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Author: Olivier Sandra Fabienne Constant Anais Vitorino Carvalho Caroline Eozénou Damien Valour Vincent Mauffré Isabelle Hue Gilles Charpigny

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HIGHLIGHTS

Development asynchrony in bovine conceptus leads to pregnancy perturbations or failure

Conceptus affects endometrial gene networks and gene expression in circulating immune cells

Lipid mediators play essential roles in embryo–uterine interactions at implantation

Endometrium as a biosensor of embryo quality was first demonstrated in cattle

MATERNAL ORGANISM AND EMBRYO BIOSENSORING: INSIGHTS FROM RUMINANTS

Olivier Sandra[1,2,3], Fabienne Constant[1,2], Anais Vitorino Carvalho[1,2], Caroline Eozénou[1,2], Damien Valour[1,2], Vincent Mauffré[1,2], Isabelle Hue[1,2], Gilles Charpigny[1,2]

1. INRA, UMR1198 Biologie du Développement et Reproduction, F-78352 Jouy-en-Josas, France

2. ENVA, UMR1198 Biologie du Développement et Reproduction, F-94704 Maisons Alfort, France

To whom correspondence should be addressed

Olivier Sandra, INRA, UMR1198 Biologie du Développement et Reproduction, F-78350 Jouy-en-Josas, France. Email: olivier.sandra@jouy.inra.fr, Phone: +33134652343.

Short title: embryo sensor and maternal environment

Abstract:

In terms of contribution to pregnancy, the mother not only produces gametes, but also hosts gestation, whose progression in the uterus is conditioned by early events during implantation. In ruminants, this period is associated with elongation of the extra-embryonic tissues, gastrulation of the embryonic disk and cross-talk with the endometrium. Recent data have prompted the need for accurate staging of the bovine conceptus and shown that asynchrony between elongation and gastrulation processes may account for pregnancy failure. Data mining of endometrial gene signatures has allowed the identification of molecular pathways and new factors regulated by the conceptus (e.g. FOXL2, SOCS6). Interferon-tau has been recognised to be the major signal of pregnancy recognition, but prostaglandins and lysophospholipids have also been demonstrated to be critical players at the conceptus–endometrium interface. Interestingly, up-regulation of interferon-regulated gene expression has been identified in circulating immune cells during implantation, making these factors a potential source of non-invasive biomarkers for early pregnancy. Distinct endometrial responses have been shown to be elicited by embryos produced by artificial insemination, in vitro fertilisation or somatic cell nuclear transfer. These findings have led to the concept that endometrium is an early biosensor of embryo quality. This biological property first demonstrated in cattle has been recently extended and associated with embryo selection in humans. Hence, compromised or suboptimal endometrial quality can subtly or deeply affect embryo development, with visible and sometimes severe consequences for placentation, foetal development, pregnancy outcome and the long-term health of the offspring.

Key words: sensor, endometrium, cell signalling, immune cells, ruminants

Introduction

In mammals, the birth of a viable and healthy progeny involves a sequence of complex biological processes and several critical points that have to be successfully overcome. In terms of the contribution to pregnancy (onset, progress, and issue), the male differs from the female. Indeed, whereas the paternal contribution is represented by the sperm, the mother not only produces the gametes (oocyte), but also hosts the whole gestation in a reproductive tract until term. Nutrition, stress, infections or endocrine disruptors have been identified as factors that affect gamete quality and fertilisation, journey of the early embryo through the oviduct, cellular interactions between endometrium and hatched blastocyst or conceptus, foeto-placental development or parturition (Fleming *et al*. 2004; Leroy *et al*. 2008). In addition, assisted reproductive technologies (ART) associated with embryo transfer have been shown to alter the biological properties of the embryo with a subsequent impact on the later stages of pregnancy (Sinclair 2008). Therefore, an inadequate maternal compartment and/or suboptimal quality of the embryo may have an impact on the two-way communication between mother and embryo, precluding completion of successful pregnancy and affecting the long-term health status of the offspring (Douglas 2010).

In vitro production of embryos and embryo transfer have demonstrated the oviduct to be a dispensable organ for supporting progression of pregnancy to term. Until now, despite several attempts to maintain early and late foetal life outside the uterus (Bulletti *et al*. 2011), no surrogate biological or artificial system has been derived for the uterus. Under normal physiological conditions, the uterus and its internal part, referred to as the endometrium, constitute the maternal site for embryo implantation. The implantation process has been recognised to be a critical step of pregnancy that is associated with a high rate of embryo loss in all mammalian species studied so far. As an illustration, whereas the calving rate of pregnancy has been estimated to be 40% in

Holstein–Friesian dairy cows, more than 70% of pregnancy failure has been associated with embryo death occurring during the early and late pre-implantation period (Diskin and Morris, 2008). Obviously, this poor reproductive performance represents a major cause of economic loss that prompts the need for a thorough investigation of the events leading to embryo mortality during the establishment of pregnancy. External events related to the environment or intrinsic maternal features may affect the biological functions of maternal organs or tissues that will in turn have an impact on endometrial physiology (Figure 1). As the ultimate and unique biological layer facing the implanting embryo in normal early pregnancy, the endometrium drives the development of the embryonic disk and extra-embryonic tissues (Hue *et al*. 2007). Complementary to this driver feature, recent data based on *in vitro* embryo manipulations have unveiled an unexpected biosensoring property of the endometrium (Sandra *et al*. 2011). While focussing on bovine and ovine species, this review aims to present recent data that have explored how the maternal environment exhibits global and subtle reactions to the conceptus at the local and systemic levels during early pregnancy in physiological or perturbed situations.

Brief overview of early pregnancy events in ruminants

In ruminants, the bicornuate uterus is covered with the endometrium, displaying two specific areas, namely the caruncles and the intercaruncular areas (Chavatte-Palmer and Guillomot 2007; Figure 1). The caruncles represent aglandular structures of limited size and are distributed over the endometrial surface. The intercaruncular areas are large and contain the endometrial glands, which produce histotroph, a collection of numerous and diverse factors including cytokines and growth factors (Spencer and Bazer 2004). Upon oocyte fertilisation and after hatching, the extra-embryonic tissue of the conceptus undergoes a progressive and critical elongation phase (Degrelle *et al.* 2005) before implanting at days 15–16 post-oestrus in the sheep and the goat (gestation period: 5 months) or at days 19–20 post-oestrus in cattle (gestation period: 9 months). Although a decidual-like process (related to haemochorial implantation) has been reported in sheep (Johnson et al. 2003), ruminant implantation is characterised by the apposition and then the adhesion between the trophectoderm and the uterine luminal epithelium, ultimately forming a synepitheliochorial placenta (Bazer *et al.* 2009).

The anatomy of the ovine uterus is advantageous for identifying the local endometrial reaction triggered by embryo-related factors *in vivo*. In the ovine model of unilateral pregnancy, the conceptus is confined to the uterine horn ipsilateral to the corpus luteum by placing a ligature proximal to the uterine body (Bazer *et al*. 1979). By comparing the gravid and the non-gravid horns, the paracrine impact of conceptus-secreted factors on the endometrium can be dissociated from their endocrine actions. In this model, dynamic changes of endometrial T lymphocyte populations were reported to be independent from embryo secretions (Majewski *et al*. 2001), whereas the localisation of macrophages was affected by the presence of the conceptus (Tekin and Hansen 2004). At the molecular level, Sandra et al. (2005) reported the paracrine influence of the conceptus on the endometrial expression of SOCS genes, a family of intracellular factors displaying major functions in the negative control of cytokine signalling pathways.

Although the first steps of uterine remodelling and the timing of the window of implantation are programmed by maternal hormones independently from the presence of the embryo, successful pregnancy will require embryo recognition by the maternal organism with a critical contribution of the uterine reaction. This reaction is promoted by the production of embryonic factors that are indispensable for initiating the implantation process through the establishment of permanent cellular interactions between the trophectoderm and the endometrium (Guillomot 1995). Embryo-derived signals vary according to mammalian species and they have been abundantly reviewed, particularly in ruminants for which the major signal of pregnancy recognition has been identified as a type I interferon (Spencer et al. 2008; Dorniak *et al*. 2013b). The production of this interferon (interferon-tau; IFNT) is unique as it is secreted by trophectoderm cells during the elongation phase and then stops when trophectoderm cells appose the luminal endometrial epithelium. IFNT is crucial for maternal recognition through the inhibition of endometrial prostaglandin-F2 alpha secretion, thus preventing luteolysis, an indispensable step for maintaining P4 luteal secretion (Martal *et al*. 1998; Roberts *et al*. 2008). Nevertheless, IFNT is not the only factor of embryo origin affecting uterine physiology and other molecules have been identified. Indeed, at the conceptus–endometrial interface of ruminants, two main categories of lipid mediators have been highlighted, namely cyclooxygenated derivatives from arachidonic acid (prostaglandins) and lysophospholipids.

Conceptus–endometrium interactions and lipid mediators

Numerous cyclooxygenated derivatives from arachidonic acid have been described in the uterine milieu (Charpigny *et al.* 1999; Ulbrich *et al.* 2009) and in the supernatant of cultured endometrial cells (Asselin *et al.* 1997; Arosh *et al.* 2002). In the ovine species, the endometrium is able to synthesise large amounts of prostaglandins in non-pregnant and pregnant females in keeping with the similar expression levels reported for the main prostaglandin generating enzyme (PTGS2, formerly COX-2) (Charpigny *et al.* 1997). A recurrent and poorly resolved question relates to the role of these high levels of prostaglandins in the uterine milieu at the time of conceptus implantation. An elegant study has demonstrated the mechanisms by which IFNT inhibits the transport of endometrial prostaglandins (Banu *et al.* 2010). Additional reports have suggested that conceptus-derived prostaglandins might regulate the endometrial function in sheep. Using intrauterine infusion of various prostaglandins or an inhibitor of cyclooxygenase activity, Bazer and Spencer's group has demonstrated that prostaglandins increased the expression of genes involved in the migration and the attachment of trophectoderm cells (Dorniak *et al.* 2012a). Moreover, in coordination with IFN or cortisol, PTGS2-derived prostaglandins have been found to regulate endometrial functions and genes that are critical for conceptus elongation (Dorniak *et al.* 2012b, 2013b).

Lysophosphatidic acid (LPA) is a naturally occurring small phospholipid. LPA has been reported to be produced by both the endometrium (Woclawek-Potocka *et al*. 2009) and the conceptus (Liszewska *et al*. 2012). The significant impact of the LPA pathway in conceptus–endometrium interactions has been strengthened by the detection of LPA receptors whose expression levels are regulated in the pregnant endometrium of bovine (Woclawek-Potocka *et al*. 2009) or ovine (Liszewska *et al*. 2012) origin as well as in the ovine conceptus (Liszewska et al. 2009). As shown in various cell models, LPA is known to modulate prostaglandin release. In the bovine species, the intrauterine administration of LPA increased PGE2 release (Woclawek-Potocka *et al*. 2010). In endometrial cultured cells, LPA was shown to stimulate the transcriptional level of PGE-synthase (Woclawek-Potocka *et al.* 2010). In addition, LPA increased the mitotic index of ovine trophoblastic cells in parallel with prostaglandin release (Liszewska *et al*. 2009). In addition to LPA, the activation of sphingosine-1–phosphate pathway has also been demonstrated in the early in the pregnancy of the ewe (Dunlap *et al*. 2010). The authors have suggested that this lysolipid mediator generated from ceramides might be necessary for conceptus implantation and critical for the angiogenesis of the uterus and placenta. Collectively, all these published data support the major biological roles played by the lipid mediators at the conceptus–endometrium interface during the establishment of pregnancy (Vilella *et al*. 2013).

Biological response of the endometrium to the conceptus: contribution of transcriptomic analyses

In ruminants, the global endometrial reaction to the presence of the conceptus has been investigated using large-scale analyses covering various days of pregnancy establishment, including implantation (Spencer *et al*. 2008). In cattle, and to a lesser extent in sheep, analyses of molecular and cellular events in the endometrium have first benefited from the development of microarray platforms, as reviewed elsewhere (Evans *et al*. 2008), before the advent of next generation sequencing (NGS), which allows the comprehensive analyses of RNA profiles based on RNA sequencing technologies (Forde *et al.* 2012; Mamo *et al.* 2012). Nevertheless, data mining in livestock remains tedious. Indeed, the current free or commercial software available is dedicated to human and rodent data and they lack records of factor interactions specifically associated with pregnancy. Large changes in endometrial gene expression – including innate immune response and associated factors – has been shown to be significantly altered early and late during the pre-implantation period compared with the equivalent day of the oestrous cycle (d*po*) in sheep (Gray *et al*. 2006; Satterfield *et al.* 2009) and cattle: 5 to 16 d*po* (Forde *et al.* 2011), day 18 (Bauersachs and Wolf 2012). Interestingly, some of the genes identified appeared to be preferentially regulated in bovine caruncles or in intercaruncular areas: 17 d*po* (Walker *et al*. 2010), 20 d*po* (Mansouri-Attia *et al*. 2009a), suggesting specific functions associated with the development of the placentomes in the caruncles and the production of histotroph in the intercaruncular areas. Regarding the immune function, variations in endometrial gene expression coincide with changes in immune cell populations. As in other mammals (Lea and Sandra, 2007), the local immune system in the endometrium of ruminants protects the uterus against infections and it has to be modulated to prevent the embryo from being rejected by the maternal organism (Hansen, 2010). In cattle, mid- and late pregnancy have been shown to affect the proportion of the various populations of endometrial and circulating leucocytes identified during the oestrus cycle, including CD4 (T helper cells), CD8 (T cytotoxic cells), CD5 (B-cells), macrophages, dendritic cells and NK cells (Cobb and Watson 1995; Oliveira and Hansen 2008, 2009; Mansouri-Attia *et al.* 2012; Oliveira *et al.* 2012).

These reports strongly suggest that the regulation of the immune function during pregnancy might be as complex in ruminants as in other mammals with an invasive type of implantation. Nevertheless, deciphering the functions of immune cells and genes in ruminants requires extensive investigation, which has just been initiated during the peri-implantation period (Mansouri-Attia *et al*. 2012; Oliveira *et al.* 2012).

Collectively, these high-throughput data have demonstrated an important global reaction of the endometrium – quantitatively and qualitatively – while interfacing with the conceptus. Data mining of bovine transcriptomic analyses have offered unique opportunities to identify novel factors, including transcription factors such as *EPAS1* (Bauersachs *et al*. 2006, Mansouri-Attia *et al*. 2009b), *NR2F2* (Bauersachs *et al*. 2009), *STAT1* (Bauersachs *et al*., 2006, Walker *et al*. 2010; Mansouri-Attia *et al*. 2009b), *MSX1* (Mansouri-Attia *et al*., 2009a) and signalling pathways, e.g. WNT-related genes (Biase *et al*. 2014), *SOCS*-related genes (Vitorino Carvalho *et al*. 2014b). Detailed investigation of gene expression and protein localisation has:

(i) Uncovered *FOXL2* (a transcription factor critical for ovarian development and function in vertebrates) as a potential regulator of endometrial physiology (Eozenou *et al*. 2012),

(ii) Unveiled new aspects of STAT1 biological functions (in association with chromatin–immmunoprecipitation analyses) during endometrium receptivity, maternal pregnancy recognition and implantation (Vitorino Carvalho *et al*. 2014a), and

(iii) Revealed *SOCS6* as an IFNT-independent gene whose expression is profoundly stimulated at implantation in the endometrial luminal epithelium (Vitorino Carvalho *et al*. 2014b).

In bovine endometrium, a major impact of IFNT on the global endometrial response has also been highlighted using a combination of *in vivo* (a two-hour infusion of recombinant IFNT into the uterus of cyclic cows at 14 d*po*) and *in vitro* (primary cultures of endometrial glandular epithelial or stromal cells treated with IFNT for 30 min, two hours or 24 hours). This strategy has led to the identification of induced or up-regulated classical type I IFN-stimulated genes (ISG), including *IFIT3*, *IFIT5*, *IRF9*, *ISG15*, *MX1*, *MX2*, *OAS1*, *OAS2*, *RSAD2*, *SOCS1*, *SOCS3*, and *STAT1*. New non-classical ISG were also discovered (*CXCL12, CXCR7, DDX58, HER6, IL12B, MCP1, MCP2, MGC127874, PARP12, PLAC8, PTX3, RNF213, TNFA, ZNFX1*) whose diversity underlines the pleiotropic biological effects of IFNT in a cell-specific manner during early pregnancy (Mansouri-Attia *et al*. 2009a; Forde *et al*. 2012; Mansouri-Attia *et al*. 2012).

Peripheral reaction of the maternal organism to the presence of the conceptus

Beyond the local reaction of the uterus to the presence of the conceptus, recent publications have also demonstrated the reaction of extra-uterine tissues to the conceptus, including circulating blood cells and the corpus luteum whose progesterone secretion is indispensable for the establishment and early maintenance of pregnancy in large animal species. Up to now, the systemic effect of the conceptus has been mainly investigated with regard to IFNT and the expression of ISG in peripheral blood leucocytes (PBL) (Oliveira *et al*. 2008; Ott *et al*. 2010).

During early pregnancy, gene expression of various ISGs (*MX1, MX2, OAS1, ISG15*) is altered in PBL collected from ewes (Yankey *et al*. 2001) or from cows (Han *et al*. 2006; Gifford *et al*. 2007; Green *et al*. 2010a, 2010b). Based on the mRNA levels of these genes, diagnosing early pregnancy has been proposed in cattle with a double objective:

(i) Managing cows that failed to be pregnant on day 18, allowing a second insemination when the next oestrus takes place,

(ii) Reducing the interval from calving to conception and thus the economic losses associated with repeated intervention.

Although gene expression of various ISGs was higher in pregnant animals than in non-pregnant ones, several studies showed that the mRNA level of *ISG15*, *MX2* or *OAS1* on day 18 was not an accurate indicator of pregnancy (or the absence of pregnancy) in dairy cows or heifers (Han *et al*. 2006; Stevenson *et al*. 2007; Green *et al*, 2010b). To improve the reliability of a pregnancy test based on *ISG15* mRNA level, serial measurements of *ISG15* mRNA levels were carried out for four or more consecutive days from day 18 (Han *et al*. 2006). This method could represent an accurate indicator of non-pregnant cows (negative predictive value was 100%), although it appears tedious to carry out in commercial herds (Han *et al*. 2006). When the ratio of the mRNA level after AI to the mRNA level before AI was calculated for three different genes at day 18, Green *et al*. obtained interesting results for *OAS1*, but only for heifers. Indeed, for heifers, an ideal cut-off was established with a 100% true-positive rate and a 0% false-positive rate (Green *et al*. 2010b). Another possibility for improving the accuracy of an early pregnancy test is to determine gene expression level in PBL subpopulations. Expression levels of *ISG15, MX1, MX2* and *OAS1* were evaluated in three subpopulations of leucocytes (granulocytes, monocytes, lymphocytes) collected from pregnant or cyclic multiparous cows of mixed breed (Japanese Black x Holstein) (Kizaki *et al*. 2013). All four genes were significantly up-regulated in the granulocyte fraction purified from pregnant cows at day 14 post-insemination compared with non-pregnant cows. Altogether, these data suggest that:

(i) Subpopulations of immune circulating cells display distinct reactions to IFNT and likely to the presence of the conceptus,

(ii) ISGs could represent a reliable source of early pregnancy biomarkers for heifers but not for multiparous females.

These data prompt the need for additional studies that could help to determine the pregnancy status using ISG (Forde *et al*. 2012) or interferon-independent genes in females, regardless of their parity.

Conceptus staging and uterine response to sexual dimorphism

During the free-life period specific to implantation in ruminants, three biological processes concomitantly take place in the conceptus: the elongation of the extra-embryonic tissues, the gastrulation of the embryonic disk and the cross-talk with the uterus. In cattle, the embryonic disk does not bear any amnion for a few days (days 14 to 17 post-insemination) and these embryonic tissues could signal to the extra-embryonic tissues or the uterus, as suggested by recent proteomic data generated from mouse epiblast cells cultured *in vitro* (Fröhlich *et al*. 2013). Most studies report the development of the embryo based on days post-insemination or days post-coitum (dpi; d*pc*). However, the elongation process is rarely synchronised after fertilisation and it appears highly advisable to combine criteria associating dpi, conceptus length (Degrelle *et al*. 2005) and developmental staging (Degrelle *et al*. 2011) to appropriately generate and interpret data related to genes driving elongation and gastrulation processes (Hue *et al.* 2012). Indeed, as reported in the pig, ovoid and filamentous conceptuses may simultaneously develop at a given d*pc* within the same cohort and exhibit gene expression differences related to their size (Blomberg *et al*. 2010). Accurate conceptus staging is of critical importance for understanding elongation and gastrulation processes, which most frequently appear coordinated after AI (Degrelle *et al.* 2012; Valour *et al.* 2014), but are seldom evaluated in experimental situations, including somatic cell nuclear transfer (SCNT; Betsha *et al*. 2013; Rodriguez-Alvarez *et al.* 2010) or in physiological conditions such as under-nutrition, low P4 circulating level, the post-partum period or alteration of immunological conditions including inflammation and infections. Last but not least, correct staging of the conceptus also matters for the cross-talk with the endometrium when potential asynchronies occur, e.g. when the conceptus lags so much behind the "uterus tempo" that the conceptus either reflects troubleshooting in early uterine signalling (or "nursing") or signals itself inappropriately to the uterus, or both.

Among the sources of gene variation in embryos, the transcriptional differences resulting from different sex chromosome complements have been unveiled as early as the blastocyst stage (Bermejo-Alvarez et al. 2010). Based on the staging of the conceptuses, a high-throughput transcriptome analysis did not underline any subsequent sex-specific differences in a late pre-implantation stage of development (day 18 post-insemination) (Degrelle *et al.* 2012), although a selection of candidate genes (*DNMT3A*, *IFNT2*, *GATA4*, *WT1*) showed a sex-specific transcriptional regulation at day 14 of pregnancy (Bermejo-Alvarez *et al*. 2011). Upon transfer of male or female embryos produced *in vitro*, the impact of the sexual dimorphism has been described in the uterine fluid of recipients after development for three days *in vivo* (Gomez *et al*. 2013). The authors have suggested that the uterine response induced by males, but not by females, would lead to a favourable uterine environment in accordance with sex selection mechanisms operating through males. Uterine fluid of recipient cows for embryos produced *in vitro* had been described to be influenced by male embryos, but not by female embryos, suggesting a sexual dimorphism in the uterine response (Gomez *et al.*, 2013).

The mammalian endometrium as a biosensor of embryo quality

In terms of the contribution to pregnancy issue, the embryo transfer procedure allows the effects of the oocyte and/or embryo quality to be separated from the impact of the genital tract. *In vitro* maturation, *in vitro* fertilisation (IVF) and subsequent *in vitro* embryo culture have been reported to significantly alter gene expression patterns in blastocysts and elongating embryos compared with their counterparts derived *in vivo* (Clemente *et al*. 2011; Gad *et al.* 2012; Marjani *et al*. 2009; Mermillod *et al*. 2010).

Although the rate of success is still very low, SCNT can lead to term development of cloned embryos when correct nuclear reprogramming takes place (Heyman 2005; Niemann *et al*. 2008; Khan *et al*., 2012). Nevertheless, severe or fatal consequences for embryo and foetal–placental development have also been reported (Chavatte-Palmer *et al*. 2012) and shown to vary considerably according to the origin of somatic cell lines and across laboratories (Yang *et al*. 2007). These different types of embryos were postulated to elicit different endometrial gene patterns that could account for the final outcome of a pregnancy.

By comparing endometrial transcriptomes of cows that are recipients of IVF-derived embryos or SCNT embryos generated with various somatic cell lines, several endometrial genes have been shown to be associated with the nuclear transfer procedure at day 18 post-oestrus (Bauersachs *et al*. 2009). Interestingly, in the uterine lumen of pregnant heifers carrying SCNT versus IVF conceptuses, abundances of ten amino acids (AA) were reduced. In this study, the mRNA level of the *SLC7A8* AA transporter solute carrier gene was also lower in the endometrium of SCNT conceptuses carrying recipients (Groebner *et al*. 2010). In these studies as in another report (Mansouri-Attia *et al.* 2009b), the variation of gene expression in the endometrium was unlikely to be attributable to differences in IFNT production as the expression of this factor was found to be similar in SCNT and IVF pregnancies. The decreased supply of numerous AA and derivatives in SCNT conceptus-carrying females may account for the placental abnormalities that have been reported in the later stages of pregnancy.

Our research group compared the impact of embryos generated *in vivo* on the endometrium with that of embryos generated by IVF-embryo transfer (IVF-ET) and SCNT (Mansouri-Attia *et al*. 2009b). SCNT embryos were produced using a unique somatic cell line of fibroblast origin (5538). Reprogramming of the efficiency of the 5538 cell line was high and was characterised across various stages of embryo development and pregnancy until term (implantation rate: 80%; calving rate: 13%) (Degrelle *et al.* 2012). Transcriptome profiles revealed distinct patterns of gene expression among the three groups; hence, the differential response of the endometrium depending on the developmental trajectory of the embryo (Mansouri-Attia *et al.* 2009b). Interestingly, SCNT embryos introduced more marked changes in the endometrium than did the IVF-ET embryos, consistent with the more severe alterations of extra-embryonic gene patterns displayed by the SCNT conceptuses produced with this 5538 somatic cell line (Degrelle *et al*. 2012). Although the mechanistic bases of the epigenetic perturbations are unknown, these data are indicative of altered epigenetic programming of embryo and endometrium. Data mining revealed profound alterations in molecular pathways (e.g. oxidative phosphorylation) and biological functions (metabolism and immune function). Differential expression of selected genes was confirmed in caruncular or intercaruncular areas during the peri-implantation period (18-42 d*po*), including *C11ORF34, FABP3*, *FBP1*, *GJA1*, *MX2* ((Mansouri-Attia *et al.* 2009b), two genes encoding extracellular matrix components (*DCN*, *COL1A2*) (Guillomot *et al*. 2014) and *SOCS3,* a major regulator of the JAK-STAT signalling pathway (Vitorino-Carvalho *et al.* 2014b). Our data led us to propose an original concept that defines mammalian endometrium as an early biosensor of embryo quality (Mansouri-Attia *et al*. 2009b; Sandra *et al*. 2012). Interestingly, recent data generated in humans have proposed that decidualising endometrial stromal cells can sense embryo quality and tailor a specific response in order to eliminate compromised embryos (Teklenburg *et al*., 2010; Brosens *et al*. 2014). Alternatively, human endometrial stromal cells with impaired decidualisation fail to discriminate between high- and low-quality human embryos (Salker *et al*. 2010). This defect of embryo sensoring results from the inappropriate preparation of the endometrium for pregnancy and appears consistent with extensive and specific endometrial deregulations present before conception in patients who experienced implantation failure or recurrent pregnancy loss (Lédée *et al*. 2011). Therefore, the endometrium as a biosensor of embryos displaying divergent developmental potencies appears to be a feature valid across mammals, despite structural differences in implantation and placentation.

Conclusion

In mammalian species, successful pregnancy has been defined as the birth of viable offspring. Nevertheless, experimental models and pathological situations have demonstrated that defining "successful" is much more complex. Indeed, several questions are raised relative to term pregnancy and offspring health: are post-natal pathological conditions related to pre-natal perturbations and can this link be identified? When the health of the progeny is affected or compromised, does it depend on embryo quality and/or endometrium quality? Detrimental events taking place in the uterine environment beyond implantation have been shown to dramatically affect the outcome of pregnancy by altering placenta functions and foetus development (Burton *et al*. 2010). Nevertheless, congenital anomalies, acquired diseases or perturbations of adult maternal physiology before and during reproductive life can affect endometrial function in a permanent or transient manner. Distinct endometrial responses can also be elicited by embryos presenting different post-implantation fates, making the endometrium an early biosensor of the ability of the embryo to develop to term (http://www.nature.com/stemcells/2009/0904/090430/full/stemcells.2009.67.html).

Hence, mammalian endometrium appears to be a dynamic and reactive tissue whose compromised or suboptimal physiology can deeply or subtly affect embryo development before implantation, with visible and sometimes severe consequences for the placentation process, foetal development and pregnancy outcome. Consequently, although term pregnancy issues incontestably rely on the quality of the embryo (the seed) (Patrizio *et al*. 2007), it is obvious that endometrium (the soil) (Salamonsen *et al*. 2009) has to be considered a critical epigenetic contributor to the embryo trajectory from the very earliest stages of pregnancy. In this context, two major issues deserve a comprehensive investigation of the maternal organism:

- Embryos can overcome the control exerted by the endometrium, as illustrated by the defect in nature's quality control proposed by Aplin and collaborators (Quenby *et al*. 2002). In other words, embryos that fail to develop to term would be able to implant and then to develop until the later stages of pregnancy. In this case, the unusual properties of these embryos (e.g. producing an excess of embryo-derived signals) or factors associated with the inability of impaired endometrial cells or tissue to prevent implantation of compromised embryos (Weimar *et al*., 2013) will have to be clearly defined.
- Diagnosing early pregnancy and predicting the issue will benefit from the identification of endometrial in addition to systemic biomarkers to improve global fertility and the proportion of term pregnancies in cattle. First insights have been provided that aim to predict reproductive capacity based on transcriptional profiles established from pre-conceptional endometrium (Salilew-Wondim *et al.* 2012; Minten *et al*. 2013). Interestingly, if endometrial quality can be determined to be suboptimal, it paves the way for the design of compensatory strategies (e.g. by optimising maternal nutrition) (Al-Gubory *et al*. 2013) aiming to restore the compromised uterine function to a level compatible with term pregnancy and the birth of a healthy progeny.

In conclusion, determining the limits of endometrial plasticity at the onset of pregnancy is a difficult task, but essential challenges for providing new insights into the contribution of the maternal environment to embryo epigenetic shaping are linked with the success, alteration or failure of pregnancy in mammals, including farm animal species.

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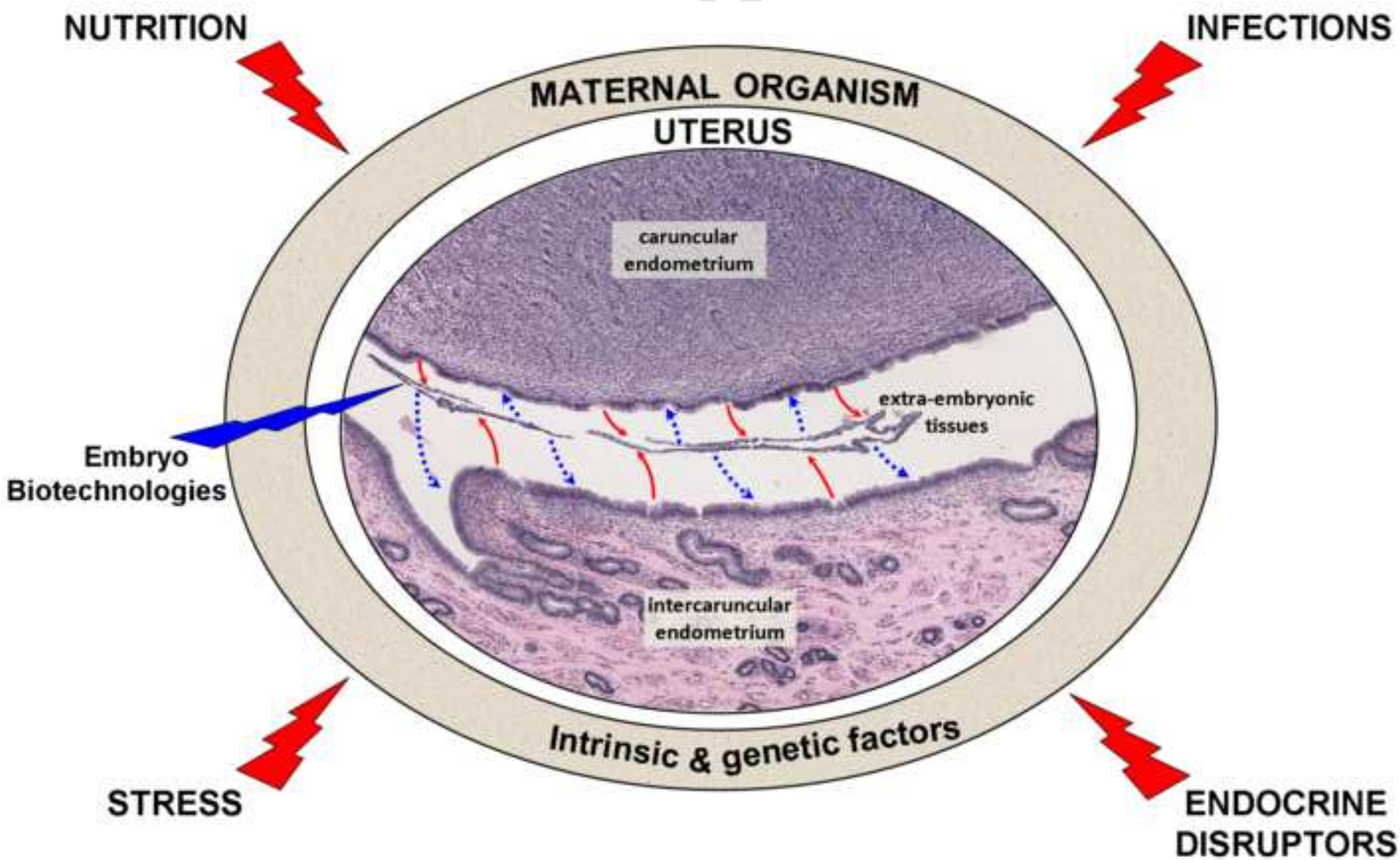
Figure legend

Fig 1. Impact of environmental insults and embryo origin on endometrium physiology. Endometrium (gland-free caruncular areas and inter-caruncular areas) and extra-embryonic tissues were sampled from a pregnant heifer at day 18 post-oestrus (microphotograph courtesy from Dr. Michel Guillomot, INRA). Embryos produced with the help of biotechnologies (*in vitro* fertilisation or somatic cell nuclear transfer followed by transfer to recipients) induce a specific response of the endometrium (sensor property, blue dashed arrows). Environmental insults (nutrition, infections, stress, endocrine disruptors) affect endometrial physiology, which in turn alters the epigenesis of extra-embryonic tissues during conceptus development, leading to pregnancy failure or perturbations with post-natal consequences (driver property, red arrows).

CONFLICT OF INTEREST STATEMENT

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

Figure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