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Deciphering early sensor and driver properties of the endometrium: contribution of the uterus to pregnancy outcome

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ABSTRACT

***Background*:** Pregnancy involves a continuum of complex biological processes. Each of them can be affected by the environment surrounding the pregnant female (e. g. chemicals, nutrition, stress, infection) and data have focused on gametes quality, early blastocyst development and placental function and their perturbations by environmental insults or embryo biotechnologies. During the pregnancy period spanning the entry of the blastocyst into the uterine cavity to implantation of the embryo, biological functions of the endometrium have also been extensively studied, namely uterine receptivity (controlled by maternal factors) and maternal pregnancy recognition (that requires conceptus-produced signals). Nevertheless, recent data based on experimental perturbations have unveiled unexpected biological properties of the endometrium whose structural organization and functionality during pre-placentation period impact embryo trajectory through epigenetic alterations with subsequent consequences on pregnancy progression and final outcome.

***Review*:** A renewed vision of the endometrium is presented in this review. Several features including uterine structure, protracted pre-implantation period, epitheliochorial implantation, a clearly identified signal for pregnancy recognition (interferon-tau), high-throughput analyses tools and original experimental models make ruminants valuable models for detailing the molecular and cellular events taking place in the endometrium before placentation occurs. In ruminants as well as other species, endometrial receptivity is required for embryo implantation and is achieved through biological actions of maternal hormones including ovarian steroids. Among them, progesterone appears as a major factor whose experimental alterations of circulating levels can significantly stimulate or inhibit conceptus elongation. Conceptus growth and survival have also been shown to fail in pregnant ewes lacking endometrial glands (UGKO). In these bovine and ovine models, endometrium exhibits altered gene expression patterns and drives embryo development independently from the quality of donor oocytes. In the context of pregnancy maternal recognition, the presence of the conceptus deeply modifies endometrial transcriptome across various stages of late pre-implantation phase. Acting as a paracrine or an endocrine factor, interferon-tau has been recognized as an indispensable factor for successful implantation through its biological actions on endometrial cells, immune cells and luteal cells. Nevertheless, beyond the global maternal reaction to conceptus secretions, distinct endometrial responses can be elicited by embryos produced by *in vivo* fertilization, *in vitro* maturation and *in vitro* fertilization or somatic cell nuclear transfer. These findings have been confirmed in human when endometrial stromal cells are incubated with normal or compromised embryos but only upon differentiation into decidual cells. Then mammalian endometrium can be considered as an early biosensor of embryos presenting different potentials of post-implantation development.

***Conclusion*:** Endometrium appears as a dynamic and reactive tissue. Its persistent or transient epigenetic modifications can dramatically affect pre-implantation embryo development with lasting consequences on later stages of pregnancy, including placentation, foetal development, pregnancy outcome and post-natal health. Developing diagnosis and prognosis tools based on endometrial factors will be valuable with the aims to estimate the reproductive capacity of the mother or to assess the developmental potential of the embryo, particularly when assisted reproductive technologies are applied.

Keywords: endometrium; biosensor; epigenetics, pregnancy, ruminants, genomics.

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I. INTRODUCTION

Pregnancy involves a continuum of complex biological processes and several checkpoints (or hurdles) that have to be passed successfully. These hurdles include production and quality of the gametes (oocytes, sperm), fertilization, luteal function rescue, early development of the embryo, implantation, development of the foeto-placental unit until term and parturition. Each of these steps is crucial for the successful delivery of a healthy offspring and their onset or progression can be affected by events taking place in the environment surrounding the parental organism. The major contributor to pregnancy is the mother who produces one of the two gametes and will host the whole gestation until term. Acute challenges, short or long periods of perturbations related to nutrition, metabolism, stress, infections or endocrine disruptors have been identified as factors that affect gametes quality and fertilization, journey of the early embryo through the oviduct, cellular interactions between endometrium and hatched blastocyst or conceptus, foeto-placental development or parturition [22,40]. In addition, biotechnologies of reproduction (or assisted reproductive technologies) associated to embryo transfer have been shown to alter biological properties of the embryo with a subsequent impact on later stages of pregnancy [64]. Therefore inadequate maternal compartment and/or suboptimal quality of the embryo may impact the two ways communication between mother and embryo, precluding completion of successful pregnancy and affecting long term health status of the offspring [19].

Although the oviduct from unstimulated animals appears to be the optimal environment for early embryonic nursing [11,26], *in vitro* embryo production and embryo transfer have demonstrated the oviduct to be a dispensable organ for supporting progression of pregnancy to term. Until now, despite several attempts in maintaining early and late fetal life outside the uterus [12], no surrogate biological or artificial system has been derived for the uterus. In normal physiological conditions, the uterus and its internal part referred as the endometrium constitute the maternal site for embryo implantation. External events (as described in the former paragraph) or intrinsic maternal features may affect biological functions of maternal organs or tissues that will in turn impact endometrial physiology. Figure 1 depicts a personal view of early pregnancy, where the maternal organism can be represented as an organs-containing funnel channelling every event affecting maternal tissues to the narrow hole represented by the endometrium. As the ultimate and unique biological layer facing the implanting embryo, endometrium drives the development of embryonic disk and extra-embryonic tissues during the establishment of pregnancy [33]. On the other hand, recent data based on *in vitro* embryo manipulations have unveiled an unexpected biological property of the endometrium since this tissue is able to differentially react to embryos displaying distinct potencies to term development [43]. Based on animal data - mainly ruminants - with references to recent results published in human, this review presents a renewed vision of the endometrium whose driver and sensor properties make this maternal tissue a major epigenetic contributor for embryo development with subsequent impact on issue of pregnancy.

II. BROAD LINES OF EARLY PREGNANCY IN RUMINANTS

In ruminants, the bicornuate uterus is covered with the endometrium displaying two specific areas, namely the caruncles and the intercaruncular areas [15]. The caruncles represent aglandular structures of limited size and distributed over the endometrial surface. The intercaruncular areas are large and contain the endometrial glands that produce histotroph, a collection of numerous and diverse factors including cytokines and growth factors [65]. Upon oocyte fertilization and after hatching, the extra-embryonic tissue of the ruminant conceptus undergoes a progressive and critical elongation phase [18] before implanting at day 15-16 post-oestrus in the sheep and the goat gestation (gestation period: 5 months) or day 19-20 post-oestrus in the cow (gestation period: 9 months). Although a decidual-like process (related to hemochorial implantation) has been reported in sheep [35], the ruminant synepitheliochorial implantation is characterized by the apposition then the adhesion between the trophectoderm and the uterine luminal epithelium [7].

The protracted pre-implantation phase (10 to 15 days) is associated to the secretion of interferon-tau (IFNT), the major signal of pregnancy recognition in ruminants. IFNT is a type I interferon whose spatio-temporal production is very specific and restricted to ruminant species. During the peri-implantation period, IFNT has been found to be secreted by trophectoderm

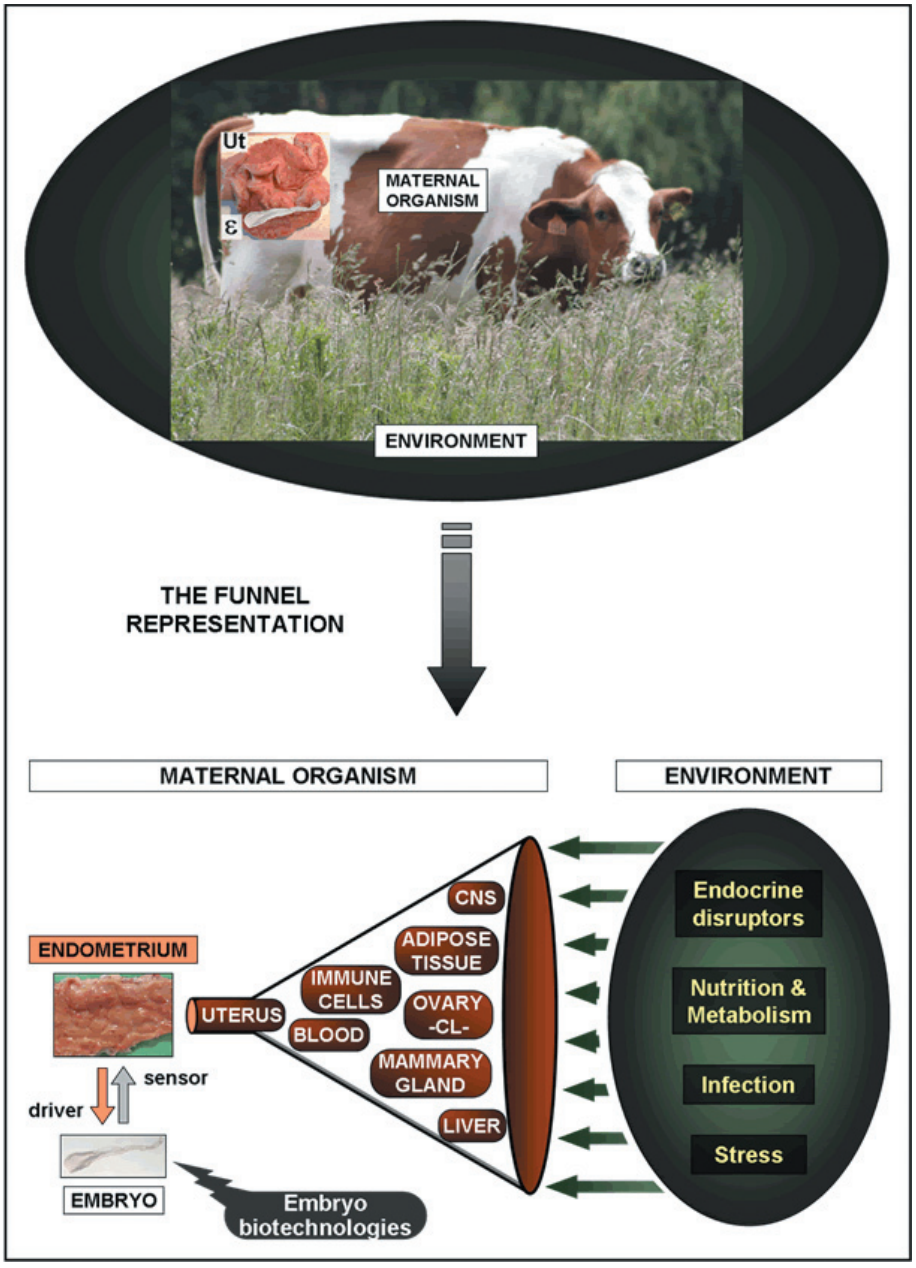

Figure 1. A personal view of the early pregnant cow. The funnel represents the maternal organism crossed by environmental insults that will target endometrium in a direct way or indirectly –by affecting biological functions of other maternal tissues-. Endometrial reaction can be modulated by the quality of the embryo as reported when assisted reproductive technologies with embryo transfer are used.
CL: corpus luteum; CNS: central nervous system; E: embryo; Ut: uterus.

cells uniquely and this factor has been shown to be indispensable for pregnancy recognition through its antiluteolytic actions [55]. IFNT biological functions were first considered to be paracrine by inhibiting the secretion of endometrial prostaglandin-F2α whose production is associated with luteolysis in the absence of conceptus [66]. Nevertheless, in addition to the impact of IFNT on the endometrial physiology, recent publications have also demonstrated endocrine and direct IFNT biological actions on extra-uterine tissues including circulating blood cells as well as corpus luteum whose progesterone secretion is indispensable for the establishment and maintenance of pregnancy in large animal species [50,51].

The long pre-implantation phase allowing the dissection of the first cellular contacts and molecular events occurring between the conceptus and the endometrium, the synepithelial implantation making it

possible the easy separation of maternal side from extra-embryonic tissue, the abundance of endometrial tissues and accessible uterine fluids as well as availability of high-throughput genomic tools dedicated to ruminants constitute solid bases for investigating driver and sensor properties of the endometrium before placentation occurs.

III. UTERINE RECEPTIVITY AND DRIVER PROPERTIES OF ENDOMETRIUM

One of the most striking evidences of embryo control by endometrium has been provided by the gland knock-out (UGKO) ovine model. The neonatal exposure of ewes to norgestomet -a P4 analogue- leads to the UGKO phenotype. In the adults, the uterus lacks middle to deep endometrial glands and exhibits a markedly reduced surface of luminal epithelium. UGKO ewes present recurrent early pregnancy loss due to failure in conceptus elongation and survival between day 12 and day 14 post-oestrus, a phenotype also present when control embryos were transferred into UGKO recipients [28]. A microarray analysis comparing cyclic and UGKO ewes has revealed 23 differentially expressed genes in the endometrium whose majority were immunoglobulin genes, likely because of the large numbers of immune cells present in the tissue [29]. The authors suggested that different populations or altered numbers of immune cells in UGKO ewes could be involved in recurrent early pregnancy loss observed in this experimental model as reported in humans. More generally, the UGKO model illustrates that alterations of the uterine tissue architecture and functionality during early post-natal life may have long-term and detrimental consequences on fertility.

Uterine receptivity can be defined as a restricted time-related period when the uterus is receptive to blastocyst attachment and implantation. Impaired uterine receptivity leads to embryo implantation failure and defining the implantation window and endometrium receptivity has represented one of the biggest challenges to increase the success of assisted reproductive technologies in both human and animals [2,20]. In sheep, embryo transfer experiments have shown that normal embryo development depends upon a sequence of changes in uterine secretions [73]. Many experiments have aimed to decipher the consequences of embryo development and uterine environment asynchrony. They have shown that embryo exposure to inappropriate uterine factors (“out of phase”) may lead to irreversible induction of abnormal development [3]. In order to define endometrial receptivity, cellular and molecular events leading to morphological and functional modifications of endometrium have been extensively investigated based on candidate gene approaches or high-throughput analyses in mammals [34,49,57]. In cattle, the highest number of differentially genes across oestrous cycle has been detected between day 7 and day 13 post-oestrus [23] and factors related to endometrial remodelling, regulation of angiogenesis, cell adhesion and embryo feeding have been identified [4,47,58]. Very interestingly, a set of endometrial genes expressed at day 7 or day 14 of the bovine oestrous cycle have been suggested to be predictors of successful or failed term pregnancy after embryo transfer in the following oestrous cycle [58]. In the same order of ideas, the quantitative homogeneous expression of six endometrial genes has been reported during the implantation window in women who became pregnant in the subsequent ICSI cycle profiles [1]. In fact, the picture is much more complex since a recent human microarray study has shown common and specific pathways of gene deregulation in endometrial biopsies collected during the mid-luteal phase from women undergoing recurrent pregnancy loss, recurrent miscarriage and implantation failure after IVF [39]. Collectively, these first published results support the notion that endometrial biomarkers during the non pregnant phase can predict the outcome of pregnancy when assisted reproductive technologies are used.

In mammalian species including ruminants, endometrial receptivity necessary for embryo implantation is driven by ovarian steroid hormones, namely estrogens (E) and progesterone (P4). Progesterone has been shown to be the major factor controlling uterine function in mammalian species [67] and bovine or ovine animal models have been derived to investigate the biological functions of this steroid hormone. Ovariectomized cows administered with either estradiol or P4 or in combination have been recently used to identify endometrial genes regulated by P4 or estrogen using a microarray approach [63]. Experimental models displaying artificially altered levels of P4 have also been reported in cattle and sheep. Elevated concentrations of circulating P4 -within physiological ranges- during the early post-conception period have been associated with the advancement of conceptus elongation in the bovine [14,27] and ovine species [61]. On the other

hand, a low P4 circulating level -obtained by repeated injections of PGF2a between day 3 and 7 of the oestrous cycle- has led to a striking reduction in conceptus size after recovery at day 13 [10]. This experimental model is consistent with the impaired ability of the maternal genital tract (oviduct and/or uterus) to support embryo development reflects in postpartum dairy cows displaying reflects low P4 blood levels [54]. In these bovine and ovine experimental models, altered P4 blood levels have been associated with modified patterns of endometrial gene expression [23,24,62] including numerous nutrients, nutrient sensing pathways, growth factors and extra-cellular molecules [8] as well as immunomodulator factors [31,38]. Very interestingly, consequences of lower or higher P4 levels on bovine embryo elongation are visible when embryos are transferred to recipient females after the treatment period and collected 6 or 7 days later [16,24]. Collectively these findings strengthen the notion that epigenetic status of the pre-implantation endometrium in adult females can be altered to an extent that totally prevents or dramatically affects embryo development at implantation, independently from the quality of the donor oocytes.

IV. MATERNAL PREGNANCY RECOGNITION AND SENSOR PROPERTIES OF THE ENDOMETRIUM

Whereas first steps of uterus remodelling and receptive window are programmed by maternal hormones independently from the presence of the embryo, successful pregnancy will require embryo recognition by the maternal organism with a major and crucial contribution of the uterine reaction. This reaction is achieved through embryonically derived factors that are indispensable for promoting implantation through the establishment of permanent cellular interactions between the trophectoderm and the endometrium [30]. Embryo derived signals vary according to mammalian species and they have been abundantly reviewed [8,36,72]. In ruminants, IFNT has been identified as the signal of trophectoderm origin crucial for maternal recognition signal and for rescuing the corpus lutem, an indispensable step for maintaining P4 luteal secretion [45,55]. Identifying IFNT-endometrial target genes has generated an exponential number of publications during the last decade based on *in vitro* endometrial cell cultures and *in vivo* experimental models [8]. Nevertheless IFNT is not the only factor of embryo origin affecting uterine physiology and identifying embryo-related factors triggering local endometrial reaction may benefit from *in vivo* experimental models such as the ovine model of unilateral pregnancy. By placing a ligature proximal to the uterine body, the conceptus is confined in the uterine horn ipsilateral to the corpus luteum [9]. By comparing the gravid and the non-gravid horns, the paracrine impact of conceptus-secreted factors on the endometrium can be dissociated from their paracrine actions. In this model, dynamic changes of endometrial T lymphocyte populations were reported to be independent from embryo secretions [41] whereas the localization of macrophages was affected by the presence of the conceptus [69]. At the molecular level, we also 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 [60].

In ruminants, global endometrial reaction to the presence of the conceptus has been recently investigated using large-scale analyses [68]. In cattle and to a lesser extend in sheep, analyses of molecular and cellular events at the endometrial level have benefited from the development of microarray platforms [21]. Still remains some limitation in data mining since the current free or priced softwares are built and dedicated to human and rodent data and they lack records of factors interactions specifically associated to pregnancy. The expression of endometrial related factors -including innate immune response and associate factors- has been shown to be significantly altered during early and late pre-implantation period compared to equivalent day of oestrous cyle in cattle (day 5 to day 16 post-oestrus [25]; day 17 [71]; day 18 [5,37]; day 20 [42]). Some of the identified genes appeared to be preferentially regulated in the caruncles or in the intercaruncular areas, suggesting specific functions related to the development of the placentomes [42]. Collectively, these data have demonstrated an important global reaction of the endometrium –quantitatively and qualitatively- while facing the conceptus. Nevertheless recent studies first carried out in cattle then supported by human data have demonstrated a subtle property of the mammalian endometrium.

In ruminants, *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 when compared to their *in vivo* derived counterparts

[17,44,46]. 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 [32,48,56]. Nevertheless, severe or fatal consequences on embryo and foetal-placental development have also been reported and shown to considerably vary according to the origin of somatic cell lines and across laboratories [74]. These embryos with distinct potentials of term development were postulated to elicit different endometrial gene patterns that could account for the final outcome of a pregnancy. Our research group [43] demonstrated that late pre-implantation endometrium shows dramatic changes in gene expression related to the way the embryo was produced, namely by *in vivo* fertilization, *in vitro* maturation and IVF or SCNT. These data have led to the original concept defining the endometrium as an early biosensor of embryo quality [43]. Data mining showed immune response and metabolism as the two most affected biological functions and differential expression of candidate genes was confirmed. By comparing endometrial transcriptomes of cows recipient for IVF-derived embryos or cloned embryos generated with various somatic cells lines, several endometrial genes have also been shown to be associated with nuclear transfer procedure at day 18 post-oestrus [6]. Very interestingly, recent human data proposed that decidualizing endometrial stromal cells can sense embryo quality and would be able to eliminate compromised embryos [59,70]. Therefore the endometrium as a biosensor of embryo displaying divergent developmental potencies appears to be a feature valid across mammals, despite structural differences in implantation and placentation.

V. CONCLUSION

In mammalian species, placentation and foetal development represent the major part of pregnancy and last much longer than the pre- and peri-implantation periods. 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 [13]. Nevertheless, current data demonstrate that congenital anomalies, acquired diseases or perturbations of adult maternal physiology during reproductive life (e. g. stress, nutrition; endocrine disruptors, infection; figure 1) 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 endometrium an early biosensor of embryo developmental potential. Hence mammalian endometrium appears as 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 on placentation process, fetal development and pregnancy outcome. Consequently, although term pregnancy issue incontestably relies on the quality of the embryo (the seed) [52], it is obvious that endometrium (the soil) has to be considered as a critical contributor for embryo developmental trajectory acting at the epigenetic level as early as pregnancy initiates. In the context of assisted reproductive technologies, developing diagnosis and prognosis tools based on endometrial factors will be valuable with the objectives to estimate the reproductive capacity of the mother, to correct suboptimal endometrial functionality or to assess the developmental potential of the embryo. On a more fundamental aspect, an essential issue will be to carefully analyse situations in which embryos can overcome the control exerted by the endometrium, as illustrated by the defect in nature's quality control proposed by Aplin and collaborators [53]. In other words, embryos that fail to develop to term would be able to implant. In this situation, the unusual properties of these embryos (e. g. producing an excess of embryo-derived signals) as well as the quality of the endometrial milieu will have to be clearly defined. Eventuallly, determining the limits of endometrial plasticity at the onset of pregnancy represents difficult tasks but essential challenges for providing new insights on the contribution of maternal environment to embryo epigenetic shaping in link with success, alterations or failure of pregnancy.

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