Oviduct: roles in fertilization and early embryo development

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Abstract
Animal oviducts and human Fallopian tubes are a part of the female reproductive tract that hosts fertilization and pre-implantation development of the embryo. With an increasing understanding of roles of the oviduct at the cellular and molecular levels, current research signifies the importance of the oviduct on naturally conceived fertilization and pre-implantation embryo development. This review highlights the physiological conditions within the oviduct during fertilization, environmental regulation, oviductal fluid composition and its role in protecting embryos and supplying nutrients. Finally, the review compares different aspects of naturally occurring fertilization and assisted reproductive technology (ART)-achieved fertilization and embryo development, giving insight into potential areas for improvement in this technology.

Introduction
Fertilization is a complex process that enables the reproduction and continuation of the species. In mammals, successful fertilization requires that sperm should survive the extremely harsh environment of the female reproductive tract and reach the site of the newly released egg(s) in the oviduct (or Fallopian tube in humans). The oviduct, a part of the female reproductive tract, is a tube-like structure that connects the ovary to the uterus. The oviduct is composed of the following three main regions ordered from the ovary toward the uterus: the infundibulum (fimbria in humans), in which most cells are ciliated epithelial cells; the ampulla, which contains large numbers of ciliated epithelial cells and is the site of fertilization; and the isthmus, which contains a large number of secretory epithelial cells. With these three distinct structures, the oviduct serves as a passage that transports gametes and the embryo as well as provides important structural, environmental and nutritional support for early embryonic development. Unlike the ovary and uterus, which have been extensively studied and relatively well understood, the oviduct is less well understood for its contribution in reproduction. Yet, dysregulation or disruption of oviductal function can result in infertility or life-threatening conditions such as ectopic pregnancy.

This review focuses on the oviductal function in establishing successful pregnancy, with new insights based on recent discoveries. This article first explains the oviductal function before fertilization during sperm and egg transport. Then, it describes oviductal function in fertilization, embryo development, embryo transport and abnormalities of the oviduct that could disrupt these processes. Because assisted reproductive technologies (ARTs), such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), can bypass the human Fallopian tubes entirely, this review also outlines the possible adverse outcomes of IVF/ICSI to emphasize...
the importance of the oviduct during fertilization and embryo development.

The path to fertilization

At intercourse, sperm entering the vagina have to survive a hostile vaginal microenvironment, including strong acidic conditions (Oberst & Plass 1936), before entering the cervix. The cervical mucus flow flushes out pathogens and removes non-motile sperm (Tung et al. 2015), as recently reviewed in Suarez (2016). The flow naturally selects for healthier sperm to advance to the uterus, where phagocytosis continues to remove weaker sperm. Then, the sperm undergo hyperactivation, a process that is required to complete their physiological change to become competent for fertilizing the egg (reviewed in Tosti & Menez 2016). Only hyperactivated sperm generate a strong counter-beating flagellum to overcome the viscoelastic mucus created by oviductal epithelial cells (Suarez et al. 1991, 1992). Details for sperm transport through the female reproductive tract are discussed in the following sections.

Oviduct guides sperm to the fertilization site

There are three potential mechanisms that guide the sperm through the oviduct, including rheotaxis, thermotaxis and chemotaxis.

Rheotaxis

Once sperm enter the oviduct, they will have direct contact with the oviductal fluid, which is generated by the transudate fluid from the systematic circulation and the secretory epithelial cells of the oviduct (Leese 1988). The fluid current is generated by ciliated epithelial cells and tubal contraction, which provides significant support to transport eggs and embryos. For sperm, the contact with epithelia and fluid to cause fertilization requires morphological changes to overcome this upcoming obstacle. One of the tubal current functions is to conduct a rheotaxis mechanism to guide the sperm to the site of fertilization (Fig. 1) (Miki & Clapham 2013). Rheotaxis is a mechanism whereby capacitated sperm can move against the direction of the current.

Soon after the sperm enter the isthmic region of the oviduct, the sperm heads attach to the oviductal epithelial cells. The studies using scanning electron micrographs of bovine sperm illustrate the physical interaction between the sperm head and cilia on the apical surface of oviductal epithelial cells (Pollard et al. 1991). Interaction between oviductal epithelial cells and sperm has also been observed in rabbit (Smith & Nothnick 1997), horse (Dobrinski et al. 1997) and human (Morales et al. 1996). In mice, the sperm attach to and detach from the oviductal epithelial cells several times before reaching the ampulla (Chang & Suarez 2012). Additionally, recent findings indicate that there are dynamic differences between the physical interactions between the sperm and the oviductal epithelial of the ampullary and isthmic regions (Ardon et al. 2016). Ultimately, the attachment of the sperm and the epithelial cells create a deposition or a reservoir of sperm within the oviduct.

Sperm–oviductal epithelial interaction causes the modification of sperm surface proteins and subsequently induces the hyperactivation of the sperm by activating the CatSper (Cation channels of Sperm) on the flagella (Ren et al. 2001, Quill et al. 2003, Chung et al. 2011, Miki & Clapham 2013). Upon CatSper activation, a large Ca\(^{2+}\) ion influx in the sperm flagella is triggered, subsequently...
altering the beating pattern from symmetrical to aggressive asymmetrical propulsion (Fig. 1). Asymmetrical beating of the flagella produces greater amplitude of force and subsequently aids its speed toward the end of the journey. Studies have demonstrated that CatSper activation and the physical rotation of the sperm flagella are the mechanisms behind rheotaxis (Miki & Clapham 2013). The sperm from male mice lacking CatSper cannot be hyperactivated, and the males are sterile (Ren et al. 2001). Therefore, the activation of the CatSper channel and the downstream physiological changes are required for sperm to become fertile.

Thermotaxis

In addition to the CatSper channel, a Ca\(^{2+}\)-sensing transient receptor potential channel (TRPM8) (De Blas et al. 2009) and a G protein-coupled receptor well known for its role in photon sensing in the retina (opsins) (Perez-Cerezales et al. 2015) are also present on the sperm. Combined with other recent studies on human sperm, it is possible that sperm have the ability to detect shallow temperature differences of less than one-hundredth of a degree (in Celsius) (Bahat et al. 2012). Thermosensing ability allows sperm to be directionally guided according to the oviduct temperature gradient, as previously observed in rabbit, mouse, pig, cow and human (Hunter et al. 2000, Bahat et al. 2003, 2005, Hunter 2012). Despite the studies conducted concerning the thermosensing channels and the temperature difference in the oviduct, some evidence suggests that the temperature gradient in the oviduct serves to influence gene expression and protein modification of the egg and embryo (Grinsted et al. 1985, Ye et al. 2007); however, thermotaxis may play a minor role in sperm guidance compared with rheotaxis.

Chemotaxis

Chemotaxis of sperm has been studied in different species and is most well known in sea urchins via resact, a peptide released from the egg (Ward et al. 1985). In Xenopus, egg jelly also produces allurin as chemotaxis for sperm attraction (Olson & Chandler 1999, Olson et al. 2001). In humans, freshly released cumulus-oocyte complexes (COCs) can secrete progesterone (P\(_4\)) as a chemoattractant for sperm (Teves et al. 2006, Oren-Benaroya et al. 2008). Chemokine receptors – CCR1, CCR5 and CCR6 – have been identified on human sperm (Isobe et al. 2002, Caballero-Campo et al. 2014). CCL20 ligands found in the follicular fluid can bind to CCR6 and alter sperm directional movement (Caballero-Campo et al. 2014). The merging of follicular fluid and the oviductal fluid after ovulation may provide sufficient chemoattractants to ensure the arrival of sperm at the ampulla.

Atrial natriuretic peptide (ANP) and its precursor A (NPPA) are found in pig, mouse, rat and rabbit oviducts (Kim et al. 1997, Zhang et al. 2006, Bian et al. 2012). ANP receptor (NPR1), however, is expressed on the sperm. Upon ANP and NPR1 binding, ANP activates the cyclic GMP-dependent protein kinase pathway (PKG) and induces acrosome reactions in pig, cow and human sperm (Zamir et al. 1995, Rotem et al. 1998, Zhang et al. 2006). This reaction might provide additional evidence regarding the role of ANP as a human sperm chemoattractant (Zamir et al. 1993, Anderson et al. 1995).

Anandamide (AEA), a phospholipid signaling molecule, acts through cannabinoid receptors 1 and 2 (encoded by Cnr1 and Cnr2 genes) and was previously identified to regulate neurological signaling and memory (Subbanna et al. 2013, Basavarajappa et al. 2014). AEA has been found in oviductal fluid, whereas CNR1 is present on the sperm (Aquila et al. 2010, Gervasi et al. 2013). AEA regulates sperm metabolism through insulin secretion (Aquila et al. 2009), implying the possibility that AEA from the oviduct externally facilitates the metabolism of sperm. Recently published studies suggested that AEA in the oviduct activates CNR2 and transient receptor potential vanilloid 1 (TRPV1) to induce Ca\(^{2+}\) influx into the sperm, which become hyperactivated and are released from oviductal epithelia (Gervasi et al. 2011, 2016, Osyczka-Salut et al. 2012, Amoako et al. 2013).

Overall, the oviduct guides sperm toward the fertilization site through various comprehensive mechanisms. Most importantly, the oviduct facilitates the hyperactivation of sperm to become fertile.

Egg entering the oviduct

Unlike sperm, eggs are released from the ovary during ovulation and enter the infundibulum (or fimbria). The cumulus cells surrounding the egg form the cumulus-oocyte complex or COC. Once inside the oviduct, the cumulus cells serve as a nutrient support for the egg. The cumulus cells use glucose for their own energy production (Sutton et al. 2003) and also produce energy sources (pyruvate and cysteine) that are needed for the cellular functions of the eggs (Tanghe et al. 2002, Sutton-McDowall et al. 2010). Cumulus cells also bridge
the communication between the environment and the egg through gap junctions (Simon et al. 1997, Li et al. 2007, Huang & Wells 2010).

The initial attachment of the egg to the oviduct epithelia is accomplished through COC-oviduct epithelia interaction. The filaments of the extracellular matrix from cumulus cells adhere to the glycocalyx at the entrance of the ciliary crowns at the epithelial cells of the infundibulum (Lam et al. 2000). Then, the COC is drawn into the oviduct and is ready for fertilization.

**Other aspects of oviduct-guided fertilization**

Sperm orient themselves by reacting to the oviduct environment to continue along the path of fertilization. The oviduct also reacts to the presence of the sperm and optimizes the microenvironment within the oviductal lumen by regulating fluid viscosity, oviductal muscular contraction and by promoting sperm–egg recognition.

**Oviductal fluid**

Sperm is bathed in oviductal fluid to advance toward the site of fertilization. The oviductal fluid is generated from secretory cells of the oviduct and is regulated by estrogen (E₂) and other hormones (discussed in detail in a later section). The oviductal protein concentration is the lowest at ovulation and highest around menstruation (Lippes et al. 1981). Changes in protein concentration and its content can alter fluid viscosity, hence influencing the flow rate of the fluid. Oviductal epithelial cells sense the change of fluid viscosity by transient receptor potential vanilloid 4 (TRPV4) channel (Andrade et al. 2005, Teilmann et al. 2005, Lorenzo et al. 2008). TRPV4 detects phospholipase in the oviductal fluid and regulates ciliary beat frequency (CBF) to enhance the fluid movement when it is too viscous, as observed in the respiratory tract. The oviduct can also sense the presence of sperm and adjust the protein content by increasing heat shock protein 70 (HSP70) and antioxidants in the oviductal fluid, possibly to help reduce sperm stress (Georgiou et al. 2005).

**Smooth muscle contraction**

The smooth muscle contraction in the oviduct is regulated by prostaglandins (PGs) through prostanoid receptors, which are modulated by E₂ (Spilman & Harper 1975, Ball et al. 2013, Huang et al. 2015). In humans, the oviductal PGs are mainly PGE and PGF produced by epithelial cells (Lindblom et al. 1983). PGE₂ and PGF₂α increase muscle contraction, whereas PGE₁ decreases muscle contraction (Wanggren et al. 2008). Evidence indicates that the contraction is possibly regulated by both E₂ and P₅α, as estrogen and progesterone receptors (ESR and PGR) are expressed in the interstitial Cajal-like cells in the muscle cell layer of the oviduct (Cretoli et al. 2009). The function of the muscle contraction is generally recognized to be for sperm transport purposes (Overstreet & Cooper 1978a). Suarez and coworkers suggested that the muscle contraction mainly helps the sperm to pass through the cervix, rather than acting as a rapid transport for sperm to reach the fertilization site (Suarez & Pacey 2006). This idea is strongly supported by experiments in rabbits, in which the sperm that reached the end of the oviduct within a few minutes were damaged (Overstreet & Cooper 1978a, b).

**Fertilization**

Recent studies demonstrated that heat shock proteins are involved with sperm–egg recognition. Heat shock protein member A2 (HSPA2) is present in the human spermatozoon and binds with arylsulfatase A (ARSA) and sperm adhesion molecule 1 (SPAM1) (Redgrove et al. 2012, 2013, Bromfield et al. 2016). Both ARSA and SPAM1 are detected in the rabbit and mouse oviduct (Vitaoli et al. 1996, Griffiths et al. 2008). HSPA2 can also bind with angiotensin-converting enzyme (ACE) and protein disulfide isomerase A6 (PDIA6) to form a complex, and then engage in sperm-zona recognition. Interestingly, oxidative stress of sperm can significantly reduce the binding ability of the ARSA/SPAM1/HSPA2 complex to the zona pellucida (Bromfield et al. 2015).

Soon after the egg and sperm convene at the ampulla, fertilization occurs. With the penetration of the sperm into the egg, ovastacin is released from the egg’s cortical granules and cleaves the zona pellucida 2 protein (ZP2) (Burkart et al. 2012), leading to zona hardening and preventing polyspermy. In a recent study, ZP2 peptide-treated beads, deployed in the mouse female reproductive tract, act as a decoy to attract sperm and form binding, resulting in female infertility (Avella et al. 2016). These ZP2 peptide beads efficiently provide a contraceptive mechanism without any pathological defect in the female reproductive tract. Another newly discovered sperm–egg interaction is that of Izumo1 and Juno. Izumo1 is present on the sperm and interacts with the Juno receptor on the egg, causing a rapid shedding of Juno to prevent
polyspermy (Bianchi et al. 2014). Juno is a species-specific protein and may contribute to the prevention of cross species sperm–egg recognition (Han et al. 2016).

After sperm–egg recognition, the gametes fuse and the pronuclei form. This event leads to embryogenesis, and the next chapter of development begins. Embryogenesis is a very early stage of the development in which the embryo undergoes a few cellular divisions before entering the uterus. It usually takes 3–4 days for human and mouse embryos to develop into the 8-cell (human) or 16-cell (mouse) stage in the oviduct. Those early cell divisions do not increase cell size but rather equally allocate the cytoplasm from the original zygote (Pelton et al. 1998). The embryo is transported from the site of fertilization toward the end of the oviduct during the early cleavage stage. At the end of the oviduct, embryos prepare to enter the uterus at the morula and blastocyst stages. The simultaneous embryo development and transport in the oviduct is an inseparable mechanism under normal physiological conditions.

Oviductal influence on embryo development

The pre-implantation embryo is housed inside the oviduct, exposed to and surrounded by oviductal secretory fluid and in contact with the oviductal epithelial cells. The microenvironment within the oviduct provides a stable temperature, optimal pH and dynamic fluid secretions to support embryo development.

Before fertilization, the oviductal fluid serves in the following three major functions: gamete protection, sperm guidance and egg guidance (Ballestero et al. 2014, Kumaresan et al. 2014). After fertilization, the oviduct assists the development of pre-implantation embryos by producing the factors required for embryo cleavage (discussed in following sections). This phenomenon was first described in the sheep model where the oviductal epithelial cells were cultured with the embryos (Gandolfi & Moor 1987). Gandolfi and coworkers found that the blastocyst cleavage rate was at 80% when the embryos were cultured with oviductal epithelial cells, in comparison to a 33% cleavage rate when cultured with fibroblast cells. This finding indicates that the presence of oviductal epithelial cells, and not just any type of somatic cell, is crucial for blastocyst development. The improvement of embryo development after culturing with oviductal epithelial cells or explanted oviduct has also been demonstrated in several species, including mouse (Sakkas & Trounson 1990), pig (White et al. 1989), cattle (Eyestone & First 1989) and human (Yeung et al. 1992). These findings indicate that the oviduct epithelia and the oviductal fluid provide the pre-implantation embryos an ideal physiological and biochemical environment to sustain development.

To determine the possible factors secreted from the oviductal epithelial cells that support and promote embryo development, we have outlined the fluid constituents during the early stage of pregnancy. Oviductal fluid is generated by two compositions: (1) the transudation of the systemic circulation and (2) the active biosynthesis from the secretory epithelial cells of the oviduct (Leese 1988). The fluid is composed of albumin, transferrin, glycoproteins, galactose, immunoglobulin, glucose, pyruvate, amino acid, lactate, cytokines, many growth factors and a monitored gas composition (Beier 1974, Leese 1988). The functions of these factors in the oviduct are discussed in the following sections.

Oviductal fluid: influence on embryo nutrient and growth

Nutrients

Early embryos usually stay in the oviduct for 3–4 days (Croxatto 2002). During this time, the fertilized embryos transition from oxidative metabolism to glycolic metabolism (Folmes & Terzic 2014). This transition is accompanied by (1) different nutrient compositions as the embryo travels through the oviduct, (2) maturation of the mitochondria to enable the embryo to establish its own metabolism and (3) a change in oxygen tension parallel to this shift in metabolism (Gardner et al. 1996, Absalon-Medina et al. 2014).

After the cumulus cells fall off in post-fertilization, the pre-implantation embryos use oxidative metabolism to acquire energy, mostly from pyruvate and lactate as the main sources of energy (Gardner & Leese 1990, Dumollard et al. 2007a, b, Absalon-Medina et al. 2014). Pyruvate, lactate, lipids and amino acids are present in the oviductal fluid, and the levels of these nutrients fluctuate through the estrous cycle in mice, rabbits, pigs and humans (Nieder & Corder 1982, 1983, Nichol et al. 1992, Leese et al. 1993, Tay et al. 1997). The carboxylic acids are processed through immature mitochondria (characterized by the short and less formed cristae, shown in Fig. 2A) (Motta et al. 2000, Trimarchi et al. 2000, Dumollard et al. 2007a). Several studies in pigs and cattle showed that fatty acids (in a form of acyl-coA) are used as an energy source for the embryo (Sturme et al. 2009,
Sutton-McDowall et al. 2012). Acyl-CoA diffuses through the mitochondrial membrane in the presence of carnitine (as a cofactor) through carnitine palmitoyl transferase 1B (CPT1B) and is used for β-oxidation, resulting in the production of acetyl-CoA (Sutton-McDowall et al. 2012).

As embryos continue developing, glycolytic metabolism slowly takes over as the mitochondria become mature. However, in human, the metabolic switch is not complete until the blastocyst stage (Sathananthan & Trounson 2000). This glycolytic metabolism requires supplies of glucose and simple sugars. Glycogen granules are present in the ampulla and isthmic secretory cells of the oviduct in monkey and human (Odor et al. 1983, Schultka & Cech 1989). In pigs, the glycogen level increases after ovulation (Lindenbaum et al. 1983, Gregoraszczuk et al. 2000). This evidence suggests that oviduct secretion prepares embryo metabolism transit during the pre-implantation stage. Additionally, enzyme amylase (AMY2B) plays a major role in converting glycogen into sugar. Several studies indicated that there is an upregulation of AMY2B in the human Fallopian tube (Mc et al. 1958, Hochberg 1974, Hayashi et al. 1986, Groot et al. 1990, Marquez et al. 2005). Therefore, it is likely that the compact polysaccharide can be converted into a simple sugar by AMY2B in the oviduct, and these simple sugar molecules can be used in cellular metabolism by both the oviduct and the cleaving embryo.

When human embryos develop into blastomeres, mitochondria are equally divided into each of the cells and are elongated and matured (Fig. 2A). The mature mitochondria can now use oxygen and glucose in glycolysis to provide ATP for further embryo development (Sathananthan & Trounson 2000). In mice, this is evident with the timing of embryo oxygen consumption, which peaks at the blastocyst stage (Leese 2012).
In addition to energy substrates, CO₂ also serves as a carbon source for RNA synthesis (Quinn & Wales 1974, Pike et al. 1975). In mouse and rabbit, CO₂ fixation has been demonstrated in early developing embryos (Quinn & Wales 1974, Pike et al. 1975). The level of CO₂ is inversely proportional the HCO₃⁻ concentration, which is modulated by the carbonic anhydrase (CA) enzyme expressed in the oviductal epithelial cells (Lutwak-Mann 1955, Ge & Spicer 1988) as well as a Cl⁻/HCO₃⁻ exchanger (solute carrier family 26) expressed in pre-implantation embryos (Lu et al. 2016). The HCO₃⁻ level in the oviduct is relatively high compared with other tissues (Vishwakarma 1962, Maas et al. 1977), and it has been previously shown that HCO₃⁻ is indispensable for the cleavage of pre-implantation embryos (Kane 1975). These studies indicate that a balance of CO₂ and HCO₃⁻ concentration needs to be fine-tuned not only for optimal pH conditions but also for RNA synthesis and the normal cleavage of the pre-implantation embryo development.

**Growth factors**

Embryotrophic factor-3 from human oviductal cells plays a significant role in enhancing pre-implantation embryo development by promoting proliferation and inhibiting apoptosis (Xu et al. 2004). Epidermal growth factor (EGF) (Adachi et al. 1995), transforming growth factor (TGF) (Chegini et al. 1994), insulin-like growth factor (IGF) (Carlsson et al. 1993, Pfeifer & Chegini 1994, Daliri et al. 1999) and fibroblast growth factor (FGF) are all detected in human Fallopian tissues (Fig. 2B). Mouse embryos cultured with EGF, TGF and IGF have an increased number of blastocyst development from 2-cell embryos (Paria & Dey 1990). The co-cultured 2-cell stage embryo and the Fallopian tubule epithelial cells significantly increase the cleavage rate and enhance blastocyst development (Takeuchi et al. 1992). However, in similar co-culture conditions, inhibition of EGF and TGF will attenuate the development of embryo from the cleavage stage to blastocyst. IGF receptor is also detected in 8-cell stage buffalo embryos (Daliri et al. 1999). This suggests that embryotrophic factors, including growth factors in the tubal fluid, can have a direct positive impact on cleavage stage embryo development.

In addition to the growth factors listed previously, hormone-like lipids such as prostaglandins also promote embryo development. In mice, the oviduct produces 10 times more prostaglandin I₂ (PGL₂) at day 2 than day 4 after coitus (Huang et al. 2004). The spiked production of PGL₂ is timed with the early cleavage stages of the embryo, and then the production drops around the time embryos hatch in the uterus. This finding suggests that PGL₂ potentially plays a role in early embryo development. Additionally, several studies demonstrated that addition of transferrin, albumin and selenium can improve bovine and goat embryonic development (Hammami et al. 2013, Wydooghe et al. 2014, Xie et al. 2015, Guimaraes et al. 2016). These findings suggest that the presence of growth factors in the tubal fluid may play an important role in promoting and enhancing embryogenesis.

**Oviductal fluid: protection against embryo stress**

Tubal fluid protects gametes from environmental stress to ensure embryo quality and pregnancy outcome. After the shedding of the cumulus cells, the embryo depends on tubal fluid and internal antioxidant activities to gain protection against reactive oxygen species (ROS)-induced stress (Fig. 3). Two major systems are involved in this process: non-enzymatic and enzymatic antioxidants (reviewed in Guerin et al. 2001).

Reduced glutathione (GSH), taurine, hypotaurine and cysteamine (CSH) are the main non-enzymatic antioxidants in the oocytes and embryos (Guerin et al. 2001). GSH reduces ROS level in the oocytes and increases the hatching rate of mouse blastocysts when added into the culture medium (Gardiner & Reed 1994). Moreover, considerable amounts of GSH are detected in the mouse oviduct and uterine flushing (Gardiner et al. 1998). CSH is
present in the secretions of the female reproductive tract (Guyader-Joly et al. 1998) and is proposed to act through the hydroxyl radical (OH•) scavenging pathway (Guerin et al. 2001). The OH• scavenging pathway may also covert CSH into hypotaurine, a sulfenic acid neutralizing OH• activity, and generate taurine as a byproduct (Guerin & Menez 1995). Both hypotaurine and taurine are present at high levels in the embryo environment and are produced by the oviduct epithelial cells of cow, sheep, goat and rabbit (Guerin et al. 1995, Guerin & Menez 1995). In addition, albumin and transferrin are highly abundant in the oviductal fluid. Transferrin acts as a metal chelator to prevent the formation of OH• from Fe2+ ions (Nasr-Esfahani & Johnson 1992, Guerin et al. 2001), whereas albumin is shown to prevent lipid peroxidation in the sperm (Alvarez & Storey 1983), suggesting that both transferrin and albumin may provide indirect protection for the embryos against oxidative stress.

In the enzymatic pathway, catalase, superoxide dismutases (SODs) and glutathione peroxidases (GPXs) are the major enzymes involved in embryo protection (Guerin et al. 2001). Catalase is one of the enzymes that converts ROS into H2O and O2 (Harvey et al. 1995). Similarly, SOD1 and SOD2 bind to ROS byproducts and turn the byproducts into O2 and hydrogen peroxide (El Mouatassim et al. 2000, Jang et al. 2010). Catalases, SOD1 and SOD2 are found in the oviduct of mice, cows and humans. Glutathione also plays a similar role in embryonic development and protection (Salmen et al. 2005, Hansen & Harris 2015). GPX4 is well known for its function in suppressing cell death and reduction in hydrogen peroxide (Imai et al. 1996, Agbor et al. 2014). Increase in E2 induces the GPX4 production in bovine oviduct (Lapointe et al. 2005). This evidence indicates that E2 and the oxidative stress preventing enzymes act in concert to protect the embryos against ROS exposure.

In addition to oxidative stress, embryos also encounter physical stress from the environment. The 1- to 2-cell stage mouse embryo expresses HSP70, which is regulated by heat shock factors (HSFs) (Christians et al. 1995, 1997). HSP70 family proteins play an important role in protecting the cell from heat stress by ensuring correct protein folding. Interestingly, the oviduct expresses HSF25 and 70 (Fig. 3), which can potentially provide heat stress protection at the molecular level to ensure correct protein folding in the newly fertilized embryo (Mariani et al. 2000, 2003). Mice lacking Hsfs had significant increases in cell death when the embryos experienced heat stress for 2 h (Le Masson & Christians 2011). A study in water buffalo indicated that when the embryos are exposed to heat stress for a prolonged period of time (24 h), the heat also decreases embryo cell numbers (Ashraf et al. 2014). These studies suggest that there is a tolerable limit of heat stress that the embryo can handle.

**Estrogen-mediated embryo protection against immune system**

E2 and its nuclear receptors (estrogen receptors) play a major role in the reproductive system, from regulating the hormonal cycle, ovulation and sexual behavior to cancer development (Couse & Korach 1999). Numbers of studies demonstrated that E2 is required for immunoprotection in the female reproductive tract, including the vagina and uterus (Wira et al. 2005, Haddad & Wira 2014). However, the actions of E2 in regulating the immune functions in the oviduct are unclear. Recently, a study indicated another important property that E2 possesses—embryo protection. E2 acts through estrogen receptor α (encoded by Esr1 gene) in the oviductal epithelial cells, which protects embryos from the attack of the maternal immune system. Loss of ESR1 in the oviductal epithelial cells in female mice results in excess protease activity and increased expression of antimicrobial peptides such as defensins. These changes, due to a lack of ESR1 in the oviduct, dampen the plasma membrane integrity of the embryos and ultimately cause embryonic death before the 2-cell stage (Winuthayanon et al. 2015). The study demonstrated that the epithelial ESR1 is required to suppress innate immune systems (Fig. 3) by changing gene expression related to inflammation responses in the oviduct during day 1 and 2 of pregnancy. This result suggests that without E2 signals through ESR1 on the oviductal epithelial cells, newly fertilized embryos will not be able to overcome the mother’s immune system. These findings reveal another infertility scenario that previously has not been demonstrated, in which the disruption of E2 signaling or ESR1 action in the Fallopian tube can cause infertility.

**Gas in the oviduct**

Little is known about the gas composition in the oviduct or the oviductal fluid. Compared with the 20% oxygen level in the atmosphere, the concentration of oxygen in the oviduct of monkeys, hamsters and rabbits is between 2% and 8% (Mastroianni & Jones 1965,
Yedwab et al. 1976, Fischer & Bavister 1993). The relatively low oxygen concentration in mammalian oviducts could result in minimal ROS levels and protect embryos from stress, as high concentrations of oxygen can lead to an increase in ROS and oxidative stress (Catt & Henman 2000).

Dysregulation of H2S gas in the Fallopian tubes has been linked to impaired embryo transport in humans. H2S is abundant in the Fallopian tube epithelial cells and synthesized intrinsically through the cell cytoplasm. This signaling pathway is upregulated during pregnancy and is mainly responsible for spontaneous oviduct muscle contraction, which provides a positive factor to the embryo movement toward the uterus (Ning et al. 2014). Nitric oxide has been identified in the Fallopian tube; it mediates tubal muscle contraction, with possible roles in the regulation of sperm motility (Ekerhovd et al. 1997, Kobayashi et al. 2016). Dimethylarginine dimethylaminohydrolase 2 (DDAH2), an enzyme regulating nitric oxide synthesis, is also expressed in the oviduct in the presence of egg and embryo (Georgiou et al. 2005). Therefore, H2S and nitric oxide could contribute to the regulation of tubal contraction during fertilization and embryo transport.

**Embryo transport**

It is necessary to mention embryo transport, as embryo development and transport occur simultaneously. Embryo transport from the oviduct to the uterus takes approximately 1–10 days, depending on the species (reviewed in Croxatto 2002). In mammals, unfertilized eggs and embryos are transported to the uterus at different rates. In horses, only embryos are transported to the uterus, whereas the unfertilized eggs are retained in the oviduct (Betteridge & Mitchell 1974, Flood et al. 1979, Freeman et al. 1992). Several studies indicated that horse embryos produced prostaglandin E2 (PGE2), which mediates an acceleration of the transit to the uterus (Weber et al. 1991a,b). In rats and hamsters, fertilized eggs reach the uterus at higher rates compared with the unfertilized eggs (Villalon et al. 1982, Ortiz et al. 1986). These findings illustrate that embryo transport is an interactive process between the embryos and the oviduct. Here, the three major elements regulating embryo transport are listed: the beating of ciliated epithelia, tubal fluid flow and tubal muscle contraction (Fig. 4).

![Embryo transport](image)

**Figure 4**

Roles of the oviduct during embryo transport. Embryo transport is composed of tubal muscle contraction and the motility of ciliated epithelial cells. Estrogen (E2) generally increases the tubal muscle contraction, tubal fluid secretion, and ciliary beat frequency (CBF), which accelerate the embryo transport rate. Opposite to E2, progesterone (P4) causes muscle relaxation and decreases CBF to reduce the embryo transport rate. In addition, P4 also inhibits E2-induced tubal fluid production. Prostaglandins (PGs) can be produced in the oviductal epithelial cells or induced by E2 treatment. PGE2 and PGF2 stimulate muscle contraction in both human and bovine oviducts. Endothelin 1 and 2 are expressed and contribute to the oviduct contraction. IP3, inositol triphosphate; PGE2, prostaglandin E2; PGF2α, prostaglandin F2α; PGR, progesterone receptor.

**Ciliary beating**

Ciliated epithelial cells have a multiciliated structure on the apical plasma membrane. The beating of the cilia generates the movement of the fluid in the oviduct, which promotes the movement of the embryo.

P4 and E2 are the key players in regulating ciliary beat frequency (CBF). P4 reduces CBF through classical progesterone receptors (PGRs) expressed on the ciliated epithelial cells in a dosage-dependent manner in humans and mice (Mahmood et al. 1998, Bylander et al. 2010, 2013). A non-classical PGR is expressed in the lower part of the cilia stalk in mouse oviducts (Teilmann et al. 2006). Recent studies in mice indicated that low dosage of P4 and short activation time (within 30 min) are sufficient to reduce oviductal CBF (Bylander et al. 2010, 2013). Therefore, extra-nuclear signaling of CBF through non-genomic actions of P4 without involving a long-delayed genomic regulation may be the cause of direct CBF regulation.

E2 is required to accelerate the transport of the eggs (Orihuela et al. 2001). E2 acts via a non-genomic pathway through protein phosphorylation of PKC and PKA in rats and cows (Orihuela & Croxatto 2001, Orihuela et al. 2003, Wen et al. 2012). Moreover, in the oviductal secretory cells, E2 induces the production of cAMP, which promotes adrenomedullin activation (Liao et al. 2013). In rats, adrenomedullin increases CBF by acting through the calcitonin-gene-related peptide (CGRP) receptor in the oviductal epithelial cells (Liao et al. 2011).
The findings from muscarinic receptor-knockout (Chrm1−/−, Chrm3−/−, Chrm4−/− and Chrm5−/−) mice suggest that the cholinergic neuromuscular system is not required for the ciliary beat function, as the particle transport rate in the oviduct remains unchanged compared with their control littermates (Noreikat et al. 2012).

**Tubal muscle contraction**

The embryos move back and forth along the oviduct due to the contraction of myosalpinx, with the net progress toward the uterus (Talo 1991). However, studies in rabbits and rats showed that inhibition of muscle contractility does not affect the transport of the embryos (Halbert et al. 1976, 1989). It suggests that at least in these species, ciliary beating alone is capable of transporting the embryos from the oviduct to the uterus. In humans, oxytocin, P4, PGs and nitric oxide participate in the tubal muscle relaxation and contraction (Ekerhovd et al. 1997, Jankovic et al. 2001, Wanggren et al. 2008). P4 through PGR-A and PGR-B relaxes muscle contraction in mice (Conneely et al. 2003). Administration of P4 in the ex vivo culture of human Fallopian tube reduces both amplitude and frequency of tubal muscle contraction (Wanggren et al. 2006); however, treatment with PGR receptor antagonist, mifepristone, has a minimal effect on the contraction. Nevertheless, the exact signaling pathway, beyond the ligand–receptor interaction, through which P4 is involved in directing muscle relaxation remains unclear.

E2 can induce the production of inositol triphosphate (IP3) to increase smooth muscle contraction and accelerate egg transport in the rat oviduct (Orhiuela et al. 2006). Additionally, E2, P4 and endothelin-1 stimulate the release of PGs (PGE2 and PGF2α) in bovine oviduct (Wijayagunawardane et al. 2001). Both PGF2 and PGF2α are shown to stimulate muscle contraction in human and bovine oviducts (Wijayagunawardane et al. 2001, Wanggren et al. 2008). Endothelin-2 alone can also induce muscle contraction in the rat oviduct through endothelin receptor type A (Al-Alem et al. 2007). These findings indicate that ovarian hormones have both direct effects on stimulating the tubal muscle contraction and indirect effects via the induction of PGs.

Cannabinoid receptor 1 and 2 (Cnr1 and Cnr2), which are G protein-coupled receptors, play crucial roles in pregnancy. Cnr1−/− and Cnr1−/−/Cnr2−/− double-knockout mice have significantly higher number of embryos retained in the oviduct (35–46% retained embryos in the oviduct) compared with WT control (0% in oviduct) (Wang et al. 2004). This suggests that CNR1 and CNR2 are important for normal embryo transport.

**Tubal fluid flow**

Studies assessing the movement of microspheres showed that the orientation of fluid is toward the uterus in rabbits, sheep, cows and guinea pigs, whereas the flow is oriented toward the ovary in the pigs (Gaddum-Rosse & Blandau 1973, 1976). These results showed that the tubal fluid flow is oriented differently depending on the species. In sheep and rabbits, the oviductal fluid secreted from secretory epithelial cells is increased by female steroid hormones, especially E2 (Hamner & Fox 1968, McDonald & Bellve 1969). The fluid production is attenuated when P4 is co-administered with E2, suggesting that P4 opposes E2-induced fluid secretion (Fig. 4). In rabbits, ovariectomy causes decreased secreted fluid in the oviduct, and this fluid level can be restored by E2 treatment (Bishop 1956), suggesting that E2 is the major regulator for secretory fluid production within the oviducts. A recent study in mice demonstrated that inhibition of prolactin, a hormone secreted from the anterior pituitary after mating, using bromocriptine severely reduced the oviductal fluid volume and flow (Miki & Clapham 2013). The increased oviductal fluid secretion is mostly due to the increased water availability, which is related to the function of the transmembrane water transport proteins, aquaporins (AQPs). In rats, expression of Aqp5, Aqp8 and Aqp9 is regulated by both E2 and P4 (Branes et al. 2005). These findings indicate that E2, P4 and prolactin play a major role in fluid production in the oviduct. In summary, an overall action of E2 is to increase the embryo transport rate by stimulating muscle contraction, inducing fluid production and flow, and increasing the CBF, whereas P4 has an opposite effect of E2 by reducing the embryo transport rate.

**Recently identified pathways affecting embryo transport**

The incidence of ectopic pregnancy is one in every 50 normal pregnancies, and 95% of ectopic pregnancies are associated with defective Fallopian tubes (Tenore 2000). Recent studies demonstrate that some novel pathways contribute to the pathological conditions of Fallopian tubes, with potential ramifications in human infertility.
Pathways crucial for normal oviduct development

In the majority of mammals, including rodents and rabbits, a healthy oviduct is a rather coiled tube with no obstructions within to ensure an open passage for eggs, sperm and embryos (Stewart & Behringer 2012). In humans and non-human primates, however, the coiled structure is not present in the Fallopian tubes. Due to difficulties in obtaining human tissues, scientists have been using genetically engineered mice as a model organism to study the roles of proteins of interest during oviduct development. The recent findings demonstrated that the morphological changes leading to a less coiled or unclored oviduct and formation of cysts within the oviduct can result in infertility. Wingless-type integration family member 4 (WNT4) signaling is critical for the development of the female reproductive tract, as female mice lacking Wnt4 expression showed a non-coiled oviduct that lacked folding (Prunksaitė-Hyyrylainen et al. 2016). Moreover, overexpression of Notch in the reproductive tract produced similar phenotypes whereby the oviduct failed to coil (Ferguson et al. 2016).

Dicer, an endonuclease responsible for microRNA (miRNA) function, is also crucial for the development of the female reproductive tract. Loss of Dicer in the mouse reproductive tract disrupts oviduct organization by reducing both the length and coiling (Nagaraja et al. 2008, Gonzalez & Behringer 2009). Additionally, mice lacking Dicer also developed oviductal cysts and severe inflammation in the oviduct at the uterotubal junction. These phenotypes lead to degeneration of the eggs and embryo transport failure. WNT7a is another critical signaling pathway involved in early female reproductive tract development, as a loss of Wnt7a results in female sterility due to an abnormal development of uterus and oviduct (Parr & McMahon 1998). These findings indicate that signaling molecules involved in WNT, Notch and miRNA regulation play critical roles in oviductal development and coiling.

Several recent studies discovered that epithelial cells in the female reproductive tract contain a subpopulation of stem-cell-like LGR5 (leucine-rich repeat-containing G-protein-coupled receptor 5)-positive cells (Ng et al. 2014). The LGR5-positive cell population has been identified in other tissues, such as the kidney (Barker et al. 2012), intestine (Barker et al. 2007) and stomach (Barker et al. 2010). LGR5-positive cells reside much like regular epithelial cells in the tissue, but the characteristics of these cells remain undifferentiated. The LGR5-positive population can be activated to divide and differentiate into designated cell types and is responsible for tissue renewal and regeneration. In the Fallopian tubes, these stem-like adult epithelial cells are concentrated at the fimbria (Paik et al. 2012, Snegovskikh et al. 2014).

Notch and WNT signals not only modulate the oviduct development but also mediate the differentiation of adult epithelial stem cells into other cells in Fallopian tubes (Kessler et al. 2015). Inhibition of Notch signaling by a γ-secretase inhibitor, dibenzazepine, in the oviduct and the stem-like epithelia leads to a genetic signature of cell differentiation into ciliated epithelium. Inhibition of Notch signaling reduces the number of stem cells in human Fallopian tube 3D organoids, while increasing ciliated cell number. LGR5 also regulates the WNT/β-catenin signaling pathway and can be used as a marker for adult oviduct epithelial stem cells (Capel 2014, Ng et al. 2014, Vieira et al. 2015). Studies in mice showed that the deletion of Lgr5 in female mice resulted in significantly fewer live births (Sun et al. 2014). These findings suggested that the number of ciliated epithelial cells in the Fallopian tubes is also controlled by the local stem-cell population via the WNT and Notch signaling pathways.

miRNAs also participate in oviductal ciliogenesis. Lacking miRNA-34 and miRNA-499 genes resulted in a loss of cilia in the trachea and oviduct epithelial cells (Wu et al. 2014). Additionally, serine/threonine protein kinase (STK36), a regulator of the hedgehog pathway, modulates a central pair construction of the cilia (the two center microtubules in the 9+2 microtubule axonemal structure). A lack of Stk36 causes an impairment of the cilia orientation and results in a failure to form the directional movement of the cilia in the oviduct (Nozawa et al. 2013). Moreover, a global deletion of Kif19a, a kinesis family member involved in cilia length regulation, causes female infertility due to elongated cilia in the oviduct (Niwa et al. 2012). Elongated cilia in the Kif19a−/− oviduct lead to excess mucus and cell debris in the oviductal lumen and blockage of the egg passage.

Pathways crucial for ciliogenesis and ciliary function

In addition to organ morphology, disruption of ciliogenesis and ciliated cell differentiation at a cellular level can lead to embryo transport defects. Ciliated epithelial cells in oviducts create tubal currents and are responsible for the transportation of the embryo (Lyons et al. 2006). A comprehensive review regarding cellular and molecular mechanisms governing ciliogenesis is provided in Choksi et al. (2014).
In addition, female mice lacking *Celsr1* (*Celsr1*−/−), a planar cell polarity gene, showed defective ciliary polarity, which resulted in a random orientation of cilia directionality. The impaired cilia were unable to transport beads in a uniform direction, which disrupted the transportation function of the oviduct (Shi et al. 2014). In conclusion, the presence, proper length, proper structure and directionality of the cilia are crucial for the oviduct transport function to support the gametes and embryos.

**In vitro fertilization and embryo development**

With recent technological advancement such as IVF and ICSI, infertility clinics can now provide solutions to allow infertile couples to conceive their own children. Procedures can bypass the Fallopian tube entirely and transfer the fertilized embryos directly into the uterus. The presence of these technologies questions the role of the oviduct and its necessity for human reproduction. ARTs are common procedure worldwide, including IVF and ICSI. However, not every couple has access to ARTs due to economical limitation. Moreover, there are several concerns regarding the use of ARTs, such as epigenetic change in the embryos due to culture conditions and controlled ovarian hyperstimulation (COH), complicated pregnancy due to multiple gestation and a lack of natural section (especially with ICSI). Therefore, the medical research community should take precaution and study possible complications with such technologies when bringing hope to many couples experiencing infertility.

For many couples with fertility issues, IVF may be their only hope to have offspring inheriting their genes. It is a technique whereby the clinicians fertilize the eggs with sperm outside the female reproductive tract and incubate the fertilized embryos in a laboratory until they are ready for implantation in the uterus. IVF has been practiced for decades throughout the world and has resulted in over 3.5–5 million newborns across the globe. The international committee for monitoring ART reported that with one million documented cases, the pregnancy rate of IVF/ICSI is 20–30% (Mansour et al. 2014) compared with 45–85% after 3–12 month conceived naturally (Luke et al. 2012).

A recent study from more than 178,000 women who went through IVF treatment suggested that an overall successful live birth rate through IVF pregnancy is 43% (McLernon et al. 2016). When a woman is over 38 years old, live birth success through IVF drops significantly to 21% (Stern et al. 2009). Compared with natural conception, IVF/ICSI-conceived embryos have a significantly higher risk of perinatal mortality, low birth weight and preterm birth (Pandey et al. 2012, Pinborg et al. 2013, Marino et al. 2014). When comparing IVF/ICSI with naturally conceived children, there is a 3–4 times higher chance for imprinting disorders, including Beckwith–Wiedemann syndrome, Prader–Willi syndrome, Angelman syndrome, Silver–Russell syndrome, transient neonatal diabetes mellitus, McCune–Albright syndrome, familial nonchromaffin paraganglioma, maternal hypomethylation syndrome and retinoblastoma in IVF/ICSI children (Owen & Segars 2009, Lazaraviciute et al. 2014).

A study comparing 7- to 8-year-old children who were conceived through IVF/ICSI and natural intercourse found that there is no difference in their cognitive ability, but there is an underlying gender difference (Punamaki et al. 2016). Naturally conceived boys showed more cognitive developmental problems than girls, whereas no differences were observed between boys and girls conceived through IVF. This study, however, is solely dependent on parental reports and may have a bias between IVF and naturally conceived parents. In terms of imprinting disorder, there is increased evidence of Beckwith–Wiedemann Syndrome in the IVF/ICSI-conceived children (4%) compared with naturally conceived children (0.7–1.2%) in a small cohort of 149 children in the UK (Maher et al. 2003). There is reduced methylation in KvDMR, an intronic CpG island in the *KCNQ1* (or *KvlQTI*) gene whose methylation status is associated with Beckwith–Wiedemann Syndrome (Smilinich et al. 1999), in the embryonic tissues conceived by the IVF method (Gomes et al. 2007).

With ever-improving biotechnology, more studies are required to understand the implications of the health and wellbeing of IVF individuals. As IVF-conceived individuals are still in their reproductive ages, long-term evaluation on transgenerational epigenetic outcomes will be needed.

**Epigenetics and environmental factors**

Embryos go through epigenetic changes and result in imprinting, which has a long-lasting effect in later development. Cell fate is not determined in the embryo before the morula stage. The early embryos repress epigenetic modification by removing DNA methylation and repressing histone modifications from the 2-cell stage until the blastocyst stage in mice (Reik et al. 2001,
Ma et al. 2012) and until the 8-cell stage in cows (Dean et al. 2001). However, the demethylation timing of DNA methylation in the embryo is on a gene-by-gene basis (Messerschmidt et al. 2014). In humans, DNA demethylation occurs much earlier compared with other mammals, from fertilization to the 2-cell stage, at which time, most genes tested have already lost their methylation status (Guo et al. 2014, Okae et al. 2014). For example, 5’ long terminal repeat-containing element is demethylated after fertilization (Smith et al. 2014). As embryos develop to the blastocyst stage, repression is slowly reversed in the inner cell mass, and methylation status becomes increased (Smith et al. 2012, Guo et al. 2014, Okamoto et al. 2016).

### Epigenetics

A recent review discussed how ARTs influence the epigenetics of early embryos and suggested that gene expression in developing embryos could be altered through the environment in which they interact (Lucas 2013). COH is one of the common hormonal regimens used to induce ovulation, either alone or as part of the IVF/ICSI procedures (Farhi & Orvieto 2010, Berker et al. 2011). Studies using in vitro maturated (IVM) human oocytes (retrieved from gonadotropin-stimulated patients) demonstrate that the widely used oocyte morphological maturation protocol may not necessarily indicate an adequate maturation of gene expression (Jones et al. 2008, Virant-Klun et al. 2013). These genes are involved in meiosis (SYCP2, SGOL2 and MSH2) and are upregulated in the IVM oocytes compared with in vivo maturated oocytes. Ovarian-stimulated IVM-derived mouse embryos express incomplete DNA demethylation at the 2-cell stage. Aberrant methylation in the mouse embryo can be an indication of failure in embryo development (Shi & Haaf 2002, Wang et al. 2010). The evidence showed that in vitro pre-implantation mouse embryo culture results in a selective loss of imprinting gene expression of imprinted maternally expressed transcript (H19) and small nuclear ribonucleoprotein polypeptide N (SNRPN) due to a reduced methylation on their control regions (Mann et al. 2004). Moreover, several studies showed that the culture of mouse embryos in different media compositions could lead to epigenetic changes and contribute to developmental defects and aberrant phenotypes in adulthood (Reik et al. 1993, Dean et al. 1998, Khosla et al. 2001). These findings indicated that media composition and COH could contribute to epigenetic alterations in the embryos.

A recent study using cord blood and placentas collected from the children conceived by ART and naturally conceived children showed that the source of alteration in DNA methylation status is a result of ART procedures, rather than the underlying fertility of the parents (Song et al. 2015). Hiura and coworkers proposed that imprinting disorders are a combination of heredity, senescent, COH, ART procedures and culture medium that potentiates the early onset of the diseases (Hiura et al. 2014). This evidence outlines the important link between ARTs and epigenetic imprinting outcomes in children.

### Oxygen tension

The physiological level of O₂ concentration is 8% (Fischer & Bavister 1993). However, IVF embryos are cultured in various oxygen concentrations in different set-ups (5–20%) (Bontekoe et al. 2012), which can impact the level of oxidative stress on the embryos. Depends on the culture media composition, the glutathione pool in human oocytes can be depleted, resulting in high ROS and causing plasma membrane damage to the oocytes (Martin-Romero et al. 2008). A recent study using post-thawed human embryos found that at 2% O₂ concentration in the culture, embryos...
Table 1  Recent literatures (published after 2010) regarding molecules and processes required for the fertilization and embryo development in the oviduct.

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Recently identified pathways affecting embryo transport

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have a decreased survival rate and increased apoptotic rate. On the contrary, when the O\textsubscript{2} level is slightly below the physiological level (5–6%), the embryos did not show any significant change in those parameters (Yang et al. 2016). The authors suggested that the culture condition at 5–6% O\textsubscript{2} concentration could improve the survival rate of the embryos. However, recent randomized control studies did not find a robust correlation between the culture of human embryos at 5–6% O\textsubscript{2} concentration and the increase in the live birth rate (Nastri et al. 2016).

Yang and coworkers also found that cell death genes (BAX), antioxidant genes (MnSOD) and stress protective genes (HSP70) are elevated and apoptosis is increased in human embryos that were cultured at a 20% O\textsubscript{2} level (Yang et al. 2016). In addition, mouse embryos cultured in 20% O\textsubscript{2} showed pre-implantation epigenetic changes that altered metabolism later in life (Donjacour et al. 2014). Specifically, male mice displayed glucose intolerance, heavier body weight and heart enlargement compared with in vivo fertilized embryos. This evidence indicates that the in vitro culture conditions during ART procedures, either at low or high O\textsubscript{2} concentration, could potentially alter embryo development and lead to metabolic disease in mammals.

Environmental exposures

The pre-implantation period is critical for embryo epigenetic control; this period normally occurs in the oviduct or Fallopian tube in humans. Factors known to be different between IVF and natural development can cause epigenetic influence, including techniques, embryo culture media and environmental exposures such as tissue culture plastics (Ventura-Junca et al. 2015). Bisphenol A (BPA) is known for its action as an endocrine-disrupting compound and a weak estrogen agonist/antagonist. However, BPA is still being used as part of the plastic containers for embryo cultures (Hunt et al. 2003, Berger et al. 2010, Varayoud et al. 2011). Studies indicated a broad range of effects on the embryos upon BPA exposure, such as altered developmental rate and cell death (Ferris et al. 2016) and toxicity to the neural progenitor cells (Yin et al. 2015). BPA-free replacement products from the industry still diffuse out estrogenic chemicals, such as BPAF (the fluorinated form of BPA) (Bittner et al. 2014), which may pose a significant hazard to the embryos.

Together, we need to take extreme precautions to monitor the in vitro environment of IVM and IVF/ICSI procedures and the possible unwanted consequences on the long-term epigenetic imprints.

Developmental factors missing in vitro

The most distinct physiological difference between IVF and naturally conceived birth is the artificial fertilization and the omission of development in the in vivo environments. IVF procedures cultivate fertilized embryos in the culture conditions until the embryos are ready for uterine implantation. Depending on the procedures and institutional protocols, it can be anywhere between 3 and 7 days of in vitro development. The culture media is pre-determined and static compared with a dynamic, interactive Fallopian tube environment. This static environment at the current state cannot provide an interactive response to ROS produced by embryos. Moreover, the viability of the IVF embryos is subjective to institutional procedures.

Of the factors influencing embryo development in vitro, the foremost is the culture media used to culture embryos. This media is meant to mimic the oviductal fluid and its nutrient composition to support early cleavage development. A data analysis study suggested that in humans, the embryo culture media could affect the birthweight of IVF babies (Zandstra et al. 2015); however, the underlying mechanism is not well understood. The oviductal environment, the fluid and the embryo interaction with the environment are difficult to replicate in vitro. This factor could lead to the difference between IVF and natural birth. A known example occurs in very early embryo development; hyaluronan acid synthase 1 (HAS1) is highly expressed in the embryo at the 2- to 4-cell stage, but then quickly fades away (Marei et al. 2013). The oviduct reacts to this change by expressing hyaluronidase-2 (HYAL-2) to degrade excessive hyaluronan acid (HA). In vitro studies in bovine demonstrated that mimicking this embryo-oviduct interaction by adding HYAL-2 to the culture media improves embryo quality (Marei et al. 2013).

Bovine embryos fertilized in vitro have lower quality than those fertilized in vivo, as indicated by the difference in the cellular junction, the presence of lipid droplets and other subcellular changes. Co-culture of human embryos with the Fallopian epithelia improved the quality of embryos (Yeung et al. 1992, Vlad et al. 1996). However, analysis in humans suggested that simply mimicking the in vivo environment using an in vitro model did not increase the baby delivery rate (Stern et al. 2009).
could be the result of a complex interaction among embryo, tubal epithelia and tubal fluid. An extensive review by Hess and coworkers discussed the important roles of the oviduct in stabilizing the very early stage of embryo development during transit (Hess et al. 2013). Therefore, the oviduct–embryo interaction is necessary for the quality of the developing embryo and lacking this interaction could result in negative health effects.

Another difference between IVF and in vivo fertilization is the zona pellucida hardening and monospermy during fertilization (Mondejar et al. 2013, Anifandis et al. 2016, Dadashpour Davachi et al. 2016). In a natural pregnancy, the female reproductive tract acts as a passage for sperm selection, by which it minimizes the number of sperm reaching the fertilization site to ensure that polyspermy occurs at a low rate. Current IVF techniques cannot effectively select the most superior sperm, which occurs naturally in the female reproductive tract. To overcome this, one could use a co-culture condition of the egg and oviduct epithelia. An experiment demonstrated that co-culture of eggs and oviductal epithelial cells is significantly better at preventing polyspermic fertilization than the standard IVF counterpart (Dadashpour Davachi et al. 2016). This suggests that fertilization in the oviduct is part of a continuing interaction between gametes and oviduct to optimize the fertilization outcome.

Lastly, gas is a factor that we have ignored in most cases. Embryos experience somewhere between 2% and 8% O2 concentrations in the human Fallopian tube (Bontekoe et al. 2012). However, in the IVF procedures, the embryos are incubated at different oxygen levels, from 5% to 20%. The effect of these O2 percentages in the culture compared with in vivo has not been well studied, but it would be wise to take precaution because high oxygen concentration is linked to oxidation stress (Fischer & Bavister 1993, Catt & Henman 2000).

The retrospective on IVF studies suggests that Fallopian tubes not only serve as the passage for the embryo to enter the uterus but also act as a cofactor to cultivate and optimize embryo quality to ensure successful implantation and later normal development.

**Conclusion**

The oviduct is essential in reproduction. Before fertilization, the oviduct primes the sperm, protects both gametes and guilds the fertilization process through distinct mechanisms, including rheotaxis, thermotaxis and chemotaxis. Increasing evidence indicates that processes that occur in the oviduct facilitate the path to fertilization, but detailed molecular mechanisms regarding each step are still largely unknown. Most importantly, the oviduct provides the optimized physical site for fertilization to occur.

After fertilization, the oviduct adjusts each of its components to ensure the survival and the normal development of the embryo, summarized in Fig. 5; the current literature regarding these aspects is included in Table 1. The oviductal fluid contains nutrients, growth factors, antioxidants, sex hormones, proteases and many other functional chemicals regulated by the presence of gametes and embryos. The oviduct also transports the embryo from the site of fertilization into the uterus. Defective embryo transport can cause infertility or ectopic pregnancy. Most recent findings suggested that a few new pathways involved in this process, along with ciliated cells, contribute a major role in this transport. Together, the oviduct fine-tunes the oviductal fluid to ensure that the embryos receive proper developmental signals and nutrients as well as helps embryos overcome environmental stress and protects embryos from our own immune system.

The oviduct has been under-appreciated with its ‘non-essential’ role in reproduction since the success of IVF over 30 years ago. However, there have been more studies probing the downside of the ARTs that link to some of the critical functionalities of the oviduct to embryo development. ARTs can be further improved with these findings. At the same time, precautions should be taken, as more research is needed for the roles and functions of the oviduct in fertilization and embryo development to benefit the health of future generations.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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