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Transcriptome analyses of bovine, porcine and equine endometrium during the pre-implantation phase☆

Stefan Bauersachs*, Eckhard Wolf

Chair for Molecular Animal Breeding & Biotechnology and Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, Ludwig-Maximilians-Universität München, Feodor-Lynen-Str. 25, 81377 Munich, Germany

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ABSTRACT

Different reproductive strategies evolved in various mammalian groups to achieve recognition, establishment and maintenance of pregnancy. The complexity of these processes is reflected by a high incidence of embryonic loss during this critical period in many mammalian species. Besides studies in mice and humans a number of transcriptome studies of endometrial tissue samples and also of early embryos have been performed during the pre-implantation phase in cattle, swine and horse to identify genes associated with embryo–maternal interaction. Results of these studies are reviewed and compared between species. The comparison of data sets from different species indicated a general role of interferons for the establishment of pregnancy. In addition to many species-specific changes in gene expression, which may reflect different pregnancy recognition signals and mechanisms of embryo implantation, a number of transcriptome changes were found to be similar across species. These genes may have conserved roles during the establishment of pregnancy in mammals and reflect basic principles of mammalian reproduction. The relevance and strategies, but also the challenges of cross-species comparisons of gene expression data are discussed.

Reproductive success depends on a number of biological processes, e.g. maturation and selection of gametes, fertilization, pre- and post-implantation embryonic development including endometrial support of embryo growth, placentation, fetal growth, and birth. A crucial process is probably embryo–maternal communication that facilitates establishment, recognition, and maintenance of pregnancy. Studies of early pregnancy in different mammalian species showed that the majority of embryo losses occur during the pre-implantation phase. For example in cattle, this corresponds to the period before day 16 following breeding (Diskin and Morris, 2008). Furthermore, a reduction in fertilization and embryo survival rates has been suggested as the most important component for decreasing reproductive efficiency in dairy cattle (Santos et al., 2004). Likewise, the pre-implantation phase is most critical in the horse (Merkt and Gunzel, 1979; Ginther et al., 1985) and also in the pig (Wilson et al., 1999; Wessels et al., 2007).

During the pre-implantation phase, the conceptus (embryo and associated extra-embryonic membranes) interacts with the uterine environment via paracrine signals, to coordinate attachment and implantation. In mammals with late implantation, the conceptus has also to signal its presence to prevent luteolysis for continuous progesterone (P4) production. Progesterone plays the most important role in uterine receptivity, i.e. the ability to support conceptus growth and development by the production of histiotroph (Spencer et al., 2007; Bazer et al., 2009). Thus,

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* Corresponding author at: Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, LMU Munich, Feodor-Lynen-Str. 25, 81377 Munich, Germany. Tel.: +49 89 2180 76701; fax: +49 89 2180 76849.
E-mail addresses: bsachs@genzentrum.lmu.de (S. Bauersachs), ewolf@genzentrum.lmu.de (E. Wolf).

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the analysis of gene expression changes during the cycle and the response of the endometrium to the conceptus at the gene expression level can be used to (i) find genes and pathways related to uterine receptivity; (ii) identify biological processes, which are stimulated or suppressed in the endometrium by the conceptus; and (iii) to evaluate the ability of embryos to elicit physiological responses in the endometrium, e.g. with regard to different types of embryos such as IVF-derived or cloned embryos.

Different mechanisms of establishment of pregnancy have evolved in various mammalian orders and families as a result of adaptations to environmental conditions leading to substantial differences between mammalian species in many aspects of reproductive biology (Bazer et al., 2009). Therefore, a comparative analysis of endometrial changes during the sexual cycle and the pre-implantation phase for different mammalian species provides a unique opportunity for identification of conserved and distinct pathways. Since most physiological processes are associated with complex changes in RNA expression profiles, transcriptome analyses are a powerful strategy for a holistic description of cellular changes at the molecular level. A number of analytical approaches, such as DNA microarrays and RNA sequencing (RNA-Seq) have been developed for systematic analyses of mammalian transcriptomes (Stanton, 2001; Hoheisel, 2006; Bauersachs et al., 2008; Wang et al., 2009). In comparison to DNA microarrays, RNA-Seq can provide information on absolute transcript levels, transcript variants, and currently not annotated transcribed regions. In general, next-generation sequencing will have an increasing impact for solving the complex biological problems in agricultural sciences (Liu, 2011) and animal sciences, e.g. for identification of yet un-annotated genes (Derrien et al., 2011; Jager et al., 2011).

1. Identification of biological themes related to endometrial remodeling and receptivity in transcriptome studies of bovine endometrium

Endometrial gene expression is mainly regulated by the complex interplay of the ovarian steroid hormones estradiol (E2) and progesterone (P4) during the estrous cycle and by progesterone during pregnancy (Goff, 2004; Spencer et al., 2004; Forde et al., 2011b). These steroid hormones act via classical nuclear steroid hormone receptors, but also via non-classical receptors such as progesterone receptor membrane component 1 and the novel family of membrane progestin receptors (Gellersen et al., 2009). P4 is the key hormone for preparation of the endometrium for embryo implantation and maintenance of pregnancy (Bazer et al., 2008) and genes with increased expression levels in the luteal phase are probably regulated by P4, directly or indirectly. The supportive role of P4 has been confirmed in a recent study in heifers where a positive influence of P4 on conceptus growth and development was found (Clemente et al., 2009; Forde et al., 2011a).

In ruminants, conceptus implantation is late (in cattle after day 18 of gestation) after trophoblast elongation, which starts on day 14 of pregnancy in cattle. An epitheliochorial placenta is formed through a relatively non-invasive placentation process. Only limited fusion of endometrial epithelial and trophoblast cells occurs in the caruncular and also in the intercaruncular areas of bovine endometrium (Wathes and Wooding, 1980; King et al., 1981). Maternal recognition of pregnancy in cattle takes place around days 16 and 17 and is mediated by conceptus interferon-tau (IFNT) that prevents induction of luteolysis (Bazer et al., 1997). IFNT has been shown to suppress estrogen receptor-alpha (*ESR1*) and oxytocin receptor (*OXTR*) genes, which results in prevention of pulsatile release of luteolytic prostaglandin F2 alpha (PGF2α) (Spencer and Bazer, 1996). In cattle, maximum secretion of IFNT was observed on day 17 (Bazer et al., 1997) coincident with the time of maternal recognition of pregnancy.

To gain deeper insight into the highly complex molecular responses of the endometrium to the ovarian hormones during the estrous cycle, several transcriptome studies of bovine endometrium using microarrays have been performed (Bauersachs et al., 2005, 2008; Mitko et al., 2008; Forde et al., 2011a). These studies revealed several hundred differentially expressed genes (DEG) between different stages of the estrous cycle. In the study of Mitko et al. (2008), two major groups of genes could be distinguished according to their expression profiles, with highest mRNA levels during the estrus phase and highest levels during the luteal phase, respectively. Genes with highest mRNA levels at estrus were characterized by overrepresented functional terms such as 'focal adhesion formation', 'cell motility', 'cytoskeleton', 'extracellular matrix' (ECM), 'ECM remodeling', and 'cell growth'. Furthermore, a number of genes showing lowest mRNA levels at diestrus were identified that have been described in the context of 'positive regulation of invasive growth'. Thus, decreased levels of these mRNAs during the luteal phase may be characteristic for the non-invasive implantation process in cattle. Accordingly, a number of genes with higher mRNA levels during the luteal phase have been described in the context of 'negative regulation of invasive growth'. Genes assigned to the functional categories 'angiogenesis', 'vascular remodeling', and 'regulation of blood flow' could also play an important role for endometrial remodeling during the estrous cycle. Several genes described to be associated with these processes were identified as differentially expressed during the estrous cycle, including members of the angiopoietin family, transcription factors controlling the expression of vascular endothelial growth factors (VEGF) and their receptors, and other genes involved, e.g. in endothelial differentiation and regulation of blood flow. Furthermore, elevated concentrations of mRNAs coding for a variety of proteins involved in metabolic and transport processes were found during the luteal phase, which could be related to increased secretion of nutrients and factors necessary for the development of the embryo (Allison Gray et al., 2000). This was also found in the study of Forde et al. (2011a) where endometrial tissue samples collected at different time points during the luteal phase were analyzed. In this study the authors found hardly any differences between days 5 and 7 but high numbers of DEG between days 7 and 13. This study also investigated the influence of exogenous supplementation of P4 and induction of low P4 concentrations in comparison to temporal changes in the expression of genes in the endometrium for normal serum