Title	The oviduct and development of the preimplantation embryo
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Citation	Reproductive Medicine Review, 2002, v. 10 n. 1, p. 21-44
Issued Date	2002
URL	http://hdl.handle.net/10722/43592
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Reproductive Medicine Review 2002; ***10****:1 21–44*
Printed in the United Kingdom DOI:10.1017/S0962279902000121

THE OVIDUCT AND DEVELOPMENT OF THE PREIMPLANTATION EMBRYO

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INTRODUCTION

Fertilization and early embryo development take place in the oviduct *in vivo*. Relative to studies in other reproductive organs, the importance of the oviduct has been ignored for many years because pregnancies can be obtained in assisted reproduction treatment using *in-vitro* fertilization (IVF) and embryo transfer to the uterus without involving the Fallopian tube. After the reports on the beneficial effect of oviductal cells on embryo development in sheep[1] and subsequently in human,[2] and a practical need to improve the success rates in clinical assisted reproduction, there was a period when more research was performed on the Fallopian tube. Many of these studies used *in vitro* coculture systems to emulate the *in vivo* environment *in vitro*, and to search for oviduct-derived embryotrophic factors. With the recent development of sequential culture[3] to improve embryo development *in vitro*, the use of coculture in assisted reproduction and its related research declined because routine use of coculture is laborious and experience-dependent.

Despite this, evidence is now accumulating to suggest that this organ is not a passive conduit for gametes and embryos. Before fertilization, it is well accepted that the oviduct serves as a sperm reservoir in several animal species.[4,5] Although there is no direct evidence for such a function in human Fallopian tube *in vivo*, *in vitro* experiments have demonstrated that human spermatozoa bind to the tubal epithelial cells.[6] In addition, human oviductal cells affect sperm functions *in vitro*.[7-9] After fertilization, the oviduct provides the microenvironment for early embryonic development. This review intends to provide an update on this postfertilization role of the oviduct, with a particular emphasis on human reproduction.

There is only limited evidence to show which of the oviductal changes are important for embryonic development because it is difficult to change each of these parameters independent of the others *in vivo* and to study the effect of such changes on embryo development. Moreover, the effect of the oviduct on embryo development

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Table 1 Expression of growth factors/cytokines and their receptors in human Fallopian tube and embryos

Growth factor/ cytokines & receptors	Oviduct	Oocyte stage	2-cell stage	3- to 4-cell stage	6- to 8-cell stage	Morula	Blastocyst
IGF1	$+t^{21},p^{19,21,94}$	$-t^{20}$	$-t^{20}$	$-t^{144}$	$-t^{20,144}$		$-t^{20,144}$
IGF2	$+p^{19}$	$+t^{20}$	$+t^{20}$		$+t^{20,144},p^{18}$	$+ p^{18}$	$+t^{20,144},p^{18,18}$
Insulin R		$+t^{20}$	$-t^{20}$		$+t^{20,144}$		$+t^{20,144}$
IGF1 R	$+p^{19,94}$	$+t^{20}$	$+t^{20}$		$+t^{20}$		$+t^{20},p^{21}$
IGF2 R	$+p^{19}$	$+t^{20}$	$+t^{20}$		$+t^{20}$		$+t^{20}$
IGFBP-1	$?t^{145}$; $+p^{94}$			$-t^{144}$			$+t^{144}$
IGFBP-2	$+t^{145},p^{94,145}$				$+t^{144}$		$+t^{144}$
IGFBP-3	$+t^{145},p^{94,145}$				$+t^{144}$		$+t^{144}$
IGFBP-4	$+t^{145},p^{94}$				$+t^{144}$		$+t^{144}$
IGFBP-5				$-t^{144}$	$+t^{144}$		$+t^{144}$
IGFBP-6				$-t^{144}$	$-t^{144}$		$-t^{144}$
TGFβ	$+t^{146},p^{146}$			$+p^{147}$			
TGFβ-RI		$+t^{148},p^{148}$		$-t^{148}$	$-t^{148}$	$-t^{148}$	$+t^{148}$
TGFβ-RII	$+t^{146},p^{146}$	$+t^{148},p^{148}$		$-t^{148}$	$-t^{148}$	$-t^{148}$	$-t^{148}$
Smad2/3		$+t^{148},p^{148}$		$+t^{148}$	$+t^{148}$	$+t^{148}$	$+t^{148}$
Inhibin α	$-p^{149}$		$-t^{150}$	$-t^{150}$	$-t^{150}$	$-t^{150}$	$-t^{150}$
Activin	$+t^{150},p^{150}$		$-t^{150}$	$-t^{150}$	$-t^{150}$	$-t^{150}$	$+t^{150}$
Activin IR			$-t^{150}$	$+t^{150}$	$-t^{150}$	$-t^{150}$	$+t^{150}$
Activin IIR			$+t^{150}$	$+t^{150}$	$+t^{150}$	$+t^{150}$	$+t^{150}$
Follistatin			$-t^{150}$	$-t^{150}$	$+t^{150}$	$+t^{150}$	$+t^{150}$
TGF-α	$+t^{14,15,74,151},p^{14\text{-}16,151\text{-}153}$	$+t^{154},p^{154}$		$+p^{16}$,	$+t^{154},p^{16,154}$		$+t^{154},p^{18,18,154}$
EGF	$+t^{14,15,74,107,151,153},p^{14,15,151,153}$	$+t^{154},p^{154}$	$+t^{154},p^{154}$		$+t^{154},p^{154}$		
EGF-R	$+t^{17,74,151,153},p^{16,17,151}$		$+p^{16}$	$+p^{16}$			
GM-CSF	$+t^{90},p^{90}$						
GM-CSF-R	$+t^{90}$						
VEGF							$+t^{155}$

PDGF-α and -β		+t[156]		−t[156]	+t[156]	+t[156]	+t[156]
PDGFR-α and -β		−t[156]		+t[156]	+t[156]	−t[156]	+t[156]
IL-1α	+p[157]			+p[147,158]			
IL-1β	+p[159]	+t[160],p[161]		+t[160],p[161,161]	+t, [160], p[161]	+t[160],p[161]	+t[160],p[161]
IL-1R tI		+t[160],p[161]		+t[160],p[161]	+t[160],p[161]	−t[160],p[161]	+t[160],p[161]
IL-1ra	+p[159]	+t[160],p[161]		+t[160],p[161]	+t[160],p[161]	+t[160],p[161]	+t[160],p[161]
CSF	+t[107]		−t[162],p[163]	−t[162],p[163]	−t[162],p[163]	−t[162]	−t[162]
CSF R			−t[162]	−t[162]	−t[162]	−t[162]	−t[162]
LIF	+t[107],p[159]		−t[162]	−t[162]	−t[162]	−t[162]	−t[162]
LIF-R		+t[164]	−t[162]	−t[162]	−t[162]	−t[162]	+t[162,164,165]
			+t[164]	+t[164]	+t[164]		
IL-6	+t[107]	+p[163]	+p[163]	+p[147,163]			+t[162]
IL-6R							+t[162]
c-fms			+t[162]	+t[162]	+t[162]	−t[162]	+t[162]
TNF-R p80			+t[162]	+t[162]	−t[162]	−t[162]	−t[162]
TNF-R p60			−t[162]	−t[162]	+t[162]	−t[162]	+t[162]
TNF-α	+p[159]		−t[162]	+t[162]	−t[162],p[163]	+t[162]	−t[162]
c-kit			+t[162]	+t[162]	+t[162]	−t[162]	+t[162]
SCF			+t[162]	−t[162]	+t[162]	+t[162]	−t[162]
gp130		+t[164]	−t[162]	+t[162]	−t[162]	+t[162]	+t[162]

t, transcript; p, protein; +, detectable; -, undetectable; ?, barely detectable; blank space, no information available.
IGF, insulin-like growth factor; R, receptor; IGFBP, insulin-like growth factor binding protein; TGF, transforming growth factor; EGF, epidermal growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; IL, interleukin; IL-1ra, Interleukin-1 receptor antagonist; CSF, colony stimulating factor; LIF, leukaemia inhibitory factor; c-fms, colony stimulating factor receptor; TNF, tumor necrosis factor; c-kit, stem cell factor receptor; SCF, stem cell factor; gp130, a component of LIF and IL-6 receptor.
Reference numbers are shown in superscript.

is facilitatory and not obligatory, making the detection of oviductal embryotrophic effects more difficult. Nevertheless, there is circumstantial evidence mainly from animal studies showing that the oviduct plays a fundamental role in early embryo development. This evidence is reviewed below.

CYCLICAL CHANGE OF OVIDUCTAL ACTIVITY

Changes in the ciliated and secretory cells of the primate oviduct are well documented across the reproductive cycle.[10] These changes are regulated by steroids, such that estrogen induces hypertrophy, maturation and an increase in cell height of nonciliated secretory oviductal epithelial cells and can partially restore ciliated phenotype *in vitro*.[11] These, together with cyclical morphological and physiological variations in the uterus, suggest that these two organs are actively involved in early reproductive events, and that they offer a dynamic microenvironment for the development of preimplantation embryos.

The presence of growth factors in the oviduct and the corresponding receptors in the preimplantation embryo or *vice versa* suggests possible mechanisms of interaction between the oviduct and the embryo. The expression of growth factors in the preimplantation embryo and oviduct has recently been reviewed.[12,13] Table 1 updates their occurrence in human oviduct and preimplantation embryos at protein and mRNA levels. Many of the growth factors in the oviduct are produced in a cyclical manner. Epidermal growth factor (EGF)/transforming growth factor-α (TGF-α) and their receptors have been extensively studied in the Fallopian tube and human embryo. The mRNA and protein expression of EGF and TGF-α are intense in the ampullary epithelium at late follicular and luteal stages but weak at the early follicular stage.[14,15] Their receptor (EGFR) at the protein level is present in 8- to 14-cell embryos.[16] On the other hand, EGFR is also found in the Fallopian tube with an expression pattern similar to its ligands.[17] Human embryos also secrete TGF-α into the culture medium; the secretion increases as the embryos develop to morula and blastocyst.[18] A similar situation exists for the insulin-like growth factor (IGF) system, with expression of the ligand and receptor found in both the Fallopian tube and the early embryo.[16,19,20] Although the addition of some of these factors stimulates embryo development *in vitro*,[21] it is uncertain whether these signaling systems are performing paracrine and/or autocrine function *in vivo*.

OVIDUCTAL MICROENVIRONMENT AND EMBRYO DEVELOPMENT

It is generally accepted that the oviduct may have evolved to provide an optimum microenvironment for early embryo development. An early preimplantation embryo is bathed in oviductal fluid. Therefore, knowledge on the content of the oviductal fluid is important. The composition of oviductal fluid has been reported in many animal species. Interested readers are referred to reviews by Leese,[22] Hamner and

Table 2 Reported composition of oviductal fluids in human and primates

Components	Species	Cycle	Concentration	Reference
Glucose	Human	Follicular	3.11 mM	[36]
		Midcycle	0.50 mM	
		Luteal	2.32 mM	
		Different stages	1.1 mM	[166]
		Different stages	0.53 mM	[167]
Pyruvate	Human	Different stages	0.24 mM	[36]
		Different stages	0.14 mM	[166]
		Different stages	0.17 mM	[167]
Lactate	Human	Follicular	4.87 mM	[36]
		Midcycle	10.50 mM	
		Luteal	6.19 mM	
		All stages	5.85 mM	[166]
		All stages	8.58 mM	[167]
Aspartic acid	Human	Proliferative	17 μM	[166]
		Secretory	41 μM	
Glutamic acid	Human	Proliferative	71 μM	[166]
		Secretory	111 μM	
Asparagine	Human	Proliferative	63 μM	[166]
		Secretory	28 μM	
Serine	Human	Proliferative	26 μM	[166]
		Secretory	38 μM	
Glutamine	Human	Proliferative	24 μM	[166]
		Secretory	51 μM	
Arginine	Human	Proliferative	151 μM	[166]
		Secretory	237 μM	
Glycine	Human	Proliferative	32 μM	[166]
		Secretory	37 μM	
Threonine	Human	Proliferative	44 μM	[166]
		Secretory	47 μM	
Alanine	Human	Proliferative	84 μM	[166]
		Secretory	139 μM	
Tyrosine	Human	Proliferative	24 μM	[166]
		Secretory	49 μM	
Tryptophan	Human	Proliferative	12 μM	[166]
		Secretory	18 μM	
Methionine	Human	Proliferative	12 μM	[166]
		Secretory	14 μM	
Valine	Human	Proliferative	22 μM	[166]
		Secretory	32 μM	
Phenylalanine	Human	Proliferative	16 μM	[166]
		Secretory	18 μM	
Isoleucine	Human	Proliferative	17 μM	[166]
		Secretory	20 μM	
Leucine	Human	Proliferative	50 μM	[166]
		Secretory	67 μM	
Lysine	Human	Proliferative	42 μM	[166]
		Secretory	61 μM	
Sodium	Human	Preovulatory	140–142 mM	[26,27]
		Postovulatory	139–148 mM	[26,27]
		Different stages	130 mM	[168]

Table 2 continued

Components	Species	Cycle	Concentration	Reference
Chloride	Human	Preovulatory	119–127 mM	26,27
		Postovulatory	112–117 mM	26,27
		Different stages	132 mM	168
Potassium	Human	Preovulatory	6.7–9.9 mM	26,27
		Postovulatory	6.7–7.7 mM	26,27
		Different stages	21.2 mM	168
Calcium	Human	Preovulatory	1.89 mM	26
		Postovulatory	2.37 mM	26
		Different stages	1.13 mM	168
Magnesium	Human	Preovulatory	0.53–0.69 mM	26
		Different stages	1.42 mM	168
pH	Rhesus monkey	Follicular	7.1–7.3	28
		Ovulation & luteal	7.5–7.8	
Bicarbonate	Rhesus monkey	Follicular	35 mM	28
		Luteal	90 mM	
pO_2	Rhesus monkey	Follicular	<10 mmHg	169
		Follicular	35 mmHg	170
		Ovulation & luteal	57–61 mmHg	

Fox,[23] and Menezo and Guerin.[24] Recently, Leese and coworkers[25] provided an excellent review on the formation of oviductal fluid. It is not the intention of this article to review all the components reported in the oviductal fluid. Table 2 contains some of the reported compositions of oviductal fluid from humans and primates.

Similar to many other species, the oviductal fluid in the primate is rich in K^+ and HCO_3^- when compared with plasma.[26–28] Estrogen appears to stimulate the production of oviductal fluid, as the greatest volume of oviductal fluid in human and monkeys was observed at midcycle, coincident with the estrogen peak.[29,30] It has been suggested that estrogen may modulate the movement of ions across the oviductal epithelium and, thereby, influence the rate of fluid secretion.[25] These data are consistent with an active regulation of oviductal function at the time of fertilization and early embryo development.

The nutrient requirement of the embryo changes as it grows. The importance of amino acids and nutrients to early preimplantation embryo development has been critically reviewed.[31,32] Similar to many other animal species, human embryos consume pyruvate throughout the preimplantation period, and they use an increasing amount of glucose at compaction and blastocyst formation.[33,34] In fact, a high level of glucose inhibits early embryonic development in a number of species including human.[35] The concentrations of nutrients in the oviduct differ from those in plasma, and vary with the endocrine state.[36] The concentration of glucose in human Fallopian tube fluid decreases sixfold between the follicular phase and midcycle.[36] It was speculated that the decrease in glucose concentration of oviductal fluid from mated pig when compared to unmated pig[37] was a strategy of the oviduct to protect embryos from exposure to a high concentration of glucose.[34] The importance of optimal

nutrient concentrations is also reflected in experiments on sheep[38] and mouse[39] showing that embryo culture media based on nutrient concentrations in oviductal fluid are superior to conventional media in supporting embryo development *in vitro*. Similarly, the embryo has a differential requirement for amino acids at different stages of development,[40] and media based on the amino acid content of oviductal fluid improve ovine blastocyst formation and hatching.[41]

Apart from simple organic and inorganic molecules, the oviduct synthesizes and releases a number of macromolecules. Interested readers are referred to reviews by Buhi and coworkers.[42,43] Like many other animal species, the biosynthetic activity of the Fallopian tube increases at midcycle in the human.[43] In pig and sheep, it has been further shown that biosynthetic activity is higher in the ampulla than it is in the isthmus.[44,45] Some secretory proteins from the human Fallopian tube fluid, including oviduct-specific glycoprotein (OGP),[46] corticosteroid-binding globulin-like protein,[47] and insulin-like growth factor-binding protein 3[48] may be involved in fertilization and early embryonic development. Three high molecular weight embryotrophic glycoprotein fractions that stimulate mouse embryo development[49] have recently been isolated from human-oviductal-cell-conditioned medium.[50] The exact identities of these factors remain to be determined, but it is believed that these molecules are responsible for maternal-embryonic cross-talk during early preimplantation development.[51]

OGP is one of the major macromolecules secreted by the oviduct. The protein has been characterized in several species, including rhesus,[52] baboon[53] and human.[54] In human, OGP is localized to the secretory granules of oviductal cells in both the ampulla and isthmus regions.[55] The human OGP gene[54] and its promoter[56] have been cloned and characterized. The presence of OGP around the time of ovulation suggests that it may play a role in fertilization and/or early embryonic development.[46,57] In hamster, the molecule attaches to the ovulated oocyte and early embryo,[46,58,59] and also associates with the blastomeres.[60,61] Although the physiological role of OGP is unclear, the chitinase-like domains of OGP are believed to interact with the oligosaccharide moieties of the zona pellucida, thereby forming a protective shield around the oocyte and early embryo.[58]

HORMONAL EFFECTS ON OVIDUCTAL EMBRYOS

It is generally believed that progesterone is essential for inducing the changes in the oviduct and uterus necessary for embryo viability and implantation. Administration of an anti-progesterone antibody to mice at 32 hours postcoitum retarded embryonic development; most of the embryos remained at the 4-cell stage whereas the control embryos were at the 8-cell to morula stage at 54–58 hours postcoitum.[62] The antibody treatment also induced a change in wheatgerm-lectin binding sites in the oviductal ampulla at days 3 and 4 of pregnancy,[63] but did not have noticeable effect on cultured mouse embryos.[62]

A similar conclusion was reached using the anti-progestin, RU486, in mice.[64,65]

However, the data on RU486 have to be interpreted with caution as this antiprogestin inhibits the development of mouse embryos *in vitro*.[66,67] RU486 also affects oviductal embryo transport in this species.[68] Using a dose that did not affect the development of cynomolgus monkey embryos *in vitro*,[69] RU486 administration on day 2 after the presumed day of ovulation retarded preimplantation embryo growth in rhesus monkey.[70] The retarded embryos had abnormal morphology[71] and did not implant after transfer to naturally synchronized surrogate recipients.[70]

Apart from progesterone, estradiol may also affect embryo development indirectly via its effect on oviductal secretion. Two-cell mouse embryos cultured in oviductal fluid from estrogen-dominated donors were significantly less able to develop to morula or blastocyst and to implant than those cultured in either control medium or the progesterone-dominated oviductal fluid.[72] No inhibitory effect was observed on embryo development when estradiol or progesterone was added directly to the control medium. Estradiol may exert its action by affecting growth factor signalling systems in the oviduct. It induces oviductal mRNA expression of IGF-I in rat[73] and EGF, TGF-α, and EGF receptor in human.[17,74]

EFFECTS OF OVIDUCTAL CELLS ON EMBRYO DEVELOPMENT *IN VITRO*

The above evidence demonstrates that the microenvironment in the oviductal fluid affects early preimplantation embryo development. However, the actual contribution of the oviductal cells on embryo development is uncertain, as the components in the oviductal fluid can be derived from plasma transudation and not solely from active secretion from the oviduct. It has been demonstrated that coculture of human embryos with human Fallopian tube cells improves the quality of the developing embryos.[2,75,75-77] As these studies provide more direct evidence that oviductal cell activities do contribute to the embryotrophic effect of the oviduct, this aspect is discussed in greater detail below.

COCULTURE STUDIES IN CLINICAL IVF

There have been a number of clinical coculture trials using oviductal cells of human or bovine origin in assisted reproduction. Table 3 summarizes the results of these studies. Most of the studies are prospective randomized clinical trials. Apart from one study, coculture with oviductal cells improved embryo quality and/or enhanced pregnancy rate/implantation rate. For some unknown reason, Tucker and coworkers[78] found no beneficial effect of oviductal cells for couples requiring intracytoplasmic sperm injection (ICSI). However, the indication for ICSI and whether couples were undergoing IVF for the first time were not mentioned. In addition to the clinical studies listed in Table 3, there are other *in vitro* experiments demonstrating that oviductal cells decreased embryo fragmentation,[76] increased blastulation rate,[76,79] the number of cells per blastocyst,[79] and the hatching rate of human embryos.[75]

Table 3 Controlled clinical trials using oviductal cells

Somatic cells origin	Patient	Study design	Coculture duration*	Cocultured embryo development	Pregnancy/ implantation#	Reference
Bovine	Non-male factors	Prospective randomized	1 day after fertilization	Less fragmentation More blastomeres	Increase PR and IR	171
	Non-male factors	Prospective randomized	1 day after fertilization	Less negative morphological characteristics	Increase PR	172
	Young first time IVF; non-male factors	Prospective randomized	2 days after fertilization	More blastomeres Less fragmentation	No difference	83
	Female $\geq$38 years; multiple failed IVF attempts	Prospective randomized; + assisted hatching	2 days after fertilization	More zona thickness variability	Increase PR	80
	Multiple failed IVF attempts; non-male factor	Previous IVF as control; + assisted hatching	2 days after fertilization	More blastomeres Les fragmentation	Not compared	81
	ICSI patients	Prospective randomized	3 days after ICSI	No difference	No difference	78
Human	Non-ICSI	Prospective randomized	1 day after fertilization	Not compared	Increase IR	77
	Unselected, non-ICSI	Non-randomized	1 day after fertilization	Not compared	Increase PR/IR**	2

ICSI, intracytoplasmic sperm injection; IR, implantation rate; IVF, *in vitro* fertilization; PR, pregnancy rate.
* Duration after fertilization check
#When compared with control
** When compared with patients performing conventional IVF in the same period

It has been suggested that oviductal cell coculture is suitable for poor prognosis patients, such as patients of advanced age,[80] patients with multiple failed IVF attempts,[80,81] and patients with a high basal level of follicle stimulating hormone.[82] Coculture may not be required for young (<35 years) first-time IVF patients as there is no difference in pregnancy rates in these patients with or without coculture, though the development of the embryos is improved after coculture.[83] This group of patients with a good prognosis is likely to produce sufficiently good quality embryos that are competent to development *in vitro*.

LIMITATIONS OF COCULTURE STUDIES

Although oviductal cell coculture intends to imitate the *in vivo* condition, this objective cannot be achieved with the current model because the culture requirements of the somatic cells and the embryos are different. In a coculture system, only one culture medium, commonly the embryo culture medium, is used. Thus, the somatic cells are grown under suboptimal conditions. Walter[84] concluded that monolayers of bovine oviduct epithelial cells could not fully substitute for the oviduct epithelium when used in coculture experiments. Thus, cultured somatic cells may not function optimally in a coculture system.

To improve the efficiency of coculture, optimization of coculture conditions is important. In our experience the best coculture system using human oviductal cells with Earles' balanced salt solution is when the cells are at the rapidly growing phase. We usually seed 5×10^4 cells in a 4-well plate and the cells will be ready to use the next day. Wiemer and coworkers[83] reported similar observations with bovine oviductal epithelial cells. They suggested that confluent cells and actively dividing nonconfluent monolayers might secrete different levels of embryotrophic factors, and that steroid treatment affected the plating efficiency of bovine oviductal cells.[83] Confluent oviductal cell culture will cause the detachment and death of epithelial cells, leading to an increase in the acidity of the medium which is deleterious to embryo development.

During coculture, the observed effects on embryo development are the consequence of two opposing activities; namely, the embryotrophic effect of somatic cells and the combined deleterious effect of excretory waste from the somatic cells, proteases released by the somatic cells as a result of cell death and inappropriate competition of nutrients between the somatic cells and the embryos. This was shown in one of our unpublished experiments (Liu LPS and Yeung WSB) in which mouse embryos were cultured in spent media after culturing the oviductal cells for different durations and in media containing only the high molecular weight embryotrophic fraction of the conditioned media. Figure 1 shows the results of the experiment. Conditioned medium had its maximal embryotrophic effect after 6–9 hours of conditioning, but became inhibitory after 24 hours. On the other hand, the embryotrophic effect of the high molecular weight factors accumulated with time, reaching a plateau after 6 hours of conditioning. It has been reported that cocultured embryos are

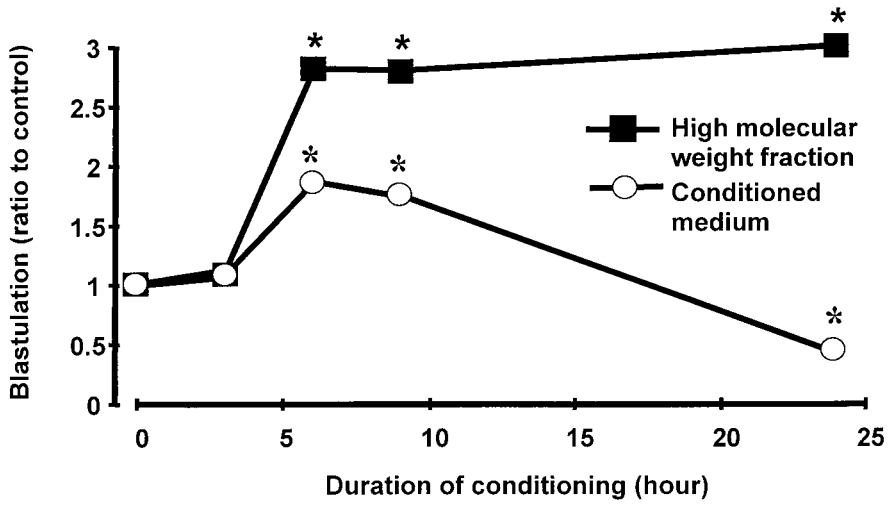

Figure 1 Effect of duration of conditioning of the embryotrophic activity of human oviductal cells on mouse embryo development. The percentage of blastulation in medium-alone culture was arbitrary taken as unity. Results of various treatments were expressed as ratios to the control. * $P<0.05$ when compared with the control (ANOVA)

subjected to dynamic changes of growth factors/cytokines released by Vero cells[85] and that the culture condition will affect the secretion of growth factors by the cells.[86]

The results of a number of reports on the use of somatic cells other than oviductal cells for coculture with human embryos are much more variable. Assisted reproduction without the involvement of the Fallopian tube can give rise to live birth, indicating that the coculture embryotrophic effect is facilitatory and not obligatory to embryo development. In many coculture studies, the embryos were cocultured for only 1–2 days. This short coculture period may not be sufficient to induce an effect that can be manifested as an increase in pregnancy rate. The embryotrophic effect is further diminished when the number of somatic cells available, e.g. granulosa cells, is limited. Therefore, it is likely to be more difficult to demonstrate an increase in pregnancy rates in these studies.[87]

Despite the general observation of enhanced embryo development after oviductal cell coculture, it is not used in most human assisted reproduction programmes because of the complexity of its implementation as a routine service and the limitations listed above. In addition, there is risk of transfer of disease from contaminated cultures to the embryos during coculture.[88] A safer and more practical approach is to produce a chemically defined embryo culture system.[89] Consequently, the recent development of the sequential culture, the use of different culture media for culturing embryos at different stages of development, has rapidly become the method of choice for improving the outcome in human assisted reproduction programmes.[3]

Coculture and sequential culture are not mutually exclusive, and in fact they can complement each another. The human Fallopian tube produces granulocyte-macrophage colony-stimulating factor (GM-CSF) with a peak expression in the preimplantation period.[90] GM-CSF has recently been shown to improve human embryo development in the G1.2/G2.2 sequential culture medium system.[91] Its receptor mRNA is present in mouse embryos at all stages of development.[92] These data suggest that the present sequential culture system is not yet optimal, and that we can further improve the system by studying the mechanisms by which oviductal cells improve embryo development. Indeed, new sequential media may obviate the need for coculture.

OVIDUCTAL EMBRYOTROPHIC MECHANISMS

The exact mechanisms of oviductal embryotrophic activity *in vivo* are unclear. Most of the information in this area is based on *in vitro* coculture studies. However, the use of coculture to study oviductal embryotrophic mechanisms in human embryo development is difficult because the availability of human embryos for research is limited for ethical reasons. Very often mouse embryos are used instead.

Characterization of oviductal embryotrophic mechanisms using coculture faces two problems. The first is that oviductal cells produce minute amount of embryotrophic factors making characterization of these factors difficult. The problem is aggravated as human oviductal cells have a limited proliferative life span in culture, posing more difficulty in isolating sufficient embryotrophic factors. We have recently established an immortalized human oviductal cell line[93] that retains most of the characteristics of primary oviductal cells including the production of OGP and some embryotrophic factors. In future, this cell line will be a valuable tool for studying the interaction between oviductal cells and embryos.

The second problem arises because the actions of oviductal embryotrophic factors are only facilitatory to embryonic development. In coculture experiments using mouse embryos, the ability to detect embryotrophic activity is often low because the development of many mouse strains does not require additional support in standard culture conditions for blastulation, the usual endpoint in these studies. In order to increase the sensitivity of detection, we use a mouse strain with low developmental potential in commonly used mouse culture media to assess the coculture embryotrophic effect. With this model, we detect the presence of three embryotrophic factors, termed ETF-1, -2, -3, in spent medium after human oviductal cell culture.[49,50]

There are three proposed mechanisms of embryotrophic activity in coculture; namely, positive conditioning, negative conditioning and cell-to-embryo contact. Positive conditioning refers to the production of embryotrophic factors by the somatic cells. During preimplantation embryo development, numerous peptide growth factors, their corresponding receptors and binding proteins are expressed in the oviduct of human, mouse and other domestic animals (Table 1). The expression of many of these growth factors, e.g. IGF-1,[19,94] IGF-2,[19] TGF-α, EGF[14,17] (full names of

IGF, TGFα and EGF can be found on p. 22), is highest from mid-cycle to the early luteal phase, a time when the preimplantation embryo is in the oviduct. Many of the corresponding growth factor receptors are also expressed in the embryos within this period. Paracrine interaction between the oviduct and human preimplantation embryos via growth factors is indicated by the observation that addition of growth factors, e.g. IGF-1[21], leukaemia inhibitory factor (LIF)[95] or heparin binding-epidermal growth factor,[96] to the culture medium facilitates human embryo development *in vitro*. Apart from growth factors, OGP binds to the zona pellucida and blastomeres of hamster embryo,[61] suggesting a possible role of this molecule in early embryo development. Hunter[97] proposed that oviduct glycoproteins might increase the viscosity of luminal fluid, thereby preventing the dispersal of essential nutrients and ions and stabilizing the microenvironment immediately surrounding the embryos. However, this is difficult to test *in vivo* as the oviduct plays only a facilitatory role in embryo development.

It is unknown how the embryotrophic factors affect embryo development. When compared to embryos cultured in medium alone, human oviductal cell coculture affects the gene expression profiles of mouse embryos,[98] and reduces the incidence of apoptosis at the morula and blastocyst stage.[99] Human[19,21,94] and bovine[100] oviductal cells express IGF-1. Exogenous IGF-I improved human embryo development partly by decreasing the incidence of blastomere apoptosis in the treated embryos,[101] leading to an increase in the number of cells within the inner cell mass.[21]

Our recent data show that human oviductal cells produce three embryotrophic factors, ETF-1, -2 and -3 *in vitro*.[50] These factors are unlikely to be common growth factors as they have much larger molecular sizes. The stimulatory activity of ETF-3 on mouse blastulation is unaffected by antibodies against TGF-α, TGF-β, IGF-II, IGFBP3 (Xu and Yeung, unpublished observations). These factors have differential spatial and temporal effects on the development of mouse embryos.[102] Thus, ETF-1 and ETF-2 treatment covering the period of 48–72 hours after human chorionic gonadotrophin injection (hCG) increased cell numbers in the inner cell mass of the resulting blastocyst, whilst ETF-3 treatment covering 96–120 hours post-hCG increased the cell number in the trophectoderm. The improved development of trophectoderm after ETF-3 treatment resulted in larger blastocysts that hatched, attached to and spread on culture dishes more often than was seen in untreated controls. These results suggest that the response of embryos to embryotrophic factors changes as the embryos develop. We are currently investigating whether the response of the embryos to ETF-3 treatment is affected by prior treatment with ETF-1 and -2. In this connection, human endometrial stromal cells enhance the expression of IGF receptors in cocultured human embryos,[103] which might then become more sensitive to subsequent IGF treatment.

It is believed that conventional embryo culture conditions are not optimal. Negative conditioning refers to the removal of the suboptimal culture condition by coculture with somatic cells, leading to improved embryo development. The level of superoxide anion in culture medium is significantly reduced by human oviductal cell coculture.[104] Bovine oviductal cells improve embryo development partly by altering

the carbohydrate concentrations in the culture medium.[105] However, this embryotrophic mechanism is likely to be an artefact *in vitro* and may not represent the situation *in vivo*. Somatic cell-to-embryo contact has been shown to be unimportant in human oviductal cells coculture.[106] However, contradictory observations have also been reported.[104]

Although various cell types exert embryotrophic activity on embryonic development *in vitro*, they may act via distinct mechanisms. Different cell types secrete different growth factors. Human oviductal cells express EGF, LIF, colony stimulating factor-1 and interleukin-6 (IL-6) while buffalo rat liver cells do not express LIF and IL-6.[107] Vero cells, but not human oviductal cells, improve mouse embryo development partly by altering the concentration of nutrients in the culture medium.[108] Therefore, it is surprising that the efficacy of coculture is different among the somatic cells used. In cattle, oviductal epithelial cell coculture produces better embryos than does coculture with granulosa cells,[109] oviductal stromal cells or endometrial epithelial cells.[110] The post-transfer development of bovine embryos is also better for embryos after coculture with oviductal cells than it is with those after coculture with bovine fetal uterine fibroblasts.[83] Although both sheep oviductal cells and fibroblast coculture stimulate embryo development *in vitro*, the development of sheep embryos after transfer following oviductal coculture is superior to that of embryos after fibroblast coculture.[1] Similarly, we also found that human oviductal epithelial cells were better than oviductal fibroblast in enhancing mouse blastulation.[49] In a prospectively randomized study, coculture with human fibroblasts did not improve embryo quality and pregnancy rate when compared to conventional medium alone.[111]

EFFECTS OF EMBRYO ON OVIDUCTAL ACTIVITY

Most of the coculture studies investigate the effect of oviductal cells on embryo development. There are some reports demonstrating that the embryos also affect oviductal physiology in animals. Although similar reports of humans are lacking, the machinery for such interaction has been demonstrated in human oviduct and embryos, suggesting that similar activity may also be present.

In mice, the vasculature of the oviduct containing 2-cell embryos is different to that in pseudopregnant mice without embryos.[112] Platelet activating factor (PAF) may be involved in these vascular changes.[112] PAF is produced by early embryos from a number of species including mouse,[113,114] sheep[115] and human.[116] It alters several aspects of the maternal physiology during early pregnancy including systemic platelet count (inducing thrombocytopenia[116]) and endometrial prostaglandin secretion.[117] The presence of the PAF receptor has recently been reported in human Fallopian tube[118] and bovine oviduct.[119] PAF increases the potential difference and short-circuit current when applied to the apical surface of human oviduct[120] and increases the intracellular free calcium concentration[121] and proliferation[122] in bovine oviductal cells in culture.

Fertilized embryos are transported down the oviduct at a faster rate than

unfertilized ova in hamsters,[123] rat,[124] bats [125] and mares.[126] Evidence suggests that the development of embryos influences the timing of their entrance to the uterus in the rat.[124] Equine embryos secrete prostaglandin E_2 immediately before and during the expected time of transport from oviduct to the uterus.[127] Prostaglandin E_2 has been shown to hasten this transport in the mare.[128] In hamsters, PAF stimulates oviductal transport of embryos,[129] probably by stimulating the frequency of the ciliary beat in oviductal cells.[130,131] Similar data are not available for humans.

TGFβ-1 mRNA expression in mice is high in the 8-cell embryo and morula, but drops significantly at the blastocyst stage.[132] However, the functional receptor for this growth factor can only be detected in the blastocyst. This suggests that the production of TGFβ-1 by the early embryo before compaction may be relevant to its paracrine action on the oviduct. This is supported by our detection of TGFβ-1 receptor expression in the oviduct throughout preimplantation embryo development. There are also indications that intra-oviductal embryos can exert a biological effect on the uterus, enhancing endometrial receptivity.[133] It is unlikely that an oviductal embryo with a few blastomeres is able to produce sufficient quantities of active components to affect the physiology of other organs. In fact, embryo-derived PAF has a systemic effect on thrombocytopenia in early pregnancy.[134] It is more likely that embryo-derived factors affect the nearby oviductal cells, which amplify the embryonic signal and modify the physiology of the maternal system. Embryos have been shown to stimulate the production of IGFBP-3 by human oviductal cells.[48] A similar conclusion was reached in a mouse embryo/human endometrial cell coculture system.[135]

CONCLUSION

The reproductive tract creates a dynamic environment for the optimal development of mammalian embryos *in vivo*. In nature, all embryos are exposed to the oviductal microenvironment after fertilization. Data suggest that the oviductal environment is important for the development of embryos and that there is complex cross-talk between embryos and the oviduct. The best available medium today contains only a small subset of the components present in the oviductal environment. Although the embryo can adapt to its environment,[34,136] the inadequacy of the medium inevitably causes stress to the cultured embryos.[137] This may be one reason for the low success rate in clinical assisted reproduction. To improve this situation, improved knowledge of the oviductal microenvironment is crucial. Unfortunately, information on this aspect is rather limited.

Coculture is a good model, though with some pitfalls, for giving clues to the possible mechanisms of interaction between the embryo and the oviduct. Its utilization in clinical assisted reproduction has generated some controversy.[138,139] The arguments against its use include the fact that coculture is not chemically defined, making interpretation difficult, and coculture embryotrophic effects may only represent a remedy for artefacts created by conventional suboptimal culture conditions.[89,140] The pursuit of specific oviduct factors in coculture and the confirma-

tion of the embryotrophic activity by adding these factors to chemically defined medium would help to solve the controversy. Future work should focus on the normal physiology and biochemistry of the oviduct and the early embryo.

A word of caution for the supplementation of only some embryotrophic factors to the embryo culture medium is necessary. Some embryotrophic factors have specific effects on certain cell types of embryos. For instance, ETF-3 affects mainly trophectoderm cells. The effect of a selective increase in the development of trophectoderm on subsequent fetal development remains to be determined. In this context, it has been reported that larger calves are born after coculture with tubal cells,[141,142] and that the percentage of male calves is increased after coculture with bovine oviduct epithelial cells and Vero cells.[143] Rigorous controlled studies will be necessary before suggesting the application of media supplemented with embryotrophic factors to clinical assisted reproduction.

Acknowledgement

This work was supported fully by grants (HKU39/91, HKU241/95M and HKU 7333/97M) to WSBY from the Research Grant Council, Hong Kong.

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