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Alternative models for the study of embryo–maternal cross-talk and signaling molecules from fertilisation to implantation

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In nature, mammalian life is conceived inside the female genital tract, more specifically in the oviduct. Because the processes of fertilisation and early preimplantation development take place in one of the most inaccessible parts of the mammalian body, it has been studied predominantly *in vitro*. In static culture platforms, the environmental conditions to which the gametes and embryos are exposed are in sharp contrast to what is observed *in vivo*. In this Research Front on embryo–maternal interactions, we present four reviews that investigate model systems for the study of embryo–maternal cross-talk and the processes that are known to be important in the period between fertilisation and implantation. The Research Front is one of the outcomes of a European Union-funded COST (European Cooperation in Science and Technology) Action on 'Maternal Interaction with Gametes and Embryos' (FA0702; http://www.cost-gemini.eu, accessed 20 September 2011), which aims to promote understanding of this topic by bringing together researchers to share data from different species.

In the female reproductive tract, embryos are surrounded by a constantly changing minimum amount of media and are constantly moved by ciliated epithelia. The preimplantation embryo, *in vivo*, develops in the absence of direct cell contact with the reproductive tract before implanting. It is free-floating, lacks a blood supply and is dependent on luminal secretions of the oviduct and uterus for its nutrition. The preimplantation embryo expresses several receptors for signalling ligands (O'Neill 2008). These signalling ligands are often paracrine factors, defined as factors that are secreted by one cell type and that execute their function on another cell type. They can originate from cells of the reproductive tract (e.g. cytokines) and have an effect on the embryo, or can be secreted by the embryo and have an effect on the oviduct or uterus (Orsi and Tribe 2008). It is clear that these paracrine factors are crucial in the embryo–maternal dialogue.

The importance of normal embryo–maternal interaction is evidenced by the finding that exposure of ruminant embryos to a suboptimal environment can lead to the so-called large offspring syndrome: affected offspring show changes in phenotype, such as having twice to five times increased birthweights. In many cases breathing difficulties, reluctance to suckle and sudden perinatal death can occur and the severity of the syndrome is influenced by culture conditions and animal species (Farin *et al.* 2010). In humans, assisted reproduction has been associated with increased risk of imprinting diseases such as Beckwith–Wiedemann syndrome (Owen and Segars 2009). Abnormal development originates from epigenetic changes in imprinted genes and epigenetically sensitive alleles (for review, see Jammes *et al.* 2011), and hypothetically this can be caused by exposure to unwanted signalling molecules during a potential window of vulnerability in development. These cases of abnormal embryonic, fetal and neonatal development illustrate the pressing need to understand what happens at the time of fertilisation and during the first days and weeks of life.

What is the best approach to study these signalling molecules and in which species should they be studied? If we first focus on the species, it seems that the mouse has traditionally been the most popular model specific for the human (or even for mammals in general). This is based on the fact that mice are highly productive (reaching sexual maturity early (6–8 weeks) and producing many offspring per litter). They exhibit a similar placentation to humans (i.e. hemochorial) (Rosenfeld 2010). However, for studying signalling molecules in connection with embryo–maternal interactions, the mouse may not be the best choice after all. Most mouse strains are inbred, genetically almost identical and therefore not comparable to humans, which are markedly diverse, with genetic and epigenetic variability. Moreover, the mouse genome is dissimilar from that of humans, in that the number of unique orthologous groups is greater for rodents than for several other mammalian species including man (Hansen 2010). In this respect, farm animals – such as cattle, pigs and even horses – are a much more interesting group of model species for research in (human) reproduction, especially when one wants to focus on signalling ligands. Another important recent development is that the advancement in molecular tools has led to the complete sequencing of the genomes of cattle (Larkin 2011), pigs (Fan *et al.* 2011) and horses (Chowdhary and Raudsepp 2008).

The horse represents a valuable model for human infertility for several reasons: (1) breeding sport horses is often postponed to later ages, and this is associated with reduced fertility, both in mares and stallions, (2) such breeding horses can successfully be treated with intracytoplasmic sperm injection and embryo

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transfer, as it is done in human subfertility and (3) such offspring are highly prized, and can be used to study long-term effects of assisted reproductive technology on postnatal development. Embryo–maternal interactions are conspicious in horses as clearly illustrated by two examples: first, the selective oviducal transportation of viable embryos to the uterus, which is linked to the stage-specific production of prostaglandin E_2 by the equine conceptus; and second, the formation of an acellular glycoprotein capsule soon after the arrival of the horse embryo in the uterus. Interestingly, this capsule formation is deficient after equine embryo culture *in vitro*, but can be upregulated by supplementing culture medium with uterocalin, an equine uterine protein typically present during early pregnancy (Smits 2010). This example shows that restoring embryo–maternal signalling can be important for embryo differentiation.

In this Research Front, two reviews describe why the horse is a valuable animal model for reproduction studies. Goudet (2011) provides an extensive overview of past and present research on the oviduct environment in the horse. The horse is in many ways different from other livestock species; the identification of signalling molecules involved in final oocyte maturation and release of sperm from the oviduct reservoir is still in its infancy, but may hold the clue to make equine fertilisation *in vitro* work, which has until now proven to be difficult. Klein and Troedsson (2011) try to unravel another equine mystery – the maternal recognition of pregnancy. Unlike in ruminants, it is not yet known which signal is secreted by the equine conceptus in order to announce the presence of an embryo to the mother, preventing the lysis of the corpus luteum and the start of a new oestrous cycle. The authors list various ways to prolong luteal function in the non-pregnant mare, together with recently published data on the transcriptome of pregnant and non-pregnant endometrium in mares. In conjunction with molecular data on different stages of horse conceptus development, they present a working model on the biological processes underlying early pregnancy in the mare. Both reviews illustrate that working with a less common mammalian model like the horse can yield valuable information, not only for horse breeders but also for researchers interested in comparative aspects of fertilisation, early embryonic development and the maternal recognition of pregnancy.

Other livestock are also used in reproductive research. There are several arguments in favour of cattle and pigs over the mouse model: (1) the amino acid sequence of most proteins is more conserved between cattle/pigs and humans than between mice and humans; (2) the chromosomal organisation is more similar between cattle/pigs and humans than between humans and mice; (3) bovine embryo production also has an economical relevance in animal agriculture; and (4) pigs serve as suitable models for human diseases, because pigs are similar in size, shape and physiology to humans. Moreover, cattle and pig embryos are, just like the human embryo, more sensitive to adverse culture conditions than the mouse embryo (Vajta *et al.* 2010). Livestock species are also much larger in size than mice, so when searching for signalling molecules, the volume of fluids obtained from the genital tract are within the range of millilitres rather than microlitres (as in the mouse). Also, because livestock are typically produced for meat production, reproductive tracts can easily be obtained from slaughterhouses for use in research involving analysis of the (epi)genome, the transcriptome and the proteome of the maternal genital tract and/or embryos.

In the third review in this Research Front, Østrup *et al.* (2011) focus on signalling during placentation in cattle and pig, and describe morphological and transcriptional changes in the endometrium accompanying placentation in these species. Oestrogen and interferon-tau are the signals for maternal recognition of pregnancy in pigs and cattle respectively, and global approaches such as microarrays have revealed that during implantation, many genes are expressed that have a role in signalling and immune response, with the interferon family being an important player. Epigenetics and imprinted genes play a particular role in implantation and placentation because paternally expressed genes act on the placenta to promote fetal growth, whereas maternally expressed genes restrict fetal growth to protect the mother from exhaustion. The authors of this review explain that porcine and bovine placentas also provide unique opportunities to investigate mechanisms underlying epigenetic regulation of oncogenes during placentation because the low DNA methylation levels in the placenta are comparable to those in cancer cells.

Finally, a word or two about the model system rather than the species (for review, see Van Soom *et al.* 2010). Whereas the first three reviews in this Research Front focus on work done *in vivo*, *in situ* or *in vitro*, the last review by Burkitt *et al.* (2011) is entirely devoted to what might be the model system of the future: the *in silico* model. Until now, predictive *in silico* or computer models aiming to enhance research have been rare or too complex, with many non-measurable adjustable parameters. This review, however, explains how such complex problems – involving too much information for the human mind to process – can be investigated using computational modelling. To simplify things, these *in silico* models contain only the parameters that are most critical in controlling the behaviour of a particular system. As an example, the modelling of sperm movement, of a 3D virtual oviduct and of sperm interactions with the oviduct is described. Of course, this approach requires the involvement of not only biologists or physiologists but also of people specialised in mathematics, physics and computer programming. For those who are looking for new approaches it is however a fascinating way to do research and it may lead to new and interesting hypotheses, preferably after confirming the predictive model with real experimental data.

Today's researchers do not have to stick to the mouse model or to *in vivo* data to study the importance of embryo–maternal interactions in mammals. As highlighted in this Research Front, researchers can now choose between different species and different approaches when they want to investigate the beginning of mammalian life, and the consequences of an adverse periconception environment on resulting offspring. They can make use of various molecular techniques to describe what happens when an embryo is present in the maternal genital tract, detect minute changes in gene expression caused by epigenetic modifications, and even predict what will happen when spermatozoa reside in the oviduct using an *in silico* model. As the four reviews in this Research Front convincingly show, livestock are providing important insights into embryo–maternal interaction.

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