Special Issue

Bovine oviductal epithelial cells: their cell culture and applications in studies for reproductive biology

Hiroyuki Abe & Hiroyoshi Hoshi

Research Institute for the Functional Peptides, Shimojo, Yamagata 990, Japan

Key words: oviductal epithelium, embryo, sperm, co-culture, secretion, reproduction

Abstract

Epithelial cells of the mammalian oviduct play an important role in reproductive and developmental events that occur there. Oviductal epithelial cells from several mammalian species can be isolated and cultured in serum or serum-free medium *in vitro* and cell culture of bovine oviductal epithelial cells (BOEC) has been described by many investigators. Cultured BOEC show a wide variety of secretory activities and these secretory factors may influence early embryonic development or sperm function. Monolayer cultures of BOEC have been widely used for *in vitro* co-culture of bovine preimplantation embryos. The use of BOEC co-culture systems has improved embryonic development in nearly all the studies conducted. In addition, interaction of bovine spermatozoa with BOEC, in a similar manner to that observed for spermatozoa *in vivo*, induced specific changes in sperm capacitation and consequently improved the fertilizing capacity of bovine spermatozoa *in vitro*. Thus co-culture systems with BOEC may not only offer an excellent model for studying the mechanisms of capacitation and acrosome reaction of bovine spermatozoa but also provide a useful tool for the improvement of embryo development *in vitro*.

Introduction

The oviduct (uterine tube and Fallopian tube) of mammals is a highly specialized structure and it assumes one of the most fundamental roles in the reproductive process. The mammalian oviduct functions as more than a simple conduit for the transport of ovulated ova, spermatozoa or developing embryos between ovary and uterus. The functional oviduct is an active organ that maintains and modulates a dynamic fluid-filled milieu. Tubal (oviductal) fluid provides a suitable environment for sperm capacitation, fertilization and early embryonic development.

The epithelium of the oviduct consists of ciliated and secretory cells. The ciliated cells play an important role in the transport of oocytes and embryos (Odor and Blandau, 1973) and possibly in the regulation of sperm progression (Hunter *et al.*, 1991), while the secretory cells produce and release specific secretory materials (Oliphant, 1986). Together with a selective transudate of serum (Leese, 1988), these secretions form the

oviductal fluid. Some of these secretory products associate with the gametes and/or the embryo and may play important roles in embryonic development and sperm function (Hunter, 1994; Malette *et al.*, 1995; Gandolfi, 1995). It has been reported in some mammals, that *in vitro* development of embryos is improved by co-culture with oviductal cells or with the conditioned medium of these cells (Thibodeaux and Godke, 1992). Therefore, it seems likely that oviductal epithelial cells are deeply involved in the reproductive and developmental events that occur in the oviduct.

Oviductal epithelial cells of several mammalian species have been isolated and cultured *in vitro* (Boulant and Greig, 1973; Ouhibi *et al.*, 1989; Bongso *et al.*, 1989; Takeuchi *et al.*, 1991). These cells have been popularly used as culture support for embryonic development either in co-culture systems or for conditioning of embryo culture media (for review, see Thibodeaux and Godke, 1992). Co-culture systems using oviductal epithelial cell monolayers have been shown to alleviate developmental blocks in the embryo and increase

the rate of blastocyst formation during extended culture periods *in vitro* (for reviews, see Rexroad, 1989; Bongso *et al.*, 1990; Thibodeaux and Godke, 1992).

In the bovine species, oviductal epithelial cell cultures have been reported by several investigators (Witkowska, 1976; Joshi, 1988; Hishinuma *et al.*, 1989; Hoshi *et al.*, 1992). BOEC co-culture enhanced bovine embryos developmental rates and/or pregnancy rates (Eyestone and First, 1989; Ellington *et al.*, 1990a). Recent studies demonstrated that cultured BOEC might also affect sperm functions, such as motility, capacitation and fertilization (Pollard *et al.*, 1991; Ellington *et al.*, 1991; Chian and Sirard, 1995). The co-culture system with BOEC could be a useful tool to investigate the mechanisms of reproductive events that occur in the oviduct. The following review focuses on the procedures for cell culture methodology of BOEC and discusses the application of these cells in studies for reproductive biology.

Isolation and cell culture of BOEC

BOEC cultures were first described by Witkowska (1976). Subsequently, isolation and culture of BOEC have been reported by other investigators (Gandolfi and Moor, 1987; Joshi, 1988; Eyestone and First, 1989; Hishinuma *et al.*, 1989; Hoshi *et al.*, 1992; Walter, 1995). The culture procedures for BOEC vary from laboratory to laboratory (Table 1). This review presents a general procedure for isolation and culture of BOEC previously published by Hoshi *et al.* (1992) and a review of other recent advances in cell culture of BOEC.

Bovine oviducts were obtained from slaughtered cows that were judged to be in the non-luteal stage of the estrous cycle based on the absence of a corpus luteum and presence of a dominant follicle. The oviducts were dissected from the uterus and were transported to the laboratory at 30–35 °C. All procedures took place within 2 h of harvesting the tissue from the cows. The oviducts from ampulla to ampullar-isthmic junction (AIJ) were saved for the study. They were freed from the associated mesentery, major blood vessels and fat and washed in Ca^{2+} , Mg^{2+} -free phosphate buffered saline (PBS) to remove the blood cells and tubal fluid and then rapidly rinsed in 70% ethanol for disinfection.

Two main procedures have been employed for the isolation of BOEC (Table 1). Cell isolation has been done by enzymatic methods using trypsin (Thibodeaux

and Godke, 1992; Walter, 1995), collagenase (Gandolfi and Moor, 1987; Joshi, 1988) or trypsin plus other enzymes (Eyestone and First, 1989; Hishinuma *et al.*, 1989). The other isolation method consists of mechanical scraping of the oviductal epithelium with a glass slide or scalpel (Witkowska, 1976; Eyestone and First, 1989; Eyestone *et al.*, 1991; Voelkel and Hu, 1992; Xu *et al.*, 1992; van Langendonck *et al.*, 1995) or squeezing of the oviduct with forceps (Walter, 1995).

In our technique, the lumen of the oviducts were treated with 0.1% collagenase in PBS at 37 °C in a humidified atmosphere of 95% air/5% CO_2 for 60 min. At the end of the incubation, the oviducts were flushed with PBS. The flushed cell aggregates were centrifuged twice at 2,000 rpm for 5 min. The single cells and cell aggregates were suspended in a growth medium of Dulbecco's modified Eagle's medium and Ham's F12 (DMEM:F12=1:1 mixture) and 10% fetal calf serum (FCS). The cells were incubated at 37 °C in a humidified atmosphere of 95% air/5% CO_2 and the growth medium was changed every 3 days until the cultures became confluent.

The isolated cells were highly viable and initially contained both ciliated and secretory cells. These cells in primary culture showed a typical epithelial morphology with highly packed polygonal cells. Ciliated cells were recognized by an active movement of cilia for the first several days of primary culture, but these cells disappeared in the secondary culture. Similar findings have been observed by several other investigators (Joshi, 1988, 1995; Hishinuma *et al.*, 1989; van Langendonck *et al.*, 1995; Walter, 1995).

Subsequent subcultures of BOEC exhibited typical epithelial morphology similar to those of the primary cultures (Figure 1a). Cultured BOEC were characterized by immunocytological staining for intermediate filament proteins. Most of the cells strongly cross-reacted with anti-cytokeratin antibody, indicating the cells to be of epithelial origin (Figure 1b). This result is consistent with the other reports (Joshi, 1988; Ouhibi *et al.*, 1989; van Langendonck *et al.*, 1995; Walter, 1995). A large number of BOEC were also positive for vimentin, but were negative for desmin. This result suggests that most of cultured BOEC co-expressed both cytokeratin and vimentin proteins. Such co-expression has also been observed in serial cultures of human oviductal epithelial cells (Henriksen *et al.*, 1990).

Previous studies have shown BOEC require serum for optimum cell attachment and proliferation (Ouhibi

Table 1. Procedures for isolation and culture of bovine oviductal epithelial cells (BOEC)

Investigators	Isolation methods	Oviductal regions	Medium	Serum	Supplement	Substrates
Witkoska (1976)	Mechanical	Isthmus	Parker-199	CS ¹ (15%)	–	Cover-glass
Witkoska (1979a, b)	Mechanical	Ampulla & Isthmus	Parker-199	CS (15%)	Hormones (E ₂ , P ⁵ , T ⁶ , LH ⁷)	Cover-glass
Joshi (1988)	Enzymatical	Ampulla & Isthmus	DMEM/F12	FCS ² (10%) or horse serum	Insulin, Transferrin, Selenium	Plastic dish
Joshi (1991)	Enzymatical	Ampulla & Isthmus	DMEM/F12	Horse serum (10%)	Insulin, Transferrin, Selenium	Matrigel
Eyestone & First (1989)	Enzymatical & Mechanical	Whole	Ham's F10 or TCM-199	FCS (10%)	–	Plastic plate
Hishinuma <i>et al.</i> (1989)	Enzymatical	Ampulla & Isthmus	DMEM/F12	CS (10%)	–	Plastic dish
Ouhibi <i>et al.</i> (1989)	Enzymatical	–	MB2 ³	FCS (7.5%)	Lactalbumin Hydrolysate Serotonin	Plastic dish
Ouhibi <i>et al.</i> (1991)	Enzymatical	Fetal oviduct	MB2	FCS (7.5%)	Lactalbumin Hydrolysate Serotonin	Plastic dish
Ellington <i>et al.</i> (1990)	Enzymatical	–	Ham's F10	FCS (10%)	Insulin, Transferrin, EGF Selenium	Plastic dish
Schmidt <i>et al.</i> (1990)	Mechanical	Ampulla & Isthmus	RPMI-1640	FCS (10%)	–	Plastic flask
Hoshi <i>et al.</i> (1992)	Enzymatical	Ampulla & AIJ ⁴	DMEM/F12	FCS (2–10%)	Growth factors (EGF, TGF- β etc.)	Plastic dish (collagen-coated)
Thibodeaux <i>et al.</i> (1992a)	Enzymatical	–	TCM-199 CMRL-1066 MB2, Ham's F12	FCS (10%)	Serotonin	Plastic flask
Tiemann and Hansen (1995)	Enzymatical & Mechanical	Whole	MEM	FCS (10%)	–	Plastic dish (Matrigel-coated)
van Langendonck <i>et al.</i> (1995)	Mechanical	Ampulla & AIJ	TCM-199	FCS (10%) or serum-free	–	Plastic flask
Walter (1995)	Enzymatical	Ampulla & Isthmus	TCM-199	FCS (10%)	–	Cover-glass or Poly carbonate membrane

¹ Calf Serum, ² Fetal Calf Serum, ³ Menezo B2 Medium (Menezo, 1976), ⁴ Ampullar-Isthmic Junction, ⁵ Progesterone, ⁶ Testosterone, ⁷ Luteinizing Hormone.

et al., 1989). However, recently, van Langendonck *et al.* (1995) reported that BOEC could proliferate in serum-free medium for as long as one week. The typical histoarchitecture of BOEC monolayers could be preserved for up to 7 days and metabolic activity was apparent for at least 6 days. These observations suggest that BOEC could be maintained in a functional state in serum-free culture for up to 7 days, although electron microscopy showed some degenerative signs, such as the less of secretory organelles (Golgi appara-

tus and rough endoplasmic reticulum) and accumulation of intermediate filaments, in the absence of FCS.

Few studies have reported on the growth requirements of oviductal epithelial cell cultures with regard to hormones and growth factors (Ouhibi *et al.*, 1989; Takeuchi *et al.*, 1991), but the growth regulatory effects of some growth factors on BOEC proliferation were examined in detail by Hoshi *et al.* (1992). Epidermal growth factor (EGF) stimulated the proliferation of BOEC in a dose-dependent manner when cultured

Figure 1. Bovine oviductal epithelial cells (BOEC) in monolayer culture (passage-5). (a) Phase-contrast microscopy of BOEC shows a typical epithelial morphology with highly packed polygonal cells. (b) Indirect immunofluorescent staining of anti-cytokeratin in the same culture. Bars = 50 μ m.

Figure 2. Effect of epidermal growth factor (EGF) on cell proliferation of BOEC. Cells (5×10^3) were inoculated into Type I collagen-precoated 24-well culture plates. Cells were incubated for 5 days at 37 °C in a humidified atmosphere of 5% CO₂/95% air and then counted by Coulter Counter. Control medium contains DMEM/F12 (1:1) and 2% fetal calf serum (FCS). Values are expressed as the mean $\pm$ SD of triplicate cultures.

in a low serum medium (Figure 2). By contrast, transforming growth factor- β_1 (TGF- β_1) strongly inhibited the growth of BOEC. The growth regulatory effects

Figure 3. Effect of fetal calf serum (FCS), epidermal growth factor (EGF), transforming growth factor- β_1 (TGF- β_1) and their combinations on the proliferation of BOEC. Control medium contains DMEM/F12 (1:1) medium alone. Concentrations of FCS (2%), EGF (10 ng/ml) and TGF- β_1 (1 ng/ml) were tested. Values are expressed as the mean $\pm$ SD of triplicate cultures.

of FCS, EGF, and TGF- β_1 on the cell proliferation of BOEC are shown in Figure 3. EGF stimulated a 42% increase in cell number in the FCS-containing medi-

um, while TGF- β_1 showed severe growth inhibition of BOEC stimulated by EGF, FCS or their combinations. Bovine insulin, bovine transferrin, estradiol-17 β (E₂), progesterone (P), human follicle stimulating hormone (FSH), human leuteinizing hormone (LH) and bovine serum albumin (BSA) did not show any proliferative or inhibitory effects on BOEC in this culture system and EGF or TGF- β_1 by itself had no significant effect on BOEC proliferation in nutrient medium alone (DMEM/F12).

Previous studies by Witkowska (1979a, b) demonstrated that E₂, P, testosterone (T) and LH modulated enzyme activities of BOEC *in vitro*. E₂ and T increased the activity of Δ^5 3 β -hydroxysteroid dehydrogenase, an enzyme which participates in the biosynthesis of steroids in cultured BOEC and also caused an increase in lipid content (Witkowska, 1979a). BOEC also responded to P and LH with decreased activity of this enzyme and accumulation of lipids into the cells. Application of E₂, P and LH to the medium caused increases of the activity of alkaline phosphatase and the amount of glycogen in cultured BOEC (Witkowska, 1979b).

³H-thymidine incorporation in cultured BOEC can be regulated by ovarian steroid hormones and growth factors. Kamwanja and Hansen (1993) reported that E₂ increased ³H-thymidine incorporation into BOEC *in vitro* while P inhibited DNA synthesis and was antagonistic to the stimulatory effects of E₂. Moreover, it was demonstrated that EGF, insulin-like growth factor-I (IGF-I) and insulin-like growth factor-II (IGF-II) also stimulated ³H-thymidine incorporation in cultured BOEC (Tiemann and Hansen, 1995).

The cell attachment efficiency of oviductal epithelial cells is generally very low because of the presence of ciliary activity in the aggregated cells introduced into the cultures. The plating efficiency of human oviductal epithelial cells is as low as 10 to 20% of the isolated cells in the absence of extracellular matrix proteins (Henriksen *et al.*, 1990). Extracellular matrix (ECM) proteins might be important to optimize the cell attachment and spreading and also the subsequent growth of BOEC. The effect of various ECM proteins, such as collagens (Type I, II, IV, V), vitronectin and laminin on BOEC proliferation in a low serum medium have been examined. The attachment efficiencies were high (70 to 80%) at 24 h after cell seeding on all substrates. However, cell proliferation on Type I and II collagen substrates were superior to those on other types of collagens (Type IV and V), vitronectin, fibronectin and laminin (Figure 4). Joshi (1988, 1991) report-

Figure 4. Effect of extracellular matrix (ECM) proteins on proliferation of BOEC. Twenty-four well culture plates were exposed to various types of ECM proteins (10 μ g/ml) such as collagens (Type I, II, IV, and V), fibronectin, vitronectin and laminin in PBS for 60 min at room temperature. The BOEC (5×10^3 /well) in DMEM/F12 plus 2% FCS were seeded into those wells precoated with ECM proteins. The cells were incubated for 5 days at 37 °C in a humidified atmosphere of 5% CO₂/95% air and the cell numbers were determined. Values are expressed as the mean $\pm$ SD of triplicate cultures.

ed that isolated BOEC seeded on plastic precoated with a matrigel containing various types of ECM proteins could fully differentiate. These cells became well polarized and their cell architecture closely resembled intact epithelial cells. BOEC growing on this substratum formed a tubal structure with a distinct lumen and microvilli were arranged at the apical plasmalemma of the epithelial cells. The differentiation of secretory cells cultured on uncoated plastic dish was incomplete, based on the fact that these cells possessed very few secretory vesicles in the apical cytoplasm as compared to cells *in vivo* (Joshi, 1988). In the intact epithelium, secretory cells possess a well-developed Golgi apparatus and a large number of secretory granules (Abe *et al.*, 1993b; Eriksen *et al.*, 1994). Non-ciliated cells cultured on matrigel-coated dishes showed the presence of abundant rough endoplasmic reticulum, well-developed Golgi apparatus and numerous secretory vesicles. Moreover, BOEC cultured on matrigel respond to E₂ and P showing an incremental uptake of ³⁵S-methionine and E₂ induced de novo synthesis of a 60 kDa protein.

Application of BOEC cultures

Co-culture of embryos with BOEC

Embryos of domestic farm animal species often become arrested in development during *in vitro* culture. In bovine, development of early embryos is generally arrested at the 8- to 16-cell stage (Wright and Bondioli, 1981). Successful culture of bovine embryos through this developmental block has required the use of intermediate hosts, such as the ligated rabbit oviduct (Boland, 1984) or sheep oviduct (Sirard *et al.*, 1988). The developmental arrest of embryos observed *in vitro* is thought to be a result of inadequate culture systems. Improved culture systems for preimplantation embryos are imperative for enhancing viability of embryos and increasing pregnancy rates. Some investigators have used various somatic cells to try and overcome these developmental blocks (for reviews, see Rexroad, 1989; Bongso *et al.*, 1990; Thibodeaux and Godke, 1992). Co-culture systems have been used with farm animal embryos to increase the rate of blastocyst formation during extended culture *in vitro*. Enhancement of embryonic development has been observed on fibroblastic feeder layers (Kuzan and Wright, 1982; Voelkel *et al.*, 1985) and in co-culture with trophoblastic tissue (Heyman *et al.*, 1987). Recently, Vero cell co-culture system was shown to increase the rate of mouse embryo viability (Lai *et al.*, 1992). The most appropriate choice of somatic cells for co-culturing would seem to be oviductal cells, since embryos in the early stages of development are located in the oviduct *in vivo* and *in vitro* oviductal cells were shown to give better support to the development of one-cell mouse embryos than uterine cells (Sakkas and Trounson, 1990).

Monolayer cultures of BOEC have been used profitably for *in vitro* development of bovine embryos (Eyestone and First, 1989; Ellington *et al.*, 1990b, 1991). Eyestone and First (1989) reported that developmental rates of bovine embryos in co-culture with BOEC and in a BOEC conditioned medium were higher than that in medium alone. Furthermore, embryos transferred to recipient animals following co-culture or incubation in conditioned medium resulted in a higher pregnancy rate.

Ellington *et al.* (1990b) compared *in vitro*-derived bovine embryos co-cultured with BOEC in a simple medium (CZB) for 5 days with that of *in vivo*-derived embryos (40 to 48 h post coitum). The developmental rate and number of nuclei per embryo did not differ between *in vivo*-derived embryos and *in vitro*-

derived embryos, suggesting that BOEC co-culture systems in a simple medium can sustain normal development of bovine embryos. Their subsequent study showed that fresh BOEC monolayers in a chemically defined medium (glucose- and serum-free CZB medium) can provide a significant benefit as assayed by a high yield of early bovine embryos (Ellington *et al.*, 1990a). In addition, they compared the developmental capacity of bovine 1–2-cell embryos in BOEC co-culture in a glucose- and serum-free CZB medium with those surgically transferred to ligated oviducts of rabbit (Ellington *et al.*, 1990c). The *in vitro* co-culture system was equivalent to the rabbit oviducts in promoting embryo development for all characteristics of embryo quality. The pregnancy rate for embryos co-cultured with BOEC was 57% compared with 58% for similar embryos transferred to rabbit oviducts. These results strongly suggest that bovine zygotes in culture with BOEC in a simple medium can develop into high quality transferrable embryos which can attain normal pregnancy rates. Other studies have also reported that BOEC co-cultures promote the developmental rates or improved the quality of *in vitro* matured and fertilized (IVM/IVF) bovine embryos (Fukui, 1989; Kitiyanant *et al.*, 1989; Aoyagi *et al.*, 1990; Shamsuddin *et al.*, 1993; Rorie *et al.*, 1994) and IVM/IVF-derived reconstructed (chimeric) bovine embryos (Kinis *et al.*, 1990). BOEC monolayers can also support the *in vitro* development of early stage embryos of other animal species (Ouhibi *et al.*, 1990; Wiemer *et al.*, 1993).

The beneficial effects of co-culture of embryos with oviductal epithelial cells have not yet been fully elucidated. However, oviductal epithelial cells probably support preimplantation embryo development by more than one mechanism. The conditioned medium of oviductal cells has been shown to be as effective as cell co-culture in supporting the development of embryos (Rexroad and Powell, 1988; Eyestone and First, 1989; Eyestone *et al.*, 1991; Mermillod *et al.*, 1993). McCaffrey *et al.* (1991) reported that physical separation by a microporous membrane inserted between BOEC monolayers and embryos did not inhibit increased developmental rates to morulae or blastocysts from 1–4 cell stage bovine embryos, suggesting that direct physical contact between embryos and oviductal cells may not be required to ensure a high rate of embryonic development. These findings provided a basis for the hypothesis that cultured BOEC produce and secrete certain soluble factors which support embryonic development (Gandolfi *et al.*, 1989; Nancarrow and Hill, 1994). Little is known about the

specific products of BOEC that are responsible for the embryonic development in most species studied. However, it has been suggested that oviductal epithelial cells secrete embryotrophic factors such as oviduct-specific glycoproteins. It has been demonstrated in sheep oviduct-specific glycoprotein influences *in vitro* development of embryos by increasing the proportion of blastocyst formation from cleaved embryos, the mean nuclei numbers of blastocysts and the time taken for blastocyst formation (Nancarrow and Hill, 1994). A recent study by Satoh *et al.* (1994) suggested the existence of embryogenesis-stimulating activities in BOEC conditioned medium.

Another possibility, in addition to the secretion of embryotrophic substances, is that the oviductal cells are capable of removing or reducing toxic components of culture medium that are detrimental to the embryo development (for review, see Bavister, 1988). It is commonly assumed that feeder layers can remove toxins (especially heavy metal ions) from the culture medium (Abramczuk *et al.*, 1977). In addition to secreting embryotrophic substances and/or removing embryotoxic substances from the medium, it is possible that oviductal epithelial cells may regulate oxygen tension in the immediate vicinity of embryo. Nagao *et al.* (1994) examined the interaction of oxygen concentration and the presence of BOEC on the development of bovine embryos *in vitro* matured and fertilized (IVM/IVF) oocytes in a protein-free medium. When embryos were cultured in the presence or absence of BOEC under 5% CO₂ in air (20% O₂) or 5% CO₂/5% O₂/90% N₂ (5% O₂), the efficiency of blastocyst formation without BOEC at 5% O₂ was almost same as that with BOEC at 20% O₂ (30 vs 33%), but blastocyst formation at 5% O₂ with BOEC was significantly suppressed (4%). These results suggest that bovine IVM/IVF embryos can efficiently develop to the blastocyst stage in a protein-free medium without BOEC at low oxygen concentration (5%) and that the beneficial role of BOEC for embryonic development may be due to reducing oxygen concentration in the culture environment.

Co-culture of spermatozoa with BOEC

Mammalian oviducts can be divided into several parts on the basis of physiological function. Biological events vary among the regions of the oviduct and oviductal epithelial cells show regional variations in cytological and functional aspects (for review, see Abe, 1996).

The oviduct provides various types of physiological support to spermatozoa. Results from co-culture experiments and from isolated oviduct cells strongly suggest that interactions between oviductal epithelial cells and spermatozoa play an important role in various sperm functions. There is some evidence from several species that the caudal isthmus of the oviduct serves as a reservoir for spermatozoa during the estrous period (Hunter and Wilmot, 1983; Smith *et al.*, 1987). Contact between spermatozoa and oviductal epithelial cells is considered to be beneficial for sperm survival and may also be necessary for sperm capacitation (Smith and Yanagimachi, 1990, 1991). In bovine, approximately 8 h may be required after insemination for sufficient spermatozoa to accumulate in the isthmic reservoir in order to ensure a high fertilization rate (Wilmot and Hunter, 1984). Since the period from onset of estrus to completion of ovulation may be as long as 30 h in bovine, spermatozoa must retain their viability and fertilizing capacity for more than 22 h in the isthmus (Hunter and Wilmot, 1984). Therefore, some mechanisms must exist which extend the life span of spermatozoa in the isthmus of the oviduct.

Technical advances in the cell culture of BOEC have made it possible to examine the interaction between spermatozoa and BOEC and the mechanisms involved in sperm storage in the bovine oviduct. Previous studies designed to evaluate interactions of spermatozoa and oviductal epithelial cells have relied mostly on indirect methods such as retrieval of spermatozoa by luminal flush (Hawk, 1987) or fixation and sectioning of oviduct post coitum (Hunter *et al.*, 1987). When bovine spermatozoa are co-cultured with BOEC monolayers, the spermatozoa attach to the BOEC within 1 h and the spermatozoa tend to attach in the intercellular troughs and amorphous mucus-like strands and granules are observed in the acrosomal region of many spermatozoa (Ellington *et al.*, 1991). Similar findings have been described by Suzuki and Foote (1995) and observed in our recent work (Figure 5). Pollard *et al.* (1991) have described the binding of bovine spermatozoa co-cultured with BOEC monolayers grown on an ECM. In this co-culture system, spermatozoa were bound to the rostral portion of the intact acrosome on the apical surface of polarized epithelial cells.

Various physiological changes in bovine spermatozoa have been induced in the presence of BOEC co-culture. The effects of BOEC, derived from ampullar and isthmic segments of oviduct, on penetration of bovine spermatozoa through the oocyte and on sperm motility have been examined in a co-culture system *in*

Figure 5. Scanning electron micrograph of bovine spermatozoa in BOEC co-culture. Spermatozoa attach to BOEC (asterisk) in intercellular troughs and mucus-like strands are present in the acrosomal regions of some spermatozoa. Bar = 10 μ m.

vitro (Chian and Sirard, 1995). Rates of sperm penetration through oocytes in a co-culture with BOEC from the ampullar segment were higher than that of spermatozoa in a co-culture with those from the isthmic segment. However, the motility of unattached spermatozoa in cultures of isthmic cells was higher than that in cultures of ampullar cells. These results suggest that sperm capacitation might be enhanced by attachment of spermatozoa to the ampullar epithelial cells and that the isthmic epithelial cells are important for maintenance of sperm motility.

Similarly, Pollard *et al.* (1991) examined maintenance of motility and the fertilizing capacity of bovine spermatozoa that had been co-cultured with BOEC on either tissue culture plastic (nonpolarizing) or Matrigel-coated Millicell (polarizing) substrata. Sperm motility was maintained for 48 h in spermatozoa that bound to cells, but to a much greater extent with polarized cells (38.4%) than with nonpolarized cells (0.8%). Moreover, spermatozoa incubated with polarized BOEC showed hyperactive motility. Fertilizing capacity was well maintained in spermatozoa cultured with BOEC, but not in spermatozoa incubated in medium alone or with tracheal cells. This study suggests that BOEC may not only store spermatozoa but may also maintain sperm motility and fertilizing capacity *in vivo*.

A study by Ellington *et al.* (1991) demonstrated additional detailed changes of bovine spermatozoa in the BOEC co-culture system. Spermatozoa attached to BOEC monolayer within 1 h of co-culture and attached spermatozoa showed vigorous tail motion. Spermatozoa unattached to BOEC monolayer had more acrosomal membrane loss, showed hyperactive motion and had an overall decrease in motility as compared to spermatozoa in BOEC-conditioned medium or control medium. Electron microscopic observations showed intimate sperm head-BOEC contact with microvilli entrapment of the spermatozoa. This investigation proposed that bovine spermatozoa in BOEC co-culture system interacted with the BOEC in a manner similar to that seen *in vivo* and specific changes in sperm capacitation were stimulated by this interaction.

Secretory products from BOEC in culture

Several proteins and glycoproteins are known to be synthesized and secreted from oviductal epithelial cells of most mammalian species studied (for reviews, see Hunter, 1994; Gandolfi, 1995; Malette *et al.*, 1995). Some of the macromolecules have been characterized biochemically and immunocytochemically and attempts have been made to clarify their biological function. In bovine, several nonserum proteins or glycoproteins have been identified in the supernatants of

oviducts in explant culture (Boice *et al.*, 1990; Gerena and Killian, 1990). The exact functional role of these oviductal secretions remains still unclear. However, results of a series of studies on cultured BOEC suggest possible functions for oviductal secretions.

It has been reported in sheep (Gutierrez *et al.*, 1993) and bovine (Ijaz *et al.*, 1994) that incubation of spermatozoa with conditioned media of oviductal epithelial cells, as well as co-culture with the oviductal epithelial cells, maintains their viability and motility for a long period of time. The results described by Gutierrez *et al.* (1993) also suggested that the two main characteristics of spermatozoa (namely, viability/motility and capacitation/acrosome reaction) might be independently regulated by oviductal epithelial cells. Intact epithelial cells can support both functions of spermatozoa but conditioned medium from oviductal epithelial cells seems to only support the survival and motility of spermatozoa. A partial biochemical characterization of bovine oviductal secretory product(s) indicated that heat-labile and nondialyzable (>8 kDa) factors may be involved in a prolongation of sperm motility (Ijaz *et al.*, 1994). Further study demonstrated that the secretion of the sperm motility factor(s) into the conditioned media of BOEC monolayers was modulated by many factors such as the oviductal region (ampulla and isthmus), the stage of the estrous cycle during which the BOEC were derived and the presence of steroid hormones in the culture media (Lapointe *et al.*, 1995). Conditioned medium from BOEC isolated from the isthmus region could maintain sperm motility *in vitro* under the influence of E₂, whereas conditioned medium from BOEC isolated from the ampullar region maintained sperm motility under the influence of P. This study suggests that maintenance of sperm motility by secretion(s) from BOEC is under hormonal control and the activity of sperm motility factor(s) is regulated under appropriate steroidal conditions. In addition, this finding suggests that there are regional differences in the hormonal responses of BOEC.

Similarly, Abe *et al.* (1995b) demonstrated that sperm viability and motility could be maintained by conditioned medium of ampullar-derived BOEC at the follicular phase of the estrous cycle, but the conditioned medium of bovine fetal artery endothelial cells had no significant effect on sperm viability and motility. Other studies demonstrated that bovine oviduct-specific glycoprotein (BOGP) was secreted by oviductal epithelial cells of ampullar and fimbrial segments at the follicular phase of the estrous cycle (Abe *et al.*, 1993). It was also shown that purified BOGP

(95 kDa) effectively improved the viability and motility of bovine spermatozoa cultured *in vitro*, indicating that BOGP might be a potent factor for the maintenance of sperm viability and motility (Abe *et al.*, 1995a; Satoh *et al.*, 1995).

The isthmus segment of the oviduct is considered to be a primary site for sperm capacitation (Hunter and Nichol, 1988). Parrish *et al.* (1988) and McNutt and Killian (1991) demonstrated that bovine oviductal fluid induced capacitation of spermatozoa and sustained their motility *in vitro*. Glycosaminoglycans have been suggested to be a capacitation factor in oviductal fluid at estrus (Parrish *et al.*, 1989). Anderson and Killian (1994) reported the effects of macromolecules from conditioned medium of bovine oviductal explants on the motility and capacitation of spermatozoa. Conditioned medium of explant culture from the isthmus at estrus caused capacitation of spermatozoa significantly more than that from the ampulla. This study suggests that secretory proteins, glycosaminoglycans and proteoglycans from the conditioned medium of oviduct may be possible capacitation factors for bovine spermatozoa. Chian *et al.* (1995) reported that BOEC monolayers secreted a capacitating factor into the culture medium and the stimulatory effect on sperm capacitation varied depending upon the stage of the estrous cycle when the BOEC were harvested. Furthermore, adding estradiol stimulated the BOEC monolayers to secrete the capacitating factor *in vitro*.

Other studies have demonstrated that droplets composed of neutral lipids are present in the epithelium of bovine oviduct (Henault and Killian, 1993a). Explants of bovine oviducts could synthesize neutral and polar lipids and also release de novo synthesized cholesterol into the culture medium (Henault and Killian, 1993b). It was postulated that lipids secreted by BOEC may be involved in fertilization and embryonic development by influencing membrane lipid contents or by providing a source of energy. In particular, it is possible that esterified cholesterol and phospholipids in the isthmus may play a role in facilitating the attachment of spermatozoa to BOEC or in the storage/survival of spermatozoa.

Endothelin is a well known vasoconstrictor substance derived from vascular endothelial cells. In the reproductive system, endometrial stromal and glandular epithelial cells of uterus produce endothelin (Economos *et al.*, 1992). Rosselli *et al.* (1994) reported the production of immunoreactive-endothelin by BOEC in serum-free culture *in vitro*. Significant amounts of endothelin were detected in the condi-

tioned medium of BOEC after 48 h in culture, without any changes in the total protein content or the number of cells. Moreover, pretreatment of BOEC with calcium ionophore A23187 increased the production of endothelin. It was speculated that endothelin produced by BOEC could influence the transport of gametes and embryos either by acting on the muscle coat of the oviduct or by interacting with the gamete surface.

Certain growth factors may also be involved in the regulation of early development of bovine embryos. Larson *et al.* (1992b) demonstrated that TGF- β_1 and basic fibroblast growth factor (bFGF) act synergistically to promote the development of bovine embryos beyond the "8-cell block" which occurs *in vitro*. In addition, they examined the effects of platelet derived growth factor (PDGF), TGF- α , and TGF- β_1 on the development of bovine embryos cultured *in vitro* during the transition from maternal to embryonic gene expression (Larson *et al.*, 1992a). This study suggests that PDGF promotes development of bovine embryos beyond the 16-cell stage, whereas TGF- α and bFGF potentially affect the blastulation of 16-cell embryos during subsequent culture. Takagi *et al.* (1995) have recently shown that insulin and bFGF stimulate bovine embryonic development to the blastocyst stage when co-cultured with bovine granulosa cells in a serum-free medium. Watson *et al.* (1992) examined gene expression of several growth factors in BOEC monolayers by using reverse transcriptase-polymerase chain reaction methods. This study showed that mRNA transcripts for PDGF-A and bFGF are present in cultured BOEC *in vitro*. More recently, it has been demonstrated that the intact bovine oviduct synthesizes transcripts for PDGF-B and bFGF in the lamina epithelialis and lamina propria, respectively, whereas IGF-I mRNA is not detected (Viuff *et al.*, 1995). These findings provide evidence that BOEC might be important in supplying growth factors for embryonic development both *in vivo* and *in vitro*.

Conclusion

Recent advances in tissue culture techniques have allowed long-term cultivation of BOEC *in vitro*. Pure cultures of these cells can be isolated by enzymatic or mechanical methods. Serum and growth factors are essential for optimum cell attachment and proliferation of BOEC. Monolayers of BOEC cultured on ECM appear to maintain a histoarchitecture similar to

intact oviductal epithelium and also maintain secretory activity *in vitro*.

Cultured BOEC are popularly used for *in vitro* co-culture of bovine preimplantation embryos and spermatozoa. The use of BOEC co-culture systems has improved the *in vitro* development of embryos in nearly all the studies conducted. Thus, BOEC cultures are a useful tool for improving procedures for bovine embryo production *in vitro*. The advances in embryo production encompass a wide range of laboratory techniques, such as micromanipulation, gene insertion, cryopreservation and nuclear transfer.

Bovine spermatozoa co-cultured with BOEC appear to interact with epithelial cells *in vitro*, in a manner similar to that described for spermatozoa *in vivo*. Bovine spermatozoa co-cultured with BOEC showed specific changes in capacitation and maintained the motility and fertilizing capacity for a long period of time. The co-culture system using BOEC and spermatozoa offers excellent possibilities for studying the mechanisms of capacitation, acrosome reaction, and subsequent fertilization. This system provides also a unique *in vitro* model to investigate the biological significance of cell to cell interactions between gametes (eg. spermatozoa) and somatic cells (oviductal epithelial cells) in the reproductive tract.

Secretory products of BOEC may influence early embryonic development in addition to sperm functions. Since BOEC appear to secrete many functional substances, such as specific glycoproteins and growth factors, further advances in culture techniques for BOEC may enhance the investigation of the critical functions of secretory products from BOEC and their role in the reproductive events.

Acknowledgments

The authors thank Drs. Takeshi Satoh, Yutaka Sendai, Takehiro Itoh, and Shoko Yamashita for their kind and helpful collaboration. The research in our institute has been funded by a grant from the Research Fund of Bio-Oriented Technology Research Advancement Institution (BRAIN) of Japan.

References

- Abe H (1996) The mammalian oviductal epithelium: regional variations in cytological and functional aspects of the oviductal secretory cells. *Histol Histopathol* 11: 743-768.

- Abe H, Numazawa C, Abe M, Onodera M and Katsumi A (1993) Immunocytochemical localization of oviduct-specific glycoproteins in the bovine oviductal epithelium at follicular and luteal phases. *Cell Tissue Res* 247: 41–47.
- Abe H, Sendai Y, Satoh T and Hoshi H (1995a) Bovine oviduct-specific glycoprotein is a potent factor for the maintenance of the viability and motility of bovine spermatozoa *in vitro*. *Mol Reprod Dev* 42: 226–232.
- Abe H, Sendai Y, Satoh T and Hoshi H (1995b) Secretory products of bovine oviductal epithelial cells support the viability and motility of bovine spermatozoa in culture *in vitro*. *J Exp Zool* 272: 54–61.
- Abramczuk J, Solter D and Koprowski H (1977) The beneficial effect of EDTA on development of mouse one-cell embryos in chemically defined medium. *Dev Biol* 61: 378–383.
- Anderson SH and Killian GJ (1994) Effect of macromolecules from oviductal conditioned medium on bovine sperm motion and capacitation. *Biol Reprod* 51: 795–799.
- Aoyagi Y, Fukui Y, Iwazumi Y, Urakawa M and Ono H (1990) Effects of culture systems on development of *in vivo* fertilized bovine ova into blastocysts. *Theriogenology* 34: 749–759.
- Bavister BD (1988) Role of oviductal secretions in embryonic growth *in vivo* and *in vitro*. *Theriogenology* 29: 143–154.
- Boice ML, Geisert RD, Blair RM and Verhage HG (1990) Identification and characterization of bovine oviductal glycoproteins synthesized at estrus. *Biol Reprod* 43: 457–465.
- Boland MP (1984) Use of the rabbit oviduct as a screening tool for the viability of mammalian eggs. *Theriogenology* 21: 126–137.
- Bongso A, Chye NS, Sathananthan H, Lian NP, Rauff M and Ratnam SS (1989) Establishment of human ampullary cell cultures. *Hum Reprod* 4: 486–494.
- Bongso A, Ng S-C and Ratnam SS (1990) Co-cultures: their relevance to assisted reproduction. *Hum Reprod* 5: 893–900.
- Boullant A and Greig AS (1973) Biological characterization of a cell line derived from the pig oviduct. *In Vitro* 9: 92–102.
- Chian R-C and Sirard M-A (1995) Fertilizing ability of bovine spermatozoa cocultured with oviduct epithelial cells. *Biol Reprod* 52: 156–162.
- Chian R-C, Lapointe S and Sirard M-A (1995) Capacitation *in vitro* of bovine spermatozoa by oviduct epithelial cell monolayer conditioned medium. *Mol Reprod Dev* 42: 318–324.
- Economos K, MacDonald PC and Casey ML (1992) Endothelin-1 gene expression and protein biosynthesis in human endometrium: potential modulator of endometrial blood flow. *J Clin Endocrinol Metab* 74: 14–19.
- Ellington JE, Carney EW, Farrell PB, Simkin ME and Foote RH (1990a) Bovine 1–2-cell embryo development using a simple medium in three oviduct epithelial cell coculture systems. *Biol Reprod* 43: 97–104.
- Ellington JE, Farrell PB and Foote RH (1990b) Comparison of six-day bovine embryo development in uterine tube (oviduct) epithelial cell co-culture versus *in vivo* development in the cow. *Theriogenology* 34: 837–844.
- Ellington JE, Farrell PB, Simkin ME, Foote RH, Goldman EE and McGrath AB (1990c) Development and survival after transfer of cow embryos cultured from 1–2-cells to morulae or blastocysts in rabbit oviducts or in a simple medium with bovine oviduct epithelial cells. *J Reprod Fertil* 89: 293–299.
- Ellington JE, Padilla AW, Vredenburg WL, Dougherty EP and Foote RH (1991) Behavior of bull spermatozoa in bovine uterine tube epithelial cells co-culture: an *in vitro* model for studying the cell interactions of reproduction. *Theriogenology* 35: 977–989.
- Eriksen T, Terkelsen O, Hyttel P and Greve T (1994) Ultrastructural features of secretory cells in the bovine oviduct epithelium. *Anat Embryol* 190: 583–590.
- Eyestone WH and First NL (1989) Co-culture of early cattle embryos to the blastocyst stage with oviductal tissue or in conditioned medium. *J Reprod Fertil* 85: 715–720.
- Eyestone WH, Jones JM and First NL (1991) Some factors affecting the efficacy of oviduct tissue-conditioned medium for the culture of early bovine embryos. *J Reprod Fertil* 92: 59–64.
- Fukui Y (1989) Effects of sera and steroid hormones on development of bovine oocytes matured and fertilized *in vitro* and co-cultured with bovine oviduct epithelial cells. *J Anim Sci* 67: 1318–1323.
- Gandolfi F (1995) Functions of proteins secreted by oviduct epithelial cells. *Microsc Res Tech* 32: 1–12.
- Gandolfi F, Brevini TAL and Moor RM (1989) Effect of oviduct environment on embryonic development. *J Reprod Fertil* (suppl.) 38: 107–115.
- Gandolfi F and Moor RM (1987) Stimulation of early embryonic development in the sheep by co-culture with oviduct epithelial cells. *J Reprod Fertil* 81: 23–28.
- Gerena RL and Killian GJ (1990) Electrophoretic characterization of proteins in oviduct fluid of cows during the estrous cycle. *J Exp Zool* 256: 113–120.
- Gutierrez A, Garde J, Garcia-Artiga C and Vazquez I (1993) Ram spermatozoa cocultured with epithelial cell monolayers: an *in vitro* model for the study of capacitation and the acrosome reaction. *Mol Reprod Dev* 36: 338–345.
- Hawk HW (1987) Transport and fate of spermatozoa after insemination of cattle. *J Dairy Sci* 70: 1487–1503.
- Henault MA and Killian GJ (1993a) Neutral lipid droplets in bovine oviductal epithelium and lipid composition of epithelial cell homogenates. *J Dairy Sci* 76: 691–700.
- Henault MA and Killian GJ (1993b) Synthesis and secretion of lipids by bovine oviduct mucosal explants. *J Reprod Fertil* 98: 431–438.
- Henriksen T, Tanbo T, Abyholm T, Oppedal BR, Claussen OP and Hovig T (1990) Epithelial cells from human Fallopian tube in culture. *Hum Reprod* 5: 25–31.
- Heyman Y, Menezo Y, Chesne P, Camous S and Garnier V (1987) *In vitro* cleavage of bovine and ovine early embryos: improved development using coculture with trophoblastic vesicles. *Theriogenology* 27: 59–68.
- Hishinuma M, Takahashi Y and Kanagawa H (1989) Isolation and monolayer culture of bovine oviduct epithelial cells. *Jpn J Vet Sci* 51: 1201–1208.
- Hoshi H, Onodera M and Oikawa T (1992) Isolation, cell characterization and growth regulation of bovine oviduct epithelial cells *in vitro*. *Tiss Cult Res Comm* 11: 5–11.
- Hunter RHF (1994) Modulation of gamete and embryonic microenvironments by oviduct glycoproteins. *Mol Reprod Dev* 39: 176–181.
- Hunter RHF, Flechon B and Flechon JE (1987) Pre- and peri-ovulatory distribution of viable spermatozoa in the pig oviduct: a scanning electron microscope study. *Tissue Cell* 19: 423–436.
- Hunter RHF, Flechon B and Flechon JE (1991) Distribution, morphology and epithelial interactions of bovine spermatozoa in the oviduct before and after ovulation: a scanning electron microscope study. *Tissue Cell* 23: 641–656.
- Hunter RHF and Nichol R (1983) Transport of spermatozoa in the sheep oviduct: preovulatory sequestering of cells in the caudal isthmus. *J Exp Zool* 228: 121–128.
- Hunter RHF and Nichol R (1988) Capacitation potential of the Fallopian tube: a study involving surgical insemination and the subsequent incidence of polyspermy. *Gamete Res* 21: 255–266.
- Hunter RHF and Wilmut I (1983) The rate of functional sperm transport into the oviducts of mated cows. *Anim Reprod Sci* 5: 167–173.

- Hunter RHF and Wilmut I (1984) Sperm transport in the cow: Peri-ovulatory redistribution of viable cells within the oviduct. *Reprod Nutr Develop* 24: 597–608.
- Ijaz A, Lambert RD and Sirard M-A (1994) *In vitro*-cultured bovine granulosa and oviductal cells secrete sperm motility-maintaining factor(s). *Mol Reprod Dev* 37: 54–60.
- Joshi MS (1988) Isolation, cell culture and immunocytochemical characterization of oviduct epithelial cells of the cow. *J Reprod Fertil* 83: 249–261.
- Joshi MS (1991) Growth and differentiation of the cultured secretory cells of the cow oviduct on reconstituted basement membrane. *J Exp Zool* 260: 229–238.
- Joshi MS (1995) Isolation, cell culture and characterization of oviduct epithelial cells of the cow. *Microsc Res Tech* 31: 507–518.
- Kamwanja LA and Hansen PJ (1993) Regulation of proliferation of bovine oviductal epithelial cells by estradiol. Interactions with progesterone, interferon- τ and interferon- α . *Horm Metab Res* 25: 500–502.
- Kinis AV, E., Gordon I, Gordon A and Gallagher M (1990) Studies in the production of chimaeric cattle embryos by aggregation of blastomeres from embryos derived from oocytes matured and fertilized *in vitro*. *Theriogenology* 33: 268.
- Kitiyant Y, Thonabulsombat C, Tochara C, Sanitwongse B and Pavasuthipaisit K (1989) Co-culture of bovine embryos from oocytes matured and fertilized *in vitro* to the blastocyst stage with oviductal tissue. *J Sci Soc Thailand* 15: 251–260.
- Kuzan FB and Wright RWJ (1982) Observations on the development of bovine morulae on various cellular and non cellular substrata. *J Anim Sci* 54: 811–816.
- Lai YM, Stein DE, Soong YK, Tang YX, Grifo J, Malter HE, Talansky BE, Cohen J, Liu HC and Rosenwaks Z (1992) Evaluation of Vero cell co-culture system for mouse embryos in various media. *Hum Reprod* 7: 276–280.
- Lapointe S, Chian R-C and Sirard M-A (1995) Effects of estrous cycle, steroids and localization of oviductal cells on *in vitro* secretion of sperm motility factor(s). *Theriogenology* 44: 119–128.
- Larson RC, Ignatz GG and Currie WB (1992a) Platelet derived growth factor (PDGF) stimulates development of bovine embryos during the fourth cell cycle. *Development* 115: 821–826.
- Larson RC, Ignatz GG and Currie WB (1992b) Transforming growth factor β and basic fibroblast growth factor synergistically promote early bovine embryo development during the fourth cell cycle. *Mol Reprod Dev* 33: 432–435.
- Leese HJ (1988) The formation and function of oviduct fluid. *J Reprod Fertil* 82: 843–856.
- Malette B, Paquette Y, Merlen Y and Bleau G (1995) Oviductins possess chitinase- and mucin-like domains: a lead in the search for the biological function of these oviduct-specific ZP-associating glycoproteins. *Mol Reprod Dev* 41: 384–397.
- McCaffrey C, McEvoy TG, Diskin MG, Gwazdauskas FC, Kane MT and Sreenan JM (1991) Successful co-culture of 1–4-cell cattle ova to the morula or blastocyst stage. *J Reprod Fertil* 91: 119–124.
- McNutt TL and Killian GJ (1991) Influence of bovine follicular and oviduct fluids on sperm capacitation *in vitro*. *J Androl* 12: 244–252.
- Menezo Y (1976) Milieu synthétique pour la survie et la maturation des gamètes et pour la culture de l'aéuf féconde. *C R Acad Sci Paris* 282: 1967–1970.
- Mermillod P, Vansteenbrugge A, Wils C, Mourmeaux J-L, Massip A and Dessy F (1993) Characterization of the embryotrophic activity of exogenous protein-free oviduct-conditioned medium used in culture of cattle embryos. *Biol Reprod* 49: 582–587.
- Nagao Y, Saeki K, Hoshi M and Kainuma H (1994) Effects of oxygen concentration and oviductal epithelial tissue on the development of *in vitro* matured and fertilized bovine oocytes cultured in protein-free medium. *Theriogenology* 41: 681–687.
- Nancarrow CD and Hill JL (1994) Co-culture, oviduct secretion and the function of oviduct-specific glycoproteins. *Cell Biol Int* 18: 1105–1114.
- Odor DL and Blandau RJ (1973) Egg transport over the fimbrial surface of the rabbit oviduct under experimental conditions. *Fertil Steril* 24: 292–300.
- Oliphant G (1986) Biochemistry and immunology of oviductal fluid. In: AM Siegler (ed) *The Fallopian Tube: Basic Studies and Clinical Contributions*. (pp. 129–145). Futura, Mount Kisco, New York.
- Ouhibi N, Hamidi J, Guillaud J and Menezo Y (1990) Co-culture of 1-cell mouse embryos on different cell supports. *Hum Reprod* 5: 737–743.
- Ouhibi N, Menezo Y, Benet G and Nicolle B (1989) Culture of epithelial cells derived from the oviduct of different species. *Hum Reprod* 4: 229–235.
- Parrish JJ, Susko-Parrish JL, Handrow RR, Sims MM and First NL (1989) Capacitation of bovine spermatozoa by oviduct fluid. *Biol Reprod* 40: 1020–1025.
- Parrish JJ, Susko-Parrish JL, Winer MA and First NL (1988) Capacitation of bovine sperm by heparin. *Biol Reprod* 38: 1171–1180.
- Pollard JW, Plante C, King WA, Hansen PJ, Betteridge KJ and Suarez SS (1991) Fertilizing capacity of bovine sperm may be maintained by binding to oviductal epithelial cells. *Biol Reprod* 44: 102–107.
- Rexroad CE Jr (1989) Co-culture of domestic animal embryos. *Theriogenology* 31: 105–114.
- Rexroad CE Jr and Powell AM (1988) Co-culture of ovine ova with oviductal cells in medium 199. *J Anim Sci* 66: 947–953.
- Rorie RW, Lester TD, Miller GF, Gliendt DW and McNew RW (1994) Effects of protein source and co-culture on bovine embryo development in synthetic oviductal fluid medium. *Theriogenology* 42: 385–395.
- Rosselli M, Imthurn B, Macas E and Keller PJ (1994) Endothelin production by bovine oviduct epithelial cells. *J Reprod Fertil* 101: 27–30.
- Sakkas D and Trounson AO (1990) Co-culture of mouse embryos with oviduct and uterine cells prepared from mice at different days of pseudopregnancy. *J Reprod Fertil* 90: 109–118.
- Satoh T, Abe H, Sendai Y, Iwata H and Hoshi H (1995) Biochemical characterization of a bovine oviduct-specific sialo-glycoprotein that sustains sperm viability *in vitro*. *Biochem Biophys Acta* 1266: 117–123.
- Satoh T, Kobayashi K, Yamashita S, Kikuchi M, Sendai Y and Hoshi H (1994) Tissue inhibitor of metalloproteinases (TIMP-1) produced by granulosa and oviduct cells enhances *in vitro* development of bovine embryo. *Biol Reprod* 50: 835–844.
- Shamsuddin M, Larsson B, Gustafsson H and Rodriguez-Martinez H (1993) *In vitro* development up to hatching of bovine *in vitro*-matured and fertilized oocytes with or without support from somatic cells. *Theriogenology* 39: 1067–1079.
- Sirard M-A, Parrish JJ, Ware GB and Leibfried-Rutledge ML (1988) The culture of bovine oocytes to obtain developmentally competent embryos. *Biol Reprod* 39: 546–552.
- Smith TT, Koyanagi F and Yanagimachi R (1987) Distribution and number of spermatozoa in the oviduct of the golden hamster after natural mating and artificial insemination. *Biol Reprod* 37: 225–234.
- Smith TT and Yanagimachi R (1990) The viability of hamster spermatozoa stored in the isthmus of the oviduct: the importance of

- sperm-epithelium contact for sperm survival. *Biol Reprod* 42: 450–457.
- Smith TT and Yanagimachi R (1991) Attachment and release of spermatozoa from the caudal isthmus of the hamster oviduct. *J Reprod Fertil* 91: 567–573.
- Suzuki H and Foote RH (1995) Bovine oviductal epithelial cells (BOEC) and oviducts: I. For embryo culture. II. Using SEM for studying interactions with spermatozoa. *Microsc Res Tech* 31: 519–530.
- Takagi Y, Kobayashi K, Hoshi H, Tsujii H, Sugawara S and Oikawa T (1995) Effect of insulin and basic fibroblast growth factor (bFGF) on bovine embryo development *in vitro* in a defined medium after *in vitro* fertilization. *J Mamm Ova Res* 12: 15–21.
- Takeuchi K, Maruyama I, Yamamoto S, Oki T and Nagata Y (1991) Isolation and monolayer culture of human Fallopian tube epithelial cells. *In Vitro Cell. Dev Biol* 27A: 720–724.
- Thibodeaux JK and Godke RA (1992) *In vitro* enhancement of early-stage embryos with co-culture. *Arch Pathol Lab Med* 116: 364–372.
- Tiemann U and Hansen PJ (1995) Steroidal and growth factor regulation of [³H]thymidine incorporation by cultured endosalpingeal cells of the bovine oviduct. *In Vitro Cell. Dev Biol* 31: 640–645.
- van Langendonck A, Vansteenbrugge A, Dessy-Doize C, Flechon J-E, Charpigny G, Mermillod CP, Massip A and Dessy F (1995) Characterization of bovine oviduct epithelial cells monolayers cultured under serum-free conditions. *In Vitro Cell. Dev Biol* 31: 664–670.
- Viuff D, Hyttel P, Greve T, Eriksen T and Alexandersen S (1995) Transcription and localization of growth factor mRNA in the bovine oviduct. *Mol Reprod Dev* 42: 28–34.
- Voelkel SA, Amborski GF, Hill KG and Godke RA (1985) Use of a uterine-cell monolayer culture system for micromanipulated bovine embryos. *Theriogenology* 24: 271–283.
- Voelkel SA and Hu YX (1992) Effect of gas atmosphere on the development of one-cell bovine embryos in two culture systems. *Theriogenology* 37: 1117–1131.
- Walter I (1995) Culture of bovine oviduct epithelial cells (BOEC). *Anat Rec* 243: 347–356.
- Watson AJ, Hogan A, Hahnel A, Wiemer KE and Schultz GA (1992) Expression of growth factor ligand and receptor genes in the preimplantation bovine embryo. *Mol Reprod Dev* 31: 87–95.
- Wiemer KE, Hoffman DI, Maxson WS, Eager S, Muhlberger B, Fiore I and Cuervo M (1993) Embryonic morphology and rate of implantation of human embryos following co-culture on bovine oviductal epithelial cells. *Hum Reprod* 8: 97–101.
- Wiley LM, Wu JX, Harari I and Adamson ED (1992) Epidermal growth factor receptor mRNA and protein increase after the four-cell preimplantation stage in murine development. *Dev Biol* 149: 247–260.
- Wilmut I and Hunter RHF (1984) Sperm transport into the oviducts of heifers mated early in oestrus. *Reprod Nutl Dev* 24: 461–468.
- Witkowska E (1976) Tissue culture of isolated epithelial cells of the bovine oviduct. *Bull Acad Pol Sci* 24: 373–376.
- Witkowska E (1979a) Reactivity of the epithelial cells of the bovine oviduct *in vitro* on the exogenic gonadotropin and steroid hormones. Part I: the effect of gonadotropic and steroid hormones on the amount of lipids and activity of dehydrogenases. *Folia Histochem Cytochem* 17: 225–238.
- Witkowska E (1979b) Reactivity of the epithelial cells of the bovine oviduct *in vitro* on the exogenic gonadotropin and steroid hormones. Part II: the effect of gonadotropic hormones on the amount of glycogen and of acid and alkaline phosphatases. *Folia Histochem Cytochem* 17: 239–250.
- Wright RW Jr and Bondioli KR (1981) Aspects of *in vitro* fertilization and embryo culture in domestic animals. *J Anim Sci* 53: 702–728.
- Xu KP, Yadav BR, Rorie RW, Plante L, Betteridge KJ and King WA (1992) Development and viability of bovine embryos derived from oocytes matured and fertilized *in vitro* and co-cultured with bovine oviductal epithelial cells. *J Reprod Fertil* 94: 33–43.

Address for Correspondence: Hiroyuki Abe, Ph.D., Research Institute for the Functional Peptides, 4–3–32 Shimojo, Yamagata 990, Japan.