# Dialogue between the preimplantation embryo and the oviduct

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## Summary

Knowledge about early embryogenesis and the role of the oviduct has gained renewed interest due to increasing awareness of the impact of animal health status and performance on the susceptibility and vulnerability of early developing embryos within their microenvironment. The significance of the oviduct is best illustrated when a glance is cast at structural as well as functional features of the oviduct which regulate the serially orchestrated and well-tuned steps in early embryogenesis. A lot of work has been done in vitro to bypass the oviduct consequently resulting in steadily increasing information about the requirements of early developing embryos. However, relatively little information is available to demonstrate direct tubal effects on gametes and embryos. There is substantial evidence that the bovine oviduct provides a source of high quality embryos, our understanding of this interaction is far from complete.

## Introduction

Worldwide activity in the area of embryo production in domestic ruminants, particularly cattle, is increasing (Perry 2013). This activity has the potential to significantly impact on animal breeding and precocious selection strategies. Furthermore, dealing with early developing embryos has made us aware of a tubal-dependent and embryo-specific orchestration which highlights the need for an increased understanding of reproductive physiology as well as pathology, and which inevitably confronts us with many unsolved issues and new scientific horizons.

Ovulation, fertilisation and embryo development are all serially connected processes which are well accepted to be complicated and sophisticated biological events in their own right. Not so long ago the oviduct was recognized as playing a major role in embryogenesis. On the one hand, it is a very small organ responsible for the transient passage of gametes and embryos. Its unimposing appearance conceals the multifaceted tasks accomplished by its extraordinary microenvironment for embryo development. The peculiarity of the oviduct is already manifest in its anatomy, which allows the gametes to separately enter from different sides.

Meanwhile, there are a plethora of scientific reports describing successful in vitro embryo production (IVP), which clearly demonstrate that the contact between embryo and oviductal epithelium is not obligatory for further development i.e. for implantation and the delivery of a calf (Hasler 2014). In addition, the oviduct can be bypassed in a variety of ways, using IVPderived embryos, or using embryos cultured in vivo in temporary host recipients of different species. This raises the question of whether the interaction of the oviduct and embryo is passive or active i.e. whether the oviduct provides an environment for optimal embryo development simply driven by the ovarian cycle and independent of the presence of an embryo. In turn, an embryo may undergo an optimal development only by receiving the components obligate to overcome this early critical period. Following this hypothesis, an embryo will undergo normal development if the components are delivered and combined in the right place at the right time and dosage. This indeed would explain why the oviduct is the only evolutionary competent organ to manage the complex biodynamics required during embryo passage. Aggravating this situation, a direct contact between embryo and tubal epithelium does not really exist; during the entire oviduct migration period the zona pellucida represents a border between the embryo and tubal epithelial cells. This matrix, adapted for tubal transportation, plays an important accessory role as mediator, biological filter, protector and guarantor of the physical intactness of the embryo (Santos et al., 2008, Van Soom et al., 2010, Held et al., 2012).

It has been comprehensively demonstrated that the effect of embryo manipulation and early culture conditions become manifest along the entire pregnancy period and even later after birth of the offspring (Merton *et al.*, 2003, Heyman 2005). Although most of the assisted reproductive technologies (ART) applied to cattle have been optimised to a level which facilitates large numbers of pregnancies and birth of calves, there is an increasing awareness of fertility problems related to in-vitro production (IVP) (Farin *et al.*, 2010), cloning (Heyman 2005) and even artificial insemination (AI) (Diskin & Morris 2008). From a quantitative point of view, many routes have been established to produce high numbers of embryos and calves but from a qualitative assessment point of view it is questionable to what extent embryo development occurs within the normal limits of its plasticity. All of these findings emphasize the significant impact of the oviduct during early embryogenesis. The dialogue between the preimplantation embryo and the oviduct can be best considered when looking at the (i) oviduct physiology, (ii) in vitro production systems emphasizing single (combined) factors as well as (iii) oviduct embryo interactions which will be discussed below.

#### Physiological events during embryo passage through the oviduct

Regnier De Graaf (1641-1673) was recognized as one of the pioneers who comprehensively described the Fallopian tubes. De Graaf depicted the oviducts as tubal elements connecting uterine horns and ovaries. He differentiated between physiologically vs. pathologically formed tubes and noted that rabbit eggs migrate through the oviduct (cited by Ankum *et al.*, 1996). Some 300 years later, the application of biotechnology to reproduction has permitted enormous progress with a main focus on in vitro technologies associated with early embryo production. In this context, it is appropriate to take up Hunter's concerns not to overlook the sophisticated control mechanisms underlying oviduct physiology and their potential influences on gametes and embryos (Hunter 2012).

The holistic role of the oviduct including its impact on early embryogenesis and implications for subsequent foetal development and offspring health is still not fully known. Nevertheless, many studies increasingly argue for a significant contribution of the oviduct to early embryo development (Hunter 2005, Leese et al., 2008, Hugentobler et al., 2010, Lonergan & Fair 2014).

Significant tasks of the oviduct are already recognizable on closer inspection of the imposing structure characterized by four main segments and four tissue layers (Kenngott & Sinowatz 2007). Although the muscular layer consists of longitudinally, circularly or spirally oriented bundles, the myosalpinx forms a unique continuous network of randomly anastomosed and multidirectional arranged smooth muscle fibres which intertwine and dichotomize along their course (Vizza *et al.*, 1995). Yániz *et al.* (2000) provided detailed insight into the microarchitecture of the bovine oviductal mucosa. The epithelial formation attracts attention by its polymorphic extensive inner surface area characterized by folds, ridges, furrows and grooves which are mainly presented in the infundibular as well as ampullar segment (Yániz *et al.*, 2000). The oviduct is lined with epithelial mucosa cells consisting mainly of cells able to secrete (non-ciliated) nutrients or convey (ciliated) fluid, gametes and embryos. Ciliated cells are predominantly present in the infundibulum and the ampulla, whereas the isthmic region, known to consist mainly of muscular elements, has barely any ciliated cells (Koelle *et al.*, 2009).

The oviductal fluid is composed of a complex mixture of energy substrates (Larose *et al.*, 2012, Leese *et al.*, 2008), ions (Hugentobler *et al.*, 2010), amino acids (Hugentobler *et al.*, 2010), and macromolecules (Buhi 2002, Aviles *et al.*, 2010), which undergo dynamic changes to temporally and spatially meet the biochemical as well as physical requirements of the developing embryo. The routes through which nutrients get into the oviduct are diverse and range from passive or active transportation from blood to oviduct, to de novo synthesis in secretory cells and release into the oviduct (Leese *et al.*, 2001). Due to the fact that the oviductal tube is in close vicinity to the uterine horn, ovary and peritoneal cavity, its fluid milieu consists not merely of substances derived from the oviduct itself, but also of follicular, uterine and peritoneal origin (Hunter *et al.*, 2007, 2011).

Oviductal activity is regulated by hormones released during the oestrous cycle (Wijayagunawardane et al., 2001). During the oestrous cycle there is a varying secretion of fluid (Roberts et al., 1975, Janson et al., 1983), macromolecules such as oviduct specific glycoproteins (Buhi 2002, Avilés et al., 2010) and hyaluronic acid (Lee & Ax 1984, Stojkovic et al., 2002) and a changing blood flow (Moor & Bruce 1976, Janson et al., 1983). These cyclic changes determine the main physical characteristics of the oviduct such as viscosity of the fluid (Hunter et al., 2011, Stojkovic et al., 2002), temperature (Hunter 2005), osmolarity (Menezo & Guerin 1997, Hunter 1994) and pH (Roberts et al., 1975) which are thought to have an effect on gamete and embryo migration, microenvironment stabilisation and immune modulation (Buhi 2002, Hunter et al., 2011). Bauersachs et al. (2003, 2004) examined key transcriptome changes in oviduct epithelium cells and illustrated that there are also marked morphological and functional changes related to the side of ovulation and to the different stages of the oestrous cycle. One of the most unsolved but intriguing issues is represented by those factors able to modulate signals such as growth factors and cytokines which act on the endocrine, paracrine, and autocrine level. Currently, there are a plethora of studies dealing with single and multiple signals; however, the oviductal course of action cannot be fully estimated and still remains an unsolved but scientifically challenging phenomenon (Wijayagunawardane & Miyamoto 2004, Hull & Harvey 2001). In this context the presence of cumulus cells and white blood cells liberated via ovulation might be seen as an extra source of factors able to activate intrinsic signaling pathways (Hunter 2002).

The passage through the oviduct is exclusively accomplished by muscular and ciliary activity, predominantly effected by the ovarian cycle. During oestrus, the phases of muscular activity become synchronized and frequency reaches its maximal strength. After three days there is a loss of intensity but not frequency, which culminates in relative inactivity (Ruckebusch & Bayard 1975). The preovulatory increase of contractions of the oviductal isthmus prevents the passage of embryos through the oviduct, whereas the postovulatory elevation of progesterone decreases oviductal motility amplitude thereby allowing the embryo to pass the uterotubal junction (Spilman et *al.*, 1978).

Besides muscular activity, ciliated mucosa cells also account for a substantial proportion of fluid movement and embryo transportation. Even during ovulation, when the follicular fluid enters the ampulla via the infundibulum, there is the first stimulus for the increase in ciliary beat frequency to optimize ovum pick-up (Lyons *et al.*, 2006). The beats of the cilia are coordinated and appear rhythmically at a local level, but have a range of frequencies along the entire tube. The ciliated cells of the infundibulum induce unidirectional flows resulting in the delivery of an ovum by their ciliary activities, although their beating periodicity is asynchronous (Shi *et al.*, 2011). The early embryo seems to be capable of down-regulating the speed of transport at a local level, which increases its length of stay (Koelle *et al.*, 2009). It was shown that in the rat oviductal ampulla, ciliary motion is capable of transporting ova in the absence of muscle contractility (Halbert *et al.*, 1989).

#### IVP – early embryo development without oviductal support

For more than two decades, huge numbers of embryos have been produced using an in vitro approach. Since then this technique has undergone significant progress resulting in increasing numbers of produced and transferred embryos annually (Perry 2013).

The total IVP procedure consists of three main parts, oocyte maturation, oocyte fertilisation and embryo culture. First successes were obtained by stepwise removing oocytes and embryos ex vivo and resuming development in vitro and vice versa (Newcomb *et al.*, 1978, Brackett *et al.*, 1982, Xu *et al.*, 1987, Lu *et al.*, 1988). In all these attempts the use of bovine oviducts provided an immediate and successful assistance to bridge those parts of embryo culture which had not been fully established in vitro at that time.

Eyestone *et al.* (1987) performed a feasibility study by collecting embryos from superovulated heifers, embedding the zygotes and two-cell stage embryos into agar chips and transferring these complexes into the ligated oviducts of sheep. It was shown that the transfer of bovine embryos to the sheep oviduct was a promising model to promote embryo development. This application was replicated by many authors using ovine and rabbit oviducts for in vivo culture of bovine embryos (Boland 1984, Sirard *et al.*, 1985, Lawson *et al.*, 1972, Lazzari *et al.*, 2010) as well as for other species to provide suitable culture conditions during embryos completely in vitro has approached a high level of optimisation yielding a steady increase in the numbers embryos of produced and transferred (Perry 2013). However, all studies share the scientific endeavour to take account of as many physiological factors as possible by simultaneously keeping the number of chemicals and components numerically manageable.

To date, it still remains unclear which components in particular alter embryo morphology and kinetics. This becomes evident especially when using biological fluids, cells or extracts. For this reason, chemically defined media do not display a biased or unsolicited effect on embryo culture; the specific effects of those chemicals which have been added to the media can be examined. These experimental designs allow the identification and characterization of single factors having a direct effect on embryo culture up to the blastocyst stage. In contrast to the use of chemically-defined media including ions, energy substrates, hyaluronic acid, amino acids or growth factors in a mixture close to the composition of tubal fluid, other protocols suggest supplementing additives like bovine serum albumin (BSA), oestrus cow serum (OCS) or fetal calf serum (FCS) that create culture conditions closer to physiological conditions (Lonergan *et al.*, 1994, Holm *et al.*, 2002, Rief *et al.*, 2002, Stojkovic *et al.*, 2003, Sagirkaya *et al.*, 2007).

Keeping in mind that the use of many tested and proven biochemical substances has substantially improved IVP results, it has also become evident that the gap between in vitro

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and in vivo development has not been filled yet (Hasler 2014). Consequently, further steps towards more accurately mimicking the tubal environment have used media conditioned by granulosa cells or Vero cells (Maeda et al., 1996), two to three dimensional coculture systems (Rief et al., 2002, Rottmayer et al., 2006, Gualtieri et al., 2013), microfluidic systems (Beebe et al., 2002, Krisher & Wheeler, 2010) or by oviduct fluid itself (Libik et al., 2002, Lloyd et al., 2009). It was also shown that the isolated mouse oviduct provides an excellent model to produce in vitro embryos. This in vitro culture model makes use of an organ system which is thought to most closely reflect embryo development obtained in the sheep or bovine oviduct.

Overall, these tremendous efforts including increasing laboratory experience and expertise has undoubtedly led to significant improvements in embryo production. However, these achievements were also associated with marked deviations from in vivo developed embryos, low predictability and reproducibility (Rizos *et al.*, 2010b). Although new developments suggesting dynamic systems such as microfluidics provide promising technologies, they lag far behind routine application (Lonergan & Fair, 2014).

# **Embryo and Oviduct**

#### Embryo-maternal communication

To date, there is not much information available about an embryo maternal crosstalk. For gametes, a direct local effect in the oviduct has been shown. Oocytes and spermatozoa that touch the tubal epithelium effectuate changes in specific gene expression profiles and protein synthesis (Einspanier *et al.*, 1997, Georgiou *et al.*, 2005). There is evidence from several species that tubal transportation depends on successful fertilization of the oocyte. It is well known, for example, that the equine embryo passes through the oviduct within 5 to 6 days, whereas the oocyte remains in the oviduct (Freeman *et al.*, 1992). In rats, the time of transportation depends on whether there is an oocyte or embryo in the oviduct. Embryos migrate much faster compared to oocytes and this phenomenon is not associated with different plasma progesterone concentrations in the blood (Villalón *et al.*, 1982). Lee *et al.* (2002) reported the identification of upregulated genes in the murine oviduct caused by the presence of embryos. It is also noteworthy in this context to remember that the rabbit oviduct envelops the embryo in an extra mucin layer. This is an obligatory coat produced during oviduct migration which is necessary for uterine implantation and which renders bypassing the oviduct impossible (Murakami & Imai, 1996).

Koelle et al. (2009) argued that the bovine oviductal epithelium is able to select viable oocytes, to generate formation of secretory cells, modify vascularization, and downregulate speed of transport. This was assessed as the first signs of embryo-maternal communication in the oviduct (Koelle et al., 2009). In contrast, Maillo et al. (2014) characterised the transcriptome of the bovine oviduct cells at the initiation of embryonic genome activation on Day 3 postoestrus in pregnant and cyclic heifers. The presence of an 8-cell stage embryo had no effect on the epithelial cells of the isthmus. However, an effect at the local site where the embryos contact the epithelial cells was not ruled out (Maillo et al., 2014), as has been shown for the endometrium (Bauersachs et al., 2009). In addition, the communication network of the oviduct constitutes something exceptional which may become evident by the presence of mediators, such as cumulus cells and granulosa-derived cells, possibly responsible for amplification of oocyte or embryonic signals to the endosalpinx and ipsilateral ovary (Hunter 2002).

#### Plasticity or misrouted development

In contrast to IVP, the use of the bovine oviduct provides per se the physiological site for optimal embryo development. There is no need to search for single factors optimizing the IVP system rather than finding factors modifying the whole "animal system". Hence, research and commercial application benefits or suffers from this system depending how far the organism is exposed to changes and disorders. The early stages of embryo preimplantation development are very sensitive to perturbation (Gad et al., 2012, Seisenberger et al., 2013). The consequence of such insults can become manifest immediately (Merton et al., 2003, Diskin & Morris 2008), during pregnancy, parturition or even later (Fleming et al., 2004, Heyman 2005, Fazeli 2011, Eckert et al., 2012). There is much evidence supporting the assumption that the oviduct recognizes the presence or even the quality of gametes and embryos leading to predetermination of both fates, that of the oviduct and of the embryo. The immediate embryo fate can be easily assessed following comparative in vivo vs. in vitro studies which have revealed deviations such as reduced cryo-resistance (Fair et al., 2001, Havlicek et al., 2010, Kuzmany et al., 2011a), morphological injury (Crosier et al., 2001, Fair et al., 2001, Rizos et al., 2002, Kuzmany et al., 2011b), altered gene expression (Lazzari et al., 2002, 2010, Rizos et al., 2002, Tesfaye et al., 2004, Smith et al., 2009, Kepkova et al., 2011, Gad et al., 2012), chromosome abnormalities (Viuff et al., 1999) and fetal and peripartal development (Lazzari et al., 2002, Farin et al., 2010) to the detriment of in vitro produced embryos. These results emphasize that any oviduct culture favours embryo development compared to IVP (Besenfelder et al., 2010).

Many studies have been performed in order to compare in vitro culture conditions with those existing in vivo. Most of the results showed that there were no differences in embryo developmental rates (Laurincik *et al.*, 2003); however, differences become obvious at the transcriptome level (Gad *et al.*, 2012, Carter *et al.*, 2010). In an extensive study, blastocyst groups were produced under alternative in vitro and in vivo culture conditions at different time points of development. The transcriptome of the blastocysts was critically influenced during the culture period. An ontological classification revealed a significant difference in expression patterns of genes related to lipid metabolism and oxidative stress response between blastocysts generated in vivo versus in vitro. This study allowed the definition of molecular mechanisms and pathways that are influenced by altered culture conditions especially during embryonic genome activation (Gad *et al.*, 2012).

These more scientifically related results could be confirmed when performing the experiments on a herd level for studying infertility in dairy cattle. The transfer of in vitro derived embryos into oviducts of heifers vs. lactating cows, or lactating vs. dried off cows illustrated that the reproductive tract of the postpartum lactating dairy cow is compromised in its ability to support early embryo development compared with heifers or non-lactating cows, which may also explain early embryo mortality (Rizos et *al.*, 2010a, Maillo *et al.*, 2012).

# Conclusion

Disturbances in early embryogenesis such as IVP, high milking performance, health problems and hormonal stimulation have an adverse impact on further embryo development resulting in sub-fertility, infertility and loss of pregnancy (Merton *et al.*, 2003, Gad *et al.*, 2011, Maillo *et al.*, 2012). It is expected that in the near future investigations will focus on physiology as well as disturbances of early embryo development with regard to maternal recognition of the embryo including short term as well as transgenerational effects as has already been shown for laboratory animals. However, studies focusing on these factors will have to be performed

on a large scale, which is expensive and time-consuming since altered phenotypes may only be evident in adult animals or even in the following generation(s) (Fleming *et al.*, 2004, Fazeli 2011, Daxinger & Whitelaw, 2012).

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