Preattachment Embryos of Domestic Animals: Insights into Development and Paracrine Secretions

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Abstract

In mammalian species, endometrial receptivity is driven by maternal factors independently of embryo signals. When pregnancy initiates, paracrine secretions of the preattachment embryo are essential both for maternal recognition and endometrium preparation for implantation and for coordinating development of embryonic and extraembryonic tissues of the conceptus. This review mainly focuses on domestic large animal species. We first illustrate the major steps of preattachment embryo development, including elongation in bovine, ovine, porcine, and equine species. We next highlight conceptus secretions that are involved in the communication between extraembryonic and embryonic tissues, as well as between the conceptus and the endometrium. Finally, we introduce experimental data demonstrating the intimate connection between conceptus secretions and endometrial activity and how adverse events perturbing this interplay may affect the progression of implantation that will subsequently impact pregnancy outcome, postnatal health, and expression of production traits in livestock offspring.

INTRODUCTION

EE: extraembryonic

MRP: maternal recognition of pregnancy

EET: extraembryonic tissues

In mammals, a complex sequence of biological processes and the successful handling of several critical points are involved in the birth of viable progeny. The period of time preceding implantation and implantation itself have appeared as critical steps of pregnancy, associated with a high rate of embryo loss leading to pregnancy failures in all mammalian species studied so far. To illustrate, reproductive efficiency in high-producing dairy cattle has been shown to be rather low, with a probability to achieve pregnancy estimated at 40% and two-thirds of pregnancies stopping during the peri-implantation period (1, 2). In addition to endogenous maternal factors (such as genetic mutations) that could be detrimental for pregnancy initiation and progression, environmental insults (stress, nutrition, infection, pollution, and endocrine disruptors) have been identified as factors that affect gamete quality, fertilization, and the journey of the early embryo through the oviduct; cellular interactions between the hatched blastocyst [or conceptus, meaning embryo and extraembryonic (EE) membranes] and the endometrium (the uterine tissue layer ensuring molecular and cellular interactions with the developing embryo); and feto-placental development and parturition (3, 4). In addition, the use of assisted reproductive technologies associated with embryo transfer has been shown to alter the biological properties of the embryo, with subsequent impacts on later stages of pregnancy (5). Therefore, an inadequate maternal compartment and/or in vitro manipulations of embryos can affect the two-way communication established between the mother and embryo when pregnancy initiates, often precluding completion of successful pregnancy or affecting long-term health status of the offspring.

Upon hatching, the critical challenge for the embryo is the maintenance of the maternal environment in such a condition that implantation, developmental progression to the feto-placental unit, and term delivery can take place. Under the actions of ovarian steroids (estrogen then progesterone), the uterus is switched to a receptive state that makes it compatible with embryo apposition and attachment or adhesion (these two steps being common across all mammalian species) and invasion of the endometrial wall (in species with endotheliochorial or hemochorial placentation) that will precede placental development (6). Endometrial receptivity is a prerequisite for supporting the progression of pregnancy beyond the blastocyst stage. Then embryo signals will be mandatory not only to ensure maternal recognition of pregnancy (MRP), which includes prolongation of the life span of the corpus luteum as a source of progesterone, but also to promote cellular interactions between embryonic cells and endometrial cells during the implantation process while preventing rejection of the semiallogenic (and even allogenic when pregnancy derives from embryos that are produced by a donor female then transferred to recipients) embryo by the mother. In the context of this review, we focus on secretions produced by the extraembryonic tissues (EET) and embryonic tissues of the preimplanting conceptus that have been associated with paracrine biological actions occurring (*a*) between the various cell populations that constitute the conceptus to coordinate its development and (*b*) between the conceptus and surrounding endometrial cells during the MRP and preattachment phase. Targeted species are domestic ungulates, with a main focus on ruminant species (mainly cattle and sheep), and we also include insights into porcine and equine species. In these species, pregnancy includes a protracted preimplantation period upon fertilization (two to three weeks, depending on the species) with an embryo that remains free-floating in the uterine cavity. This feature constitutes a unique opportunity to investigate the complex network of secretions that drives the development of the conceptus and its interactions with the endometrium.

DEFINITION OF THE PREATTACHMENT EMBRYO: MAIN STEPS OF DEVELOPMENT

ICM: inner cell mass
TE: trophectoderm
PS: primitive streak

During the free-life period in the uterus specific to implantation in ungulates, three concomitant biological processes take place at the conceptus level: the elongation of the EET, the gastrulation of the embryonic disc, and cross talk with the uterus.

The embryo enters the uterus at different times postfertilization depending on the species, though generally at a stage where the blastocyst is described as spherical (7). It then elongates in most ungulates, except for horses, and harbors sequential shapes usually named, in bovine, sheep, goat, and pig, ovoid, tubular, and filamentous. Though it has been observed for decades, the elongation process remains largely elusive. Its extent varies according to litter, dam's physiological status, breed, and study (**Figure 1**). Its arrest depends on implantation and the first cellular contacts that take place at the time of apposition, whereas its onset relies on both extrinsic and intrinsic parameters, the former relating to uterine secretions, early P4 circulating levels, and uterine/embryonic synchrony, and the latter to developmental cues that could be set up much earlier and relate to blastocyst or oocyte competence (8, 9). Regardless, elongation corresponds to the differentiation of the tissues that contribute to implantation and placenta formation (chorion, yolk sac, allantois) and are thus called extraembryonic (EE), as opposed to those contributing to fetal organs, which are called embryonic (10). Nonetheless, two EE layers derive from the embryonic lineage at the time of embryonic differentiation (pre- and postgastrulation), the EE endoderm and EE mesoderm, whereas one of these, the EE endoderm, partly contributes to the embryonic tissue at later stages [mouse (11)]. Thus, reciprocal interactions between embryonic and EE tissues play essential roles in conceptus development and thus in embryonic/EE differentiation owing to paracrine signaling and transcriptional regulatory networks [mouse (12)]. Originally, however, at the blastocyst stage—when the embryos of eutherian mammals enter the uterine cavity (13)—two lineages are formed: the embryonic one, recognizable as an inner cell mass (ICM), and the EE one, recognizable at first by a trophectoderm (TE) layer of polarized epithelial cells and later by the formation of a primitive endoderm, these differentiations resulting from a complex interplay of molecular and cellular cues (14, 15). Conversely to in rodents and primates, in ungulates the preattachment embryo does not implant at the blastocyst stage (16) but develops far beyond the spherical stage (**Figure 2**), implanting at the somite stages (bovine, sheep, goat) or later (e.g., at the limb buds stage in the horse). However, mouse and human embryos are able to develop and self-organize prior to primitive streak (PS) formation in the absence of uterine tissues using optimized in vitro culture conditions (17–19).

PARACRINE SECRETIONS OF CONCEPTUS ORIGIN: DESCRIPTION AND BIOLOGICAL ACTIONS

Definition of Paracrine Signals in the Context of Early Pregnancy

Historically, one way to classify cell secretions has been based on their biological actions: autocrine (the target cell is the secreting cell), paracrine (the secreting cell acts on a nearby target cell), or endocrine (cell productions are secreted into the blood, then carried by blood and tissue fluids to the target cells). With the considerable amount of work carried out to decipher the nature of secretions of preattachment embryos, it has become clear that factors originally defined as paracrine secretions can also exert endocrine actions. One of the most striking examples is interferon-tau

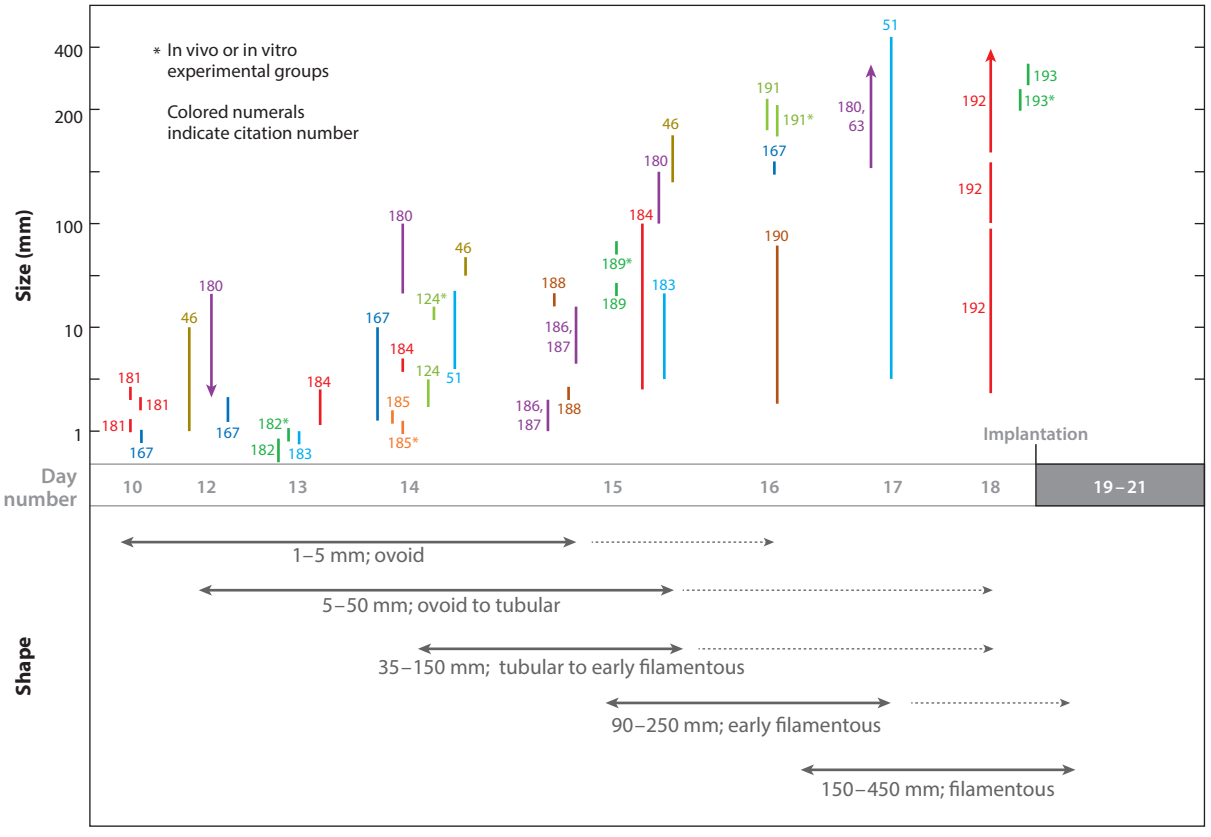


Figure 1

Overview of conceptus elongation in cattle. Conceptus size (mm) and shape have been recorded here for cattle, but similar data exist for sheep (58, 159–165), pigs (57, 63, 166–176), and horses (177–179). As for bovines, we compiled data from day 10 to day 18 (initial implantation steps: I, day 19–day 21), based on reports using different breeds (beef, dairy, crossbred), different dam physiologies (heifers, lactating, nonlactating), in vivo development after artificial insemination or in utero transfers of in vitro–produced embryos, or in vivo or in vitro experimental groups (*). Obviously, day 14 and day 15 were the most frequent flushes analyzed so far, and conceptus size (or shape) is a confounding criterion. Whatever the shape or the day, size records appear so variable that small-sized embryos recovered one to three days after their initial descriptions by other reports may correspond to slightly or severely growth-retarded concepti (180), prompting some authors to sort them by stage (e.g., shape, size, and embryonic development; see **Figure 2*a***) instead of days (63, 180, 192), whereas others preferred recording the length of the embryonic tissues (epiblast: 100–800 μm) to evaluate embryo development consistently from day 11 to day 16, after embryo transfers at day 7 (33). Colored bars correspond to the indexed minimal to maximal sizes from the referenced studies, and corresponding colored numerals indicate the citation numbers of the referenced studies.

(IFNT), a ruminant-specific protein secreted by the preimplanting conceptus and considered as a typical paracrine factor. Since the molecular characterization of IFNT in the mid-1980s, its paracrine actions on endometrial physiology have been extensively investigated (see next section), but more recent data in cattle and sheep have also reported the induction of interferon-stimulated genes (ISGs) in peripheral blood leukocytes (20) and peripheral tissues, including corpus luteum (21, 22) and liver (23). Using a specific and sensitive radioimmunoassay, Romero et al. (22) eventually detected IFNT in the blood of early-pregnant ewes, a finding that at least partially accounts for the systemic actions of IFNT in ruminants. Therefore, factors secreted by the preattachment conceptus can no longer be classified based on the type of cell-signaling mechanism.

IFNT: interferon-tau

ISG: interferon-stimulated gene

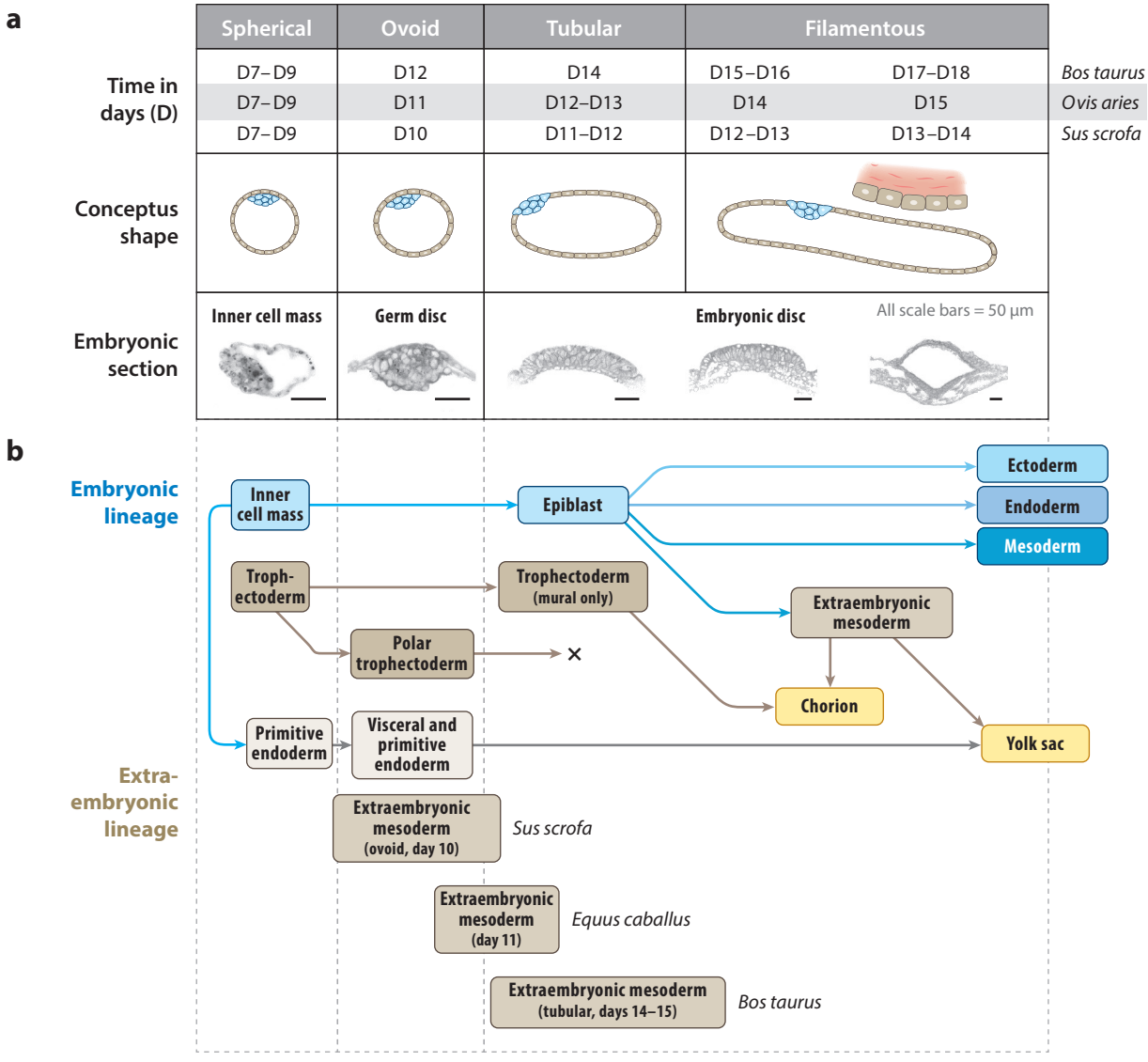


Figure 2

Developing conceptus in cattle. Conceptus shapes evolve along time according to different schedules in cattle (*Bos taurus*), sheep (*Ovis aries*), and pigs (*Sus scrofa*). Illustrated are typical embryonic sections at the three main stages observed for the embryo proper: inner cell mass, germ disc, and embryonic discs (panel *a*), differentiating tissues along each lineage, (in panel *b*) embryonic (*blue boxes*) and extraembryonic (*brown boxes*). Whatever the species, an early landmark of gastrulation is the epithelialization of the epiblast, as in the mouse. Specific to these livestock species (cow, sheep, pig, horse) are the following features: the early loss of the polar trophoblast lineage (or Rauber's layer), the early detection of extraembryonic mesoderm cells that appear prior to a visible primitive streak, and the whole gastrulation process that precedes implantation. Graded blue (embryonic tissue) or brown (extraembryonic tissue) colors indicate differing cell types within each same-colored lineage.

Paracrine Signals and Interactions Between Embryonic and Extraembryonic Tissues

ExE: extraembryonic ectoderm

EPC: ectoplacental cone

PGC: primordial germ cell

VE: visceral endoderm

DVE: distal visceral endoderm

AVE: anterior visceral endoderm

ExVE: visceral endoderm facing the ExE

EmVE: visceral endoderm facing the epiblast

TSC: trophoblast stem cell

Paracrine secretions and signaling have long been studied in the mouse and are thus briefly and schematically described here to highlight how they might proceed differently in livestock species (**Figure 3**), leaving aside other amniotes (marsupials, monotremes, birds, or reptiles), though they are essential to discussions about (non)conserved features (13, 24–26). These models are indeed helpful in considering the mouse as a unique case of highly deciphered mechanisms, in which quickly evolving methods helped solve functional questions in vivo and in vitro (27). However, one should be aware of its differences from other mammals. For instance, the mouse has a specific morphogenesis in which the epiblast organizes as an egg cylinder (inverted, cup-shaped, isolated from the uterine milieu)—which is different from the epiblast morphogenesis observed in most mammals [flat, facing the uterine cavity (17)]—and leads to mouse-specific embryonic/EE tissue boundaries and vicinities. Also, it features a specific expansion of the trophoblast lineage that covers the ICM at the blastocyst stage (polar trophoblast), which gives rise to the mouse extraembryonic ectoderm (ExE), ectoplacental cone (EPC), and chorioallantoic placenta (28), whereas it regresses in most mammals (29–33) and does not exist in others [marsupials (34)], the polar lineage being a possible eutherian innovation (13). Finally, the EE mesoderm spreads out of the posterior pole earlier in several mammalian species than in the mouse (32).

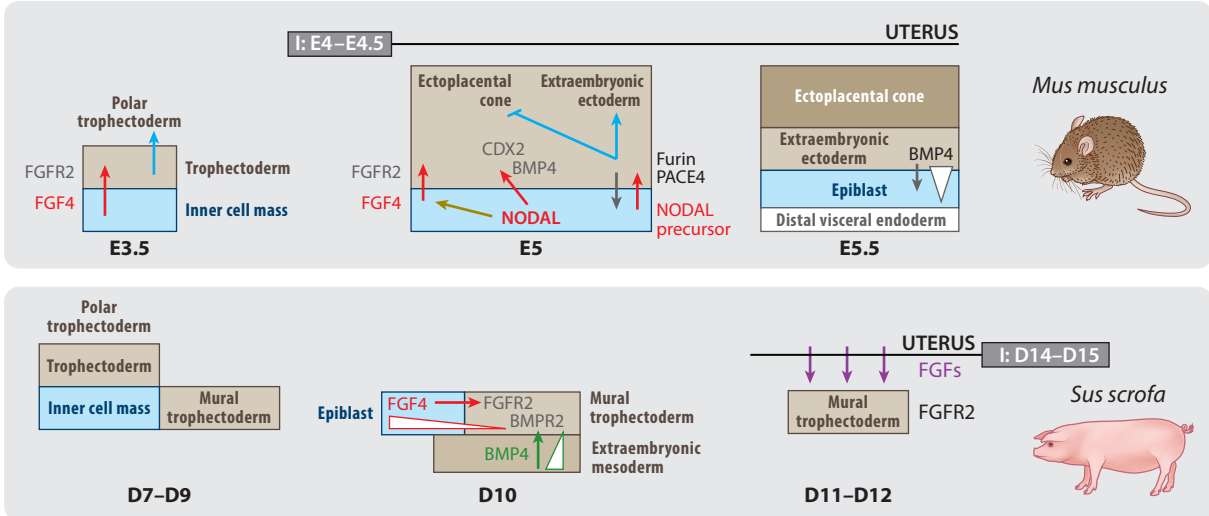
Nonetheless, reciprocal interactions between ET and EET have been described in the mouse, involving signaling molecules that are conserved across vertebrates, including the FGF, TGFB, and WNT families. Based on the egg cylinder morphology, these interactions, in which the EET are sources of signals for lineage specification and embryonic patterning (35, 36), play essential roles in pre- and postgastrulation stages to transiently maintain epiblast pluripotency, establish the embryonic axes (proximo-distal first, then antero-posterior), position the PS, restrict it to one axis, and sort the primordial germline cells (PGCs) out of the epiblast (12). These EET include the polar TE, the ExE, and the visceral endoderm (VE) and its subtypes, depending on time [distal and anterior visceral endoderm (DVE, AVE)] or neighbors [ExVE, columnar cells associated with the ExE; EmVE, squamous cells associated with the epiblast (37)]. These tissues, though similar, display distinct transcriptional networks and patterns that provide a mosaic of EE cell types (38) less studied in other species.

Reciprocal interactions in which the embryonic tissue (ICM, epiblast) are the source of signals that prevent the precocious differentiation of the EET (TE and ExE, respectively) are known as well (39). The data are valuable for deciphering the in vivo and in vitro molecular nature of the trophoblast stem cell (TSC) niche at early or late stages in mouse as in human [mouse: TE at day 3.5, ExE at day 6.5, or chorionic ectoderm at day 7.5 (40, 41)].

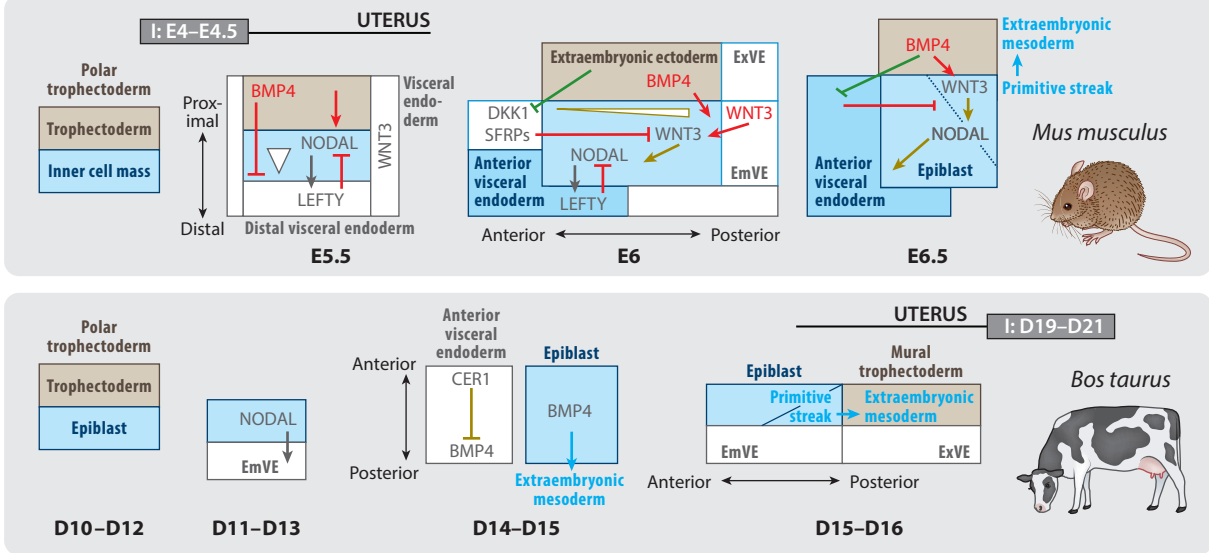
Figure 3

Paracrine secretions within the conceptus. In the mouse, implantation (I) starts at E4.5 and precedes the differentiation of the embryonic/extraembryonic lineages once established. Blue (embryonic tissue, ET) and brown (extraembryonic tissue, EET) boxes indicate differing cell types within each lineage. The trophoblast subtypes that form the placenta originate from the polar lineage in the mouse but from the mural lineage in cows, sheep, pigs, and horses. In the mouse, trophectoderm and extraembryonic ectoderm identities are maintained through transcription factor networks, downstream of the paracrine signaling emanating from the embryonic lineage (inner cell mass, epiblast; panel *a*: ET→EET), most of which are differing in the bovine or porcine cell types (31, 45, 47), due to different precursors and/or to the lack of extraembryonic ectoderm equivalents in these species. On the other hand, the setup of the embryonic axes and the positioning of the primitive streak result in the mouse, as in the cow, from paracrine signaling emanating from the extraembryonic lineage (trophectoderm, visceral endoderm; panel *b*: EET→ET) and involve conserved molecules, the asymmetry of which is not similarly established (33). Not shown here, though essential (17), is the central lumen that starts forming by E5.0 and extending by E5.5 (mouse, panels *a* and *b*). Abbreviation: D, day.

a Embryonic tissue–extraembryonic tissue paracrine secretions



b Extraembryonic tissue–embryonic tissue paracrine secretions



Gradient | Inhibition | Activation/induction

- Paracrine signaling
- Feedback signaling
- Signaling between two embryonic or extraembryonic cell types
- Signaling within same cell type
- Signaling in livestock
- New differentiation process

EmVE Visceral endoderm facing the epiblast
ExVE Visceral endoderm facing the extraembryonic ectoderm

EEM: extraembryonic mesoderm

Embryonic tissue secretions acting on extraembryonic tissue. In the mouse, the signal that prevents the precocious differentiation of the ExE and sustains the self-renewal and multipotency of the TSC progenitors is the FGF4 growth factor, secreted by the epiblast. Acting through an FGF receptor (FGFR2) expressed by the ExE, this signal inhibits progenitor differentiation toward the EPC trophoblast subtypes (42) while maintaining an ExE identity (39). FGF4 expression is promoted in the epiblast by NODAL (TGFB family member), which, synthesized as a precursor by the epiblast, is cleaved by the proprotein convertases secreted by the ExE at the epiblast-ExE interface, namely, FURIN (or PCSK3) and SPC4 (or PCSK6, or PACE4). Reciprocal ET/EET interactions are thus at work. Conversely to the polar lineage, no such signaling maintains proliferation of the mural lineage, so that it quickly differentiates, likely from other TSC progenitors (43).

Below FGFR2, the FGF4 signaling cascade (FRS2α-ERK) leads to the induction of BMP4 expression in the ExE through the expression of the CDX2 transcription factor (44). Together with a network of transcription factors, CDX2 contributes to TSC maintenance in the ExE, whereas BMP4 (a member of the TGFB family) acts through another inductive cascade (SMADS 1/5/8) to promote epiblast cell proliferation, mesoderm formation, and PGC fate induction in the epiblast (17). Therefore, although reciprocal, ET/EET interactions often auto-amplify.

As stated earlier, owing to the quick regression of the polar TE in bovine and porcine conceptuses, the proliferating and implanting trophoblast in these species is the mural lineage (30). Moreover, it has long seemed unlikely that this mural lineage proliferates through a similar signaling (FGF4/NODAL) emanating from the embryonic lineage (ICM, epiblast) (**Figure 3*a***) or relies on a similar transcriptional network, owing to (*a*) an adjacent, flat, and tiny embryonic disc; (*b*) a slow restriction in the expression of the OCT4 transcription factor to the epiblast lineage [bovine (45)]; (*c*) a lack of CDX2, ELF5, or EOMES in the mural TE at early ovoid stages, e.g., when proliferation exponentially increases to achieve the elongation process [bovine or porcine (31, 46, 47)]; and (*d*) a limited need for early FGF signaling, because addition of FGF to in vitro–produced blastocysts was not reported to promote trophoblast maintenance (48–50).

It was thus hypothesized that trophoblast proliferation is sustained beyond the ovoid stage by uterine secretions, and indeed, FGF ligands and FGF receptors have been identified at the uterus/conceptus interface [bovine: FGF1, FGF2, FGF10 versus FGFR1, FGFR3 at D13, FGFR2 at D16–D19 (51–53); porcine: FGF7, FGF9 versus FGFR2 (47, 54, 55)], but so far with no in vivo functional evidence of an active uterine signaling in elongating embryos. Nevertheless, FGF2 and FGF10 stimulate trophoblast cell migration in vitro [bovine CT1 cells and ovine oTr1 cells, respectively (48)], as does FGF4 on the porcine trophoblast, but through a different cascade [pTr cells (56)]. Conversely, in pigs, a gradient of FGF4- and BMP4-inducing signals has been evidenced prior to elongation. FGF4 is secreted by epiblast cells at the early ovoid stage and acts upon neighboring trophoblast cells, whereas BMP4 is produced by the nascent extraembryonic mesoderm (EEM) and acts upon trophoblast proliferation through a SMADS 1/5/8 cascade (47). Indeed, in pigs, as in horses (32), EEM is formed earlier than in sheep or cattle (at the ovoid instead of tubular stage; 10, 57, 58) (**Figure 2**). However, this BMP signaling argues instead for EET secretions acting on EET (from EEM to trophoblast, see **Figure 3*a***).

Extraembryonic tissue secretions acting on embryonic tissue. In the mouse, the two main pathways that signal from the EET to the ET are the WNT and TGF (NODAL, BMP) pathways: (*a*) a NODAL gradient in the epiblast to establish a proximo-distal axis and allow the egg-cylinder elongation in this axis; (*b*) an antero-posterior gradient of NODAL and Wnt in the epiblast, once the anterior side has been defined, to position and restrict the PS formation; and (*c*) dose-dependent BMP-SMAD signals to specify the germ cell lineage (12).

As described earlier, the active NODAL form upregulates the level of BMP4 in the ExE, then activating CDX2, but also signaling backward to the epiblast to enhance WNT3 expression. In the meantime, a NODAL and WNT gradient is established through the secretion of extracellular antagonists (CER1 and LEFTY1 inhibit NODAL) by a small subset of VE cells that acquired a distal fate (DVE), followed by the setup of an anterior secretion of WNT antagonists (SFRP1, SFRP5, DKK1) by the AVE to restrict WNT3 to the posterior side of the epiblast, thus reinforcing an earlier WNT3 signaling from the posterior EmVE (EET, E5.5) that precedes its own expression in the epiblast (ET, E6.5; 59). The DVE-to-AVE transition, which involves a set of asymmetric expressions as well as collective cell movements, guiding cues, cell-shape changes, and neighboring cell exchanges, has been much refined recently (60, 61) but is not described here. Then, at the ET/EET boundary, PS formation results from a conjunction of signaling: BMP4 from the ExE (BMP4–SMADS 1/5/8), active NODAL from the proximal epiblast (NODAL–SMAD 2/3), and canonical WNT3 from the posterior EmVe and posterior epiblast (WNT3–β-catenin). In this case, two BMP signaling cascades (BMP4–SMADS 1/5/8 from the ExE and BMP–ALK2 from the VE) induce a germ cell switch in epiblast cells (36). Through an EMT process, multiple mesoderm lineages emerge from the PS, according to time and site of ingression. The EEM emerges first (posterior end of the streak) followed by the embryonic ones, paraxial mesoderm (middle streak) and axial mesendoderm (anterior end of the streak).

A/P: antero/posterior

Of main interest for other eutherian species, the VE resembles the hypoblast (birds, rabbit), the antero/posterior (A/P) polarity of the VE precedes that of the epiblast, and paracrine signals from the EET maintain pluripotency (extrinsic signals) and pattern the epiblast during a short time window (25, 35, 62). As stated above for ET/EET signaling, the molecules involved in EET/ET signaling appear similar, but "the mechanisms of asymmetry establishment, which relies on EE tissues such as the hypoblast and trophoblast, is more difficult to reconcile both in gene expression and morphological terms" (33, p. 23) when comparing mouse, rabbit, porcine, and bovine data.

The setup of an A/P axis and the restriction of the PS at the posterior pole of the embryonic disc have been suggested in these species, based on altered features such as enlarged streaks or ectopic posterior poles [bovine (63)] or specific gene expression patterns [ovine (58), pig (57)], but were not functionally assessed in the embryo, except for in rabbits. The graded ablation of the hypoblast, leading to the graded lack of NODAL and WNT inhibitors in the AVE (CER1, LEFTY, DKK1; 64), generated similarly altered features of PS positioning or restriction. At earlier stages, in cattle and pigs, expression patterns for CER1 suggested an A/P asymmetry similar to the situation reported in mice, although no DVE equivalent was observed (absence of centrally positioned CER1+ cells as observed in the rabbit embryonic disc; 33). This might be due to the small size of these embryonic discs in porcine and bovine species, thus keeping active the inhibitory influence of the circumferential trophoblast cells. Then, NODAL expression in the bovine epiblast is lost, whereas BMP4 is expressed in the whole epiblast, which is opposite to the situation described in mice, in which NODAL represses BMP4 expression in the epiblast while sustaining it in the ExE (**Figure 3*b***).

Interactions across extraembryonic tissue or across embryonic tissue. Last but not least, interactions across EET (EET on EET) are known to occur at perigastrulating stages, often mediated (or possibly so) through the basement membrane, extracellular matrix components, and signaling cues trapped (or released) by this meshwork [integrins, growth factors, cytokines (65, 66)], such as those in which (*a*) trophoblast cells do not fall apart or form vesicles while adhering to neighbors and to extracellular matrix substrates [bovine (10), mouse (11)]; (*b*) EE endoderm cells adopt different morphologies depending on their facing tissues [mouse (37), sheep (67)]; and (*c*) EE endoderm/ectoderm forming the allantois, chorion, or yolk sac differentiate according

to instructive cues emanating from the EE mesoderm [mouse (28), human (41), bovine (10)]. Homotypic interactions across ET occur as well, when the embryonic mesoderm, endoderm, or ectoderm is formed and involved in fetal organogenesis [e.g., limb bud development (68)].

PG: prostaglandin

AAs: amino acids

OTP1: ovine trophoblast protein 1

Diversity of Conceptus Secretions and Actions on Endometrial Cells

Although uterine remodeling and the timing of the window of implantation are programmed by female hormones independently from the embryo, establishment and progression of pregnancy will be dependent upon embryo recognition by the maternal organism, with critical contribution from the endometrial tissue (69). This reaction is governed by a set of embryonic factors that are indispensable for promoting the implantation process through the establishment of permanent cellular interactions between theTE and the endometrium (70). Factors released by the conceptus have been intensively investigated in mammalian species, particularly in domestic ungulates, for which a wealth of available data has clarified the nature of the signals and provides comprehensive information about their biological actions necessary for successful MRP and implantation (71). A type I interferon in cattle, sheep, and goats and a steroid (estradiol) and interferons in pigs have been recognized as critical signals acting in a paracrine manner for regulating MRP and implantation; no such signal has been clearly identified in horses because the luteotrophic equine chorionic gonadotropin is secreted after MRP has occurred. Nevertheless, in this species, as in the aforementioned ones, prostaglandins (PGs) are secreted by the conceptus and act as paracrine factors on the endometrium. Here we focus on interferons and PGs at the conceptus-endometrial interface.

Reproductive interferons: the saga of interferon-tau. Among the embryonic factors that have been investigated during the establishment of pregnancy in ungulates, IFNT and its biological actions have generated a large amount of literature. The story starts with an antiluteolytic factor (trophoblastin) that was identified in the ewe by means of intrauterine injections of homogenates produced with conceptuses collected at days 14–16 of pregnancy (72). By using gel filtration chromatography and conditioned culture medium of ovine conceptuses incubated in the presence of radiolabeled amino acids (AAs), an abundant de novo synthesized protein with a low molecular weight was isolated and named protein X and later ovine trophoblast protein 1 [OTP1 (73)]. It appeared that trophoblastin and protein X/OTP1 were the same protein encoded by a gene that was cloned and sequenced by three independent groups (74–76). The gene, as well as its product, was named interferon-tau (IFNT) because it was found to be a new member of the type I interferon family, which includes interferon alpha, beta, delta, epsilon, kappa, and omega, all of which share a high degree of structural homology (77). IFNT was subsequently isolated from other ruminant species, including cattle and goat, and it appeared that IFNT genes are specifically found in artiodactyles. Very interestingly, interferon delta and gamma (a member of the type II interferon family) are secreted by the TE of porcine conceptuses during the peri-implantation period of pregnancy. There is no evidence they act as antiluteolytic or luteotrophic factors, but regulation of endometrial genes by interferon delta and/or gamma may be required for blastocyst attachment (71).

Biological features of IFNT are unique because (*a*) this factor is not virus inducible and, unlike interferon alpha and beta, displays high antiviral and antiproliferative activities across species, with a lack of cytotoxicity at high concentrations in vitro (78); (*b*) this factor is secreted in massive amounts only by mononuclear TE cells of elongating conceptuses between day 10 and days 22–25 of pregnancy in ruminants, and its production ceases with the attachment of trophectodermic cells to luminal endometrial epithelium (79); and (*c*) this factor represents the major signal

of pregnancy recognition in ruminants through the inhibition of endometrial prostaglandin-F2 alpha (PGF2a) secretion, thus preventing luteolysis and allowing maintenance of P4 luteal secretion (71). The pivotal role of IFNT in MRP has been reviewed extensively, and the molecular mechanisms can be summarized as follows: Upon binding of type I interferon receptors and associated transduction pathways (e.g., JAK-STAT and MAPK signaling proteins) present in the endometrium (80, 81), IFNT inhibits the transcription of estrogen receptor alpha in sheep and of oxytocin receptor in sheep and cattle, which ultimately prevents the oxytocin-induced pulsatile secretion of PGF2a and luteolysis, thereby maintaining the corpus luteum in a functional state and a progesterone secretion compatible with conceptus development and implantation (81). In addition to its antiluteolytic actions, IFNT acts on the endometrium of ruminants to induce or enhance expression of ISGs that are hypothesized to promote conceptus implantation by acting on the endometrium during the late preimplantation phase. Numerous ISGs have been identified using candidate gene approaches or high-throughput profiling analyses (82). Experimental models combine in vivo intrauterine infusion of recombinant IFNT into cattle and sheep as well as in vitro experiments that include primary cell lines of various endometrial cell subpopulations, human and ruminant established cell lines, and endometrial explants. Together, these approaches have led to the identification of induced or upregulated classical type I ISGs (e.g., IFIT3, IFIT5, IRF9, ISG15, MX1, MX2, OAS1, OAS2, RSAD2, SOCS1, SOCS3, and STAT1) as well as nonclassical ISGs (e.g., CXCL12, CXCR7, DDX58, HER6, IL12B, MCP1, MCP2, MGC127874, PARP12, PLAC8, PTX3, RNF213, TNFA, and ZNFX1). A comprehensive list of IFNT-stimulated genes is presented in **Supplemental Table 1** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**), which illustrates the pleiotropic nature of paracrine IFNT biological effects in a cell-specific manner. Further investigations are necessary to delineate the specific functions of each ISG in the endometrium of ruminants. Loss-of-function studies based on the intrauterine infusion of morpholinos during the preattachment period represent an elegant, relatively fast, and easy-to-use in vivo approach when the targeted factor is present only in the endometrium. When expression of the factor is detected in the endometrium and in the conceptus, interpretation of data can be more tedious, as was recently illustrated for a study aiming to determine the role of type I IFN receptor in elongation of ovine conceptus (83).

Supplemental Material

Prostaglandins at the conceptus-endometrium interface.

Production of prostaglandins by porcine and equine conceptus. Davis et al. (84) demonstrated the synthesis of PGs by porcine conceptus, but the pattern of PG synthesis enzymes during conceptus development has been described more recently (85). Both *PTGS2* transcript and PGE synthase are developmentally upregulated during the peri-implantation period (85). PGE synthase is upregulated during trophoblast elongation (85) and coincides with elevated PGE amounts measured in the blastocyst (84) and in the uterine lumen (86). Production of PGs may contribute to the increases in the PGE2/PGF ratio that are essential for MRP. Autocrine action of conceptus-secreted PGE2 is supported by the increase of PTGER2 expression in conceptus following PGE2 administration. In addition, PGE2 induces aromatase mRNA expression and secretion of the porcine embryonic signal estrogen by the conceptus (87). Altogether, the parallel increase in *PGE2* and *PTGER2* expression has been shown to induce adhesive capacity of the trophoblast via integrins (87).

Peri-implantation embryos of many species produce a variety of PGs when they are incubated in vitro (88). In the mare, the conceptus secretes PGs as early as day 5 (89, 90), and this secretion lasts up to day 30 (89, 91). PGF2a and PGE2 have been the two main PGs identified in equine conceptus. Because the embryonic signal for pregnancy recognition has not been identified in the

horse (92), it has been tempting to question whether PGs released by the conceptus may play this role. From day 18 of pregnancy, PGE2 is the main PG formed, and it has been speculated that PGE2 participates in luteal maintenance. But no clear effect of PGs has been demonstrated, nor those of any other equine conceptus-derived factors on luteal function (89, 93). Thus, the genuine biological functions of PGs produced by the equine conceptus have to be clarified, even though possible local functions important for conceptus development, movement, and nutrition have been evoked (91). PGs from the conceptus may stimulate uterine contractions required for conceptus mobility (94), a process known to be critical for the maintenance of pregnancy in this species (95). PGs could also act as autocrine factors for promoting rapid expansion of the equine blastocysts between day 7 and day 16 (91). As in other domestic species, the conceptus must signal its presence to the mother to initiate pregnancy and then support it to term. In mares, incubation of endometrial tissues with embryos reduces the amount of PGF2a released by the endometrium, suggesting that paracrine factors from the conceptus act on uterine PG production (89, 96, 97). Conceptus secretions have also been shown to modulate *PTGS2* mRNA abundance in the mare endometrium (97), and it seems that preventing upregulation of PTGS2 expression is a key event regulated by the equine conceptus (98). Similarly to the porcine conceptus, the equine conceptus produces large amounts of estrogens before definitive implantation, which occurs 40 days postfertilization in this species, but the role of estrogens as paracrine factors modulating endometrial function and/or conceptus development is still poorly understood. Unlike in porcine species, estrogens from equine conceptus are not involved in the maintenance of luteal activity because estrogen administration does not extend luteal function in nonpregnant mares (93, 99).

Paracrine roles of prostaglandins in growth and elongation of the ruminant conceptus. In ruminants, conceptus elongation is coincident with *PTGS2* expression in both endometrium and EET, suggesting that PGs are involved in conceptus growth (100–102). By infusing a PTGS2 inhibitor into the uterine lumen of ewes, Spencer's group (103) has demonstrated that PTGS2-derived PGs released by conceptus and/or by endometrium are critical regulators of conceptus elongation during the peri-implantation step of pregnancy in sheep. Whether endometrial or conceptus PGs account for inhibiting conceptus elongation remains unclear. Because inhibition of PG synthesis partly reduces the expression of various progesterone-induced endometrial genes (104), PGs could control conceptus elongation through the modification of endometrial histotroph. When exogenous PGs are infused into the uterine lumen of ewes, expression of genes is upregulated in the endometrial epithelium (105). These genes encode proteins that are secreted into the uterine milieu and are related to proliferation, migration, and attachment processes essential for conceptus growth, elongation, and implantation (105). Other genes critical for MRP, such as ISGs, are also regulated by PG potentially secreted by the conceptus (106). Collectively, available data strongly support the idea of an essential interplay between progesterone, IFNT, and PGs for the regulation of endometrial genes (107). In contrast, few data have indicated a direct effect of PGs on conceptus development either as justacrine factors or through an indirect mechanism involving paracrine actions on endometrium. Nevertheless, the fact that both membrane and nuclear PG receptors are expressed in ovine (104, 108) and bovine (109, 110) conceptuses supports a potential role for a PG signaling pathway in conceptus development. The description of each specific PG synthase, receptor, and transporter (108, 110–114) has moderately improved our understanding of the respective paracrine and autocrine functions of the different signaling pathways recruited by PGs. In cattle and sheep, comparing uterine flushing from pregnant and nonpregnant females to pinpoint the contribution of the conceptus for PGs production (110, 115, 116) appears weakly relevant because it postulates that endometrial PG synthesis is not modified in the presence of

the conceptus. Based on this postulate, PGI2, identified as the principal PG in the uterine lumen of pregnant cows, was assigned to the conceptus (110). However, in vitro–produced blastocysts secrete considerable amounts of PG, primarily PGF2a and PGE2 (117–120), whereas ovine embryos produce mainly 6-keto PGF1a, PGE2, and PGD2 (101, 121, 122). As Ulbrich et al. (110) pointed out, the main criticism of these in vitro approaches is that they may not strictly account for in vivo situations. Additional experimental models (e.g., in vivo transient invalidation of genes; specific inhibitors of PG receptor action) will be necessary to clarify the genuine and respective contribution of PGs at the conceptus-endometrium interface.

IVF: in vitro fertilization

SCNT: somatic cell nuclear transfer

In conclusion, the diversity of conceptus signals and strategies developed by large domestic species to initiate MRP and implantation is fascinating. Very interestingly, despite this variety in signals and actions, integrative analyses of transcriptome studies carried out in bovine, porcine, and equine species have unveiled similar regulation of endometrial genes across these species (7), a finding consistent with molecular and cellular features that define superficial implantation common to artiodactyles.

CONCEPTUS-PRODUCED PARACRINE SECRETIONS: SITUATIONS OF PERTURBED PREGNANCY AND BIOLOGICAL CONSEQUENCES

In cattle, in vitro maturation, in vitro fertilization (IVF), and subsequent in vitro embryo culture have been reported to significantly alter gene expression patterns in blastocysts and elongating embryos compared with their in vivo–derived counterparts (123, 124). Although the rate of success is still very low, somatic cell nuclear transfer (SCNT) can lead to term development of cloned embryos when correct nuclear reprogramming takes place (125–127). Nevertheless, during the preattachment period, SCNT embryos also display severe alterations of embryonic and EE gene patterns (63), including perturbations in molecules secreted by the EET (128).

Considering these alterations in conceptus gene expression reported during the preattachment period, our group and the laboratory of E. Wolf and S. Bauersachs tested the hypothesis that bovine endometrium could react to these perturbations. Using bovine embryos generated by artificial insemination, IVF, or SCNT, two pioneer studies used bovine microarrays to provide the first evidence of a tailored response of the endometrium to embryos displaying distinct rates of term development (129, 130). Altered mRNA expression of conceptus-regulated endometrial genes was detected at day 18 and day 20 of pregnancy (e.g., *C11ORF34*, *COL1A2*, *DCN*, *FABP3*, *GJA1*, and *SOCS3*), including classical ISGs (*BST2*, *IFIT1*, *MX2*, *OAS1*, and *RSAD2*) (129–132). Collectively, these data generated from in vivo models have demonstrated the endometrium as a dynamic and reactive tissue that can be qualified as a biosensor of embryo quality (133); this biological property is likely shared by all mammalian species because it was also found to be applicable to the human endometrium (134).

Differences in intrauterine essential and nonessential AA amounts were also reported at day 18 of pregnancy in bovine dams carrying a SCNT conceptus compared with females carrying an IVF conceptus (135). At the same stage of pregnancy, 15-keto metabolites of PGF2a and PGE2 were increased in the uterine fluids of recipient females carrying SCNT embryos, indicating a reduced capacity of SCNT embryos to produce sufficient amounts of PG (Ulbrich et al., unpublished observations cited in Reference 136). Thus, bovine SCNT embryos may contribute to the altered PG environment in the uterine lumen either by synthesizing insufficient amounts of PG or by inducing inappropriate endometrial PG production and metabolism. During the preattachment phase of pregnancy, the abnormal supply of AAs, PGs, and their derivatives might deregulate early development of SCNT conceptuses, eventually leading to abnormalities of the fetoplacental unit and pathologies detected during later stages of pregnancy (137).

EXTRACELLULAR VESICLES AND CONCEPTUS-ENDOMETRIUM INTERACTIONS

Strong efforts have been deployed to investigate which factors (proteins, RNA, miRNA, lipids) in the uterine luminal fluids could function as messengers between the conceptus and the endometrium. Since the mid-2000s, the presence of extracellular vesicles (EVs) has been revealed in many bodily fluids (153). These vesicles, in particular exosomes, protect their cargo from degradation and target cells through specific cell surface molecules. Several publications have reported the presence of EVs and their content (RNA, small RNA, proteins) in uterine luminal fluids, first in human (154), then in sheep (155). In addition, in sheep EVs are produced by both the endometrium and ex vitro–cultured conceptuses (156). Moreover, ovine uterine EVs have been shown to stimulate IFNT produced by ovine conceptus trophectoderm cells (157), illustrating for the first time an exchange of EV-mediated cargo from endometrium to trophoblast cells. A very recent report has shown that cargo of EVs is hormonally regulated through the menstrual cycle in humans and that these EVs are internalized by human trophoblast cells, enhancing their adhesive capacity (158). Together these data illustrate that conceptus-endometrium interactions via EVs exist and must be considered and further investigated as critical components of histotrophy.

Very interestingly, such perturbations in AAs and PGs in uterine fluids observed during early pregnancy of SCNT embryos raise a more global comment: Productions of the preattachment conceptus require essential nutrients of maternal origin that are present in the uterine lumen. Indeed, conceptus elongation is driven by uterine histotroph, which includes proteins (signaling factors or enzymes), ions, mitogens, AAs, lipids, carbohydrates, vitamins, and substances protected and carried by exosomes and microvesicles (see sidebar titled Extracellular Vesicles and Conceptus-Endometrium Interactions). This complex mixture of molecules is produced by luminal and glandular epithelial glands. Ablation of uterine glands in sheep (UGKO model) is associated with preimplantation mortality because it abrogates conceptus elongation and consequently IFNT secretion (138). However, progesterone supplementation of heifers during a short postestrus period of time accelerates conceptus elongation, a phenomenon indirectly mediated by progesterone stimulation of various endometrial genes involved in the production of embryotrophic substances. Increased IFNT secretion was also reported as the consequence of enhanced EET elongation (9). Collectively, these results demonstrate that conceptus development and its secretions are tightly dependent on maternal supply of nutrients. Using physiological and experimental porcine and ovine models, F.W. Bazer and his group have focused extensively on disentangling the role of AAs, glucose, fructose, and their endometrial transporters in conceptus development. Among major outcomes, they have shown that the MTOR cell-signaling pathway, a central pathway that controls mRNA translation for protein synthesis and cell proliferation, is modulated by variations in AAs and glucose present in uterine luminal fluids. Complementary to the biosensor property identified for the endometrium, a driver property can thus be proposed for this tissue (139). Indeed, alterations in maternal physiology, such as variations in metabolism and nutrition, perturb endometrial function, including availability of various nutrients in the uterine fluids. This can bring about dramatic global changes (e.g., retarded elongation) associated with detrimental impacts on conceptus production that will end in pregnancy failure. Nevertheless, even in the absence of morphologically detectable changes, subtle yet essential perturbations of conceptus development can be induced, including quantitative and qualitative alterations in conceptus secretions that will not induce proper reaction from the endometrium, therefore amplifying the loop that will compromise pregnancy or will lead to perturbations with postnatal consequences. Because conceptus secretions are intimately linked to endometrial activity, investigating those products and their actions in normal or adverse situations requires taking maternal physiology into account.

CONCLUSION

In mammalian species, successful pregnancy has long been defined as the birth of viable offspring. Since the concept of developmental origins of health and disease (DOHAD) has been proposed (140), experimental models have demonstrated that restricting pregnancy success to viability of progeny is obsolete. This black-and-white vision has been moving toward a fifty-shades-of-gray picture with the delivery of progeny that are viable but are unable to express expected zootechnical performance or suffer from health problems consecutive to epigenetic alterations driven by prenatal programming (141, 142). Adverse periconceptional and early-postconceptional events have been shown to alter early steps of pregnancy that will subsequently lead to mild or substantial perturbations of the placentation process and fetal development, affecting pregnancy outcome and postnatal development (143). Health concerns and suboptimal production traits encountered in offspring may then result from altered embryo quality, from perturbed endometrial function, or from both. Inadequate embryo quality is also reflected in the nature of its secretions, which can be aberrant owing to (*a*) genetic mutations, (*b*) the use of assisted reproductive technologies, or (*c*) an altered composition of uterine products. Consequently, molecular and cellular processes taking place in the posthatching embryo between EET and ET cannot be analyzed without considering the uterus and particularly the endometrium as the biological interface collecting, treating, and integrating biological signals present in conceptus secretions and in endometrial cell products and conveyed by maternal fluids. In the context of secretions produced by the preattachment conceptus in ungulates, two major issues deserve attention.

First, some embryos can implant before developing to later stages of pregnancy while failing to reach term. These pregnancy losses (which when repeated result in a dramatic pathology named recurrent pregnancy loss or recurrent miscarriage in humans) may result from an inappropriate endometrial function (144), but they could also stem from embryos endowed with the ability to bypass the control exerted by the endometrium. This concept, defined as "a defect in nature's quality control" (145), could also apply to pregnancy failures occurring long after implantation in cattle or pigs. Very interestingly, bovine SCNT embryos that were produced with the 5538 cell line displayed an implantation rate much higher than the one reported for in vitro–produced embryos [up to 90% versus 45%, respectively, when transferred to recipient heifers (130)] but with only a 13% term pregnancy rate (52). This case feeds the notion that biological actions of EE and embryonic genes critical for successful implantation are distinct from genes essential for postimplantation development of the conceptus. Making use of these superimplanting embryos and generating alternative experimental approaches, including the derivation of in vitro primary cells of EET and endometrial origin (10, 146), will provide major tools to identify factors and decipher the complex networks of their actions during the course of the peri-implantation process.

Second, improving reproductive performance and optimizing production in domestic animals imply the reliable diagnosis of early pregnancy and prediction of the issue as early as possible. In ungulates, ultrasonic imaging and determination of maternal progesterone or placental hormone levels (e.g., pregnancy-associated proteins in the bovine and ovine species, eCG and estrone sulphate in the equine species) have been used to reliably assess pregnancy and predict viability from the early postimplantation period onward (147, 148). In domestic ruminants, quantifying ISG expression in maternal circulating cells during MRP has been proposed as an early pregnancy diagnostic tool, but accuracy appears to be conditioned by the physiological status of the female, including parity and health (20, 149). Therefore, there is a need to identify alternative markers based on embryonic factors, as early pregnancy can be reliably determined in nonhuman primates and humans by detecting chorionic gonadotropin in urine and blood (150). Collecting preattachment secretions from the uterine cavity appears unrealistic without compromising progression of

pregnancy; therefore, investigating the presence of embryo-specific biomarkers in maternal fluids represents a reasonable and sensible option to assess early pregnancy. Very interestingly, recent data in cattle have presented preimplantation factor, an embryo-derived peptide detectable in early maternal circulation from day 10 of pregnancy onward, as an accessible and relevant biomarker to diagnose viable early pregnancy at day 20 post artificial insemination (151). Further investigation will be required to confirm these findings, and efforts should be intensified (*a*) to identify embryonic secretions in the uterine luminal milieu (152) as well as in maternal fluids and (*b*) to investigate their biological effects, not only to improve phenotyping of pregnancy from earlier stages but also to yield a comprehensive view of the impact the conceptus exerts on the female organism to help it adapt to pregnancy at the local and peripheral levels.

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