*Journal of Assisted Reproduction and Genetics, Vol. 19, No. 12, December 2002 (© 2002)*

Physiology

# Variable Expression of Oviductin mRNA at Different Stages of Human Reproductive Cycle

**Ingrid H. Lok,[1,2] Christine M. Briton-Jones,[1] Pong M. Yuen,[1] and Christopher J. Haines[1]**

*Submitted May 1, 2002; accepted June 5, 2002*

***Purpose*:** To examine the in vivo expression of oviductin mRNA at different stages of the human female reproductive cycle including pregnancy and after menopause.
***Methods*:** Oviducts were obtained from 25 women in normal menstrual cycle, 5 in early pregnancy, 5 undergoing postpartum sterilization, and 4 menopausal women. The oviductal mucosal tissue was isolated and oviductin mRNA was assessed using reverse-transriptase-polymerase chain reaction (RT-PCR); its correlation with various hormones was assessed.
***Results*:** Oviductin mRNA was detected throughout the menstrual cycle, highest in the periovulatory period. It continued to be expressed in early pregnancy but was absent in the postpartum period and after menopause.
***Conclusions*:** The production and function of oviductin at different stages of human reproductive cycle including pregnancy is not well known. Its highest expression at the time of ovulation is consistent with a supportive role in fertilization and early embryo development.

**KEY WORDS:** Fallopian tubes; humans; oviduct; oviductin; RT-PCR.

## INTRODUCTION

The mammalian oviduct is the site of a number of crucial events that precede implantation including gamete transport, fertilization, and the initial stages of embryo development. In-vivo fertilization studies using animal models have shown that fertilization occurs more efficiently in the oviduct than with fertilization in vitro (1). This suggests that factors present in the oviduct facilitate fertilization and that these are missing from the in-vitro culture media (2).

A number of chemical factors produced by the oviduct have been shown to affect gamete binding and early embryo development (3). Of specific interest is a heavily glycosylated, high molecular weight glycoprotein termed oviductin, which is exclusively synthesized and secreted by the oviduct. Oviductin has been shown to bind to both bovine and hamster sperm (4,5). It also binds to the zona pellucida and is present in the perivitelline space of baboon and human oocytes (6,7). Boatman and Magnoni have also reported that the incubation of ovarian oocytes with purified hamster oviductin increases sperm penetration (3). These findings suggest that oviductin may play a role in gamete binding preceding fertilization. In addition, oviductin has been found inside blastomeres in baboons (6) and the cleavage rate of sheep embryos appears to be enhanced by oviductin (8). Other in-vitro studies also suggested that oviductin may have embryotrophic effects (9–12).

Oviductin has been identified in many different species (13), including hamsters (14), rabbits (15,16), sheep (17), pigs (18), cows (19,20), baboons (21), and humans (22). However, the regulation of oviductin appears to differ among species (2). In the golden hamster, it has been found in the oviductal epithelium

[1] Department of Obstetrics and Gynaecology, Prince of Wales Hospital, Chinese University of Hong Kong, Shatin, Hong Kong SAR, People's Republic of China.
[2] To whom correspondence should be addressed; e-mail: ingridlok@cuhk.edu.hk.



1058-0468/02/1200-0569/0 © 2002 Plenum Publishing Corporation

at all stages of the menstrual cycle (23,24) and also during pregnancy (24,25). On the other hand, it has been shown to be only temporally associated with concentrations of estrogen during the estrous cycle and in early pregnancy in pigs (18,26), cows (19,27), and sheep (17). Studies performed in humans are limited. It appears that oviductin is estrogen-dependent as it has been detected around the time of ovulation but has been found to be either markedly reduced or absent at other stages of the menstrual cycle (22,28). Unexpectedly, it has not been detected in early human pregnancy even though this is a high estrogen state (28). Other studies, however, have been contradictory. An in-vitro study using bovine oviductal cells showed that estrogen had no effect on oviductin production but luteinizing hormone (LH) had a stimulatory effect (29). The hormonal regulation of oviductin production in humans has not been thoroughly examined. In addition, the potential role, if any, of oviductin at other stages of the female reproductive cycle has not been well explored.

The aim of this study was to examine the in-vivo expression of oviductin mRNA at different stages of the human female reproductive cycle. In particular, oviductin expression was examined in early pregnancy, the postpartum period, and in postmenopausal women.

## MATERIALS AND METHODS

### Tissue Samples

Oviduct tissue was obtained from 25 parous women with regular ovulatory menstrual cycles at the time of either a laparoscopic sterilization or else a hysterectomy for uterine fibroids. Women with a history of infertility, tubal disease, or exogenous hormone intake were excluded. The stage of the cycle was determined by the date of commencement of the last menstrual period and blood was taken for the measurement of LH, follicule stimulating hormone (FSH), progesterone (P), and oestradiol ($E_2$) on the day of operation to confirm the stage of the menstrual cycle. Oviduct tissue was also obtained from 5 women undergoing legal termination of pregnancy and tubal sterilization at 7–10-weeks gestation and 5 other women undergoing postpartum sterilization within 3 days of delivery. Samples were also collected from 4 menopausal women undergoing laparoscopic bilateral salpingo-oophorectomy for benign ovarian cysts. Written consents were obtained from all women and the study was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong.

The methodology of oviductal mucosal tissue extraction, mRNA extraction, and analysis of the products of the reverse transcriptase-polymerase chain reaction (RT-PCR) has been described previously (30). In brief, the excised oviduct tissue was immediately placed and rinsed in Hepes buffered Quinn's Human Tubal Fluid (Irvine Scientific, Santa Ana, CA). The mucosal layer was then dissected off and minced macroscopically.

### RNA Extraction and cDNA Synthesis

mRNA was extracted from the oviductal mucosal tissue by using the Oligotex direct mRNA kit (Qiagen, Hilden, Germany). All mRNA samples were treated with 1 unit of DNAse (Boehringer Mannheim/Hoffman-La Roche, Basel, Switzerland). The DNAse was then inactivated by incubating at 90°C for 5 min. The mRNA was precipitated with isopropanol at −70°C overnight, then washed with 70% ethanol, and dissolved in Tris EDTA buffer. A total of 100 ng of mRNA was used for cDNA synthesis with Multiscribe reverse transcriptase (PE Biosystems, Foster City CA). All the resultant cDNA were stored at −20°C or used directly for PCR.

### RT-PCR

PCR was performed using 5$\mu$L cDNA with Amplitaq Gold DNA polymerase (PE Biosystems, Foster City CA). Primers specific to human oviductin were designed from the published cDNA sequence (28) (forward, 5′TAG GTA CCA AGG AGA GGA ACA GAG AG-3′; reverse, 5′TAG GTA CCC CTT TCC CAA CTT CCA TG-3′). The primers for $\beta$-actin were derived from the published sequences (31) (forward, 5′ ATC GTG GGG CGC CCC AGG CAC3′; reverse, 5′CTC CTT AAT GTC ACG CAC GAT TTC3′) (Mwgag Biotech, Ebersberg, Germany). Each PCR cycle consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. Thirty-five cycles were performed, followed by a final extension at 72°C for 4 min.

### Analysis of PCR Products

Twenty percent of each PCR product was separated by gel electrophoresis on a 2% agarose gel containing 0.5 $\mu$g/mL ethidium bromide in TBE buffer.

The separated PCR products were visualized under ultraviolet illumination. The integrated optical density (IOD) was determined for each PCR product by the image analyzer, Gel Doc System. The level of oviductin mRNA was measured as the IOD ratio between the PCR amplified oviductin product and its simultaneously amplified $\beta$-actin control.

The PCR product generated by the oviductin primers was digested with the restriction enzyme *Sac* 1 (Boehringer Mannheim/ Hoffman-La Roche, Basel, Switzerland) to confirm that it was oviductin. This restriction enzyme cuts the PCR derived oviductin product resulting in two bands, one at 235 bp and the other at 175 bp.

### Statistics

Statistical analysis was performed using the Statistical Packages of Social Sciences for Windows (SPSS, Inc.). Nonparametric tests were used to compare the expression of oviductin mRNA at different stages of the menstrual cycle. The Spearman's rank test ($r_s$) was used to assess correlations between individual hormones and oviductin mRNA expression. Multiple linear regression using the backward stepwise regression model was used to assess the relative effects of the various hormones on oviductin mRNA expression.

## RESULTS

### Oviductin Expression Throughout the Menstrual Cycle

Of the 25 women with ovulatory cycles, 4 were in early follicular phase (Day 1–5), 6 in midfollicular phase (Day 6–11), and 5 each in the periovulatory (Day 13–16), early luteal (Day 17–20), and late luteal phases (Day 23–28), respectively. In all women, serum concentrations of FSH, LH, $E_2$, and P were consistent with the stages of the cycle estimated by date of last menstrual period. Oviductin mRNA was expressed in all oviduct mucosal cell samples examined throughout the menstrual cycle (Fig. 1).

The expression of oviductin mRNA was significantly higher in the periovulatory stage (median = 4.0, interquatile range [IQR] 3.4–4.7, $p < 0.001$) than in any other stages of the menstrual cycle (Fig. 2). The level of oviductin mRNA expression in the follicular phase was significantly higher than that in the luteal phase (median 1.6, IQR 1.0–2.3 vs. median 0.7, IQR 0.2–0.9, $p < 0.005$).

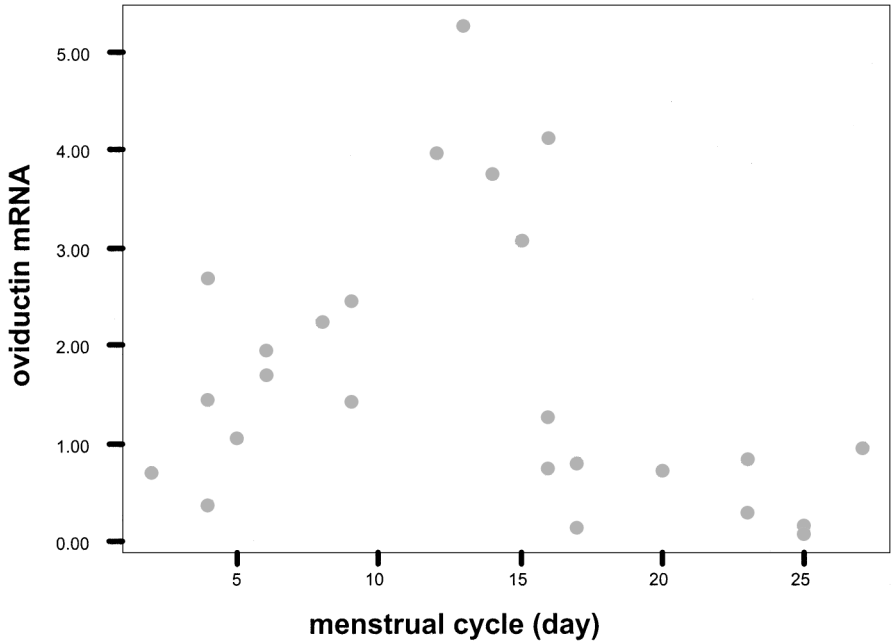


**Fig. 1.** Scatterplot showing the expression of oviductin mRNA throughout the menstrual cycle. The expression of oviductin mRNA was measured as the relative intensity of oviductin to $\beta$-actin control on densitometry reading.

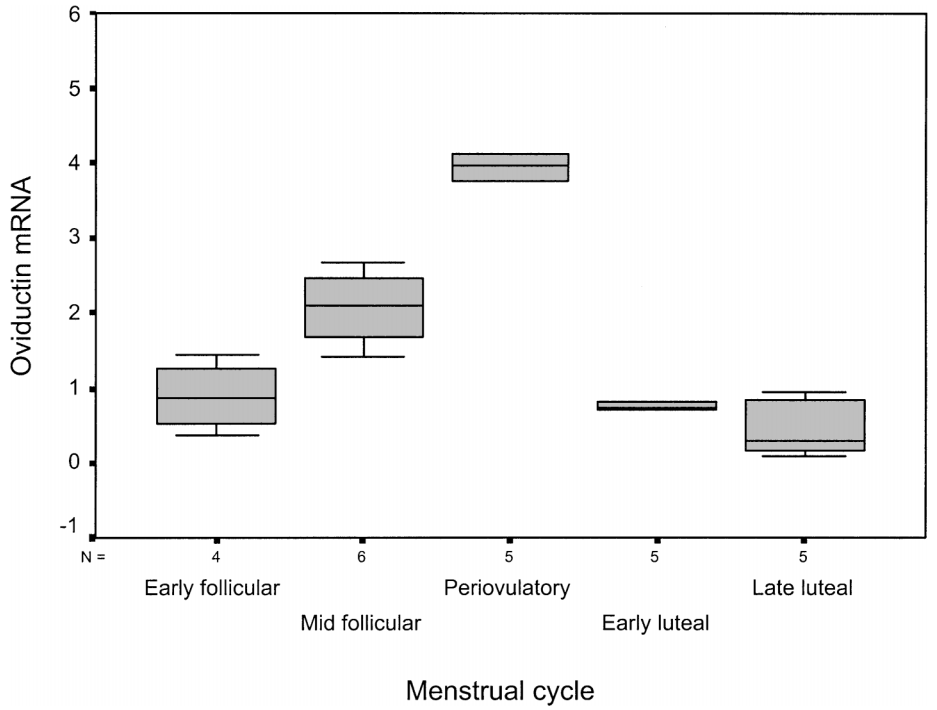


**Fig. 2.** Illustration of the median and IQR of oviductin mRNA expression at different phases of the menstrual cycle: early follicular ($n = 4$); midfollicular ($n = 6$); periovulatory ($n = 5$); early luteal ($n = 5$); and late luteal ($n = 5$). The expression of oviductin mRNA was significantly higher in the periovulatory phase (median = 4.0, IQR 3.4–4.7, $p < 0.001$) than at any other phases of the menstrual cycle.

### Correlation Between Oviductin mRNA Expression and Hormone Concentrations

Throughout the menstrual cycle, the correlation coefficients of serum concentrations of LH and FSH with oviductin mRNA expression were 0.56 and 0.58, respectively ($p < 0.01$). The correlation coefficient of serum $E_2$ concentrations with oviductin mRNA expression was 0.27. When samples collected at the time of P dominance (i.e. luteal phase) were excluded, the correlation coefficient increased to 0.76 ($p < 0.01$) (Fig. 3). On the other hand, the serum P concentration was negatively correlated with oviductin mRNA expression ($r_s = -0.61$, $p < 0.01$) (Fig. 4). Using multiple regression analysis, $E_2$ and P were the only two hormones found to have significant effects on oviductin mRNA expression, with a regression coefficient of 0.82 ($p < 0.01$).

### Expression of Oviductin mRNA in Pregnancy and After Menopause

Oviductin mRNA was detected in all five samples collected in early pregnancy. However, it was absent in the immediate postpartum period and in postmenopausal samples (Fig. 5).

## DISCUSSION

Oviductin mRNA was detected in all samples throughout the menstrual cycle with the highest level being detected in the periovulatory phase. These findings are similar to the findings of other studies performed in humans, except that in those studies oviductin was almost nondetectable except at the time of ovulation (22,28). In baboons, oviductin was detected only in the follicular phase but not in the luteal phase (32). This discrepancy may be due to species differences or it may have resulted from the different methods used to analyze the mRNA as the RT-PCR method employed in our study is regarded as being more sensitive (33) than the other tests used such as the Northern Blot (28,32).

It is difficult to assess the hormonal regulation of oviductin mRNA expression in vivo as the hormones vary in concentration at different stages of the

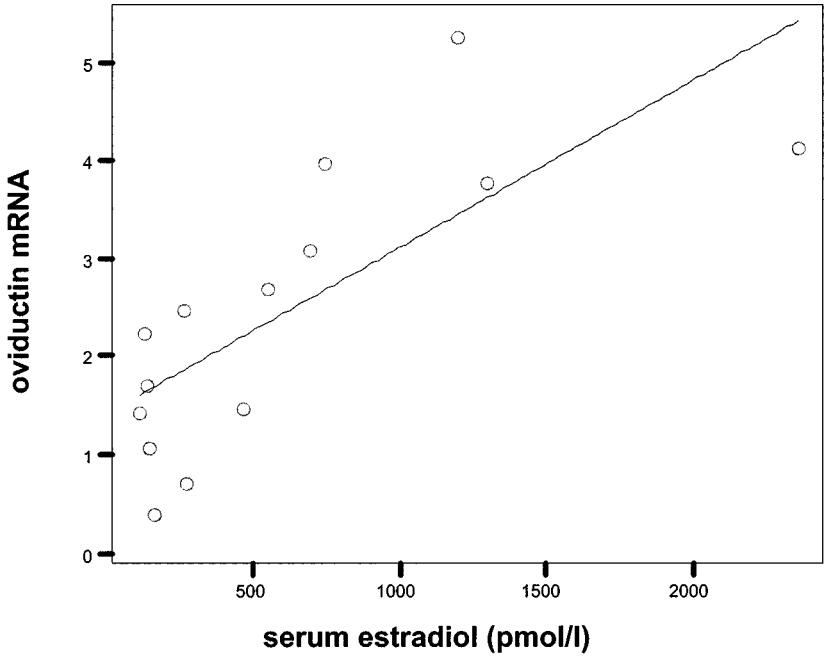


**Fig. 3.** A scatterplot showing the correlation between serum estradiol concentration and oviductin mRNA expression in the follicular phase and periovulatory phase ($r_s = 0.76 p < 0.01$).

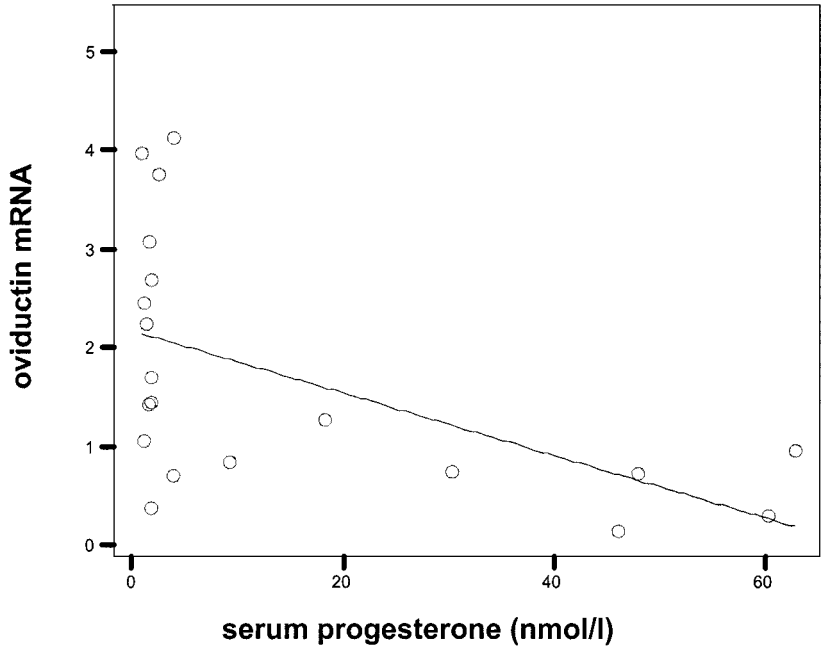


**Fig. 4.** A scatterplot showing the negative correlation between serum progesterone concentration and oviductin mRNA expression throughout the menstrual cycle ($r_s = -0.61$, $p < 0.01$).

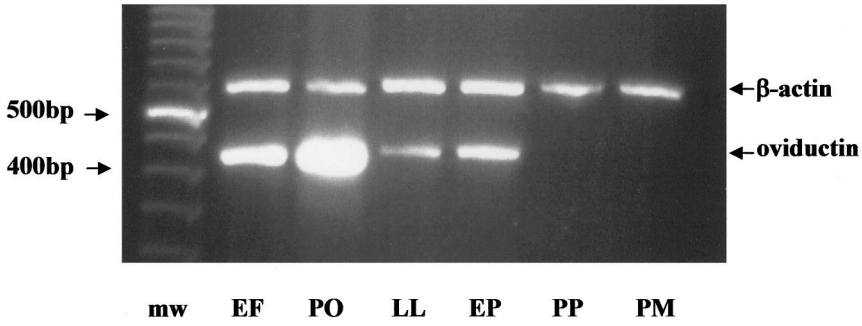


**Fig. 5.** Gel densitometry of the RT-PCR products: early follicular phase (EF); periovulatory phase (PO); late luteal phase (LL); early pregnancy (EP); immediate postpartum period (PP); postmenopausal (PM); and a 50-bp ladder, molecular weight marker (mw). The oviductin (410 bp) band was present in all stages except in the immediate postpartum and postmenopausal samples where $\beta$-actin band used as a control was present in all samples. The relative intensity of oviductin to $\beta$-actin control was highest in the periovulatory sample (PO).

menstrual cycle and their production is interrelated. In an in-vitro study using a bovine model, LH was reported to have a stimulatory effect on the production of oviductin while other hormones including estrogen had no effect (29). This apparently is consistent with the fact that oviductin expression is highest in the periovulatory phase as shown in our study. However, using a multiple regression model, LH was not found to have any independent effect. In fact, $E_2$ and P were the only hormones that had an independent effect. Progesterone exerts a negative effect on oviductin mRNA expression throughout the cycle. While oestradiol exerts a positive effect in the follicular and periovulatory phase, its effect is markedly reduced in the luteal phase when the progesterone level is elevated. This suggests that the stimulatory effect of $E_2$ is blocked or counteracted by the inhibitory effect of progesterone. This finding is consistent with the finding of the study performed in ovariectomized rhesus macaques treated with exogenous $E_2$ and P (34).

Of interest, oviductin mRNA expression was present in all five samples collected in early pregnancy. We believe that this is the first time that this has been reported in humans. Our finding is contrary to that of Arias *et al.* (28) who failed to demonstrate the presence of oviductin mRNA in early pregnancy. There are a number of reasons that may account for this discrepancy. First, this may be because the Northern Blot method used in the Arias study is less sensitive than RT-PCR. Second, the oviducts collected in the Arias study were those contralateral to an ectopic pregnancy, where preexisting tubal damage may have reduced oviductin production. The presence of oviductin in our normal preganant participants and its absence in women with ectopic pregnancy may also be an indication of the protective role of oviductin against tubal infection and ectopic pregnancy (35).

The human oviductin sequence shares characteristics of epithelial mucins (35). In studies using the hamster model, it has been proposed that oviductin could be a protective epithelial type of mucin (35,36) and it has been classified as MUC9 (37). Some authors have also speculated that the highly glycosylated and charged oviductin molecules may prevent ectopic implantation of the embryo by providing repulsive forces that allow the free movement of the embryo within the oviduct (38). However, the role of oviductin in protecting the oviduct from infection and ectopic pregnancy needs further elucidation involving direct examination of the normal and pathogenic oviducts. In addition, whether its presence in early pregnancy may signify an extension of its role after implantation is unknown.

Nevertheless, the presence of oviductin in early pregnancy but its absence in postpartum participants is consistent with high estrogen levels in first trimester and reduced estrogen but predominant progesterone levels in the postpartum period. The absence of oviductin in postmenopausal women is consistent with low circulating estrogen levels and failure of reproduction in menopausal women.

In conclusion, oviductin mRNA expression increases throughout the follicular phase, peaks at around the time of ovulation, and then reduces markedly in the luteal phase. This is consistent with a supportive role of oviductin in fertilization and possibly in early embryo development. Oestradiol has a stimulatory effect while progesterone has

an inhibitory effect on the expression. Oviductin mRNA expression is also present in early pregnancy but not in the immediate postpartum period and after menopause. Whether oviductin has a role in protection against ectopic pregnancy or in embryonic development after implantation is worthy of further investigation.

## REFERENCES


1. Boatman DE: Oviductal modulators of sperm fertilizing ability. *In* Fertilization in Mammals, BD Bavister, JM Cummins, E Roldan (eds), Norwell, MA, Serono Symposia USA, 1990, pp 223–238
2. Boatman DE: Responses of gametes to the oviductal environment. Hum Reprod 1997;12:133–149
3. Boatman DE, Magnoni GE: The role of an estrogen dependent glycoprotein, oviductin, in fertilization in the golden hamster. *In* Proceedings of the Twenty Sixth Annual Conference, Australia Society of Reproductive Biology, Brisbane, Australia, 1994, p 105
4. Anderson SH, Killian GJ: Effect of macromolecules from oviductal conditioned medium on bovine sperm motion and capacitation. Biol Reprod 1994;51:795–799
5. King RS, Killian GJ: Purification of bovine estrus-associated protein and localization of binding on sperm. Biol Reprod 1994;51:4–42
6. Boice ML, McCarthy TJ, Mavrogianis PA, Fazleabas AT, Verhage HG: Localisation of oviductal glycoproteins within the zona pellucida and perivitelline space of ovulated ova and early embryos in baboons (*Papio anubis*). Biol Reprod 1990;43:340–346
7. O'Day-Bowman MB, Mavrogianis PA, Reuter LM, Johnson DE, Fazleabas AT, Verhage HG: Association of oviduct specific glycoproteins with human and baboon (*Papio anubis*) ovarian oocytes and enhancement of human sperm binding to human hemizonae following *in vitro* incubation. Biol Reprod 1996;54:60–69
8. Hill JL, Walker SK, Brown GH, Nancarrow CD: The effects of an ovine oviductal estrus-associated glycoprotein on early embryo development. Theriogenology 1996;46:1367–1377
9. Bongso TA, Fong CY, Ng SC, Ratnam SS: Blastocyst transfer in human in vitro fertilization: The use of embryo co-culture. Cell Biol Int 1994;18:1181–1189
10. Kaufman RA, Menezo Y, Hazout A: Cocultured blastocyst cryopreservation: Experience of more than 500 transfer cycles. Fertil Steril 1995;64:1125–1129
11. Hill JL, Walker SK, Brown GH, Nancarrow CD: The effects of an estrus-associated oviductal glycoprotein on the in vitro fertilization and development of ovine oocytes matured *in vitro*. Theriogenology 1996;46:1379–1388
12. Vansteenbrugge A, Van Langendonckt A, Massip A, Dessy F: Effect of estrus-associated glycoprotein and tissue inhibitor of metalloproteinase-1 secreted by oviduct cells on *in vitro* bovine embryo development. Mol Reprod Dev 1997;46:527–534
13. Malette B, Paquette Y, Merlen Y, Bleau G: Oviductins possess chitinase- and mucin-like domains: A lead in the search for the biological function of these oviduct-specific ZP-associatins glycoproteins. Mol Reprod Dev 1995;41:384–397
14. Leveille MC, Roberts KD, Chevalier S, Shapdelaine V, Bleau G: Uptake of an oviductal antigen by the hamster zona pellucida. Biol Reprod 1987;36:227–238
15. Oliphant G, Ross PR: Demonstration of production and isoloation of three sulfated glycoproteins from the rabbit oviduct. Biol Reprod 1982;26:537–544
16. Oliphant G, Reynolds AB, Smith PF, Ross PR, Marta JS: Immunocytochemical localization and determination of hormone-induced synthesis of the sulfated oviductal glycoproteins. Biol Reprod 1984;31:165–174
17. Sutton R, Nancarrow CD, Wallace ALC, Rigby NW: Identification of an oestrus-associated glycoprotein in oviducal fluid of the sheep. J Reprod Fertil 1984;72:415–422
18. Buhi WC, Alvarez IM, Suldhipong V, Donnes-Smith MM: Identification and characterization of de-novo synthesized of porcine oviductal secretory proteins. Biol Reprod 1990;43:929–938
19. Boice ML, Geisert RD, Blair RM, Verhage HG: Identification and characterization of bovine oviductal glycoproteins synthesized at estrus. Biol Reprod 1990;43:457–565
20. Malayar JR, Hansen PJ, Buhi WC: Secretion of proteins by cultured bovine oviducts collected from estrus through early diestus. J Exp Zool 1988;238:345–353
21. Verhage HG, Fazleabas AT: The *in-vitro* synthesis of estrogen-dependent proteins by the baboon (*Papio anubis*) oviduct. *Endocrinology* 1988;123:552–558
22. Verhage HG, Fazleabas AT, Donnelly K: The *in vitro* synthesis and release of proteins by the human oviduct. Endocrinology 1988;122:1639–1645
23. Roux E, Kan FWK: Stage-specific immunolabelling for oviductin in the secretory granules of the oviductal epithelium of the golden hamster during the estrous cycle. Anat Rec 1995;241:369–376
24. Komiya H, Onuma T, Hiroi M, Araki Y: In situ localization of messenger ribonucleic acid for an oviduct-specific glycoprotein during various hormonal conditions in the golden hamster. Biol Reprod 1996;55:1107–1118
25. Roux E, Bleau G, Kan FWK: Fate of hamster oviductin in the oviduct and uterus during early gestation. Mol Reprod Dev 1997;46:306–376
26. Buhi WC: Cyclic changes in the oviduct during fertilization and early cleavage-stage embryonic development. Arch Anim Breeding 1996;39:15–25
27. Geisert RD, Buhi SC, Fields M: Synthesis and release of polypeptides by bovine oviductal tissue in culture. Biol Reprod 1987;36(Suppl):84
28. Arias EB, Verhage HG, Jaffe RC: Complementary deoxyribonucleic acid cloning and molecular characterization of an estrogen-dependent human oviductal glycoprotein. Biol Reprod 1994;51:685–694
29. Sun T, Lei ZM, Rao CV: A novel regulation of the oviductal glycoprotein gene expression by luteinizing hormone in bovine tubal epithelial cells. Molec Cell Endocrin 1997;131:97–108
30. Briton-Jones C, Lok IH, Yuen PM, Chiu TTY, Cheung LP, Haines C: Regulation of human oviductin mRNA expression in-vivo. Fertil Steril 2001;75:942–946
31. Soutar RL, Dillon J, Ralston SH: Control genes for reverse-transcription-polymerase chain reaction: A comparison of beta-actin and glyceraldehyde phosphate dehydrogenase. Br J Haematol 1997;97:247–248

32. Jaffe RC, Arias EB, O'Day-Bowman MB, Donnelly KM, Mavrogianis PA, Verhage HG: Regional distribution and hormonal control of estrogen-dependent oviduct-specific glycoprotein messenger ribonucleic acid in the baboon (*Papio anubis*). Biol Reprod 1996;55:421–426
33. Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual, 2nd edn. New York, Cold Spring Harbour Laboratory Press, 1989
34. Verhage HG, Mavrogianis PA, Boomsma RA, Schmidt A, Brenner RM, Slayden OV: Immunologic and molecular characterization of an estrogen-dependent glycoprotein in the Rhesus (*Macaca mulatta*) oviduct. Biol Reprod 1997;57:525–531
35. Lapensee L, Paquette Y, Bleau G: Allelic polymorphism and chromosomal localization of the human oviductin gene (MUC9). Fertil Steril 1997;68:702–708
36. Paquette Y, Merlen Y, Malette B, Bleau G: Allelic polymorphism in the hamster oviductin gene is due to a variable number of mucin-like tandem repeats. Mol Reprod Dev 1995;42:388–396
37. Lagow E, DeSouza MM, Carson DD: Mammalian reproductive tract mucins. Hum Reprod Update 1999;5:280–292
38. Malette B, Bleau G: Biochemical characterization of hamster oviductin as a sulphated zona pellucida-binding glycoprotein. Biochem J 1993;295:437–445