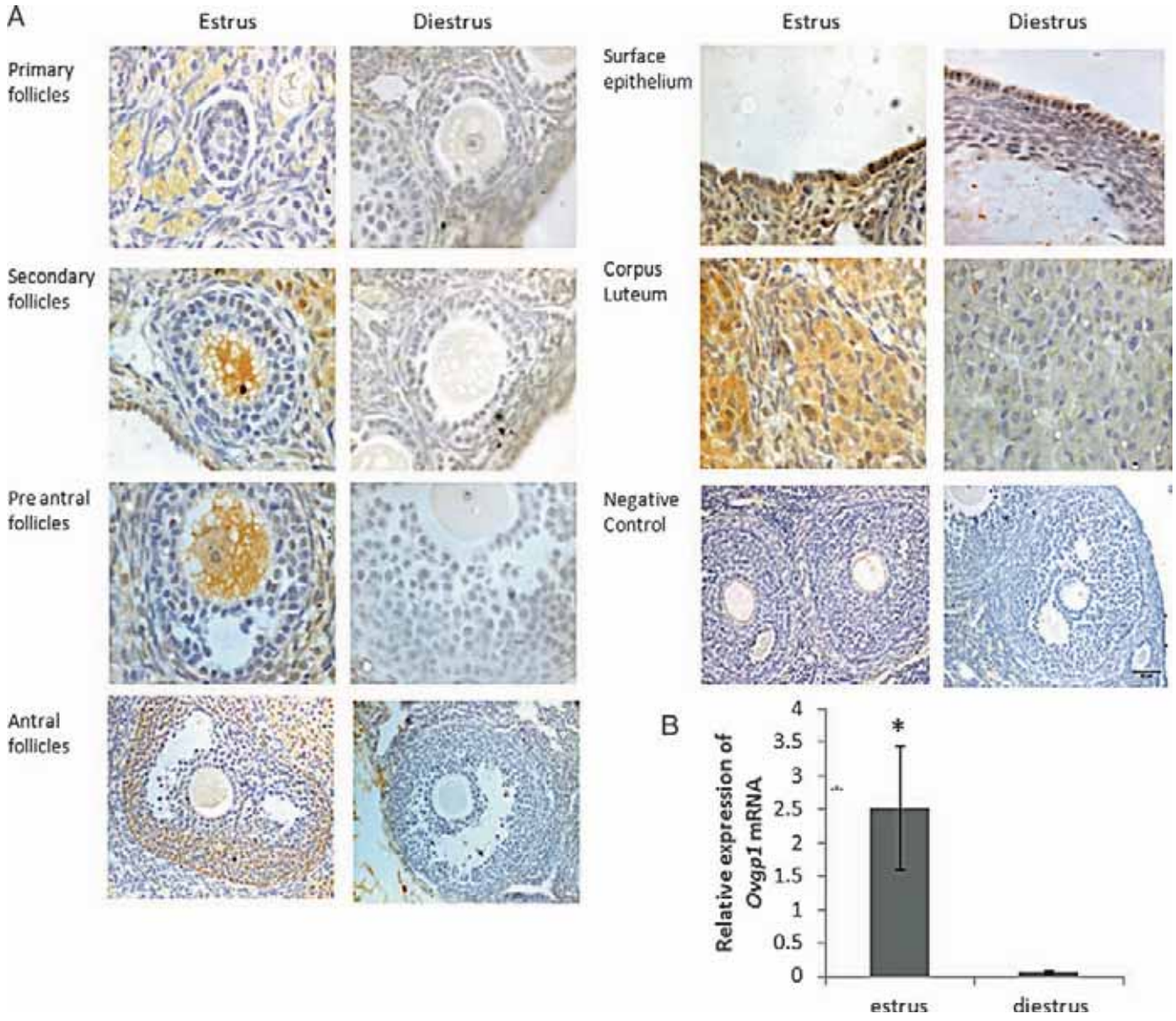

Figure 7. Cyclic regulation of OVGP1 in ovary. (**A**) Immunohistochemistry of OVGP1 in different segments (primary, secondary, pre-antral, antral follicles, surface epithelium, corpus luteum) of the mouse ovary in estrus and diestrus stages. Brown staining indicated positive reaction, while blue staining is indicative of counterstaining by haematoxylin. (**B**) Relative expression of *Ovgp1* mRNA in estrus and diestrus phase in ovary (n=3). Data was analysed using one-tailed Student's *t*-test. *$p<0.05$.

found that the expression of OVGP1 does not seem to be cycle-dependent in all the segments of mouse oviduct. While in the infundibulum the expression of OVGP1 did not differ in the estrus vs. diestrus stages, in the ampulla and the isthmus, the expression is higher in estrus as compared to diestrus. However, in cow, as compared to the luteal phase (diestrus of mice), in the follicular phase (estrous of mice) the expression of OVGP1 is higher in ampulla and the infundibulum but not in the isthmus (Abe *et al.* 1993). These observations imply that although OVGP1 is hormonally regulated in the oviductal cells, there might be species specific difference in its segmental regulation.

Within the ovary, OVGP1 was detected in the granulosa cells of the pre-antral and the antral follicles, and the corpus luteum, and in all these compartments, the expression was higher in the estrus as compared to the diestrus stage. This increased expression of OVGP1 protein at the estrus stage is not because of protein accumulation, but is due to increased transcription of *Ovgp1* mRNA in ovary at the estrus stage, as compared to the diestrus stage. The expression of OVGP1 by

the growing follicles and its differential regulation during the estrous cycle implies that its regulation may be hormonally controlled. As the granulosa cells of the pre-antral and antral follicles are FSH and oestrogen-responsive, it is possible that both these hormones independently or in concert may regulate OVGP1 expression. The persistent expression of OVGP1 in the granulosa cells until luetinization and its cyclic variation suggest that OVGP1 may have some roles in folliculogenesis.

The functional significance of the expression of *Ovgp1* by the gonads is difficult to speculate. At present, it is not known if the human testis and ovary express *Ovgp1*. However, *Ovgp1* is reported to be expressed by the serous ovarian carcinoma in a stage-dependent manner (Woo *et al.* 2004a), suggesting that *Ovgp1* might have some functions in cancer. It is imperative to study the expression of *Ovgp1* in various endocrine and non-endocrine pathological conditions to understand the functional significance of *Ovgp1* beyond the oviduct.

In summary, the present study for the first time has demonstrated the extra-oviductal expression of *Ovgp1*. The discovery of *Ovgp1* expression in the gonads will have relevance in expanding our understanding of the regulation of gonadal physiology. The association of *Ovgp1* expression in cancer with our findings indicates that we must revisit this molecule and explore its role beyond fertilization.

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