Aus dem Zentrum für klinische Tiermedizin

der Tierärztlichen Fakultät

der Ludwig-Maximilians-Universität München

Arbeit angefertigt unter der Leitung von:

Univ.-Prof. Dr. Johannes Handler

und Dr. Stefan Bauersachs

Microarray Analysis of the Equine Endometrium at Days 8 and 12 of Pregnancy

Inaugural-Dissertation

zur Erlangung der tiermedizinischen Doktorwürde

der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität

München

von

Maximiliane Christina Merkl

aus Weiden in der Oberpfalz

München 2011

Gedruckt mit der Genehmigung der Tierärztlichen Fakultät

der Ludwig-Maximilians-Universität München

Dekan:	Univ.-Prof. Dr. Braun
Berichterstatter:	Univ.-Prof. Dr. Handler
Korreferenten:	Univ.-Prof. Dr. Gelen
	Univ.-Prof. Dr. Braun
	Priv.-Doz. Dr. Schneider
	Univ.-Prof. Dr. Wolf

Tag der Promotion: 12. Februar 2011

meinen Eltern

Table of contents

1 Introduction

The horse exhibits a number of unusual features during early pregnancy which are unique to the genus *Equus* and differ considerably from corresponding events in other large domestic animal species. Moreover, the establishment and maintenance of pregnancy in the mare are only partially understood. Successful gestation in mammals critically relies on an intact embryo-maternal dialogue. Unlike domestic ruminants and pigs, the nature of maternal recognition of pregnancy by which the embryo prevents cyclical luteolysis still remains unknown in the mare.

The objective of this study was to systematically analyze the maternal endometrial response to the presence of a conceptus in the mare, in order to gain new insights into the early events underlying pregnancy and the complex embryo-maternal dialogue in equids. Therefore, a transcriptome study of endometrium samples from six mares at days 8 and 12 of pregnancy and the corresponding non-pregnant stages was performed by using Agilent 4x44k Horse Gene Expression microarrays.

2 Review

2.1 The early events of pregnancy in the mare

Horses exhibit a number of features during early pregnancy that differ considerably from corresponding events in other large domestic animal species. Although establishment and maintenance of pregnancy in the mare are not yet completely understood, some early embryo-maternal interactions have been investigated to play a substantial role for successful gestation.

2.1.1 The estrous cycle

Horses are polyestric seasonal breeders. Normal breeding season usually starts in late spring and lasts until fall, depending on the duration of daylight. Stimulation of the pineal gland, either by natural or artificial light, results in a reduction of melatonin secretion, which in turn allows gonadotropin releasing hormone (GnRH) to be secreted in pulses from the hypothalamus. GnRH thereby stimulates and regulates the production and release of the gonadotropic hormones FSH (follicle stimulating hormone) and LH (luteinising hormone) from the anterior pituitary.

The average length of the estrous cycle in the mare is 21 ± 2 days but it is very variable depending on season. During the follicular phase, interfering actions of FSH, LH and estrogens result in follicular maturation and ovulation. The rise of estrogens from a dominant follicle induces a period of sexual receptivity (estrus) characterized by typical estrus behavior and physiological signs such as endometrial edema and relaxation of the cervix. Ovulation in the mare occurs spontaneously at the end of a follicular phase, determining by definition day 0 of the estrous cycle. In the mare, the LH surge leading to ovulation is considered to be longer than in most other animals and to be rather a plateau then a peak.

After ovulation, formation of the corpus luteum (CL) marks the beginning of the luteal phase. Under the influence of progesterone, the major steroid hormone secreted by the CL, cyclical ovarian activity and estrus behavior are diminished and cervical tone increases. In the non-pregnant mare, regression of the CL (luteolysis) is initiated at about day 14 by prostaglandin F2alpha ($PGF_{2\alpha}$) released from the endometrium, followed by an immediate decline in circulating progesterone levels, which terminates the luteal phase and permits a new ovarian cycle to begin [1, 2].

2.1.2 Oviductal transport

After ovulation, the ovum is released into the oviduct for potential fertilization. As a special feature in the mare, fertilized and unfertilized oocytes are differentially transported within the oviduct. While in most mammals both, fertilized and unfertilized oocytes enter the uterus at similar times after ovulation, in the mare, unfertilized oocytes are retained in the oviduct near the ampullary-isthmic junction, where they degenerate over months [3]. Horse oviducts therefore typically yield multiple degenerated oocytes, accumulated from sterile ovulations in preceding estrous cycles [4]. However, after successful fertilization, embryos are transported all the way down the oviduct, bypassing the unfertilized oocytes, and enter the uterus via the prominent uterotubal papilla at the expected time of gestation.

It has been shown that the embryo itself initiates its oviductal transport by a stage dependent secretion of prostaglandin E_2 (PGE_2). Therefore, the embryo begins to secrete appreciable quantities of PGE_2 when it reaches the compact morula stage of development on day 5 after conception [5, 6]. This embryonic PGE_2 acts locally on the wall of the oviduct by relaxing the circular smooth muscle fibers, thus allowing a rapid onward passage towards the uterus [7]. Furthermore, treatment of pregnant mares with PGE_2 has been shown to hasten oviductal transport of equine embryos [8]. The differential transport of embryos in the equine oviduct impressively illustrates very early embryo-maternal interactions, essential for the establishment of pregnancy in the mare.

The time taken for the cleaving embryo to traverse the oviduct has been shown to be 144 to 156 h [9]. The last part of the journey – trough the isthmus – is accomplished quite rapidly. By the time the embryo enters the uterus, development has progressed to the late morula or early blastocyst stage [10].

2.1.3 Pre-fixation period

The pre-attachment phase of the early conceptus within the uterus is an outstanding feature of equine pregnancy as it occurs over a considerably longer period than it has been observed in many other mammalian species [11], and further includes a highly mobile conceptus, surrounded by an acellular glycoprotein capsule. Moreover, embryo survival and maintenance of pregnancy during this time critically rely on mutual interactions between the pre-attachment conceptus and the maternal organism.

In coincidence with the time of blastulation, the acellular glycoprotein capsule first becomes visible between the trophectoderm and the zona pellucida from around day 6.5 [10]. During the next 24 h, the zona decreases markedly in thickness before it literally

bursts open to allow the expanding blastocyst, now completely enclosed within the capsule, to hatch [12, 13]. This glycoprotein capsule, which is one of the most unusual features of the equine embryonic development, is secreted initially by the trophectoderm cells and subsequently hardens to a thin, elastic membrane, which completely envelops the embryo during the second and third week of gestation [14, 15]. Due to its close fitting, the trophoblast is not able to elongate, as it does in pigs and ruminants to bring the trophoblast in direct contact with the endometrium and to maximize local transmission of molecules. Instead, the equine conceptus remains spherical, completely unattached and highly mobile within the uterine lumen until days 16/17 [16].

Conceptus mobility marks another prominent feature during early gestation in the horse and is assumed to be of great importance since its restriction results in failure of pregnancy [17]. Driven by strong, peristaltic myometrial contractions, the conceptus is moved through the uterine lumen many times per day [11]. The embryonic capsule thereby probably provides strength and elasticity to the conceptus and enables it to withstand the rigorous contractions. Importantly, between days 11 – 14, the time of its maximal mobility, the conceptus is thought to act on the endometrium to prevent secretion of the luteolytic pulses of $PGF_{2\alpha}$ which would otherwise lead to regression of the CL [11]. It is supposed that the constant movement allows the pre-attachment embryo to get in contact with most of the endometrial surface, thereby enabling it to signal its presence uniformly to the entire endometrium [18].

In view of these facts it seems surprising that mobility is virtually abolished when pregnant mares are treated with the cyclooxigenase-inhibitor flunixin meglumine, thus implicating prostaglandins as the primary stimulus for the uterine contractions required for conceptus mobility [19]. It is assumed that these prostaglandins arise from the conceptus itself as it secretes both, PGE_2 and $PGF_{2\alpha}$, when cultured in vitro [20]. This prostanoic synthetic capacity probably enables the conceptus to locally stimulate the peristaltic contractions and relaxations of the myometrium that propel it around. However, it remains to be determined whether the conceptus is the only source of prostaglandins or if these prostaglandins are supplemented by the endometrium abutting the conceptus, possibly under the influence of the latter [21, 22].

Besides facilitating mobility and providing mechanical protection, the embryonic capsule is further essential for embryonic survival, as it plays a crucial role in mediating nutrition and development for the unattached conceptus [13, 23]. During its mobile phase, the conceptus embarks upon a period of rapid expansion that is also accompanied by a steady increase in size and dry-weight of the capsule [10, 24]. Due to its negative electrostatic charge, the outer surface of the capsule is very “sticky” towards other

proteins and thus binds endometrial secretions onto its surface as it moves through the uterus [15]. One of the major capsule-bound proteins is maternally derived uterocalin (lipocalin p19), a progesterone-dependent, 19-kDa protein, which is implicated in transport of biologically important lipids like polyunsaturated fatty acids and retinol across the capsule [25-27]. This is of great importance, since uterine gland secretions (histotrophe) are presumed to be the only source of nutrients for the rapidly growing conceptus before a direct contact between maternal and fetal tissues is established [16].

Finally, the embryonic capsule also contains insulin-like growth factor binding protein 3 (IGFBP3), which might concentrate maternal insulin-like growth factors (IGFs) in the capsule and eventually releases them in a controlled manner, therefore regulating the influence of maternal IGFs on the conceptus [28].

2.1.4 Fixation of the conceptus

At about days 16/17, intrauterine migration of the conceptus suddenly ceases as it becomes immobilized at the site of subsequent placentation [11, 29]. Since the embryo is still surrounded by its glycoprotein capsule, there is only a “fixation” of the conceptus, but no implantation at this time. Except for a short period starting around day 35 (formation of the endometrial cups), the implantation in equids is non-invasive, and a stable microvillous attachment to the luminal cells of the endometrium is not established before approximately day 40 after ovulation [30].

A study of the temporal relationship between the diameter of the uterine horns, uterine tone and size of the embryonic vesicle throughout the fixation period [31] showed, that fixation occurs when the mobile and growing conceptus attains, on the average, a diameter equivalent to the distance between opposite inner walls of the myometrium. The uterus becomes turgid by this time and presumably does not expand adequately to accommodate continued motility of the expanding conceptus. The high frequency of fixation at the caudal proportion of the uterine horn might be attributed to its flexure which may act as the greatest impediment to continued embryo mobility [29].

But fixation may occur not only as a result of increased conceptus diameter and uterine tone, but also because of changes in the embryo’s capsule and environment. Coincidently with fixation, the conceptus becomes flaccid [23] and the capsule surface looses sialic acid [15, 24]. The loss of sialic acid and the subsequent decrease of negatively charged galactose and N-acetylgalactose residues of the major core type 1 O-glycan exposed on the capsule [32] might be important in changing the permeability or ‘stickiness’ of the capsule thus suggesting an important role in normal fixation of the conceptus.

Fixation also coincides with alterations in several capsule-bound proteins, for example maternally-secreted uterocalin, which is proteolytically converted to smaller fragments [33] at the time of fixation and β2-microglobulin (β2M) which as well undergoes limited proteolysis during the fixation period [33, 34] and is subsequently degraded. However, the role of this conversion still needs to be determined.

2.2 Maternal recognition of pregnancy

Progesterone produced by a viable corpus luteum is essential for the establishment of pregnancy in many, if not all, mammalian species. During the estrous cycle, the CL undergoes cyclical luteolysis, which is characterized by an initial decline of progesterone secretion, and terminates the female reproductive cycle to permit a new ovarian cycle to begin. In the large domestic animal species, $PGF_{2\alpha}$, which is known as the uterine luteolysin, is synthesized and released from the endometrium in a pulsatile pattern during late diestrus. This appears to have evolved as a mechanism to increase reproductive efficiency, as, in this way, a further opportunity is provided for the female to conceive within a relatively short interval of time if she has not conceived following ovulation [35].

However, during pregnancy, the CL is sustained over its cyclical lifespan, thereby ensuring the ongoing supply of progesterone, which does not only reduce cyclical ovarian activity, but also provides a uterine environment suitable for embryonic survival and development. The conceptus must therefore somehow prevent cyclical regression of the CL, a process commonly referred to as “maternal recognition of pregnancy” (MRP) [36]. Several different mechanisms exist in mammalian species to achieve this objective, e.g. by suppressing the pulsatile release of luteolytic $PGF_{2\alpha}$ from the endometrium, or by protecting the CL against its luteolytic action. Indeed, there is evidence that one or both effects may occur in large domestic animal species.

2.2.1 Maternal recognition of pregnancy in the horse

In the mare, a primary CL formed at the time of conception is the only source of progesterone for at least the first month of pregnancy [37]. In non-pregnant mares, luteolysis is triggered by an oxytocin-dependent pulsatile release of $PGF_{2\alpha}$ from the endometrium between days 13 and 16 after ovulation [38-41]. Unlike in ruminants or pigs, $PGF_{2\alpha}$ is thought to reach the ovaries of the mare only via the peripheral circulation [42] where it promptly exerts its luteolytic effect. However, in the presence of a conceptus, the cyclical release of luteolytic $PGF_{2\alpha}$ is suppressed to maintain a viable CL [18]. Co-incubation of conceptus membranes with endometrial tissue has been shown to block $PGF_{2\alpha}$ production in vitro [43] and measurements of $PGF_{2\alpha}$ concentrations in uterine flushings recovered from cyclic mares reached high values during days 14–16 after ovulation, the expected time of luteolysis, but were negligible in pregnant mares at this time [20]. The conceptus must therefore somehow prevent production of $PGF_{2\alpha}$ while it

traverses the uterus. However, the embryonic signal by which luteostasis is achieved in the mare still remains unknown.

Oxytocin

Oxytocin is thought to play a central role in luteal regression in the mare. Oxytocin is a nonapeptide hormone produced mainly by the hypothalamic magnocellular neurons [44]. It is stored in secretory vesicles of the posterior pituitary along with its “carrier protein” neurophysin and released into the peripheral circulation in a pulsatile manner during the estrous cycle [45, 46].

Besides this classical hypothalamo–neurohypophyseal axis, oxytocin has also been reported to be produced by other organs such as the ovary, placenta or testis. In contrast to ruminants, the ovary of the mare does not appear to be a source for oxytocin during the estrous cycle [47]. However, it is of interest that, like in the pig, locally synthesized uterine oxytocin is implicated an important role in control of cyclical luteolysis in the mare [48]. Oxytocin-mRNA has been identified in the equine endometrium and oxytocin has been detected in secretory vesicles of the secretory (nonciliated) cells of the uterine luminal and glandular epithelium, and is thought to be secreted into the uterine lumen, where it binds to its receptor on luminal epithelial cells and thereby stimulates the pulsatile release of $PGF_{2\alpha}$ leading to luteolysis [48-50].

Furthermore it has been demonstrated that the response of $PGF_{2\alpha}$ to oxytocin is maximal at the time of luteolysis in non-pregnant mares and that this response cannot be induced during early pregnancy, neither with endogenous nor with exogenous oxytocin, thus implicating an important role for this process in MRP in the horse [40, 51]. However, the mechanisms underlying the decreased oxytocin responsiveness in the pregnant mare are controversially discussed [41, 51].

Prostaglandin $F_{2\alpha}$ synthesis

When $PGF_{2\alpha}$ was identified as the mediator of luteolysis in the horse, it seemed likely that the embryo prevented luteolysis by suppressing the uterine production of prostaglandins. Prostaglandins are synthesized from arachidonic acid, an essential fatty acid stored in form of membrane phospholipids of the cell. Arachidonic acid is released from phospholipids via phospholipase action and converted into the common intermediate, prostaglandin H_2 (PGH_2) by prostaglandin G/H synthases (PTGS). While PTGS1 (also known as cyclooxygenase-1, COX-1) is constitutively expressed in most tissues, PTGS2 (also known as cyclooxygenase-2, COX-2) expression is inducible. Terminal prostaglandins are subsequently produced by specific prostanoid synthases like PGE

synthase (PTGES) and PGF synthase (PTGFS), which catalyze the isomerization of PGH_2 to PGE_2 and $PGF_{2\alpha}$.

Particular attention regarding MRP in the mare has been paid to PTGS2, as it is a rate-limiting enzyme in prostaglandin synthesis. PTGS2 mRNA and protein have been shown to be up-regulated at days 14 and 15 of the estrous cycle, but not at corresponding days in pregnant mares [52, 53]. Moreover, *PTGS2* mRNA abundance and $PGF_{2\alpha}$ concentrations have been shown to be reduced by conceptus secretions in an equine endometrial explant culture system [53]. Therefore it has been suggested that the conceptus blocks endometrial $PGF_{2\alpha}$ synthesis at least in part by repressing the induction of PTGS2 expression during early pregnancy.

Other proposed mechanisms for MRP

Although the equine conceptus is known to produce a number of different secretory products during early pregnancy, including steroids, prostaglandins, different proteins, and peptides [19] such as interferon delta (IFNδ), a member of the type I interferon family [20], the nature of the embryonic pregnancy recognition signal which effects luteostasis still remains unclear. Finally, the application of small intrauterine devices, e.g. water-filled plastic balls, has been demonstrated to prolong the luteal phase in the mare, indicating that a form of mechanotransduction by the migrating conceptus may also prevent the endometrial cells from releasing $PGF_{2\alpha}$ [21].

2.2.2 Maternal recognition of pregnancy in the pig

In the pig, cyclical luteolysis occurs during late diestrus in response to a pulsatile release of $PGF_{2\alpha}$ from the endometrium on days 15 and 16 after ovulation. $PGF_{2\alpha}$ is subsequently transported to the ovary by a countercurrent transfer between the uterine venous system and the ovarian artery and via the lymphatic pathways where it exerts its luteolytic effect [54, 55].

Estrogen as a pregnancy recognition signal in the pig

Pregnancy recognition in pigs is thought to occur between days 11 and 12 after ovulation [56]. During this time, the blastocyst undergoes marked morphological changes and elongates from a spherical to a tubular and filamentous form [57]. The conceptus also begins to secrete substantial amounts of estrogens which are known to function as the primary pregnancy recognition signal in pigs [58]. These conceptus-derived estrogens have been implicated in causing a shift in endometrial $PGF_{2\alpha}$ secretion from an endocrine

(towards the uterine venous drainage) to an exocrine (towards the uterine lumen) direction. Luteolytic $PGF_{2\alpha}$ is consequently sequestered within the uterine lumen where it is unavailable to exert its luteolytic effect on the CL [59-61]. Additionally, the retrograde transfer of $PGF_{2\alpha}$ from the venous blood and uterine lymph into the uterus, and the ability of the uterine vein and artery wall to accumulate $PGF_{2\alpha}$, could also constitute part of the putative mechanism of CL protection during early pregnancy in pigs [55, 62].

Application of exogenous estrogens has been shown to induce pseudo-pregnancy in cycling gilts when administered from days 11 to 15 of the estrous cycle [63], thus confirming an involvement of estrogens in MRP in the sow. Furthermore, estrogens, either of conceptus origin or injected, are thought to stimulate the endometrial release of calcium into the uterine lumen, followed by its re-uptake by endometrial and/or conceptus tissues within the next 12 hours. This period of release and re-uptake of calcium by the endometrium has been shown to be closely associated with redirection of $PGF_{2\alpha}$ in pregnant and pseudo-pregnant gilts [64]. However, the specific role for this uterine secretory response to estrogen in the maintenance of pregnancy still needs to be determined. Furthermore, estradiol itself has been suggested to have a direct luteotropic effect [65].

Prostaglandin E_2

Another supportive mechanism by which the conceptus is thought to inhibit luteolysis in the pig is by changing prostaglandin synthesis in favor of luteoprotective PGE_2. Indeed, a luteoprotective effect of PGE_2 has been frequently demonstrated in pigs [66-68]. It has been suggested that estrogens (and PGE_2) produced by the porcine conceptus modulate the expression of key enzymes in PG synthesis in the trophoblast and the endometrium, resulting in a changing pattern of $PGF_{2\alpha}$ and PGE_2 secretion during early pregnancy [66]. Indeed, increased mRNA levels of microsomal *PTGES-1* with simultaneous down-regulation of *PTGFS* and carbonyl reductase-1 (*CBR1*), which converts PGE_2 into $PGF_{2\alpha}$, has been observed in day 10-13 pregnant pigs [68, 69]. This may cause predomination of endometrial PGE_2 secretion and therefore be an effective agent in increasing the PGE_2:$PGF_{2\alpha}$-ratio.

Oxytocin

The porcine CL also synthesizes oxytocin, although its ability to do so is much lower than it is in ruminants. It is believed that the neurohypophysis is the primary source of oxytocin in the sow, probably supplemented by locally produced oxytocin from the uterus [35]. However, the role for oxytocin and its receptor during luteolysis and early pregnancy in

pigs is controversially discussed. On the one hand, the increase in circulating concentrations of oxytocin during luteolysis is associated with an increase in uterine secretion of $PGF_{2\alpha,}$ and exogenous oxytocin stimulates the secretion of $PGF_{2\alpha}$ in cyclic and early pregnant pigs [70]. On the other hand, blocking of oxytocin receptors did not prevent luteolysis or change duration of the estrous cycle [71], which does not suggest a mandatory role for oxytocin in MRP in the pig.

Interferons

The porcine trophoblast also secrets interferons (IFNs) between days 12 and 20 of gestation [72], e.g. the major type II interferon (interferon gamma, IFNγ), which is secreted in substantial amounts with a peak of synthesis being observed on days 15-16 of pregnancy [73], and a novel Type I interferon (interferon delta, IFNδ) [74]. In contrast to the ruminant interferon tau, IFNγ does not appear to exhibit antiluteolytic properties in the pregnant sow [75]. However, recent studies support a role for porcine trophoblast interferons in conceptus implantation as they may stimulate the remodeling and/or depolarization of the uterine endometrial epithelium as a prerequisite for blastocyst attachment and establishment of a functional placenta [76, 77].

2.2.3 Maternal recognition of pregnancy in domestic ruminants

Cyclical luteolysis in domestic ruminants is induced by an oxytocin-dependent pulsatile release of endometrial $PGF_{2\alpha}$ during late diestrus. The ongoing exposure to progesterone thereby negatively autoregulates the expression of the progesterone receptor in the endometrial epithelium, closely followed by increases in epithelial estrogen receptor (ESR1) and oxytocin receptor (OXTR) [78, 79]. This allows oxytocin to induce the uterine release of $PGF_{2\alpha}$ pulses. In ruminants, the posterior pituitary acts as the central oxytocin pulse generator. Moreover, a positive feedback-loop has been described between luteal oxytocin and uterine PGs, hence amplifying the luteolytic pulses of $PGF_{2\alpha}$ [80].

Due to the unique structure of its vascular utero-ovarian plexus, $PGF_{2\alpha}$ is then transported directly from the uterus to the ovary, possibly by a prostaglandin transporter-mediated mechanism, where it exerts its luteolytic effect [81]. In addition, $PGF_{2\alpha}$ is also supposed to act partly via the systemic circulation in the cow [35].

Interferon tau

Interferon tau (IFNτ), a ruminant-specific member of the type I IFN family, which is synthesized and secreted in substantial amounts by the mononuclear cells of the

conceptus trophectoderm, is well established as the primary pregnancy recognition signal in ruminants [82, 83],

The expression of IFNτ occurs during a defined period of conceptus development in cattle and sheep. IFNτ mRNA and protein are first detected as trophectoderm forms at the late morula to early blastocyst stage of development [84, 85] and increase with advancing age of the spherical conceptus. Coincident with the time of MRP, the conceptus changes from a spherical to a tubular and filamentous form and IFNT secretion increases dramatically at days 14–15 of pregnancy in cattle and at days 12–13 of pregnancy in ovine conceptuses [86]. IFNτ subsequently acts on the endometrium in a paracrine manner to prevent generation of the luteolytic cascade leading to endometrial secretion of $PGF_{2\alpha}$.

In pregnant ewes, it has been supposed that IFNτ suppresses transcription of the *ESR1* gene and thereby prevents estrogen to induce expression of the *OXTR* gene, as it would normally occur during the estrous cycle [87, 88]. In cows, although much of the available data are consistent with this hypothesis in sheep, evidence for a similar mechanism operating is less clear, since *OXTR* is up-regulated prior to *ESR1* expression during the estous cycle. Therefore it is implicated that the bovine conceptus probably exerts a rather direct effect on endometrial *OXTR* gene expression [89-91].

Prostaglandin E_2

It seems likely that PGE_2, produced by the blastocyst or the endometrium, may also counteract the luteolytic effects of $PGF_{2\alpha}$ in pregnant ruminants. As a stimulator of cAMP and a vasodilator, PGE_2 has properties that are opposite to $PGF_{2\alpha}$ [92]. In support of this view, the infusion of PGE_2 into the uterus of non-pregnant ewes delays luteolysis [93]. Similar effects have been observed in cows [94]. Moreover, an increase of PGE_2 in uterine venous blood has been reported during early pregnancy in ewes [95]. Because of its structural similarity to $PGF_{2\alpha}$, a small amount of PGE_2 may be transported locally from the uterine vein to the ovarian artery by a countercurrent transfer. With the use of cultured bovine endometrial cells, it has been proposed that IFNτ may transform the response of the endometrium to oxytocin from stimulating $PGF_{2\alpha}$ to stimulating PGE_2 [96, 97]. Furthermore, it has also been reported that recombinant bovine IFNτ reduces $PGF_{2\alpha}$ synthesis by blocking the oxytocin-induced expression of COX-2 and prostaglandin F synthase [98]. Thus IFNτ may act via multiple pathways to protect the CL from luteolysis during early gestation in ruminants.

IFNτ -stimulated genes

IFNτ is further known to stimulate expression of a number of so-called IFNτ-stimulated genes (ISGs) that are hypothesized to play a role in endometrial differentiation and concepus implantation [61, 99, 100]. A systematic study of maternal transcriptome changes in response to the presence of an embryo on day 18 of pregnancy in cattle revealed 87 genes up-regulated in pregnant animals during this time. Almost one half of these genes were known to be stimulated by type I IFNs. A functional classification of the identified genes revealed several different biological processes involved in the preparation of the endometrium for the attachment and implantation of the embryo such as genes involved in modulation of the maternal immune system and genes relevant for cell adhesion and for remodeling of the endometrium. Furthermore, the ISG15ylation system has been assumed to play an important role in IFNτ signaling [101].

Other factors important for embryo-maternal interaction

However, although many experimental findings indicate a pivotal role for IFNτ in pregnancy recognition of ruminants, a number of other systems (e.g. growth factors) may be involved in the embryo-maternal dialogue [100] and subsequently, overlapping actions of progesterone, interferon tau, placental lactogen, and growth hormones regulate endometrial gland morphogenesis and terminal differentiated function to maintain pregnancy.

3 Publications

3.1 Publication 1

Microarray analysis of equine endometrium at days 8 and 12 of pregnancy

M. Merkl,[1,4] S.E. Ulbrich,[5] C. Otzdorff,[2] N. Herbach,[3] R. Wanke,[3] E. Wolf,[4] J. Handler,[1,6] and S. Bauersachs[4]

Clinic for Horses,[1] Clinic for Small Animal Surgery and Gynecology,[2] and Institute of Veterinary Pathology,[3] Center for Clinical Veterinary Medicine, and Laboratory for Functional Genome Analysis (LAFUGA),[4] Gene Center, Ludwig-Maximilian University Munich, Munich; Germany Physiology Weihenstephan,[5] Technical University Munich, Freising-Weihenstephan, Germany; Clinic for Horses,[6] Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

BIOLOGY OF REPRODUCTION 2010 Nov; 83(5):874-86.

Epub 14 July 2010

BIOLOGY OF REPRODUCTION **83,** 874–886 (2010)
Published online before print 14 July 2010.
DOI 10.1095/biolreprod.110.085233

Microarray Analysis of Equine Endometrium at Days 8 and 12 of Pregnancy[1]

M. Merkl,[3,6] S.E. Ulbrich,[7] C. Otzdorff,[4] N. Herbach,[5] R. Wanke,[5] E. Wolf,[6] J. Handler,[3,8] and S. Bauersachs[2,6]

Clinic for Horses,[3] Clinic for Small Animal Surgery and Gynecology,[4] and Institute of Veterinary Pathology,[5] Center for Clinical Veterinary Medicine, and Laboratory for Functional Genome Analysis (LAFUGA),[6] Gene Center, Ludwig-Maximilians University of Munich, Munich, Germany
Physiology Weihenstephan,[7] Technical University Munich, Freising-Weihenstephan, Germany
Clinic for Horses,[8] Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

ABSTRACT

Establishment and maintenance of pregnancy in equids is only partially understood. To provide new insights into early events of this process, we performed a systematic analysis of transcriptome changes in the endometrium at Days 8 and 12 of pregnancy. Endometrial biopsy samples from pregnant and nonpregnant stages were taken from the same mares. Composition of the collected biopsy samples was analyzed using quantitative stereological techniques to determine proportions of surface and glandular epithelium and blood vessels. Microarray analysis did not reveal detectable changes in gene expression at Day 8, whereas at Day 12 of pregnancy 374 differentially expressed genes were identified, 332 with higher and 42 with lower transcript levels in pregnant endometrium. Expression of selected genes was validated by quantitative real-time RT-PCR. Gene set enrichment analysis, functional annotation clustering, and cocitation analysis were performed to characterize the genes differentially expressed in Day 12 pregnant endometrium. Many known estrogen-induced genes and genes involved in regulation of estrogen signaling were found, but also genes known to be regulated by progesterone and prostaglandin E2. Additionally, differential expression of a number of genes related to angiogenesis and vascular remodeling suggests an important role of this process. Furthermore, genes that probably have conserved functions across species, such as *CRYAB*, *ERRFI1*, *FGF9*, *IGFBP2*, *NR2F2*, *STC1*, and *TNFSF10*, were identified. This study revealed the potential target genes and pathways of conceptus-derived estrogens, progesterone, and prostaglandin E2 in the equine endometrium probably involved in the early events of establishment and maintenance of pregnancy in the mare.

embryo-maternal communication, equus caballus, *female reproductive tract, gene regulation, horse, pregnancy, steroid hormones, uterus*

INTRODUCTION

Progesterone produced from a viable corpus luteum is essential for establishment and maintenance of pregnancy. In the mare, cyclical luteolysis takes place between Days 14 and 16 after ovulation. The equine conceptus must therefore prevent luteal regression, a process commonly referred to as maternal recognition of pregnancy. In contrast to other large domestic animal species, the nature of embryo-maternal communication and maternal recognition of pregnancy in equids is still not completely understood. Furthermore, a number of features of equine pregnancy are unique to the genus *Equus* and differ from corresponding events in other mammals.

The equine blastocyst enters the uterus between 144 and 156 h after ovulation [1]. Between Day 7 and Day 21, the embryo is completely enveloped by a tough glycoprotein capsule, which prevents the trophoblast from elongating and provides its typical spherical shape [2, 3]. Furthermore, the capsule is thought to play a protective role, to ensure nutrition, and to facilitate migration of the equine conceptus [4]. The capsule may also concentrate growth factors at the embryo-maternal interface and eventually release them in a controlled manner [5]. Until Day 16, the equine conceptus remains completely unattached within the uterus and migrates continuously throughout the uterine lumen driven by peristaltic myometrial contractions [6, 7]. The constant movement allows the embryo to get in contact with most of the endometrial surface, likely serving to signal its presence uniformly to the entire endometrium and to garner uterine secretions [8]. At Day 17, not only as a result of increased conceptus diameter and increased uterine tone, but also because of changes in the embryo's capsule and uterine environment, the conceptus becomes immobilized (''fixed'') at the base of one of the uterine horns [6, 9, 10].

Although the mechanisms of luteal rescue in the mare are still unknown, the role of prostaglandins is undisputed. In cyclic mares luteolysis is triggered by an oxytocin-dependent pulsatile release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the endometrium from Day 14 after ovulation [11]. However, in the presence of a conceptus, the synthesis and secretion of $PGF_{2\alpha}$ in the mare is abrogated [8]. Furthermore, coincubation of conceptus membranes with endometrial tissue has been shown to block $PGF_{2\alpha}$ production in vitro [12]. Although the signal that accomplishes this effect is not known, the presence of a conceptus seems to uncouple the oxytocin-induced release of $PGF_{2\alpha}$ [8, 13]. It has been demonstrated that the $PGF_{2\alpha}$ response to oxytocin is maximal at the time of luteolysis in nonpregnant mares and that this response cannot be induced during early pregnancy either with endogenous or with exogenous oxytocin [13–15]. These data suggest that maternal recognition of pregnancy, which in the mare is commonly

[1]Supported by the German Ministry for Education and Research (BMBF, FUGATO-plus, COMPENDIUM) and the German Research Foundation (DFG, Research Training Unit 1029 Functional Genome Research in Veterinary Medicine). The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE21046.
[2]Correspondence: Stefan Bauersachs, Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, LMU Munich, Feodor Lynen Str. 25, 81377 Munich, Germany. FAX: +49 89 2180 76701/76849; e-mail: bsachs@lmb.uni-muenchen.de

Received: 8 April 2010.
First decision: 6 May 2010.
Accepted: 18 June 2010.

eISSN: 1529-7268 http://www.biolreprod.org
ISSN: 0006-3363

believed to occur between Days 14 and 16 [16], may be as early as Days 11–13 [13].

Another hypothesis is that the antiluteolytic signal produced by the equine conceptus targets prostaglandin biosynthesis in order to prevent luteolysis. Prostaglandin G/H synthase 2 (PTGS2; also known as cyclooxygenase 2), a rate-limiting enzyme in prostaglandin synthesis, has been shown to be up-regulated at Day 14/15 of the estrous cycle, but not at corresponding days in pregnant mares [17, 18]. Moreover, *PTGS2* mRNA abundance and $PGF_{2\alpha}$ concentrations have been shown to be reduced by conceptus secretions in an equine endometrial explant culture system [18]. Therefore it has been suggested that the conceptus blocks endometrial $PGF_{2\alpha}$ synthesis at least in part by repressing the induction of *PTGS2* expression.

What also remains unknown is the nature of the embryonic pregnancy recognition signal to prevent luteolysis. The equine conceptus produces a number of different secretory products during early pregnancy, including steroids, prostaglandins, different proteins, and peptides [19], such as interferon delta, a member of the type I interferon family [20]. Moreover, the application of intrauterine devices has been demonstrated to prolong the luteal phase in the mare, indicating that a form of mechanotransduction by the migrating conceptus may prevent the endometrial cells from releasing $PGF_{2\alpha}$ [21].

In order to systematically analyze the maternal response, i.e., the changes in the equine endometrium, to the presence of a conceptus a transcriptome study of endometrium samples from six mares at Days 8 and 12 of pregnancy and the corresponding nonpregnant stages was performed.

MATERIALS AND METHODS

Sample Collection and Experimental Design

In this study, two experiments were performed. Endometrial biopsy samples were collected from inseminated mares 1) on Day 8 and 2) on Day 12 after ovulation. In both experiments one pregnant and one control (nonpregnant) sample were taken from every mare by random order. Only one endometrial biopsy was taken per estrous cycle.

Samples were collected from six normal cycling Bavarian Warmblood mares belonging to the Bavarian principal and state stud of Schwaiganger, Germany. Follicular development and ovulation were monitored routinely by daily transrectal palpation and ultrasound examination. When mares developed an ovarian follicle of approximately 35 mm in diameter, accompanied by prominent endometrial edema, they were treated with 1500 IU human chorionic gonadotropin i.v. (Ovogest; Intervet Deutschland GmbH, Unterschleissheim, Germany) to induce ovulation. All mares were inseminated artificially with $>500 \times 10^6$ freshly collected, progressively motile, extended spermatozoa from one fertile stallion. Insemination was performed 24 h after induction of ovulation and was repeated if ovulation had not occurred after 48 h. Endometrial samples were obtained by transcervical biopsy. Samples were collected 1) on Day 8 and 2) on Day 12 after flushing of the uterus. On Day 8, mares were rated pregnant if embryo recovery was successful. On Day 12, pregnancy was additionally proved by ultrasonographic detection of an embryonic vesicle in the uterine lumen before flushing. Embryos were flushed transcervically without sedation using up to four times 1.5 L prewarmed and sterile filtered phosphate buffered saline (Lonza Verviers Sprl, Verviers, Belgium). The fluid was recovered directly into sterile glass bottles and subsequently, if necessary, filtered with an embryo filter system and examined under a microscope (in the case of Day 8 embryos) for the presence of an embryo.

For determination of peripheral plasma progesterone (P4) concentrations, blood samples were collected in ethylenediaminetetraacetic acid tubes from the jugular vein on Day 0 and directly after biopsy. Blood samples were centrifuged at $2000 \times g$ for 10 min and plasma was decanted and stored at −20°C until assay.

In order to analyze tissue composition, the biopsy samples were cut transversely into six equal and plane-parallel slices. For quantitative stereological analyses, every second slice was transferred into embedding capsules with their right cut surface facing downwards, covered with a foam sponge to avoid distortion of the tissue samples, and fixed by immersion in 4% buffered formaldehyde. The remaining pieces of the biopsy samples were immediately transferred into vials containing 4 ml RNAlater (Ambion, Huntingdon, U.K.) for mRNA expression analysis. The vials were cooled on ice and incubated overnight at 4°C. Samples were stored at −80°C until further processing. All experiments with animals were conducted with permission from the local veterinary authorities and in accordance with accepted standards of humane animal care.

Quantitative Stereological Analysis

For qualitative histological and quantitative stereological analyses, three formalin-fixed slices of each biopsy sample were routinely processed and embedded in paraffin with their right cut surface facing downwards. Histological sections were cut at a nominal thickness of 3 μm with a rotary microtome, transferred onto glass slides, and stained with hematoxylin and eosin (H&E). Quantitative stereological analyses were carried out with newCAST software (Visiopharm A/S, Hoersholm, Denmark). Slides were displayed on a monitor at 400× final magnification via a camera (universal camera DP72, Olympus Deutschland GmbH, Hamburg, Germany) coupled to a microscope (standard laboratory microscope BX41, Olympus Deutschland GmbH) and images were superimposed by an adjustable point counting grid. More than 7000 points were evaluated per biopsy sample to determine the volume densities of surface epithelium, glandular epithelium, blood vessels, and remaining tissue. The volume densities (Vv) of the different tissue compartments were obtained by dividing the number of points hitting a compartment ($P_{(compartment)}$, e.g., points hitting blood vessels, $P_{(blood\ vessels)}$) by the total number of points hitting the biopsy sample ($P_{(sample)}$): $Vv_{(compartment/sample)} = P_{(compartment)}/P_{(sample)}$.

Microarray Analysis

Total RNA was isolated from the 12 endometrial biopsy samples using Trizol reagent (Invitrogen GmbH, Karlsruhe, Germany) according to the manufacturer's instructions. Quantity and purity of RNA were measured with a NanoDrop 1000 (PEQLAB Biotechnologie GMBH, Erlangen, Germany). Quality of total RNA was determined electrophoretically with an Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). RNA integrity values ranged from 8.3 to 9.2. Microarray analysis was performed using Agilent 4x44k Horse Gene Expression microarrays (AMADID 021322). Cy3-labeled cRNA was produced with the Quick Amp Labeling Kit, one-color (Agilent Technologies), and hybridized to the microarrays according the manufacturer's instructions. Hybridized and washed slides were scanned at 3-μm resolution with an Agilent DNA Microarray Scanner (G2505C; Agilent Technologies). Image processing was performed with Feature Extraction Software 10.5.1.1 (Agilent Technologies). Processed signals were filtered based on ''Well above background'' flags (detection in four of six samples in either one of the two experimental groups) and subsequently normalized with the BioConductor package vsn [22]. For quality control normalized data was analyzed with a distance matrix and a heatmap based on pair-wise distances (BioConductor package geneplotter). Significance analysis was performed using the Microsoft Excel add-in ''Significance analysis of microarrays'' (SAM, two-class paired) [23]. Significance thresholds were set as follows: 1) false discovery rate (FDR) <5% and fold change at least 1.5-fold and 2) ratio fold change/q-value ≥0.75 to have higher confidence for smaller differences. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE21046.

Functional Analysis of Array Data

The Agilent horse microarray was reannotated based on Ensembl 55, Entrez Gene, and BLAST analyses to obtain equine and human (putative orthologous genes) Entrez Gene identifiers and the corresponding gene information. For gene set enrichment analysis (GSEA) [24], genes were preranked based on fold change pregnant vs. control and SAM q-value (log2(fold change + 2) * −log10(q-value)). This preranked gene list was compared with GSEA gene sets c2.all.v2.5.symbols.gmt (curated) and our own published and unpublished gene sets (see *Results*). Functional classification of differentially expressed genes (DEGs) was done with the ''Functional annotation clustering'' and ''Functional annotation chart'' tools of the Database for Annotation, Visualization, and Integrated Discovery (DAVID) [25] and the text-mining tool CoPub [26], which finds biomedical concepts from Medline that are significantly linked to the gene set. Both analyses were performed on the basis of Entrez Gene IDs of the putative human orthologous genes. Interaction networks were drawn with the Pathway Architect software (version 3.0.1; Stratagene, Heidelberg, Germany).

Quantitative Real-Time RT-PCR

The same RNA samples as for microarray analysis were used for quantitative real-time RT-PCR (qPCR). First-strand cDNA was synthesized

starting from 1 μg total RNA with the Sprint RT Complete-Double PrePrimed Kit (Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France). The two-step quantitative real-time PCR experiments were performed as described previously [27] in accordance with the MIQE guidelines [28]. The LightCycler DNA Master SYBR Green I protocol (Roche, Mannheim, Germany) was applied. Primer sequences, annealing temperatures (AT), the appropriate fluorescence acquisition (FA) points for quantification within the fourth step of the amplification segment, and the melting points (MP) are shown in Supplemental Table S1 (all Supplemental Data are available online at www.biolreprod.org). The cycle number (CT) required to achieve a definite SYBR Green fluorescence signal was calculated by the second derivative maximum method (LightCycler software version 3.5.28). The CT is correlated inversely with the logarithm of the initial template concentration. The CT determined for the target genes were normalized against the geometric mean of the housekeeping genes histone (H3F3A), ubiquitin (UBQ3), and 18S rRNA (ΔCT) [29]. Finally, with respect to the paired design, the relative expression difference between the nonpregnant and pregnant state was calculated for each animal (ΔΔCT). All amplified PCR fragments were sequenced to verify the resulting PCR product.

Progesterone Assay

Progesterone concentrations in peripheral blood plasma were measured with a mini VIDAS (bioMérieux Deutschland GmbH, Nürtingen, Germany) and VIDAS Progesterone kits, a system based on the enzyme-linked fluorescent assay technique. A detection limit of 0.25 ng/ml and a correlation coefficient of 0.89 towards radio immune assay are certified for the assay by the manufacturer.

RESULTS

To characterize endometrial responses to the early embryo in the mare, microarray analyses of Day 8 and Day 12 endometrial biopsy samples were performed in two separate experiments. A paired design was used, i.e., RNA samples derived from the same mare were hybridized on the same slide (4x44k array) to reduce technical and biological variation. The paired design was chosen to take into account potential interindividual differences related to genetic background and other actors. Additional sources for variation were tried to rule out with the measurement of P4 concentrations and the analysis of the composition of the endometrial biopsy samples.

Peripheral Plasma Progesterone Concentrations

P4 values showed basal levels on Day 0. On Day 8, plasma progesterone concentrations ranged from 12.6 to 27.7 ng/ml and on Day 12 from 12.0 to 35.3 ng/ml. Plasma progesterone concentrations were not significantly different between pregnant and nonpregnant mares on Day 8 and on Day 12, respectively (*t*-test: $P > 0.05$; data not shown).

Quantitative Stereological Analysis

Tissue composition of all endometrial biopsy samples, i.e., the volume fractions of luminal epithelium (LE), blood vessels (BV), glandular epithelium (GE), and remaining tissue (Rest), was determined by using quantitative stereological techniques (Supplemental Figs. S1 and S2). Overall, tissue composition was quite consistent within the biopsy samples (see examples in Supplemental Fig. S2).

In endometrial biopsy samples collected on Day 8, volume fractions of the different structures were 0.23%–0.91% (LE), 2.4%–3.9% (BV), 25.8%–35.8% (GE), and 59.3%–71.3% (Rest). Maximal deviation was 0.41 percentage points (pp) (LE), 1.3 pp (BV), 4.8 pp (GE), and 3.6 pp (Rest) within pregnant and control samples of one mare.

In endometrial biopsy samples collected on Day 12, volume fractions of the different structures were 0.24%–1.82% (LE), 2.7%–3.9% (BV), 22.9%–33.3% (GE), and 62.4%–73.7% (Rest). Maximal deviation was 0.54 pp (LE; excluding mare #3), 0.8 pp (BV), 7.7 pp (GE), and 8.6 pp (Rest) within pregnant and control samples of one mare. In mare #3, volume fraction of LE was 1.5 pp higher (5.6-fold) in the control sample than in the pregnant sample.

Microarray Analysis

After data processing and normalization the microarray data sets were initially analyzed with correlation heatmaps in order to cluster the data sets of the individual samples according to their pair-wise correlations. Then statistical analysis was done to identify DEGs. For the endometrial tissue samples derived from Day 8 pregnant mares vs. Day 8 control mares, statistical analysis did not reveal any significant expression differences (data not shown), even after exclusion of mare #3 (aberrant expression differences for immune response genes in pregnant sample).

In contrast to Day 8, differential gene expression was identified at Day 12 of pregnancy. A heatmap of pair-wise correlations based on normalized microarray data sets is shown in Figure 1a for analysis of Day 12 of pregnancy. Samples from the same mares clustered together, but no grouping could be observed within samples collected during pregnancy or during the estrous cycle. The control sample of mare #3 (Fig. 1a, M3 co) showed the lowest correlation to all other samples. A second heatmap was generated based on a limited number of hybridization probes, which showed at least 1.5-fold difference between pregnant and control samples (Fig. 1b). Based on this reduced data set a clear separation of pregnant and control samples was obtained. Figure 1c shows a heatmap of log2 fold changes pregnant vs. control for the six mares. Except for mare #3, similar expression patterns were observed between mares. For mare #3, many genes showed inverse expression differences. Because of the 5.6-fold higher proportion of luminal epithelium in the control sample compared to the pregnant sample (Supplemental Fig. S2) and the results of the heatmap analysis (Fig. 1c), data from mare #3 were excluded from further analysis. Statistical analysis of Day 12 microarray data of the remaining five mares revealed 374 DEGs in endometrial tissue samples of pregnant vs. control mares (Supplemental Table S2). Of these genes, 332 transcripts showed at least 1.5-fold higher expression values (in the following referred to as up-regulated genes) and 42 transcripts showed lower expression values (in the following referred to as down-regulated genes) in biopsy samples from pregnant endometrium compared to control samples. Figure 1c shows a cluster analysis of log2 fold changes of the DEGs for all six mares. Whereas similar pregnant to control expression differences were observed for five of the mares, mare #3 showed for many of these genes either no expression differences or even inverse differences (Fig. 1c, M3).

Differential expression was in addition analyzed between Day 8 and Day 12 control samples (see Supplemental Table S2). Of the Day 12 DEGs (pregnant vs. control), 34 genes were also differentially expressed in Day 12 compared to Day 8 control samples (fold change >1.5-fold, FDR 5%): 6 of the Day 12 down-regulated genes and 28 of the up-regulated genes. Most of the Day 12 of pregnancy down-regulated genes (5 of 6) showed lower mRNA levels in Day 12 vs. Day 8 control samples. Likewise there were a number of genes up-regulated from Day 8 to Day 12 in the control samples that were additionally up-regulated in Day 12 pregnant samples. Furthermore, there were some genes down-regulated from Day 8 to Day 12 of the estrous cycle but with higher mRNA levels in Day 12 pregnant compared to Day 12 control samples.

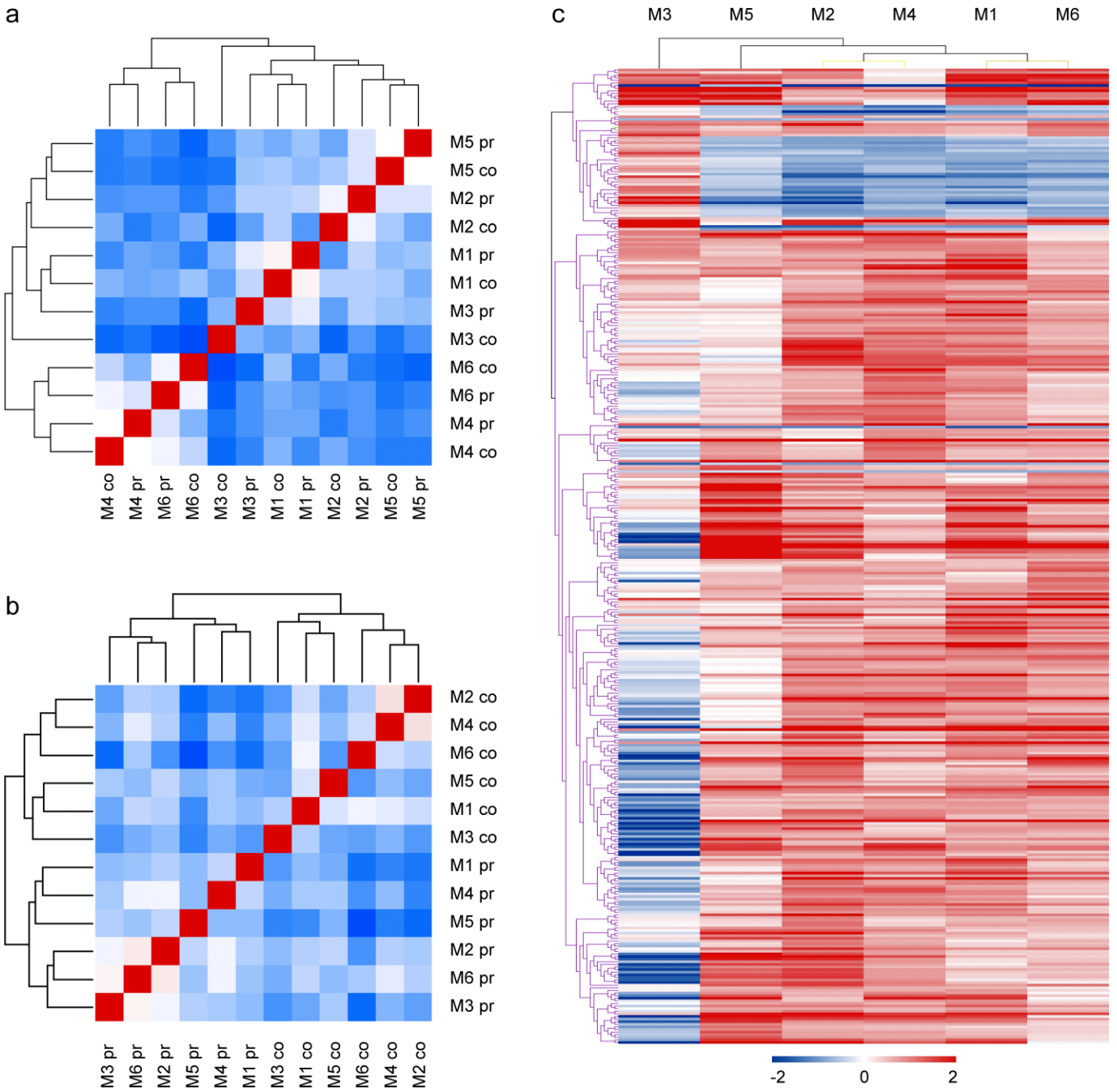


FIG. 1. Microarray analysis of Day 12 pregnant vs. nonpregnant endometrium. Normalized expression data was clustered based on pair-wise correlation using all detectable probes (**a**) and after filtering for probes with at least 1.5-fold mean difference between pregnant and control samples (**b**) (red: correlation = 1; blue: lowest observed correlation). After statistical analysis a hierarchical cluster analysis of the log2 fold changes of the single mares limited to the significant genes was performed (**c**). Mare #3 is also shown but was excluded from the statistical analysis. M, mare #; pr, pregnant; co, control.

Validation of Microarray Results by Quantitative Real-Time RT-PCR

To validate microarray results, 13 of the DEGs were selected for quantification with real-time RT-PCR (Table 1). Overall, expression differences found by microarray analysis were confirmed. For some of the analyzed genes *t*-test *P*-values were not significant (>0.05) because of variations in expression differences between mares. For most of those genes, expression differences were significant between Day 8 and Day 12 pregnant samples (Table 2). The comparison of qPCR data between Days 8 and 12 corresponded well to the array data and showed that four of the analyzed genes (*CTSL1*, *FGF9*, *PTGR1*, *SLC36A2*) were also differentially expressed between Days 8 and 12 of the estrous cycle (Table 2). Interestingly, *FGF9* was down-regulated at Day 12 of the estrous cycle compared to Day 8 of the estrous cycle. Samples derived from mare #3 were also analyzed, and the findings of the microarray experiment that for many of the DEGs expression differences were much lower or even inverse were confirmed (data not shown).

TABLE 1. Quantification of selected genes with quantitative real-time RT-PCR: Day 12 pregnancy vs. control.

Gene name	Gene symbol	Entrez gene ID	Ensembl gene ID	Hsa Gene symbol	Hsa Entrez gene ID	Qpcr		Array	
						Pr/Co[a]	*P*-value	Pr/Co[a]	q-value
Cathepsin L	*LOC100061532*	100061532	ENSECAG00000007210	*CTSL1*	1514	−2.7	0.002	−2.2	0.012
ERBB receptor feedback inhibitor 1	*ERRFI1*	100052062	ENSECAG00000017104	*ERRFI1*	54206	1.7	0.012	2.6	0.009
Fibroblast growth factor 9	*LOC100050353*	100050353	ENSECAG00000018716	*FGF9*	2254	7.9	0.017	8.8	0.001
Hedgehog-interacting protein	*HHIP*	100062868	ENSECAG00000024485	*HHIP*	64399	−1.6	0.090	−1.7	0.001
Kinase insert domain receptor	*KDR*	100033959	ENSECAG00000019429	*KDR*	3791	1.8	0.061	1.7	0.012
Krueppel-like factor 9	*KLF9*	100050300	ENSECAG00000024925	*KLF9*	687	1.3	0.179	1.5	0.018
Oxytocin receptor	*LOC100058848*	100058848	ENSECAG00000017844	*OXTR*	5021	1.8	0.050	1.6	0.018
Progestin and adipoQ receptor family member V	*LOC100064749*	100064749	ENSECAG00000008154	*PAQR5*	54852	4.7	0.001	2.0	0.002
Prostaglandin E2 receptor EP4 subtype	*LOC100053208*	100053208	ENSECAG00000011145	*PTGER4*	5734	2.2	0.001	2.0	<0.001
Prostaglandin reductase 1	*PTGR1*	100058059	ENSECAG00000004698	*PTGR1*	22949	3.0	0.069	2.7	0.018
Secreted frizzled-related sequence protein 1	*LOC100055845*	100055845	ENSECAG00000021358	*SFRP1*	6422	1.5	0.147	1.7	0.012
Solute carrier family 36 (proton/amino acid symporter), member 2	*LOC100071541* 3′-UTR[b]	100071541	ENSECAG00000011961	*SLC36A2*	153201	53.1	<0.001	84.3	<0.001
Solute carrier family 36 (proton/amino acid symporter), member 2	*LOC100071541* ORF[c]	100071541	ENSECAG00000011961	*SLC36A2*	153201	32.2	<0.001	2.5	<0.001
Solute carrier organic anion transporter family member 2A1	*SLCO2A1*	100065438	ENSECAG00000024948	*SLCO2A1*	6578	1.8	0.102	2.0	0.021

[a] Pr: pregnant; Co: control.
[b] UTR, untranslated region.
[c] ORF, open reading frame.

Bioinformatics Analysis of Microarray Data

In order to get a first characterization of the DEGs, the Day 12 expression data set was ranked according to the expression fold change and the SAM q-value (see *Materials and Methods*), resulting in a ranked gene list containing the most significantly up-regulated genes on Day 12 of pregnancy at the top and the most significantly down-regulated genes at the bottom of the list. This preranked list was compared to gene sets of the GSEA Molecular Signature Database, of selected published studies, and of our own published and unpublished studies. Table 3 shows a number of significantly enriched gene sets, i.e., sets with genes occurring toward the top of the preranked Day 12 gene list. The corresponding enrichment plots are shown in Supplemental Figure S3. The gene set with the highest enrichment score and 24 (of 63) overlapping genes in ranks 1–500 of the Day 12 preranked gene list contains genes up-regulated in equine endometrium at Day 13.5 of pregnancy [30]. This gene set is followed by a set of genes up-regulated in human endometrium 7 days after the LH surge (the window of implantation) compared to 2 days after the LH surge [31] (29 of 129 genes in top 500). The gene set with the largest number of overlapping genes within ranks 1–500 was Boquest_CD31$^+$_vs_CD31$^-$_up (75 of 540 genes). Significant enrichment was also found for the corresponding gene set Boquest_CD31$^+$_vs_CD31$^-$_dn (38 of 215 genes). These gene sets were obtained from a comparison of two populations of CD45$^-$CD34$^+$CD105$^+$ adipose tissue-derived adult stromal stem cells that were either CD31 (PECAM1) positive or negative [32]. In addition, gene sets containing hypoxia-induced genes, genes of the RAS pathway, TGF-beta-induced genes, targets of the transcription factor TCF21, vascular endothelial growth factor (VEGF)-induced genes, estrogen-induced genes [33–36], genes up-regulated in ovine endometrium between Days 9 and 12 of pregnancy [37], and prostaglandin E2 (PGE2)-induced genes were found as significantly enriched. The analysis of gene sets from our own studies of bovine and porcine endometrium revealed best enrichment scores for genes up-regulated at Day 14 of pregnancy in porcine endometrium [38] and at Day 18 of pregnancy in bovine endometrium (our unpublished data) but the number of genes in ranks 1–500 of the Day 12 preranked list was rather small (23 and 25 genes, respectively). Higher numbers of genes in ranks 1–500 were found for the gene sets ''up-regulated at estrus in bovine endometrium'' (58 genes) and ''up-regulated at diestrus in bovine endometrium'' (44 genes). Additional information for the gene sets and the genes overlapping with the top 500 of the Day 12 preranked gene list can be found in Supplemental Table S3.

In the next step the up-regulated genes of ranks 1–500 were sorted based on their frequencies: 1) in the gene sets ''Up-regulated in human endometrium during the window of implantation'' (two human gene sets were combined), ''Up-regulated at Day 14 of pregnancy in porcine endometrium,'' and ''Up-regulated at Day 18 of pregnancy in bovine endometrium''; 2) in the gene sets ''Up-regulated in ovine endometrium between Days 9 and 12 of pregnancy,'' and ''Up-regulated at diestrus in bovine endometrium''; and 3) in the gene sets ''Up-regulated at estrus in bovine endometrium'' and ''Estrogen-induced genes'' to find genes that have conserved functions across mammalian species regarding establishment and maintenance of pregnancy. The genes anterior gradient homolog 2 (*AGR2*, Pr/Co = 1.6, q-value = 0.0348, rank 433), G protein-coupled receptor, family C, group 5, member B (*GPRC5B*, Pr/Co = 1.13, q-value = 0.0264, rank 480), ubiquitin D (*UBD*, Pr/Co = 1.4, q-value = 0.025, rank 395), and ubiquitin-conjugating enzyme E2L 6 (*UBE2L6*, Pr/Co = 1.2, q-value = 0.021, rank

TABLE 2. Quantification of selected genes with quantitative real-time RT-PCR: Day 12 vs. Day 8.[a]

	qPCR Co 12/8		Array Co 12/8		qPCR Pr 12/8		Array Pr 12/8	
Gene symbol	FC	*P*-value	FC	q-value	FC	*P*-value	FC	q-value
CTSL1	−2.6	0.025	−2.7	0.010	−8.4	<0.001	−7.0	<0.001
ERRFI1	1.3	0.489	1.5	0.148	2.3	0.002	3.6	<0.001
FGF9	−2.2	0.007	−1.6	0.019	4.4	0.040	5.9	<0.001
HHIP	−1.1	0.589	1.1	0.435	−1.6	0.063	−1.3	0.137
KDR	1.1	0.490	1.1	0.341	2.3	0.011	1.9	0.003
KLF9	1.1	0.596	1.1	0.272	1.5	0.108	1.5	0.229
OXTR	1.1	0.896	1.2	0.339	2.2	0.032	1.9	0.006
PAQR5	2.1	0.370	1.0	0.511	14.3	0.012	2.2	0.007
PTGER4	1.1	0.829	1.1	0.359	2.6	0.003	2.4	<0.001
PTGR1	4.2	0.020	1.7	0.054	12.9	0.001	3.9	<0.001
SFRP1	1.1	0.855	1.2	0.245	1.5	0.126	1.9	0.014
SLC36A2 3′-UTR[b]	2.9	0.029	3.6	0.012	144.2	<0.001	198.0	<0.001
SLC36A2 ORF[c]	2.2	0.024	−1.1	0.312	93.7	<0.001	2.1	0.001
SLCO2A1	1.2	0.567	1.1	0.384	2.4	0.032	2.0	0.026

[a] Pr: pregnant; Co: control; FC: fold change.
[b] UTR, untranslated region.
[c] ORF, open reading frame.

401) matched two gene sets containing up-regulated genes during pregnancy and one genes set up-regulated by progesterone, but these genes showed no significant up-regulation according to the thresholds of the significance analysis. B-cell CLL/lymphoma 6 (*BCL6*, Pr/Co = 1.7, q-value = 0.039, rank 453), crystallin, alpha B (*CRYAB*, Pr/Co = 2.2, q-value = 0.003, rank 85), insulin-like growth factor binding protein 2 (*IGFBP2*, Pr/Co = 1.9, q-value = 0.002, rank 74) and stanniocalcin 1 (*STC1*, Pr/Co = 3.1, q-value = 0.0001, rank 10) matched two gene sets containing up-regulated genes during pregnancy. Insulin-like growth factor binding protein 1 (*IGFBP1*, Pr/Co = 5.8, q-value = 0.004, rank 50) matched one gene set containing up-regulated genes during pregnancy and two gene sets up-regulated by progesterone. A list of all genes and their frequencies in the gene sets is shown in Supplemental Table S4.

To find quantitatively enriched functional terms for the Day 12 up-regulated genes, the DAVID functional annotation clustering tool was used. This method clusters significantly enriched functional terms, i.e., significantly more differential genes were found for a given term than expected, which contain similar sets of genes. This analysis resulted in a relatively large number of significant clusters of related functional terms that represented a variety of biological themes (Supplemental Table S5). These quantitatively enriched biological themes or processes included glycoproteins, secretory proteins, membrane proteins, development, differentiation, angiogenesis, calcium ion binding, carbohydrate binding, wound healing, apoptosis, cell migration, tissue remodeling, neurogenesis, cell growth, and proliferation. The text mining tool CoPub that identifies biological keywords from the Medline database significantly linked to a given gene set from a microarray data analysis [26] also highlighted a list of keywords that were significantly correlated with the genes up-regulated at Day 12 of pregnancy (Supplemental Table S6). The obtained keywords confirmed the results of DAVID functional annotation clustering and included a number of additional terms such as chemotaxis, inflammation, cell adhesion, cell invasion, cytoskeleton, different reproduction-related terms, and endocytosis.

Expression of Genes Involved in Prostaglandin Signaling and Metabolism

Microarray analysis revealed several up-regulated genes in Day 12 pregnant endometrium with a significant fold change ranging from 1.6 to 2.7 that are known to play a role in prostaglandin signaling and metabolism. In particular, transcripts for prostaglandin E receptors 3 and 4 (PTGER3, PTGER4), genes similar to prostaglandin F synthase (LOC100070491, LOC100070501), a prostaglandin transporter, and a prostaglandin reductase were found (Table 4). In addition to these differentially expressed prostaglandin-related genes, many more transcripts of genes involved in prostaglandin signaling and metabolism were found to be expressed in equine endometrium on Day 12 but were not differentially expressed according to the thresholds applied in the statistical analysis (Supplemental Table S7).

Angiogenesis and Steroid Hormone/Prostaglandin Signaling Interaction Networks

Putative interaction networks for genes related to the process of angiogenesis (Fig. 2) and genes described in context of steroid hormone and prostaglandin signaling (Fig. 3), were generated based on a literature search, CoPub results, and interactions from the Pathway Architect database and other public protein interaction databases. For the process of angiogenesis, genes representing different levels of angiogenesis regulation were found, such as members of the angiopoietin family, members of the VEGF system, hypoxia-induced genes, and genes regulating endothelial cell fate (Fig. 2 and Supplement to Fig. 2). The interaction network related to steroid hormone and prostaglandin signaling was clearly dominated by estradiol (E2) with many E2-regulated genes (Fig. 3 and Supplement to Fig. 3). There were also a number of genes described as negative regulators of estrogen receptor 1 (ESR1), genes involved in regulation of growth and differentiation, and genes involved in E2 metabolism. A considerable number of genes were involved in both networks.

Day 12 of Pregnancy Down-Regulated Genes

For the down-regulated genes, quantitatively enriched functional terms were obtained neither with DAVID Functional Annotation Clustering nor with CoPub. The down-regulated genes belonged to very different functional classes. The five most down-regulated genes were FXYD domain-containing ion transport regulator 4 (FXYD4, −3.4), keratin 4 (KRT4, −2.8), cartilage acidic protein 1 (CRTAC1, −2.4), RELT-like 2 (RELL2, −2.2), and cathepsin L1 (CTSL1, −2.2).

TABLE 3. Selected results of Gene Set Enrichment Analysis.

Gene set	Size[a]	NES[b]	FDR q-value[c]	FWER *P*-value[d]	Rank at max	Rank in top 500[e]	Rank in top 250[f]
Genes up-regulated at Day 13.5 of pregnancy in equine endometrium [30]	63	3.17	0.0000	0.0000	634	24	21
Genes up-regulated in human endometrium LH+7 vs. LH+2 [31]	122	2.90	0.0000	0.0000	1283	29	17
Boquest_CD31^{+}_vs_CD31^{-}_up — genes associated with endothelium, related to MHC class II complex and antigen presentation, genes for cytokines and cytokine receptors, and genes involved in signal transduction and transcription	540	2.69	0.0000	0.0000	2046	75	37
Boquest_CD31^{+}_vs_CD31^{-}_dn — genes involved in cell cycle arrest, stem cell biology and development, and in biology of adipose tissue, bone, cartilage, muscle, and neuronal tissue	215	2.64	0.0000	0.0000	2465	38	20
Manalo_hypoxia_up — genes up-regulated in human pulmonary endothelial cells under hypoxic conditions	84	2.60	0.0000	0.0000	2169	16	8
Genes up-regulated at Day 14 of pregnancy in porcine endometrium [38]	131	2.40	0.0000	0.0000	1746	23	14
Genes up-regulated at Day 18 of pregnancy in bovine endometrium[g]	226	2.37	0.0001	0.0010	2815	25	12
RAS_oncogenic_signature — gene expression signature that reflects the activity of the RAS-induced pathway	200	2.30	0.0003	0.0080	2601	25	14
TGFbeta_all_up — up-regulated by TGF-beta treatment of skin fibroblasts	73	2.30	0.0003	0.0090	1447	16	10
Genes up-regulated at estrus in bovine endometrium[h]	462	2.29	0.0004	0.0100	1815	58	34
Estrogen-induced genes[i]	400	2.29	0.0004	0.0100	1575	47	21
Genes up-regulated in receptive (LH+8) vs. pre-receptive (LH+3) human endometrium [41]	44	2.27	0.0000	0.0000	2778	11	6
Pod1_KO_dn — down-regulated in glomeruli isolated from Pod1 (TCF21)$^{-/-}$ mice versus wild-type controls	592	2.24	0.0005	0.0180	2576	53	27
Genes up-regulated at diestrus in bovine endometrium[h]	466	2.19	0.0009	0.0400	2731	44	25
VEGF_MMMEC_all_up — VEGF-induced genes in human myometrial microvascular endothelial cells	84	2.17	0.0011	0.0550	1949	14	7
Genes up-regulated in ovine endometrium between Days 9 and 12 of pregnancy [37]	358	2.07	0.0004	0.0010	2947	27	12
PGE2 up-regulated genes in human monocyte-derived dendritic cells [j]	121	1.92	0.0004	0.0060	2439	16	9

[a] Number of genes in a gene set that matched with the ranked gene list.
[b] NES, normalized enrichment score.
[c] FDR, false discovery rate.
[d] FWER, family-wise error rate.
[e] Genes in top 500 of pre-ranked gene list.
[f] Genes in top 250 of pre-ranked gene list.
[g] Affymetrix analysis of bovine endometrium from Day 18 pregnant animals vs. Day 18 controls.
[h] Affymetrix analysis of bovine endometrium estrus vs. diestrus.
[i] Estrogen-induced genes derived from different data sets (see *Results* [33–36]).
[j] Gene set derived from GEO gene expression series GSE8539.

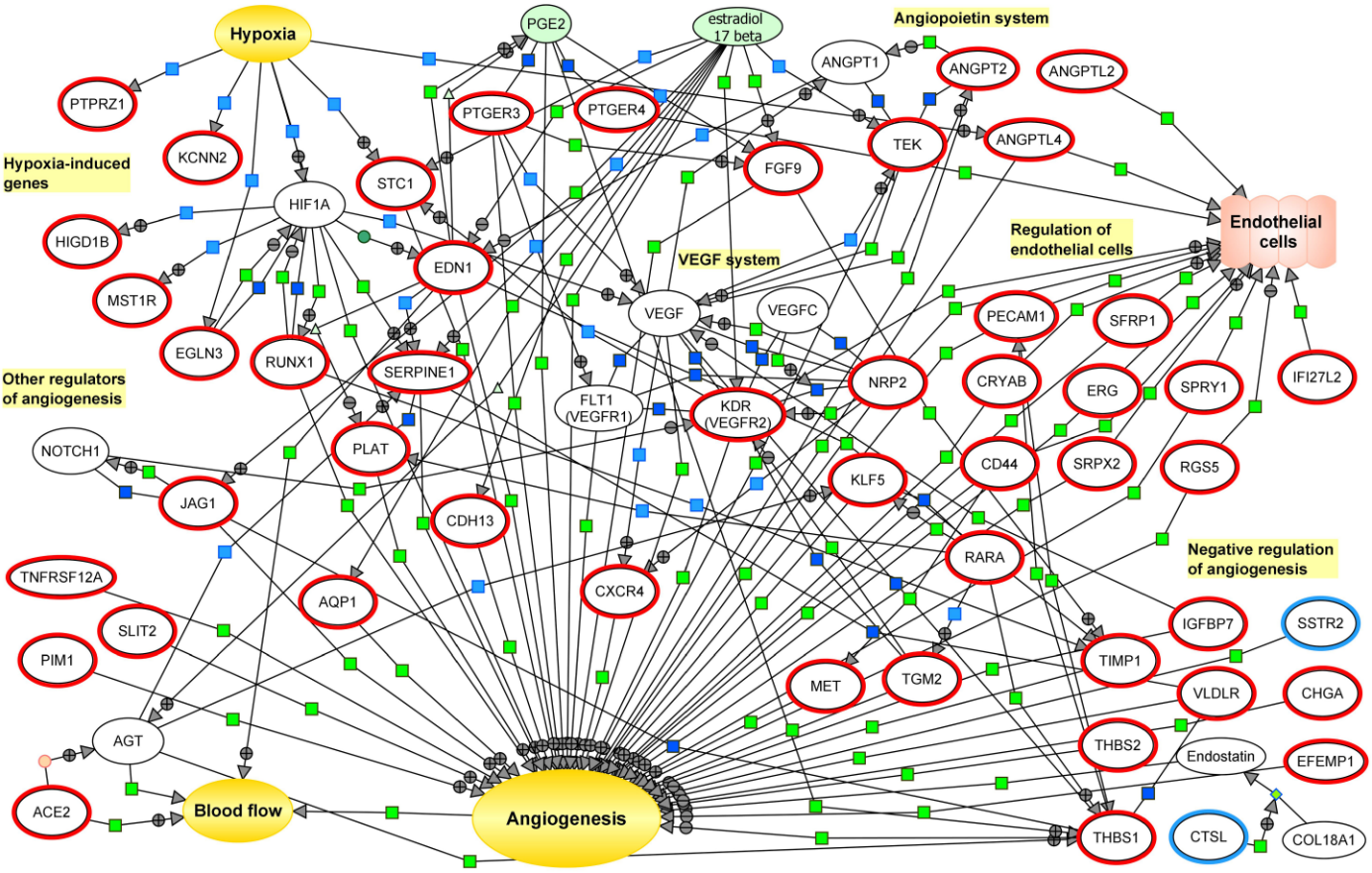


FIG. 2. Interaction network of genes related to the process of angiogenesis. Genes with higher mRNA levels in pregnant endometrium are highlighted in red, genes with lower levels in blue. Genes/proteins are in white, small molecules in green, and biological processes in yellow. Interaction types: dark blue squares: binding; light blue squares: expression; green squares: regulation; green circles: promoter binding; cyan triangles: transport; cyan diamonds: metabolism. Further information on nodes and interactions can be found in Supplement to Figure 2 (navigable HTML).

DISCUSSION

Biological Model and Quantitative Stereological Analysis of the Biopsy Samples

In order to reduce biological noise due to genetic variability in our biological model, pregnant and nonpregnant samples were obtained from the same mare (paired design) so that each animal served as its own control. The heatmap in Figure 1a demonstrates the relevance of genetic variability between animals by grouping the corresponding pregnant and control sample of each mare, thus confirming the importance of paired analysis. Potential effects by the order of sampling were excluded by randomization, i.e., for some animals the pregnant samples and for other animals the nonpregnant samples were taken first.

Because the endometrial tissue is composed of different cell types, such as surface epithelium, glandular epithelium, stromal cells, and blood vessels, all biopsy samples were analyzed by

TABLE 4. Differentially expressed genes involved in prostaglandin signaling and metabolism.

Eca gene symbol	Eca gene name	Eca Entrez gene ID	Hsa gene symbol	Hsa gene name	Hsa Entrez gene ID	FC Pr/Co[a]	q-value (%)
LOC100053557	similar to protaglandin receptor EP3E	100053557	*PTGER3*	prostaglandin E receptor 3 (subtype EP3)	5733	1.8	1.6
LOC100053208	similar to prostaglandin E2 receptor EP4 subtype	100053208	*PTGER4*	prostaglandin E receptor 4 (subtype EP4)	5734	2.0	0
LOC100070491	similar to prostaglandin F synthase	100070491	*AKR1CL1*	aldo-keto reductase family 1, member C-like 1	340811	2.3	1.3
LOC100070501	similar to prostaglandin F synthase	100070501	*AKR1CL1*	aldo-keto reductase family 1, member C-like 1	340811	2.2	2.1
PLA2G1B	phospholipase A2, group IB (pancreas)	100033889	*PLA2G4A*	phospholipase A2, group IVA (cytosolic, calcium-dependent)	5321	1.6	0.6
LOC100065438	hypothetical LOC100065438	100065438	*SLCO2A1*	solute carrier organic anion transporter family, member 2A1 (prostaglandin transporter)	6578	2.0	2.1
	ENSECAG00000004698		*PTGR1*	prostaglandin reductase 1	22949	2.7	1.8

[a] FC, fold change; Co, control; Pr, pregnant.

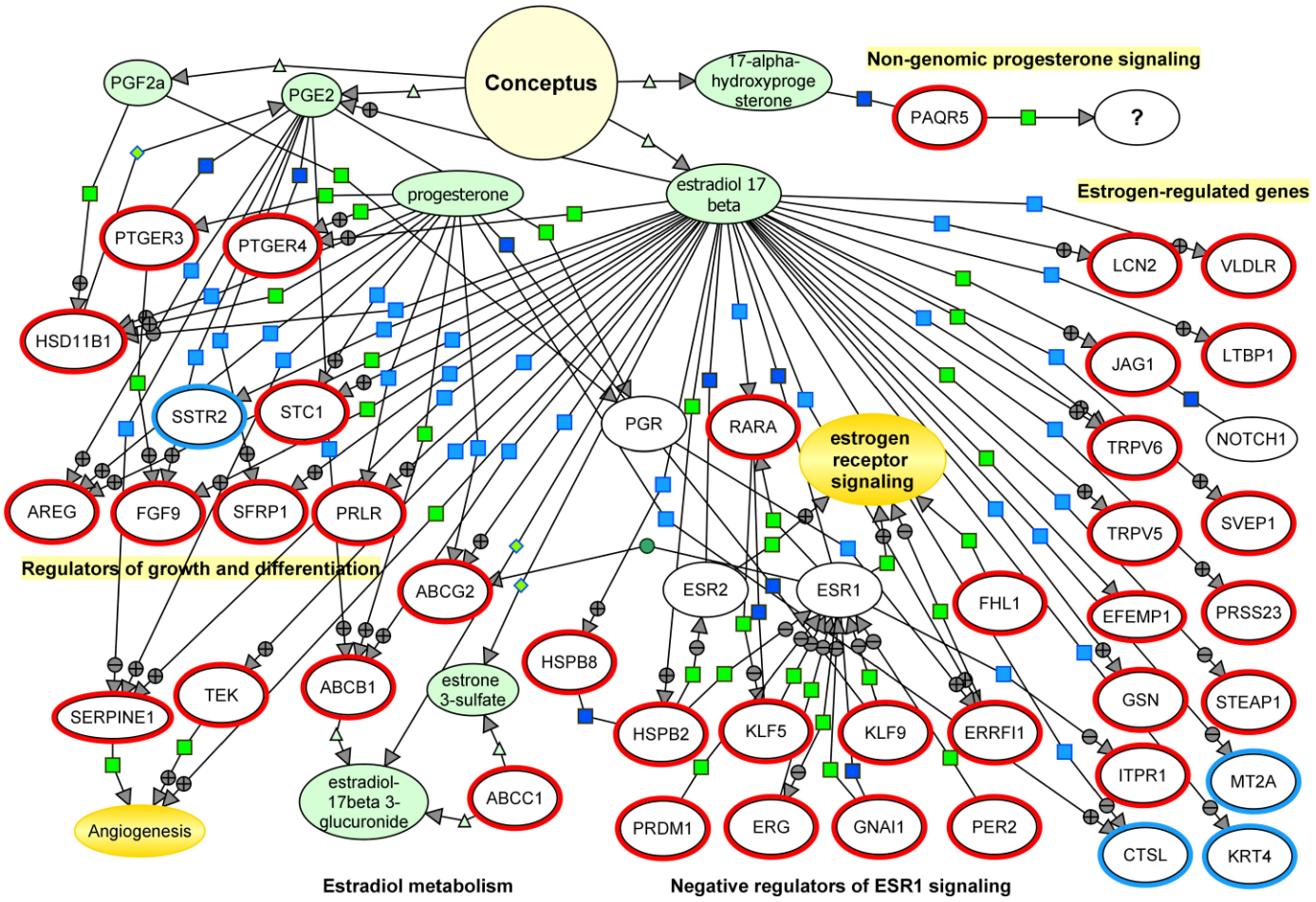


FIG. 3. Interaction network of genes related to steroid hormone and prostaglandin signaling. Genes with higher mRNA levels in pregnant endometrium are highlighted in red, genes with lower levels in blue. Genes/proteins are in white, small molecules in green, and biological processes in yellow. Interaction types: dark blue squares: binding; light blue squares: expression; green squares: regulation; green circles: promoter binding; cyan triangles: transport; cyan diamonds: metabolism. Further information on nodes and interactions can be found in Supplement to Figure 3 (navigable HTML).

quantitative stereological analysis to determine quantitative information about their tissue composition. Overall, tissue composition was very consistent within the biopsy samples. This is an important feature because biopsy sample composition can have a strong influence on microarray findings because of different mRNA concentration changes in different cell types. The 5.6-fold higher proportion of luminal epithelial cells in the Day 12 control sample of mare #3 indicated a different biopsy sample composition, most probably causing the lower or even inverse gene expression differences observed for many of the DEGs for this mare (Supplemental Table S2 and Fig. 1c). This finding underlines the importance to verify similar biopsy sample composition because this may have a strong influence on microarray results.

Differential Gene Expression at Days 8 and 12 and Between Days 8 and 12 of the Estrous Cycle

Microarray analysis of endometrial biopsy samples collected from Day 8 pregnant mares in comparison to corresponding control samples did not reveal any DEGs. Also, the exclusion of data from mare #3 because of an aberrant up-regulation of immune response genes in the pregnant sample did not result in identification of DEGs. Validation of 13 selected genes by qPCR confirmed the microarray data for these genes. This result suggests that there are no detectable changes in mRNA concentrations in endometrial biopsy samples on Day 8 of pregnancy in response to the early conceptus, which is in line with the beginning secretion of appreciable amounts of steroid metabolites by the equine embryo at around Day 10 of gestation [39, 40].

In contrast, significant expression differences were observed at Day 12 of pregnancy. For these genes, gene expression was also compared between the control samples of Days 8 and 12 and between pregnant samples of Days 8 and 12. Although the microarray analyses of Days 8 and 12 were performed at different times and slight technical biases influencing comparability of Day 8 and Day 12 data sets cannot be excluded, the results of the qPCR validation showed good agreement with the array results. The additional analysis of the expression between Day 8 and Day 12 control samples showed that most of the Day 12 (pregnant vs. control) down-regulated genes are down-regulated from Day 8 to Day 12 in the control samples as well, indicating an enhancement of down-regulation of these genes at Day 12 by the presence of a conceptus. Some of the genes up-regulated at Day 12 of pregnancy are also down-regulated from Day 8 to Day 12 in the control samples, i.e., the higher mRNA levels in Day 12 pregnant compared to Day 12 control samples are rather due to a prevention of down-regulation in response to the conceptus except for *FGF9* and *FGF9*-antisense transcripts, which are additionally up-regulated in Day 12 pregnant samples. Finally, an increased

expression from Day 8 to Day 12 in the control samples is further enhanced by the presence of a conceptus for some genes. These relatively complicated expression changes may be caused by the complex interactions of steroid hormone regulations in the equine endometrium.

Characterization of the DEGs by GSEA

GSEA revealed a number of enriched gene sets that provided a first characterization of the obtained DEGs and helped to identify genes that could have conserved functions across species. Overall, the number of genes overlapping with the top 500 genes of the ranked Day 12 gene list that contain the up-regulated genes was rather low for most of the identified gene sets. The gene set with the highest enrichment score was derived from the recently published study by Klein et al. of Day 13.5 pregnant endometrium in comparison to nonpregnant endometrium [30]. Similar to our results, more genes with higher expression levels in pregnant endometrium were found in this study. The overlap of the Day 13.5 up-regulated genes with the top 500 of our study was 24 (of 63) but only 2 for the down-regulated genes (in top 100 down-regulated genes). This could be an indication that there are different responses to the conceptus at these two time points of early pregnancy. However, comparability of the microarray results is limited because different Agilent microarrays (Klein et al. used a custom array) and different techniques (Klein et al.: dual-color hybridization and Axon scanner resulting in lower sensitivity) were used, and many of the probes on the custom array of Klein et al. are not well annotated. The significant overlap with gene sets containing genes up-regulated in human endometrium during the window of implantation [31, 41] indicates that there are similarities in gene expression changes in equine und human endometrium during early pregnancy. Furthermore, significant enrichment was found for genes induced at Day 14 of early pregnancy in porcine endometrium [38] and at Day 18 of early pregnancy in bovine endometrium (our unpublished data), but the number of genes overlapping with the top 500 of the Day 12 ranked gene list was relatively low. Higher numbers of overlapping genes with the top 500 were found for genes regulated during the estrous cycle in bovine endometrium and estrogen-induced genes in general. The gene set with the highest overlap with the top 500 genes (Boquest $CD31^+$ vs. $CD31^-$ [32]) comprised genes differentially expressed between two types of CD45 $(PTPRC)^-$ $CD34^+$ CD105 $(endoglin)^+$ stromal stem cells distinguished by the expression of CD31 (PECAM1). At first glance, the relatively high overlap with this gene set seems somewhat unexpected but can be explained by the different cell types present in the endometrium. For example, bovine endometrial stromal cells have been characterized to have similarities to mesenchymal progenitor cells [42]. Furthermore, the mRNA coding for CD31 (PECAM1), a marker of endothelial cells that has also been described in context of angiogenesis [43], was found as 1.6-fold up-regulated in the samples of Day 12 pregnant endometrium. Boquest et al. [32] described the $CD31^+$ cells as closely related to microvascular endothelial cells based on their up-regulated transcripts, which agrees well with the results of DAVID and CoPub where terms related to angiogenesis were found as quantitatively enriched. A substantial overlap was also found for the $CD31^+$ down-regulated gene set (38 genes in the top 500) that contains transcripts associated with extracellular matrix, transcripts that have been shown as expressed in early osteoblast differentiation, osteoclast-related transcripts, and transcripts typical of neuronal tissue [32]. Again, related terms were found with DAVID and CoPub, such as extracellular region, tissue remodeling, bone remodeling, neurogenesis, and inflammation. Overall, the identification of biologically very different gene sets could reflect 1) differential gene expression in different compartments of the endometrium and 2) a response to different embryonic signals. This corresponds to the fact that the equine conceptus produces different molecules [19], such as progesterone, E2, and prostaglandins.

Genes with Conserved Roles Across Species

The analysis of the endometrium-related gene sets from different species revealed a number of genes that could have conserved regulatory roles in the endometrium across species. Stanniocalcin 1 (*STC1*) has been described in multiple species, e.g., as a marker for implantation in pigs [44]. In sheep, *STC1* mRNA and protein are up-regulated in the uterine glands after Day 16 of pregnancy, probably regulating growth and differentiation of the fetus and placenta [45]. Increase of *STC1* expression has also been shown in rat uterus during embryo implantation and decidualization [46] and during the window of implantation in human endometrium [31]. In our gene expression study of bovine endometrium during the estrous cycle, highest expression levels were found at estrus, suggesting an up-regulation by E2 [47]. Crystallin, alpha B (*CRYAB*), coding for a member of the small heat shock protein (HSP20) family, is also up-regulated in human endometrium during the window of implantation [31, 41] and in bovine endometrium at Day 18 of pregnancy, as well as at estrus compared to diestrus (our unpublished data). In human myometrium CRYAB interacts with HSP27 (HSPB) and decreased CRYAB expression at the time of labor is thought to liberate HSP27 (HSPB) that participates in cytoskeletal remodeling in myometrial cells [48]. Up-regulation of *IGFBP2* was also found in porcine endometrium at Day 14 of pregnancy [38] and at Day 18 of pregnancy [49] as well as at estrus in bovine endometrium [47]. IGFBP2 expression has also been shown to be regulated by E2 and progesterone in human endometrial stromal cells [50]. Furthermore, *IGFBP1* has been reported as a common endometrial marker of conceptus elongation in sheep and cattle [51] and to mediate progesterone-induced decidualization in human endometrium [52]. In addition, IGFBP1 and TIMP metallopeptidase inhibitor 1 (TIMP1) have been demonstrated to inhibit trophoblast invasiveness in human endometrium [53, 54]. Tumor necrosis factor (ligand) superfamily member 10 (*TNFSF10*, *TRAIL*) mRNA has been shown to be up-regulated in human endometrium during the window of implantation [31] and in bovine endometrium at Day 18 of pregnancy [55]. Furthermore, a role of *TNFSF10* in the modulation of the cytokine milieu at the implantation site has been suggested based on the differential regulation of cytokines and chemokines in human endometrial stromal cells by TNFSF10 [56]. In addition to the genes at the top of Supplemental Table S4, a literature search revealed further genes described in the context of pregnancy in other species. Namely, amphiregulin (*AREG*), a member of the epidermal growth factor family, has been attributed a function in embryonic attachment in humans [53]. Abundant expression of insulin-like growth factor binding protein 7 (*IGFBP7*) has been found in human glandular epithelial cells during the secretory phase, and an in vitro knockdown revealed a role of IGFBP7 protein in differentiation of these cells [57]. In porcine endometrium induction of prolactin receptor (*PRLR*) mRNA by estradiol was shown, whereas coadministration of progesterone abolished this effect [58]. Expression of the PGE2 receptors *PTGER3* and *PTGER4* was investigated in the mouse uterus, and the observed

expression patterns in the preimplantation and postimplantation period indicated a role in uterine preparation for implantation and in the process of decidualization, respectively [59]. Moreover, a number of genes (e.g., *STC1*, *ATP2A3*, *TRPV5*, *TRPV6*) have been described in the context of calcium ion binding and regulation of calcium homeostasis that has been implicated in establishment and maintenance of pregnancy in pigs [60]. Finally, genes are up-regulated at Day 12 of pregnancy in equine endometrium that have been described as essential for successful pregnancy in the mouse, such as ERBB receptor feedback inhibitor 1 (*ERRFI1*) [61], a negative regulator of *ESR1* and nuclear receptor subfamily 2, group F, member 2 (*NR2F2*, *COUP-TFII*) [62–64]. *NR2F2* has been shown to repress the oxytocin gene promoter in human uterine epithelial cells [65] and to regulate stromal cell differentiation (decidualization) and, indirectly, the suppression of estrogen activity required for establishing a receptive uterus in the mouse [63]. In bovine endometrium we found increased expression at Day 18 of pregnancy [55] and decreased *NR2F2* transcript levels in endometrium from clone pregnancies vs. IVF pregnancies at Day 18 of pregnancy [66].

Genes Related to Angiogenesis and Vascular Remodeling

The search for quantitatively enriched functional terms (DAVID) and biological keywords (CoPub) associated with the Day 12 up-regulated genes revealed the highly enriched functional term angiogenesis. In the context of this process, increased endometrial vascular perfusion has been shown on Days 12–16 in both uterine horns of pregnant mares compared to nonpregnant mares by transrectal color Doppler ultrasonography [67]. Also, dysregulation of angiogenesis in the endometrium during early pregnancy has been found in the context of pregnancy failure [68]. To get an overview of the angiogenesis-related genes represented in the DEGs and their putative interactions, an interaction network was drawn (Fig. 2). DEGs were found for many regulatory systems of the complex process of angiogenesis, namely the VEGF system (receptors *KDR*, *NRP2*), the angiopoietin family (*ANGPT2*, *ANGPTL2*, *ANGPTL4*, *TEK*), different regulators of endothelial cells, and hypoxia-induced genes. There are also negative regulators of angiogenesis up-regulated in Day 12 pregnant endometrium, such as thrombospondins 1 and 2 (*THBS1*, *THBS2*), known inhibitors of endothelial cells and angiogenesis [69]. The complex regulation of angiogenesis and the results of the quantitative stereology (no difference in the proportion of blood vessels between pregnant and control samples) indicate that there is a remodeling of vascularization rather than neoangiogenesis or that neoangiogenesis is not yet microscopically detectable in Day 12 pregnant endometrium. This remodeling of vascularization is likely to play a role in maternal support of conceptus growth and in preparing the uterus for the prospective pregnancy.

Genes Related to Steroid Hormone and Prostaglandin Signaling

Furthermore, many genes were found that are probably regulated by the steroid hormones E2 and progesterone in Day 12 pregnant endometrium. This is in line with the finding that the embryo begins to secrete significant amounts of estrogens as early as Day 10 after ovulation [70, 71] and progesterone is the key hormone that prepares the endometrium for establishment and maintenance of pregnancy [72]. Conceptus estrogens are also supposed to have multiple effects on early pregnancy, such as stimulation of early conceptus migration and changes in uterine tonicity, blood flow, and endometrial secretory activity important to the nutrition of the preimplantation conceptus [73]. An important mediator of estrogen signaling in equine endometrium could be *FGF9* (microarray 9-fold, qPCR 8-fold up-regulated in Day 12 pregnant endometrium) that has been described as an autocrine endometrial stromal growth factor induced by E2 in human endometrial stroma [74]. Induction of *FGF9* expression by PGE2 through the EP3 receptor was also demonstrated in human endometrium [75]. In contrast to the localization in human endometrium, FGF9 protein expression in the porcine endometrium has been detected in the glandular epithelium at Day 14 of pregnancy [38]. The complex expression pattern of *FGF9* mRNA (see above) and the up-regulation of a putative antisense transcript (8-fold, Supplemental Table S2) make this gene an especially interesting candidate.

In addition to genes up-regulated by E2, a number of negative regulators of estrogen signaling, e.g., *KLF5*, *ERRFI1*, and *HSPB2* (Fig. 3), were found as up-regulated that could be indications for either a negative feedback regulation in response to the E2 signal or the result of progesterone action on the endometrium. A study of steroid metabolites produced by the equine conceptus revealed 17-alpha-OH-progesterone as the major steroid metabolite [39]. Interestingly, this metabolite binds to the progestin and adipoQ receptor family member V (PAQR5) [76], also known as membrane progestin receptor gamma, which is up-regulated in Day 12 pregnant endometrium (qPCR: 4.7-fold). PAQR5 is one of the receptors mediating nongenomic effects of progesterone. The equine conceptus is also known to secrete prostaglandins E2 and F2-alpha [12] that could play a role in pregnancy recognition and prevention of luteolysis. A number of genes that function in context of prostaglandin signaling and metabolism were found as up-regulated. Furthermore, mRNAs of PGE2 receptors EP3 (*PTGER3*) and EP4 (*PTGER4*) were up-regulated, similar to findings in the pig, in which *PTGER2* is up-regulated in early pregnancy [77]. However, in contrast to studies in porcine endometrium, mRNA levels of prostaglandin E synthases did not differ between pregnant and nonpregnant equine endometrium. There was also no difference in mRNA levels for the known $PGF_{2\alpha}$ synthases; only two predicted $PGF_{2\alpha}$ synthases that have homology to *AKR1CL1* (pseudogene in humans) were approximately 2-fold up-regulated. Unlike in ruminants, where up-regulation of mRNA for oxytocin receptor (*OXTR*) is prevented by the signaling of interferon tau [78], *OXTR* mRNA was slightly up-regulated in equine endometrium at Day 12 of pregnancy.

Genes Possibly Related to the Process of Mechanotransduction

Although the results of this study suggest an endometrial response to different signaling molecules, this does not exclude a mechanical signaling induced by the migrating conceptus. In a recent study a small intrauterine device (water-filled plastic ball with a diameter of 20 mm) was shown to induce prolonged luteal function [21], further supporting the concept of pregnancy recognition via mechanosensation. A study in sheep also described changes at the maternal-conceptus interface and uterine wall during pregnancy reflecting an increased mechanosensation and mechanotransduction [79]. Possibly, changes in mRNA expression levels at Day 12 of pregnancy in the mare could in part reflect mechanosensation responses to the conceptus. Some of the up-regulated genes of our study were already described in the context of mechanotransduction: a direct response to mechanical force has been shown for PECAM1 protein [80]; up-regulation of IGFBP1 secretion in

response to mechanical stretch was found by Harada et al. [81] in decidualized endometrial stromal cells; two members (*RND1*, *RND3*) of the Rho GTPase family (key regulators of cytoskeletal signaling) and a Rho GTPase activating protein (*ARHGAP29*) are up-regulated in Day 12 pregnant endometrium; and Rho activation has been described in the context of mechanotransduction-associated alveolar epithelial cell differentiation [82].

In conclusion, this study is the first systematic analysis of maternal transcriptome changes in response to the presence of an embryo in the mare on Days 8 and 12 of pregnancy. The stereological analysis of the biopsy samples showed that the homogenous composition of endometrial biopsies is an important issue for endometrial transcriptome analysis. No changes in endometrial gene expression were detectable at Day 8 of pregnancy. The DEGs identified on Day 12 in response to the early embryo evidence the orchestrated roles of estrogens, progesterone, and prostaglandin E2 in regulating gene expression in the equine endometrium in context of establishment and maintenance of pregnancy. Additionally, a form of mechanotransduction by the migrating conceptus is likely of importance. A large number of interesting candidate genes and biological processes were identified as potentially important for endometrial remodeling in response to the early embryo and need further detailed analysis.

ACKNOWLEDGMENTS

We would like to thank Dr. E. Senckenberg from the Bavarian principal and state stud of Schwaiganger, Germany, for providing mares for this study.

REFERENCES

1. Battut I, Colchen S, Fieni F, Tainturier D, Bruyas JF. Success rates when attempting to nonsurgically collect equine embryos at 144, 156 or 168 h after ovulation. Equine Vet J Suppl 1997; 29:60–62.
2. Betteridge KJ, Eaglesome MD, Mitchell D, Flood PF, Beriault R. Development of horse embryos up to twenty two days after ovulation: observations on fresh specimens. J Anat 1982; 135:191–209.
3. Oriol JG, Betteridge KJ, Clarke AJ, Sharom FJ. Developmentally regulated changes in the glycoproteins of the equine embryonic capsule. Mol Reprod Dev 1993; 34:255–265.
4. Sharp DC, McDowell KJ, Weithenauer J, Thatcher WW. The continuum of events leading to maternal recognition of pregnancy in mares. J Reprod Fertil Suppl 1989; 37:101–107.
5. Herrler A, Pell JM, Allen WR, Beier HM, Stewart F. Horse conceptuses secrete insulin-like growth factor-binding protein 3. Biol Reprod 2000; 62: 1804–1811.
6. Ginther OJ. Mobility of the early equine conceptus. Theriogenology 1983; 19:603–611.
7. Leith GS, Ginther OJ. Mobility of the conceptus and uterine contractions in the mare. Theriogenology 1985; 24:701–712.
8. Sharp DC. The early fetal life of the equine conceptus. Anim Reprod Sci 2000; 60–61:679–689.
9. Ginther OJ. Fixation and orientation of the early equine conceptus. Theriogenology 1983; 19:613–623.
10. Betteridge KJ. Equine embryology: an inventory of unanswered questions. Theriogenology 2007; 68(suppl 1):S9–S21.
11. Stout TA, Lamming GE, Allen WR. Oxytocin administration prolongs luteal function in cyclic mares. J Reprod Fertil 1999; 116:315–320.
12. Watson ED, Sertich PL. Prostaglandin production by horse embryos and the effect of co-culture of embryos with endometrium from pregnant mares. J Reprod Fertil 1989; 87:331–336.
13. Goff AK. Oxytocin stimulation of plasma 15-keto-13,14-dihydro prostaglandin F-2a during the oestrus cycle and early pregnancy in the mare. J Reprod Fertil Suppl 1987; 35:253–260.
14. Sharp DC, Thatcher MJ, Salute ME, Fuchs AR. Relationship between endometrial oxytocin receptors and oxytocin-induced prostaglandin F2 alpha release during the oestrous cycle and early pregnancy in pony mares. J Reprod Fertil 1997; 109:137–144.
15. Starbuck GR, Stout TA, Lamming GE, Allen WR, Flint AP. Endometrial oxytocin receptor and uterine prostaglandin secretion in mares during the oestrous cycle and early pregnancy. J Reprod Fertil 1998; 113:173–179.
16. Hershman L, Douglas RH. The critical period for the maternal recognition of pregnancy in pony mares. J Reprod Fertil Suppl 1979:395–401.
17. Boerboom D, Brown KA, Vaillancourt D, Poitras P, Goff AK, Watanabe K, Dore M, Sirois J. Expression of key prostaglandin synthases in equine endometrium during late diestrus and early pregnancy. Biol Reprod 2004; 70:391–399.
18. Ealy AD, Eroh ML, Sharp DC III. Prostaglandin H synthase Type 2 is differentially expressed in endometrium based on pregnancy status in pony mares and responds to oxytocin and conceptus secretions in explant culture. Anim Reprod Sci 2010; 117:99–105.
19. Betteridge KJ. Comparative aspects of equine embryonic development. Anim Reprod Sci 2000; 60–61:691–702.
20. Cochet M, Vaiman D, Lefevre F. Novel interferon delta genes in mammals: cloning of one gene from the sheep, two genes expressed by the horse conceptus and discovery of related sequences in several taxa by genomic database screening. Gene 2009; 433:88–99.
21. Rivera Del Alamo MM, Reilas T, Kindahl H, Katila T. Mechanisms behind intrauterine device-induced luteal persistence in mares. Anim Reprod Sci 2008; 107:94–106.
22. Huber W, von Heydebreck A, Sultmann H, Poustka A, Vingron M. Variance stabilization applied to microarray data calibration and to the quantification of differential expression. Bioinformatics 2002; 18(suppl 1): S96–S104.
23. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proc Natl Acad Sci U S A 2001; 98:5116–5121.
24. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005; 102: 15545–15550.
25. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol 2003; 4:P3.
26. Frijters R, Heupers B, van Beek P, Bouwhuis M, van Schaik R, de Vlieg J, Polman J, Alkema W. CoPub: a literature-based keyword enrichment tool for microarray data analysis. Nucleic Acids Res 2008; 36:W406–W410.
27. Ulbrich SE, Schulke K, Groebner AE, Reichenbach HD, Angioni C, Geisslinger G, Meyer HH. Quantitative characterization of prostaglandins in the uterus of early pregnant cattle. Reproduction 2009; 138:371–382.
28. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 2009; 55:611–622.
29. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001; 25:402–408.
30. Klein C, Scoggin KE, Ealy AD, Troedsson MH. Transcriptional profiling of equine endometrium during the time of maternal recognition of pregnancy. Biol Reprod 2010; 83:102–113.
31. Riesewijk A, Martin J, van Os R, Horcajadas JA, Polman J, Pellicer A, Mosselman S, Simon C. Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology. Mol Hum Reprod 2003; 9:253–264.
32. Boquest AC, Shahdadfar A, Fronsdal K, Sigurjonsson O, Tunheim SH, Collas P, Brinchmann JE. Isolation and transcription profiling of purified uncultured human stromal stem cells: alteration of gene expression after in vitro cell culture. Mol Biol Cell 2005; 16:1131–1141.
33. Li RW, Capuco AV. Canonical pathways and networks regulated by estrogen in the bovine mammary gland. Funct Integr Genomics 2008; 8: 55–68.
34. Kwon YS, Garcia-Bassets I, Hutt KR, Cheng CS, Jin M, Liu D, Benner C, Wang D, Ye Z, Bibikova M, Fan JB, Duan L, et al. Sensitive ChIP-DSL technology reveals an extensive estrogen receptor alpha-binding program on human gene promoters. Proc Natl Acad Sci U S A 2007; 104:4852–4857.
35. Li RW, Meyer MJ, Van Tassell CP, Sonstegard TS, Connor EE, Van Amburgh ME, Boisclair YR, Capuco AV. Identification of estrogen-responsive genes in the parenchyma and fat pad of the bovine mammary gland by microarray analysis. Physiol Genomics 2006; 27:42–53.
36. Lin CY, Vega VB, Thomsen JS, Zhang T, Kong SL, Xie M, Chiu KP, Lipovich L, Barnett DH, Stossi F, Yeo A, George J, et al. Whole-genome cartography of estrogen receptor alpha binding sites. PLoS Genet 2007; 3: e87.
37. Satterfield MC, Song G, Kochan KJ, Riggs PK, Simmons RM, Elsik CG, Adelson DL, Bazer FW, Zhou H, Spencer TE. Discovery of candidate

genes and pathways in the endometrium regulating ovine blastocyst growth and conceptus elongation. Physiol Genomics 2009; 39:85–99.
38. Ostrup E, Bauersachs S, Blum H, Wolf E, Hyttel P. Differential endometrial gene expression in pregnant and nonpregnant sows. Biol Reprod 2010; 83:277–285.
39. Goff AK, Leduc S, Poitras P, Vaillancourt D. Steroid synthesis by equine conceptuses between days 7 and 14 and endometrial steroid metabolism. Domest Anim Endocrinol 1993; 10:229–236.
40. Allen WR. Fetomaternal interactions and influences during equine pregnancy. Reproduction 2001; 121:513–527.
41. Mirkin S, Arslan M, Churikov D, Corica A, Diaz JI, Williams S, Bocca S, Oehninger S. In search of candidate genes critically expressed in the human endometrium during the window of implantation. Hum Reprod 2005; 20:2104–2117.
42. Donofrio G, Franceschi V, Capocefalo A, Cavirani S, Sheldon IM. Bovine endometrial stromal cells display osteogenic properties. Reprod Biol Endocrinol 2008; 6:65.
43. Woodfin A, Voisin MB, Nourshargh S. PECAM-1: a multi-functional molecule in inflammation and vascular biology. Arterioscler Thromb Vasc Biol 2007; 27:2514–2523.
44. Song G, Dunlap KA, Kim J, Bailey DW, Spencer TE, Burghardt RC, Wagner GF, Johnson GA, Bazer FW. Stanniocalcin 1 is a luminal epithelial marker for implantation in pigs regulated by progesterone and estradiol. Endocrinology 2009; 150:936–945.
45. Song G, Bazer FW, Wagner GF, Spencer TE. Stanniocalcin (STC) in the endometrial glands of the ovine uterus: regulation by progesterone and placental hormones. Biol Reprod 2006; 74:913–922.
46. Xiao LJ, Yuan JX, Song XX, Li YC, Hu ZY, Liu YX. Expression and regulation of stanniocalcin 1 and 2 in rat uterus during embryo implantation and decidualization. Reproduction 2006; 131:1137–1149.
47. Mitko K, Ulbrich SE, Wenigerkind H, Sinowatz F, Blum H, Wolf E, Bauersachs S. Dynamic changes in messenger RNA profiles of bovine endometrium during the oestrous cycle: focus on mammalian embryogenomics. Reproduction 2008; 135:225–240.
48. MacIntyre DA, Tyson EK, Read M, Smith R, Yeo G, Kwek K, Chan EC. Contraction in human myometrium is associated with changes in small heat shock proteins. Endocrinology 2008; 149:245–252.
49. Klein C, Bauersachs S, Ulbrich SE, Einspanier R, Meyer HH, Schmidt SE, Reichenbach HD, Vermehren M, Sinowatz F, Blum H, Wolf E. Monozygotic twin model reveals novel embryo-induced transcriptome changes of bovine endometrium in the preattachment period. Biol Reprod 2006; 74:253–264.
50. Giudice LC, Milkowski DA, Fielder PJ, Irwin JC. Characterization of the steroid-dependence of insulin-like growth factor-binding protein-2 synthesis and mRNA expression in cultured human endometrial stromal cells. Hum Reprod 1991; 6:632–640.
51. Simmons RM, Erikson DW, Kim J, Burghardt RC, Bazer FW, Johnson GA, Spencer TE. Insulin-like growth factor binding protein-1 in the ruminant uterus: potential endometrial marker and regulator of conceptus elongation. Endocrinology 2009; 150:4295–4305.
52. Matsumoto H, Sakai K, Iwashita M. Insulin-like growth factor binding protein-1 induces decidualization of human endometrial stromal cells via alpha5beta1 integrin. Mol Hum Reprod 2008; 14:485–489.
53. Giudice LC. Genes associated with embryonic attachment and implantation and the role of progesterone. J Reprod Med 1999; 44:165–171.
54. Zhang J, Salamonsen LA. Tissue inhibitor of metalloproteinases (TIMP)-1, -2 and -3 in human endometrium during the menstrual cycle. Mol Hum Reprod 1997; 3:735–741.
55. Bauersachs S, Ulbrich SE, Gross K, Schmidt SE, Meyer HH, Wenigerkind H, Vermehren M, Sinowatz F, Blum H, Wolf E. Embryo-induced transcriptome changes in bovine endometrium reveal species-specific and common molecular markers of uterine receptivity. Reproduction 2006; 132:319–331.
56. Fluhr H, Sauter G, Steinmuller F, Licht P, Zygmunt M. Nonapoptotic effects of tumor necrosis factor-related apoptosis-inducing ligand on interleukin-6, leukemia inhibitory factor, interleukin-8, and monocyte chemoattractant protein 1 vary between undifferentiated and decidualized human endometrial stromal cells. Fertil Steril 2009; 92:1420–1423.
57. Kutsukake M, Tamura K, Yoshie M, Tachikawa E. Knockdown of IGF-binding protein 7 inhibits transformation of the endometrial gland in an in vitro model. Mol Reprod Dev 2010; 77:265–272.
58. Trott JF, Horigan KC, Gloviczki JM, Costa KM, Freking BA, Farmer C, Hayashi K, Spencer T, Morabito JE, Hovey RC. Tissue-specific regulation of porcine prolactin receptor expression by estrogen, progesterone, and prolactin. J Endocrinol 2009; 202:153–166.
59. Yang ZM, Das SK, Wang J, Sugimoto Y, Ichikawa A, Dey SK. Potential sites of prostaglandin actions in the periimplantation mouse uterus: differential expression and regulation of prostaglandin receptor genes. Biol Reprod 1997; 56:368–379.
60. Choi Y, Seo H, Kim M, Ka H. Dynamic expression of calcium-regulatory molecules, TRPV6 and S100G, in the uterine endometrium during pregnancy in pigs. Biol Reprod 2009; 81:1122–1130.
61. Kim TH, Lee DK, Franco HL, Lydon JP, Jeong JW. ERBB receptor feedback inhibitor 1 regulation of estrogen receptor activity is critical for uterine implantation in mice. Biol Reprod 2010; 82:706–713.
62. Takamoto N, Kurihara I, Lee K, Demayo FJ, Tsai MJ, Tsai SY. Haploinsufficiency of chicken ovalbumin upstream promoter transcription factor II in female reproduction. Mol Endocrinol 2005; 19:2299–2308.
63. Kurihara I, Lee DK, Petit FG, Jeong J, Lee K, Lydon JP, DeMayo FJ, Tsai MJ, Tsai SY. COUP-TFII mediates progesterone regulation of uterine implantation by controlling ER activity. PLoS Genet 2007; 3:e102.
64. Petit FG, Jamin SP, Kurihara I, Behringer RR, DeMayo FJ, Tsai MJ, Tsai SY. Deletion of the orphan nuclear receptor COUP-TFII in uterus leads to placental deficiency. Proc Natl Acad Sci U S A 2007; 104:6293–6298.
65. Chu K, Zingg HH. The nuclear orphan receptors COUP-TFII and Ear-2 act as silencers of the human oxytocin gene promoter. J Mol Endocrinol 1997; 19:163–172.
66. Bauersachs S, Ulbrich SE, Zakhartchenko V, Minten M, Reichenbach M, Reichenbach HD, Blum H, Spencer TE, Wolf E. The endometrium responds differently to cloned versus fertilized embryos. Proc Natl Acad Sci U S A 2009; 106:5681–5686.
67. Silva LA, Gastal EL, Beg MA, Ginther OJ. Changes in vascular perfusion of the endometrium in association with changes in location of the embryonic vesicle in mares. Biol Reprod 2005; 72:755–761.
68. Tayade C, Fang Y, Hilchie D, Croy BA. Lymphocyte contributions to altered endometrial angiogenesis during early and midgestation fetal loss. J Leukoc Biol 2007; 82:877–886.
69. Iruela-Arispe ML, Luque A, Lee N. Thrombospondin modules and angiogenesis. Int J Biochem Cell Biol 2004; 36:1070–1078.
70. Zavy MT, Vernon MW, Sharp DC III, Bazer FW. Endocrine aspects of early pregnancy in pony mares: a comparison of uterine luminal and peripheral plasma levels of steroids during the estrous cycle and early pregnancy. Endocrinology 1984; 115:214–219.
71. Choi SJ, Anderson GB, Roser JF. Production of free estrogens and estrogen conjugates by the preimplantation equine embryo. Theriogenology 1997; 47:457–466.
72. Bazer FW, Spencer TE, Johnson GA, Burghardt RC, Wu G. Comparative aspects of implantation. Reproduction 2009; 138:195–209.
73. Stout TA, Allen WR. Oestrogens and pregnancy maintenance in the mare: for or against? Pferdeheilkunde 2001; 17:579–582.
74. Tsai SJ, Wu MH, Chen HM, Chuang PC, Wing LY. Fibroblast growth factor-9 is an endometrial stromal growth factor. Endocrinology 2002; 143:2715–2721.
75. Chuang PC, Sun HS, Chen TM, Tsai SJ. Prostaglandin E2 induces fibroblast growth factor 9 via EP3-dependent protein kinase Cdelta and Elk-1 signaling. Mol Cell Biol 2006; 26:8281–8292.
76. Smith JL, Kupchak BR, Garitaonandia I, Hoang LK, Maina AS, Regalla LM, Lyons TJ. Heterologous expression of human mPRalpha, mPRbeta and mPRgamma in yeast confirms their ability to function as membrane progesterone receptors. Steroids 2008; 73:1160–1173.
77. Waclawik A, Jabbour HN, Blitek A, Ziecik AJ. Estradiol-17beta, prostaglandin E2 (PGE2), and the PGE2 receptor are involved in PGE2 positive feedback loop in the porcine endometrium. Endocrinology 2009; 150:3823–3832.
78. Wolf E, Arnold GJ, Bauersachs S, Beier HM, Blum H, Einspanier R, Frohlich T, Herrler A, Hiendleder S, Kolle S, Prelle K, Reichenbach HD, et al. Embryo-maternal communication in bovine—strategies for deciphering a complex cross-talk. Reprod Domest Anim 2003; 38:276–289.
79. Burghardt RC, Burghardt JR, Taylor JD II, Reeder AT, Nguen BT, Spencer TE, Bayless KJ, Johnson GA. Enhanced focal adhesion assembly reflects increased mechanosensation and mechanotransduction at maternal-conceptus interface and uterine wall during ovine pregnancy. Reproduction 2009; 137:567–582.
80. Fujiwara K. Platelet endothelial cell adhesion molecule-1 and mechanotransduction in vascular endothelial cells. J Intern Med 2006; 259:373–380.
81. Harada M, Osuga Y, Takemura Y, Yoshino O, Koga K, Hirota Y, Hirata T, Morimoto C, Yano T, Taketani Y. Mechanical stretch upregulates IGFBP-1 secretion from decidualized endometrial stromal cells. Am J Physiol Endocrinol Metab 2006; 290:E268–E272.
82. Foster CD, Varghese LS, Gonzales LW, Margulies SS, Guttentag SH. The Rho pathway mediates transition to an alveolar type I cell phenotype during static stretch of alveolar type II cells. Pediatr Res 2010; 67:585–590.

3.2 Publication 2

Identification of differentially expressed genes in equine endometrium at day 12 of pregnancy

M. Merkl,[1,4] C. Otzdorff,[2] N. Herbach,[3] R. Wanke,[3] E. Wolf,[4] J. Handler,[1,5] and S. Bauersachs[4]

Clinic for Horses,[1] Clinic for Small Animal Surgery and Gynecology,[2] and Institute of Veterinary Pathology,[3] Center for Clinical Veterinary Medicine, and Laboratory for Functional Genome Analysis (LAFUGA),[4] Gene Center, Ludwig-Maximilian University Munich, Munich, Germany; Clinic for Horses,[5] Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

ANIMAL REPRODUCTION SCIENCE 2010; 121S: 288-290

Animal Reproduction Science 121S (2010) S288–S290

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Abstract

Identification of differentially expressed genes in equine endometrium at day 12 of pregnancy☆

M. Merkl[a,d], C. Otzdorff[b], N. Herbach[c], R. Wanke[c], E. Wolf[d], J. Handler[a], S. Bauersachs[d,*]

[a] Clinic for Horses, Center for Clinical Veterinary Medicine, LMU Munich, Germany
[b] Clinic for Small Animal Surgery and Gynecology, Center for Clinical Veterinary Medicine, LMU Munich, Germany
[c] Institute for Animal Pathology, Center for Clinical Veterinary Medicine, LMU Munich, Germany
[d] Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, LMU Munich, Feodor Lynen Str. 25, 81377 Munich, Germany

1. Introduction

Progesterone produced from a viable corpus luteum (CL) is essential for maintenance of early pregnancy in the mare. During the estrous cycle luteolysis takes place between days 14 and 16 after ovulation, due to an oxytocin-dependent pulsatile release of prostaglandin F2α (PGF2α) from the endometrium (Stout et al., 1999). The equine conceptus must therefore prevent luteal regression, a process commonly referred to as maternal recognition of pregnancy. Unlike in other large domestic animal species, the nature of embryo–maternal communication and maternal recognition of pregnancy in equids is still not well understood. To obtain a systematic overview of transcriptome changes in the equine endometrium underlying this complex embryo–maternal dialogue, a microarray study of endometrial biopsy samples from six mares at day 12 of early pregnancy and the corresponding non-pregnant stage was performed.

2. Materials and methods

Endometrial samples were collected from six warmblood mares belonging to the Bavarian principal and state stud of Schwaiganger, Germany. All mares were inseminated artificially with $>5 \times 10^8$ freshly collected, extended stallion spermatozoa. Follicular development and ovulation were monitored by daily transrectal palpation and ultrasound examination. Pregnancy was determined by transrectal ultrasonography and endometrial biopsies were obtained on day 12 of pregnancy. Non-pregnant control samples were obtained from the same mares on day 12 of a different estrous cycle within breeding season. Blood samples were collected for measurement of peripheral plasma progesterone concentrations. To estimate composition of the biopsies, they were cut transversely into six pieces, and every second piece was used for quantitative stereology to calculate the proportion of surface and glandular epithelium. From the remaining pieces of the biopsy samples total RNA was isolated using Trizol® Reagent (Invitrogen, Karlsruhe, Germany). Microarray analysis was performed using Agilent 4x44k Horse Gene Expression microarrays (AMADID 021322, Agilent Technologies, Waldbronn, Germany). Gene expression signals were filtered based on 'well above background' flags and normalized. Statistical analysis was performed with the Microsoft Excel add-in 'Significance analysis of microarrays' (SAM, two-class paired) (Tusher et al., 2001). Significance thresholds were set as follows: (1) false discovery rate (FDR) <5% and fold change at least 1.5-fold; (2) ratio fold change/q-value $\geq$0.75 to have greater confidence for smaller differences. The Agilent Horse microarray was re-annotated based on Ensembl 55, Entrez Gene and BLAST analyses to obtain equine and human (putative orthologous genes) Entrez Gene identifiers and the corresponding gene information. Functional analysis of the array data was performed using bioinformatics tools like Gene Set Enrichment Analysis (GSEA) (Subramanian et al., 2005) and the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Dennis et al., 2003).

☆ This paper is part of the supplement entitled "Proceedings of the Tenth International Symposium on Equine Reproduction", Guest Edited by Margaret J. Evans.

* Corresponding author.
E-mail address: bsachs@lmb.uni-muenchen.de (S. Bauersachs).

0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2010.04.088

Table 1
Results of Functional Annotation Clustering of genes up-regulated at day 12 of pregnancy.

Functional group description	Enrichment score[a]	# Genes[b]
Glycoprotein/signal peptide/extracellular region/disulfide bond	14.52	150
Developmental process/cell differentiation	10.57	121
Anatomical structure morphogenesis/blood vessel development/angiogenesis	8.34	50
EGF-like domain/EGF-like calcium-binding/calcium ion binding	5.54	37
Glycoprotein/membrane/plasma membrane	5.38	178
Carbohydrate binding/glycosaminoglycan binding	4.24	15
Response to external stimulus/response to stress/blood coagulation/wound healing	3.94	38
Cell differentiation/apoptosis/regulation of apoptosis/negative regulation of apoptosis	3.76	63
Anatomical structure formation/cell motility	3.63	28
Tissue development/tissue remodeling/bone remodeling	2.38	15
Nervous system development/cell morphogenesis/neurogenesis	2.01	34
Cell morphogenesis/cell growth	1.91	18
Signal transducer activity/receptor activity/transmembrane receptor activity	1.91	80
Cell proliferation/positive regulation of cell proliferation	1.79	38
Regulation of apoptosis	1.76	31
Cytoplasmic vesicle	1.69	14
Di-, tri-valent inorganic cation homeostasis	1.59	11
Enzyme regulator activity/endopeptidase inhibitor activity	1.54	22

[a] Geometric mean (in −log scale) of member's *p*-values of the corresponding annotation cluster.
[b] Total number of different genes in a functional group.

3. Results

Statistical analysis of microarray data revealed 374 differentially expressed genes in endometrial tissue samples of pregnant and control mares on day 12. Of these genes, 332 transcripts showed at least 1.5-fold greater gene expression values, and 42 transcripts showed lesser gene expression values in biopsy samples from pregnant endometrium compared to control biopsy samples. The gene expression data set was compared to a number of different gene sets (mainly derived from our unpublished data) containing, for example, genes up-regulated in endometrium of ovariectomized cows after estradiol treatment, genes up-regulated in bovine endometrium during estrus and diestrus, respectively, and genes up-regulated in endometrium of early pregnant pigs (data not shown). Significant enrichment was found for all of these gene sets in the data set but no predominant gene set could be found. DAVID Functional Annotation Clustering of the up-regulated genes resulted in a number of clusters of quantitatively enriched functional terms such as extracellular region, angiogenesis, calcium ion binding, cell growth, cell proliferation and differentiation (Table 1).

4. Discussion

Microarray analysis of endometrial biopsy samples collected from day 12 pregnant mares in comparison to corresponding control samples revealed several hundred differentially expressed genes, most of them with greater mRNA levels in pregnant samples. To reduce biological noise due to genetic variability, pregnant and non-pregnant samples were obtained from the same mare (paired design) so that each animal served as its own control.

Gene set enrichment analysis did not reveal clearly enriched gene sets corresponding to the obtained gene expression differences between day 12 pregnant and non-pregnant endometrium. However, there were significant overlaps with genes induced during early pregnancy in porcine endometrium, during estrus and after estrogen treatment in bovine endometrium but also with genes induced during the luteal phase in bovine endometrium. This finding corresponds to the different potential signaling molecules produced by the equine conceptus (Betteridge, 2000) and suggests a composition of different pregnancy recognition signals.

Analysis of the known or inferred functions of the identified up-regulated genes revealed a number of significantly enriched biological themes. One highly enriched functional group contains genes related to the process of angiogenesis. Dysregulation of angiogenesis in the endometrium during early pregnancy has been found in context with pregnancy failure (Tayade et al., 2007). Furthermore, a number of genes have been described in context of calcium ion binding and regulation of calcium homeostasis that has been implicated in establishment and maintenance of pregnancy in pigs (Choi et al., 2009). Genes related to cell growth, cell proliferation, and differentiation may reflect the endometrial remodeling needed for the support of embryo growth and development. Finally, several genes related to PGE_2 signaling and prostaglandin metabolism were regulated that could have a role for prevention of luteolysis.

This study is the first systematic analysis of maternal transcriptome changes in response to the presence of an embryo in the mare on day 12 of gestation and provides the basis for in-depth analyses of the complex changes in the equine endometrium in response to the early embryo.

Conflict of interest

None.

Acknowledgements

We would like to thank Dr. E. Senckenberg from the Bavarian principal and state stud of Schwaiganger, Germany for providing mares for this study. This study was supported by the German Ministry for Education and

Research (BMBF, FUGATO-plus, COMPENDIUM) and the German Research Foundation (DFG, Research Training Group 1029 Functional Genome Research in Veterinary Medicine).

References

Betteridge, K.J., 2000. Comparative aspects of equine embryonic development. Anim. Reprod. Sci. 60–61, 691–702.

Choi, Y., Seo, H., Kim, M., Ka, H., 2009. Dynamic expression of calcium-regulatory molecules, TRPV6 and S100G, in the uterine endometrium during pregnancy in pigs. Biol. Reprod. 81, 1122–1130.

Dennis Jr., G., Sherman, B.T., Hosack, D.A., Yang, J., Gao, W., Lane, H.C., Lempicki, R.A., 2003. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol. 4, P3.

Stout, T.A., Lamming, G.E., Allen, W.R., 1999. Oxytocin administration prolongs luteal function in cyclic mares. J. Reprod. Fertil. 116, 315–320.

Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., Mesirov, J.P., 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. U.S.A. 102, 15545–15550.

Tayade, C., Fang, Y., Croy, B.A., 2007. A review of gene expression in porcine endometrial lymphocytes, endothelium and trophoblast during pregnancy success and failure. J. Reprod. Dev. 53, 455–463.

Tusher, V.G., Tibshirani, R., Chu, G., 2001. Significance analysis of microarrays applied to the ionizing radiation response. Proc. Natl. Acad. Sci. U.S.A. 98, 5116–5121.

4 Discussion and Perspectives

In addition to the data discussed in Publication 1 “Microarray analysis of equine endometrium at days 8 and 12 of pregnancy” and Publication 2 “Identification of differentially expressed genes in equine endometrium at day 12 of pregnancy”, further aspects will be discussed regarding the early events underlying establishment and maintenance of pregnancy in the mare.

Uterocalin (P19 lipocalin)

The unusual long pre-attachment period of the conceptus is a special feature in equine pregnancy. Therefore, nutrition by endometrial gland secretions seems obviously necessary for survival and development of the rapidly growing conceptus before a direct contact between maternal and fetal tissues is established [16, 102].

One of the major progesterone-dependent endometrial proteins secreted by the endometrial glands in the mare is uterocalin (P19 lipocalin). Uterocalin sticks to the embryonic capsule as the conceptus moves through the uterine lumen and is thought to transport a range of biologically important lipids to the conceptus. Equids appear to need particularly large quantities of this protein during early pregnancy, and a limited ability of the endometrium to properly secrete P19 is supposed to be one cause for subfertility in mares. Furthermore the cessation of P19 secretion coincides with the beginning of capsule dissolution (days 20/21), suggesting uterocalin an important role in supplying the embryo before a direct contact is established between the maternal and fetal tissues [27, 103]. Furthermore, uterocalin has been detected in large amounts in the equine endometrium during diestrus and early pregnancy [104]. According to these results, *P19* mRNA yielded high expression levels in equine endometrium at day 12, independently of the presence of a conceptus.

Solute carrier family 36, member 2 (SLC36A2)

Solute carrier family 36, member 2 (*SLC36A2*) is likely of interest as it displayed the greatest expression fold change on day 12 (84.3-fold) and on day 13.5 [105] of pregnancy in equine endometrium, compared to the non-pregnant stage. *SLC36A2* encodes a transporter protein also known as tramdorin1 or PAT2 and is broadly expressed in mammalian tissues. PAT2 belongs to the SLC36 transporter family and is known to mediate the symport of protons and small amino acids. The more intensively studied family member PAT1 (SLC36A1) plays a dual role in mammals, depending on its cell-

specific subcellular localization. In brain neurons, its localization adjunct to lysosomes implies a role in the export of small amino acids generated by lysosomal proteolysis. On the other hand, in small intestinal epithelial cells, it is involved in the absorption of small amino acids and their derivatives at the apical membrane [106]. Although the physiological significance of PAT2 is yet not known, due to the tremendous up-regulation of *SLC36A2* in pregnant mares, it seems quite possible that it may contribute to the increasing histotrophe support essential for survival and development of the early conceptus.

Progestin and adipoQ receptor family, member 5 (PAQR5)

The steroid hormone progesterone is indispensable for mammalian reproduction by controlling key female reproductive events that range from ovulation to implantation, maintenance of pregnancy and mammary development. Not all effects of progesterone, however, can be explained by the classical model of steroid action and, like other steroid hormones, progesterone also elicits a variety of rapid signaling, independently of transcriptional or genomic regulation [107].

A candidate of special interest in the horse is progestin and adipoQ receptor family member-5 (*PAQR5*), which was significantly up-regulated in day 12 pregnant endometrium (qPCR: 4.7-fold). PAQR5, also known as membrane progestin receptor gamma (mPRγ) is one of the receptors mediating non-genomic effects of progesterone. What makes it even more interesting is that 17-alpha-OH-progesterone, which is the major steroid metabolite produced by the conceptus between days 7 and 14 [21], has been shown to bind to PAQR5 [108].

Membrane progestin receptors (mPRs) are thought to mediate rapid physiological functions in a variety of tissues, and several observations have sparked enormous interest in the role of these novel receptors in female reproduction [109]. In fish, for example, mPRs have been shown to mediate progesterone-dependent oocyte maturation and play an important role in stimulating sperm hypermotility [110, 111]. In mammals, mPRs have been implicated in the regulation of GnRH secretion in mice, and in the onset of parturition in humans [112]. Furthermore recent studies suggest that regulation of gamete transport in the oviduct is mediated by mPRβ and mPRγ, via non-genomic receptor mechanisms, in several species [113, 114].

However, information about endometrial membrane progestin receptors is still limited, but, in view of the large quantities of 17-alpha-OH-progesterone secreted by the conceptus and the up-regulation of *PAQR5* mRNA during early pregnancy, this signaling pathway needs to be further investigated, especially regarding its function in the pregnant uterus.

Estrogens and Estrogen receptor 1 (ESR1)

Microarray analysis also revealed a number of negative regulators of estrogen signaling, e.g., kruppel-like factor 5 (*KLF5*), ERBB receptor feedback inhibitor 1 (*ERRFI1*), and heat shock 27kDa protein 2 (*HSPB2*). However, it did not show differential expression of *ESR1* on day 12 of pregnancy, which coincides with the findings that *ESR1* expression levels do not differ at day 10, but are significantly decreased by days 13.5 and 15 of pregnancy in equine endometrium compared to the corresponding non-pregnant stages [105, 115]. These data indicate that there is a down-regulation or suppression of up-regulation of *ESR1* in pregnant mares during the time when cyclical luteolysis would normally occur, which is of particular interest because of the potential involvement of estrogens in suppressing luteal regression.

In face of the large quantities of estrogen synthesized by the equine conceptus from day 10 after ovulation [21], it might be possible that interfering actions of progesterone and conceptus-derived estrogens cause a down-regulation of *ESR1* from day 13.5 in pregnant mares. Indeed, the amount of steroid receptor mRNA has been shown to change with the fluctuating steroid environment in the equine endometrium. Furthermore, estrogens are known to regulate expression of its receptor, both positively and negatively, in various tissues including the uterus (42–44).

However, in view of these facts, and since estrogens are known to be the primary pregnancy recognition signal in pigs and regulation of its receptor via IFNτ plays a central role in inhibition of luteolysis in ruminants, further investigations are needed to discover the precise role of *ESR1* and its regulation in the context of MRP in the horse.

Additionally, conceptus estrogens are also supposed to have multiple effects on early pregnancy, such as stimulation of early conceptus migration [19], and changes in uterine tonicity, blood flow and endometrial secretory activity important for nutrition of the pre-implantation conceptus [116].

Oxytocin receptor (OXTR)

Oxytocin and its receptor play an important role in luteal regression in mares and ruminants by inducing the pulsatile release of luteolytic $PGF_{2\alpha}$ from the endometrium during late diestrus. In ruminants, the suppression of *ESR1* and *OXTR* by IFNτ is a central event in inhibiting luteolysis during early pregnancy [40]. Likewise, the response of $PGF_{2\alpha}$ to oxytocin in the mare is maximal at the time of luteolysis, but is completely diminished during early pregnancy and cannot be induced neither with endogenous nor with exogenous oxytocin [40, 51].

However, the decreased oxytocin responsiveness preventing luteolysis in days 11 – 14 pregnant mares [40] is controversially discussed. In one study, the measurement of endometrial OXTR protein concentrations revealed significantly increased amounts on day 14 of the estrous cycle, but no such increase was evident during pregnancy, suggesting that up-regulation of endometrial OXTR is suppressed by the conceptus during early pregnancy [51]. In another study, the oxytocin receptor density in pregnant mares was similar to that in non-pregnant mares but affinity of the oxytocin receptors was lower [41]. In our study, *OXTR* mRNA was up-regulated 1.6-fold in eindometrial biopsy samples from day 12 pregnant mares. Regarding the decreased responsiveness to oxytocin during this time, these results suggest that inhibition of $PGF_{2\alpha}$ release in pregnant mares may occur rather due to a lower affinity of OXTR towards oxytocin or an uncoupling of the oxytocin-induced release of $PGF_{2\alpha}$, than due to a suppression of *OXTR* expression as it has been reported in ruminants. However, other mechanisms cannot be excluded to regulate OXTR expression and information on how the abundance of both mRNA and protein fluctuate is required to gain a more complete understanding of its regulation during early pregnancy.

Prostaglandin $F_{2\alpha}$ synthesis

Since endometrial $PGF_{2\alpha}$ production is largely suppressed during early pregnancy in mare, several approaches were made to investigate whether the equine conceptus directly suppresses uterine $PGF_{2\alpha}$ synthesis.

One approach targets cytosolic phospholipase A2 (PLA2G4A; cPLA2). cPLA2 is activated by increased intracellular calcium (Ca^{2+}) levels, resulting in its translocation from the cytosol and nucleus to perinuclear membrane vesicles. It is thought to mediate endometrial $PGF_{2\alpha}$ production in the horse endometrium as its expression has been shown to be negatively correlated with peripheral plasma progesterone concentrations and it is therefore highly expressed in the endometrium at the expected time of luteolysis. Measurement of *cPLA2* mRNA expression levels during the estrous cycle reported basal levels on day 8, reaching its maximum at day 15, the expected time of luteolysis. Furthermore, equal to lower expression levels have been reported for pregnant mares at day 15 compared to day 15 of the estrous cycle, depending on plasma progesterone concentrations [117]. In our studies day 8 and day 12 controls also showed similar expression levels, and microarray analysis revealed slightly higher levels of *cPLA2* in pregnant mares on days 12 (1.6-fold) and 16 (1.7-fold, our own unpublished data), compared to day 12 of the estrous cycle, indicating that *cPLA2* expression is not regulated during diestrus prior to the expected time of luteolysis. However, in view of the slightly up-regulated *cPLA2* levels in pregnant endometrium, further analysis needs to provide a

better understanding for its role during early pregnancy, although it has to be kept in mind that cPLA2 only generates the first intermediate of prostaglandin synthesis,.

Furthermore, studies were completed to establish whether the conceptus influences PTGS2, a rate-limiting enzyme in prostaglandin synthesis. It has been suggested that the presence of a conceptus blocks endometrial $PGF_{2\alpha}$ synthesis, at least in part, by repressing induction of *PTGS2* expression at days 14/15 of pregnancy and in this way contributes an important mechanism for preventing luteolysis [52, 53]. Importantly, *PGHS2* expression in day 14 and day 15 cyclic endometrium has been shown to be significantly increased (54-fold; 6-fold) in relation to the corresponding days of early pregnancy and to other time points of the diestrus. Additionally, *PGHS2* expression levels in day 15 pregnant endometrium were similar to those observed in Day 10 and Day 13 cyclic animals [52]. In our study, *PGHS2* did not show significant expression differences between pregnant and cyclic mares on day 12, thus confirming the previous findings that *PGHS2* may not be induced before day 14 of the estrous cycle.

Finally, mRNA expression of *PTGFS* has recently been studied, reporting greater expression levels in both, cyclic and pregnant mares at days 14-18, compared to *PTGFS* expression levels day 0 of the estrous cycle. Furthermore, *PTGFS* expression levels were significantly higher in cyclic compared to pregnant mares ($p<0.05$) on day 14, the expected time of luteolysis [118]. However, in another study, both, *PTGFS* mRNA and PTGFS protein levels were found to be invariant throughout days 10-15 of the estrous cycle and unaffected by pregnancy at day 15 [52]. Microarray analysis as well did not detect differences in mRNA levels for the known *PTGFS* in pregnant and control mares on day 8 or on day 12, indicating that *PTGFS* is at least not targeted by the conceptus until day 12 of pregnancy.

Prostaglandin transporter (PGT)

Microarray analysis also revealed a number of genes up-regulated in day 12 pregnant endometrium that function in the context of prostaglandin signaling and metabolism, such as *SLCO2A1* (solute carrier organic anion transporter family, member 2A1), commonly known as prostaglandin transporter (PGT). PGT is an uptake carrier of prostaglandins with high affinity for PGE_2 and $PGF_{2\alpha}$, and constitutes an important part of the prostaglandin signal transduction cascade as it contributes to the regulation of local prostaglandin concentrations, signal termination and metabolic clearance [119, 120]. In human females, PGT have been shown to mediate regulation of prostaglandin action in reproductive processes. In endometrial stromal cells, for example, PGT and its mRNA are up-regulated during decidualization to mediate the higher uptake of prostaglandins,

required for the initiation and maintenance of decidualization [121]. Therefore it is likely that the up-regulation of *SLCO2A1* detected in our study in early pregnant horses (2.0-fold) also contributes to the regulation of prostaglandin actions in the endometrium, probably involved in preparing the uterus for the upcoming pregnancy.

Prostaglandin E_2 synthesis and Prostaglandin E_2 receptors

In many species, both, the conceptus and the endometrium, also synthesize PGE_2, which is thought to counteract the luteolytic effects of $PGF_{2\alpha}$, thereby playing a luteoprotective role. PGE_2 has also been shown to have a luteoprotective effect in early pregnant pigs. Moreover, the porcine blastocyst is supposed to change the PGE_2:$PGF_{2\alpha}$-ratio secreted from the uterus in favor of luteoprotective PGE_2 by modulating expression of the key enzymes in endometrial PG synthesis [66-68]. Similar effects have also been proposed in ruminants [96, 97].

The equine conceptus is also known to secrete PGE_2 [43], which possibly plays a luteoprotective role in the mare. However, although expression differences have been reported for day 14 pregnant compared non-pregnant mares, mRNA levels for both, *PTGFS* and *PTGES*, did not differ between pregnant and non-pregnant mares on day 12. Additionally, the mRNA encoding *CBR1*, which converts PGE_2 into $PGF_{2\alpha,}$ was not differentially expressed in pregnant mares as well. Thus, the analysis of mRNA expression indicates that i) endometrial PGE_2 synthesis is not increased during early pregnancy and that ii) the equine conceptus does not affect the endometrial PGE_2:$PGF_{2\alpha}$ ratio at least until day 12 of pregnancy.

Furthermore, an important role has been suggested for endometrial PGE_2 receptors (PTGER) in the embryo-maternal dialogue in mammals, as they mediate local effects of PGE_2. Four subtypes of G protein-coupled receptors (PTGER1-4), which are encoded by four separate genes, are known, but distribution and function varies among species.

In early pregnant pigs, PGE_2 is known to act mainly through endometrial PTGER2, resulting in local increase of endometrial vascular permeability and preparation for angiogenesis and implantation. Moreover, PTGER2 mRNA and protein, localized in luminal and glandular epithelium and blood vessels of porcine endometrium, were significantly up-regulated during early pregnancy and it has been suggested that estrogens, PGE_2 and endometrial PTGER2 are involved in a PGE_2 positive feedback loop [69].

In our study, microarray analysis revealed up-regulation of *PTGER3* and *PTGER4* mRNA, similar to findings in the pig, in which *PTGER2* is up-regulated in endometrium during

early pregnancy. Expression of *PTGER3* and *PTGER4* have also been investigated in the mouse uterus, and the observed expression patterns in the pre-implantation and post-implantation period indicated a role in uterine preparation for implantation and in the process of decidualization [122]. However, the specific roles of these receptors in the equine endometrium remain to be elucidated.

Mechanosensation

In a recent study small intrauterine devices (water-filled plastic ball with a diameter of 20 mm) were shown to induce prolonged luteal function in the mare [123], further supporting the concept of pregnancy recognition via mechanosensation, since the hypothesis that an IUD might achieve luteostasis through mild inflammation of the endometrium could not be confirmed. It is suggested that the close contact to or pressure of an IUD on the uterine wall may induce changes in the endometrial cells and therefore prevent them from releasing luteolytic pulses of $PGF_{2\alpha}$.

Mechanosensation has also been reported to play a role in reproduction in other mammals. In humans, for example, the initial contact between the blastocyst and maternal tissues is by adhesion of the trophoblast to the uterine epithelium. This event is hormonally controlled and requires a certain degree of pressure between the cell surfaces [124]. Furthermore, a recent study in sheep has also described changes at the maternal-conceptus interface and uterine wall during pregnancy, reflecting an increased mechanosensation or mechanotransduction [125].

In our study, some DEGs in day 12 pregnant endometrium have already been described in context with mechanosensation and could in part reflect a response to a form of mechanotransduction by the migrating conceptus. Therefore, although the results of our study show an endometrial response to different signaling molecules, a mechanical signaling induced by the migrating conceptus is not excluded.

5 Summary

The horse exhibits a number of unusual features during early pregnancy, which are unique to the genus *Equus* and differ considerably from corresponding events in other large domestic animal species. Moreover, the establishment and maintenance of pregnancy in the mare are only partially understood. In order to provide new insights into the early events of pregnancy in the horse, a systematic analysis of maternal transcriptome changes in equine endometrium in response to the presence of a conceptus on days 8 and 12 of pregnancy was performed.

Endometrial biopsy samples were collected from six Bavarian Warmblood mares on days 8 and 12 of pregnancy and the corresponding non-pregnant stages. Pregnant and non-pregnant samples were taken from the same mare respectively (paired design) in order to reduce biological noise due to genetic variability. The proportions of surface epithelium, glandular epithelium and blood vessels in the biopsy samples were determined with quantitative stereological techniques to ensure homogenous tissue composition. Microarray analysis was performed using Agilent 4x44k Horse Gene Expression microarrays, and expression of selected genes was validated by quantitative real-time RT-PCR.

Microarray analysis did not reveal significant changes in endometrial gene expression in day 8 pregnant mares compared to day 8 of the estrous cycle, whereas 374 genes were differentially expressed in endometrium from day 12 of pregnancy, 332 with higher and 42 with lower transcript levels than in day 12 non-pregnant mares.

Gene set enrichment analysis (GSEA), functional annotation clustering and co-citation analysis were performed to characterize the DEGs in day 12 pregnant mares in response to the presence of a conceptus. Furthermore, two interaction networks of i) genes related to steroid hormone and prostaglandin signaling, and ii) of genes related to angiogenesis and vascular remodeling were generated.

Many known estrogen-induced genes and genes involved in regulation of estrogen signaling were found, but also genes known to be regulated by progesterone and PGE_2, that evidence their orchestrated roles in regulating gene expression in the pregnant mare. Additionally, some differentially expressed genes possibly reflect a form of mechanotransduction by the migrating conceptus. Further, a number of genes related to endometrial remodeling, in particular regarding angiogenesis and vascular remodeling were found. Finally, GSEA revealed genes that probably have conserved functions across species, such as *CRYAB*, *ERRFI1*, *FGF9*, *IGFBP2*, *NR2F2*, *STC1*, and *TNFSF10*.

In conclusion, this study is the first systematic analysis of maternal transcriptome changes in response to the presence of an embryo in the mare on days 8 and 12 of pregnancy. This study revealed the potential target genes and pathways of conceptus-derived estrogens, progesterone, and PGE_2 in the equine endometrium probably involved in the early events of establishment and maintenance of pregnancy in the mare. A large number of interesting candidate genes and biological processes were identified as potentially important for endometrial remodeling in response to the early embryo, providing the basis for continuative in-depth analyses.

6 Zusammenfassung

Pferde zeigen während der Frühträchtigkeit eine Reihe ungewöhnlicher Merkmale, die eine Besonderheit der Gattung *Equus* sind und sich beträchtlich von den entsprechenden Ereignissen anderer großer Haussäugetierspezies unterscheiden. Darüber hinaus sind die Etablierung und auch der Erhalt der Trächtigkeit bei der Stute nur teilweise verstanden. Um die maternalen Genexpressionsänderungen als Reaktion auf die Anwesenheit eines Konzeptus zu erfassen und somit neue Einblicke in die frühe Tächtigkeit beim Pferd zu bekommen, wurde eine systematische Transkriptomanalyse des Endometriums trächtiger Stuten an Tag 8 und 12 durchgeführt.

Endometriumproben wurden von sechs Bayerischen Warmblutstuten an Tag 8 und Tag 12 der Trächtigkeit und an den entsprechenden Tagen des Zyklus entnommen. Trächtige und nicht-trächtige Proben stammten jeweils von denselben Stuten (gepaartes Design), um Schwankungen aufgrund der genetischen Variabilität zu verringern. Um eine homogene Gewebszusammensetzung zu gewährleisten, wurden die Volumenanteile von Oberflächenepithel, Drüsenepithel und Blutgefäßen der Biopsieproben mithilfe quantitativ stereologischer Techniken bestimmt. Die Mikroarray-Analysen wurden mittels Agilent 4x44k Horse Gene Expression Mikroarrays durchgeführt und die Expression ausgewählter Gene durch quantitative real-time RT-PCR validiert.

Die Mikroarray-Analyse zeigte keine signifikanten Änderungen der Genexpression an Tag 8 der Trächtigkeit im Vergleich zu Tag 8 des Zyklus. An Tag 12 dagegen konnten 374 differentiell exprimierte Gene (DEGs) im Endometrium identifiziert werden, 332 mit höheren und 42 mit niedrigeren mRNA-Konzentrationen in trächtigen im Vergleich zu nicht-trächtigen Stuten.

Gene Set Enrichment-Analysen (GSEA), Functional Annotation Clustering und Co-Zitations-Analysen wurden durchgeführt, um die DEGs im equinen Endometrium an Tag 12 der Trächtigkeit zu charakterisieren. Desweiteren wurden zwei Interaktionsnetzwerke von Genen erstellt die im Zusammenhang mit i) Steroidhormon- und Prostaglandin Signalwegen und ii) Angiogenese und vaskulärem Umbau stehen.

Viele Östrogen-induzierte Gene und Gene die in Östrogen-Signalwege involviert sind, aber auch eine Reihe von Genen, die von Progesteron und PGE_2 reguliert werden, konnten detektiert werden, was deren Einfluss auf die Regulierung der Genexpression im Endometrium der trächtigen Stute widerspiegelt. Darüber hinaus deuten einige DEGs möglicherweise auf eine Form der Mechanotransduktion durch den mobilen Konzeptus hin. Weiter wurden viele Gene gefunden die im Zusammenhang mit den

Umbauprozessen des Endometriums stehen, vor allem bezüglich Angiogenese und vaskulärer Umstrukturierung. Letztlich deckte die GSEA Gene auf, die höchstwahrscheinlich speziesübergreifend eine konservierte Funktion innehaben, wie *CRYAB*, *ERRFI1*, *FGF9*, *IGFBP2*, *NR2F2*, *STC1*, und *TNFSF10*.

Zusammenfassend ist diese Studie die erste systematische Analyse der Transkriptomänderungen im Endometrium der Stute als Reaktion auf die Anwesenheit eines Embryos an Tag 8 und 12 der Frühträchtigkeit. Die Untersuchungen veranschaulichen die potentiellen Zielgene und Signalwege der vom Konzeptus sezernierten Östrogene, Progesteron und PGE_2 im equinen Endometrium, die vermutlich in die frühen Geschehnisse der Etablierung und den Erhalt der Trächtigkeit der Stute involviert sind. Eine große Anzahl interessanter Kandidatengene und biologischer Prozesse, die für Umbauvorgänge im trächtigen Endometrium von Bedeutung sind, konnten aufgezeigt werden und bieten so die Basis für weiterführende, detaillierte Untersuchungen.

7 References

1. Aurich C. Reproduktionsmedizin beim Pferd. Parey Verlag; 2005: 15-28.
2. Engelhardt von W, Breves G. Physiologie der Haustiere. Enke Verlag; 2005: 495-517.
3. Flood PF, Jong A, Betteridge KJ. The location of eggs retained in the oviducts of mares. J Reprod Fertil 1979; 57:291-294.
4. Betteridge KJ, Mitchell D. Direct evidence of retention of unfertilized ova in the oviduct of the mare. J Reprod Fertil 1974; 39:145-148.
5. Weber JA, Freeman DA, Vanderwall DK, Woods GL. Prostaglandin E2 secretion by oviductal transport-stage equine embryos. Biol Reprod 1991; 45:540-543.
6. Freeman DA, Weber JA, Geary RT, Woods GL. Time of embryo transport through the mare oviduct. Theriogenology 1991; 36:823-830.
7. Weber JA, Woods GL, Freeman DA, Vanderwall DK. Prostaglandin E2-specific binding to the equine oviduct. Prostaglandins 1992; 43:61-65.
8. Weber JA, Freeman DA, Vanderwall DK, Woods GL. Prostaglandin E2 hastens oviductal transport of equine embryos. Biol Reprod 1991; 45:544-546.
9. Battut I, Colchen S, Fieni F, Tainturier D, Bruyas JF. Success rates when attempting to nonsurgically collect equine embryos at 144, 156 or 168 hours after ovulation. Equine Vet J Suppl 1997:60-62.
10. Betteridge KJ, Eaglesome MD, Mitchell D, Flood PF, Beriault R. Development of horse embryos up to twenty two days after ovulation: observations on fresh specimens. J Anat 1982; 135:191-209.
11. Ginther OJ. Mobility of the early equine conceptus. Theriogenology 1983; 19:603-611.
12. Flood PF, Betteridge KJ, Diocee MS. Transmission electron microscopy of horse embryos 3-16 days after ovulation. J Reprod Fertil Suppl 1982; 32:319-327.
13. Stout TA, Meadows S, Allen WR. Stage-specific formation of the equine blastocyst capsule is instrumental to hatching and to embryonic survival in vivo. Anim Reprod Sci 2005; 87:269-281.
14. Albihn A, Waelchli RO, Samper J, Oriol JG, Croy BA, Betteridge KJ. Production of capsular material by equine trophoblast transplanted into immunodeficient mice. Reproduction 2003; 125:855-863.
15. Oriol JG, Betteridge KJ, Clarke AJ, Sharom FJ. Mucin-like glycoproteins in the equine embryonic capsule. Mol Reprod Dev 1993; 34:255-265.
16. Allen WR. Fetomaternal interactions and influences during equine pregnancy. Reproduction 2001; 121:513-527.
17. McDowell KJ, Sharp DC, Grubaugh W, Thatcher WW, Wilcox CJ. Restricted conceptus mobility results in failure of pregnancy maintenance in mares. Biol Reprod 1988; 39:340-348.
18. Sharp DC. The early fetal life of the equine conceptus. Anim Reprod Sci 2000; 60-61:679-689.
19. Stout TA, Allen WR. Role of prostaglandins in intrauterine migration of the equine conceptus. Reproduction 2001; 121:771-775.
20. Stout TA, Allen WR. Prostaglandin E(2) and F(2 alpha) production by equine conceptuses and concentrations in conceptus fluids and uterine flushings recovered from early pregnant and dioestrous mares. Reproduction 2002; 123:261-268.
21. Goff AK, Leduc S, Poitras P, Vaillancourt D. Steroid synthesis by equine conceptuses between days 7 and 14 and endometrial steroid metabolism. Domest Anim Endocrinol 1993; 10:229-236.
22. Vernon MW, Zavy MT, Asquith RL, Sharp DC. Prostaglandin F2alpha in the equine endometrium: steroid modulation and production capacities during the estrous cycle and early pregnancy. Biol Reprod 1981; 25:581-589.
23. Betteridge KJ, Waelchli RO. Equine embryo encapsulation; ephemeral, essential and enigmatic. Havemeyer Found Monogr Ser 2005; 16:59-61.

24. Oriol JG, Sharom FJ, Betteridge KJ. Developmentally regulated changes in the glycoproteins of the equine embryonic capsule. J Reprod Fertil 1993; 99:653-664.
25. Crossett B, Suire S, Herrler A, Allen WR, Stewart F. Transfer of a uterine lipocalin from the endometrium of the mare to the developing equine conceptus. Biol Reprod 1998; 59:483-490.
26. Kennedy MW. Uterocalin - provider for essential lipids and amino acidsto the pre-placentation equine conceptus. Havemeyer Found Monogr Ser 2005; 16:53-56.
27. Stewart F, Kennedy MW, Suire S. A novel uterine lipocalin supporting pregnancy in equids. Cell Mol Life Sci 2000; 57:1373-1378.
28. Herrler A, Pell JM, Allen WR, Beier HM, Stewart F. Horse conceptuses secrete insulin-like growth factor-binding protein 3. Biol Reprod 2000; 62:1804-1811.
29. Ginther OJ. Fixation and orientation of the early equine conceptus. Theriogenology 1983; 19:613-623.
30. Allen WR, Stewart F. Equine placentation. Reprod Fertil Dev 2001; 13:623-634.
31. Gastal MO, Gastal EL, Kot K, Ginther OJ. Factors related to the time of fixation of the conceptus in mares. Theriogenology 1996; 46:1171-1180.
32. Arar S, Chan KH, Quinn BA, Waelchli RO, Hayes MA, Betteridge KJ, Monteiro MA. Desialylation of core type 1 O-glycan in the equine embryonic capsule coincides with immobilization of the conceptus in the uterus. Carbohydr Res 2007; 342:1110-1115.
33. Hayes MA, Quinn BA, Keirstead ND, Katavolos P, Waelchli RO, Betteridge KJ. Proteins associated with the early intrauterine equine conceptus. Reprod Domest Anim 2008; 43 Suppl 2:232-237.
34. Quinn BA, Hayes MA, Waelchli RO, Kennedy MW, Betteridge KJ. Changes in major proteins in the embryonic capsule during immobilization (fixation) of the conceptus in the third week of pregnancy in the mare. Reproduction 2007; 134:161-170.
35. McCracken JA, Custer EE, Lamsa JC. Luteolysis: a neuroendocrine-mediated event. Physiol Rev 1999; 79:263-323.
36. Moor RM. Effect of embryo on corpus luteum function. J Anim Sci 1968; 27 Suppl 1:97-118.
37. Squires EL, Douglas RH, Steffenhagen WP, Ginther OJ. Ovarian changes during the estrous cycle and pregnancy in mares. J Anim Sci 1974; 38:330-338.
38. Neely DP, Kindahl H, Stabenfeldt GH, Edqvist LE, Hughes JP. Prostaglandin release patterns in the mare: physiological, pathophysiological, and therapeutic responses. J Reprod Fertil Suppl 1979:181-189.
39. Ginther OJ, Rodrigues BL, Ferreira JC, Araujo RR, Beg MA. Characterisation of pulses of 13,14-dihydro-15-keto-PGF2alpha (PGFM) and relationships between PGFM pulses and luteal blood flow before, during, and after luteolysis in mares. Reprod Fertil Dev 2008; 20:684-693.
40. Goff AK. Oxytocin stimulation of plasma 15-keto-13,14-dihydro prostaglandin F-2a during the oestrus cycle and early pregnancy in the mare. J Reprod Fertil Suppl 1987; 35:253-260.
41. Sharp DC, Thatcher MJ, Salute ME, Fuchs AR. Relationship between endometrial oxytocin receptors and oxytocin-induced prostaglandin F2 alpha release during the oestrous cycle and early pregnancy in pony mares. J Reprod Fertil 1997; 109:137-144.
42. Ginther OJ, First NL. Maintenance of the corpus luteum in hysterectomized mares. Am J Vet Res 1971; 32:1687-1691.
43. Watson ED, Sertich PL. Prostaglandin production by horse embryos and the effect of co-culture of embryos with endometrium from pregnant mares. J Reprod Fertil 1989; 87:331-336.
44. Melrose PA, Knigge KM. Topography of oxytocin and vasopressin neurons in the forebrain of Equus caballus: further support of proposed evolutionary relationships for proopiomelanocortin, oxytocin and vasopressin neurons. Brain Behav Evol 1989; 33:193-204.
45. Vanderwall DK, Silvia WJ, Fitzgerald BP. Concentrations of oxytocin in the intercavernous sinus of mares during luteolysis: temporal relationship with concentrations of 13,14-dihydro-15-keto-prostaglandin F2 alpha. J Reprod Fertil 1998; 112:337-346.
46. Tetzke TA, Ismail S, Mikuckis G, Evans JW. Patterns of oxytocin secretion during the oestrous cycle of the mare. J Reprod Fertil Suppl 1987; 35:245-252.

47. Stevenson KR, Parkinson TJ, Wathes DC. Measurement of oxytocin concentrations in plasma and ovarian extracts during the oestrous cycle of mares. J Reprod Fertil 1991; 93:437-441.
48. Stout TA, Lamming GE, Allen WR. The uterus as a source of oxytocin in cyclic mares. J Reprod Fertil Suppl 2000:281-287.
49. Bae SE, Watson ED. A light microscopic and ultrastructural study on the presence and location of oxytocin in the equine endometrium. Theriogenology 2003; 60:909-921.
50. Behrendt-Adam CY, Adams MH, Simpson KS, McDowell KJ. Oxytocin-neurophysin I mRNA abundance in equine uterine endometrium. Domest Anim Endocrinol 1999; 16:183-192.
51. Starbuck GR, Stout TA, Lamming GE, Allen WR, Flint AP. Endometrial oxytocin receptor and uterine prostaglandin secretion in mares during the oestrous cycle and early pregnancy. J Reprod Fertil 1998; 113:173-179.
52. Boerboom D, Brown KA, Vaillancourt D, Poitras P, Goff AK, Watanabe K, Dore M, Sirois J. Expression of key prostaglandin synthases in equine endometrium during late diestrus and early pregnancy. Biol Reprod 2004; 70:391-399.
53. Ealy AD, Eroh ML, Sharp DC, 3rd. Prostaglandin H synthase Type 2 is differentially expressed in endometrium based on pregnancy status in pony mares and responds to oxytocin and conceptus secretions in explant culture. Anim Reprod Sci 2010; 117:99-105.
54. McCracken JA, Carlson JC, Glew ME, Goding JR, Baird DT, Green K, Samuelsson B. Prostaglandin F 2 identified as a luteolytic hormone in sheep. Nat New Biol 1972; 238:129-134.
55. Krzymowski T, Stefanczyk-Krzymowska S. The role of the endometrium in endocrine regulation of the animal oestrous cycle. Reprod Domest Anim 2008; 43:80-91.
56. Geisert RD, Renegar RH, Thatcher WW, Roberts RM, Bazer FW. Establishment of pregnancy in the pig: I. Interrelationships between preimplantation development of the pig blastocyst and uterine endometrial secretions. Biol Reprod 1982; 27:925-939.
57. Geisert RD, Brookbank JW, Roberts RM, Bazer FW. Establishment of pregnancy in the pig: II. Cellular remodeling of the porcine blastocyst during elongation on day 12 of pregnancy. Biol Reprod 1982; 27:941-955.
58. Bazer FW. Establishment of pregnancy in sheep and pigs. Reprod Fertil Dev 1989; 1:237-242.
59. Bazer FW, Thatcher WW. Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F2alpha by the uterine endometrium. Prostaglandins 1977; 14:397-400.
60. Gross TS, Mirando MA, Young KH, Beers S, Bazer FW, Thatcher WW. Reorientation of prostaglandin F secretion by calcium ionophore, estradiol, and prolactin in perifused porcine endometrium. Endocrinology 1990; 127:637-642.
61. Spencer TE, Bazer FW. Conceptus signals for establishment and maintenance of pregnancy. Reprod Biol Endocrinol 2004; 2:49.
62. Krzymowski T, Kotwica J, Stefanczyk-Krzymowska S. Uterine and ovarian countercurrent pathways in the control of ovarian function in the pig. J Reprod Fertil Suppl 1990; 40:179-191.
63. Geisert RD, Thatcher WW, Roberts RM, Bazer FW. Establishment of pregnancy in the pig: III. Endometrial secretory response to estradiol valerate administered on day 11 of the estrous cycle. Biol Reprod 1982; 27:957-965.
64. Geisert RD. Maternal recognition of pregnancy. Anim Reprod Sci 1992; 28:287-298.
65. Conley AJ, Ford SP. Direct luteotrophic effect of oestradiol-17 beta on pig corpora lutea. J Reprod Fertil 1989; 87:125-131.
66. Christenson LK, Farley DB, Anderson LH, Ford SP. Luteal maintenance during early pregnancy in the pig: role for prostaglandin E2. Prostaglandins 1994; 47:61-75.
67. Ford SP, Christenson LK. Direct effects of oestradiol-17 beta and prostaglandin E-2 in protecting pig corpora lutea from a luteolytic dose of prostaglandin F-2 alpha. J Reprod Fertil 1991; 93:203-209.
68. Waclawik A, Blitek A, Kaczmarek MM, Kiewisz J, Ziecik AJ. Antiluteolytic mechanisms and the establishment of pregnancy in the pig. Soc Reprod Fertil Suppl 2009; 66:307-320.

69. Waclawik A, Jabbour HN, Blitek A, Ziecik AJ. Estradiol-17beta, prostaglandin E2 (PGE2), and the PGE2 receptor are involved in PGE2 positive feedback loop in the porcine endometrium. Endocrinology 2009; 150:3823-3832.
70. Geisert RD, Ross JW, Ashworth MD, White FJ, Johnson GA, DeSilva U. Maternal recognition of pregnancy signal or endocrine disruptor: the two faces of oestrogen during establishment of pregnancy in the pig. Soc Reprod Fertil Suppl 2006; 62:131-145.
71. Kotwica G, Franczak A, Okrasa S, Kotwica J. Effect of an oxytocin antagonist on prostaglandin F2 alpha secretion and the course of luteolysis in sows. Acta Vet Hung 1999; 47:249-262.
72. La Bonnardiere C, Martinat-Botte F, Terqui M, Lefevre F, Zouari K, Martal J, Bazer FW. Production of two species of interferon by Large White and Meishan pig conceptuses during the peri-attachment period. J Reprod Fertil 1991; 91:469-478.
73. Lefevre F, Martinat-Botte F, Guillomot M, Zouari K, Charley B, La Bonnardiere C. Interferon-gamma gene and protein are spontaneously expressed by the porcine trophectoderm early in gestation. Eur J Immunol 1990; 20:2485-2490.
74. Lefevre F, Guillomot M, D'Andrea S, Battegay S, La Bonnardiere C. Interferon-delta: the first member of a novel type I interferon family. Biochimie 1998; 80:779-788.
75. Lefevre F, Martinat-Botte F, Locatelli A, De Niu P, Terqui M, La Bonnardiere C. Intrauterine infusion of high doses of pig trophoblast interferons has no antiluteolytic effect in cyclic gilts. Biol Reprod 1998; 58:1026-1031.
76. Cencic A, Guillomot M, Koren S, La Bonnardiere C. Trophoblastic interferons: do they modulate uterine cellular markers at the time of conceptus attachment in the pig? Placenta 2003; 24:862-869.
77. Cencic A, La Bonnardiere C. Trophoblastic interferon-gamma: current knowledge and possible role(s) in early pig pregnancy. Vet Res 2002; 33:139-157.
78. Spencer TE, Becker WC, George P, Mirando MA, Ogle TF, Bazer FW. Ovine interferon-tau regulates expression of endometrial receptors for estrogen and oxytocin but not progesterone. Biol Reprod 1995; 53:732-745.
79. Spencer TE, Becker WC, George P, Mirando MA, Ogle TF, Bazer FW. Ovine interferon-tau inhibits estrogen receptor up-regulation and estrogen-induced luteolysis in cyclic ewes. Endocrinology 1995; 136:4932-4944.
80. Wathes DC, Lamming GE. The oxytocin receptor, luteolysis and the maintenance of pregnancy. J Reprod Fertil Suppl 1995; 49:53-67.
81. Lee J, McCracken JA, Banu SK, Rodriguez R, Nithy TK, Arosh JA. Transport of prostaglandin F(2alpha) pulses from the uterus to the ovary at the time of luteolysis in ruminants is regulated by prostaglandin transporter-mediated mechanisms. Endocrinology 2010; 151:3326-3335.
82. Spencer TE, Ott TL, Bazer FW. tau-Interferon: pregnancy recognition signal in ruminants. Proc Soc Exp Biol Med 1996; 213:215-229.
83. Roberts RM, Ealy AD, Alexenko AP, Han CS, Ezashi T. Trophoblast interferons. Placenta 1999; 20:259-264.
84. Kubisch HM, Larson MA, Roberts RM. Relationship between age of blastocyst formation and interferon-tau secretion by in vitro-derived bovine embryos. Mol Reprod Dev 1998; 49:254-260.
85. Hernandez-Ledezma JJ, Sikes JD, Murphy CN, Watson AJ, Schultz GA, Roberts RM. Expression of bovine trophoblast interferon in conceptuses derived by in vitro techniques. Biol Reprod 1992; 47:374-380.
86. Ealy AD, Yang QE. Control of interferon-tau expression during early pregnancy in ruminants. Am J Reprod Immunol 2009; 61:95-106.
87. Spencer TE, Bazer FW. Ovine interferon tau suppresses transcription of the estrogen receptor and oxytocin receptor genes in the ovine endometrium. Endocrinology 1996; 137:1144-1147.
88. Spencer TE, Johnson GA, Bazer FW, Burghardt RC, Palmarini M. Pregnancy recognition and conceptus implantation in domestic ruminants: roles of progesterone, interferons and endogenous retroviruses. Reprod Fertil Dev 2007; 19:65-78.

89. Robinson RS, Mann GE, Lamming GE, Wathes DC. Expression of oxytocin, oestrogen and progesterone receptors in uterine biopsy samples throughout the oestrous cycle and early pregnancy in cows. Reproduction 2001; 122:965-979.

90. Robinson RS, Mann GE, Lamming GE, Wathes DC. The effect of pregnancy on the expression of uterine oxytocin, oestrogen and progesterone receptors during early pregnancy in the cow. J Endocrinol 1999; 160:21-33.

91. Fuchs AR, Rollyson MK, Meyer M, Fields MJ, Minix JM, Randel RD. Oxytocin induces prostaglandin F2 alpha release in pregnant cows: influence of gestational age and oxytocin receptor concentrations. Biol Reprod 1996; 54:647-653.

92. Henderson KM, McNatty KP. A biochemical hypothesis to explain the mechanism of luteal regression. Prostaglandins 1975; 9:779-797.

93. Magness RR, Huie JM, Hoyer GL, Hueckstead TP, Reynolds LP, Seperich GJ, Whysong G, Weems CW. Effect of chronic ipsilateral or contralateral intrauterine infusion of prostaglandin E2 (PGE2) on luteal function of unilaterally ovariectomized ewes. Prostaglandins Med 1981; 6:389-401.

94. Reynolds LP, Robertson DA, Ford SP. Effects of intrauterine infusion of oestradiol-17 beta and prostaglandin E-2 on luteal function in non-pregnant heifers. J Reprod Fertil 1983; 69:703-709.

95. Silvia WJ, Ottobre JS, Inskeep EK. Concentrations of prostaglandins E2, F2 alpha and 6-keto-prostaglandin F1 alpha in the utero-ovarian venous plasma of nonpregnant and early pregnant ewes. Biol Reprod 1984; 30:936-944.

96. Asselin E, Bazer FW, Fortier MA. Recombinant ovine and bovine interferons tau regulate prostaglandin production and oxytocin response in cultured bovine endometrial cells. Biol Reprod 1997; 56:402-408.

97. Asselin E, Lacroix D, Fortier MA. IFN-tau increases PGE2 production and COX-2 gene expression in the bovine endometrium in vitro. Mol Cell Endocrinol 1997; 132:117-126.

98. Xiao CW, Murphy BD, Sirois J, Goff AK. Down-regulation of oxytocin-induced cyclooxygenase-2 and prostaglandin F synthase expression by interferon-tau in bovine endometrial cells. Biol Reprod 1999; 60:656-663.

99. Kim S, Choi Y, Bazer FW, Spencer TE. Identification of genes in the ovine endometrium regulated by interferon tau independent of signal transducer and activator of transcription 1. Endocrinology 2003; 144:5203-5214.

100. Wolf E, Arnold GJ, Bauersachs S, Beier HM, Blum H, Einspanier R, Frohlich T, Herrler A, Hiendleder S, Kolle S, Prelle K, Reichenbach HD, et al. Embryo-maternal communication in bovine - strategies for deciphering a complex cross-talk. Reprod Domest Anim 2003; 38:276-289.

101. Klein C, Bauersachs S, Ulbrich SE, Einspanier R, Meyer HH, Schmidt SE, Reichenbach HD, Vermehren M, Sinowatz F, Blum H, Wolf E. Monozygotic twin model reveals novel embryo-induced transcriptome changes of bovine endometrium in the preattachment period. Biol Reprod 2006; 74:253-264.

102. Spencer TE, Bazer FW. Uterine and placental factors regulating conceptus growth in domestic animals. J Anim Sci 2004; 82 E-Suppl:E4-13.

103. Suire S, Stewart F, Beauchamp J, Kennedy MW. Uterocalin, a lipocalin provisioning the preattachment equine conceptus: fatty acid and retinol binding properties, and structural characterization. Biochem J 2001; 356:369-376.

104. Stewart F, Gerstenberg C, Suire S, Allen WR. Immunolocalization of a novel protein (P19) in the endometrium of fertile and subfertile mares. J Reprod Fertil Suppl 2000:593-599.

105. Klein C, Scoggin KE, Ealy AD, Troedsson MH. Transcriptional Profiling of Equine Endometrium During the Time of Maternal Recognition of Pregnancy. Biol Reprod 2010.

106. Boll M, Daniel H, Gasnier B. The SLC36 family: proton-coupled transporters for the absorption of selected amino acids from extracellular and intracellular proteolysis. Pflugers Arch 2004; 447:776-779.

107. Gellersen B, Fernandes MS, Brosens JJ. Non-genomic progesterone actions in female reproduction. Hum Reprod Update 2009; 15:119-138.

108. Smith JL, Kupchak BR, Garitaonandia I, Hoang LK, Maina AS, Regalla LM, Lyons TJ. Heterologous expression of human mPRalpha, mPRbeta and mPRgamma in yeast confirms their ability to function as membrane progesterone receptors. Steroids 2008; 73:1160-1173.

109. Zhu Y, Bond J, Thomas P. Identification, classification, and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progestin receptor. Proc Natl Acad Sci U S A 2003; 100:2237-2242.

110. Tan Q, Zagrodny A, Bernaudo S, Peng C. Regulation of membrane progestin receptors in the zebrafish ovary by gonadotropin, activin, TGF-beta and BMP-15. Mol Cell Endocrinol 2009; 312:72-79.

111. Tubbs C, Thomas P. Progestin signaling through an olfactory G protein and membrane progestin receptor-alpha in Atlantic croaker sperm: potential role in induction of sperm hypermotility. Endocrinology 2009; 150:473-484.

112. Karteris E, Zervou S, Pang Y, Dong J, Hillhouse EW, Randeva HS, Thomas P. Progesterone signaling in human myometrium through two novel membrane G protein-coupled receptors: potential role in functional progesterone withdrawal at term. Mol Endocrinol 2006; 20:1519-1534.

113. Bylander A, Nutu M, Wellander R, Goksor M, Billig H, Larsson DG. Rapid effects of progesterone on ciliary beat frequency in the mouse fallopian tube. Reprod Biol Endocrinol 2010; 8:48.

114. Nutu M, Weijdegard B, Thomas P, Thurin-Kjellberg A, Billig H, Larsson DG. Distribution and hormonal regulation of membrane progesterone receptors beta and gamma in ciliated epithelial cells of mouse and human fallopian tubes. Reprod Biol Endocrinol 2009; 7:89.

115. McDowell KJ, Adams MH, Adam CY, Simpson KS. Changes in equine endometrial oestrogen receptor alpha and progesterone receptor mRNAs during the oestrous cycle, early pregnancy and after treatment with exogenous steroids. J Reprod Fertil 1999; 117:135-142.

116. Stout TA, Allen WR. Oestrogens and pregnancy maintenance in the mare: for or against? Pferdeheilkunde 2001; 17:579–582.

117. Ababneh MM, Ababneh H, Shidaifat F. Expression of Cytosolic Phospholipase A2 in Equine Endometrium during the Oestrous Cycle and Early Pregnancy. Reprod Domest Anim 2010.

118. Atli MO, Kurar E, Kayis SA, Aslan S, Semacan A, Celik S, Guzeloglu A. Evaluation of genes involved in prostaglandin action in equine endometrium during estrous cycle and early pregnancy. Anim Reprod Sci 2010.

119. Schuster VL. Prostaglandin transport. Prostaglandins Other Lipid Mediat 2002; 68-69:633-647.

120. Nomura T, Chang HY, Lu R, Hankin J, Murphy RC, Schuster VL. Prostaglandin signaling in the renal collecting duct: release, reuptake, and oxidation in the same cell. J Biol Chem 2005; 280:28424-28429.

121. Kang J, Chapdelaine P, Laberge PY, Fortier MA. Functional characterization of prostaglandin transporter and terminal prostaglandin synthases during decidualization of human endometrial stromal cells. Hum Reprod 2006; 21:592-599.

122. Yang ZM, Das SK, Wang J, Sugimoto Y, Ichikawa A, Dey SK. Potential sites of prostaglandin actions in the periimplantation mouse uterus: differential expression and regulation of prostaglandin receptor genes. Biol Reprod 1997; 56:368-379.

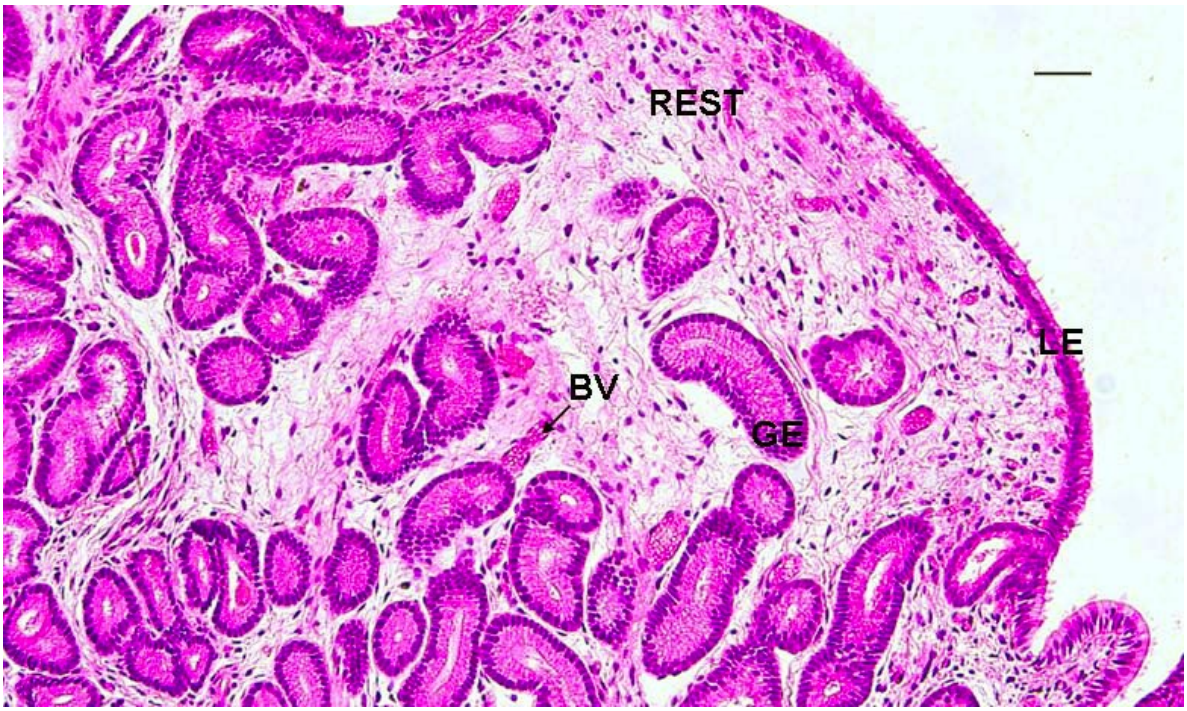
123. Rivera Del Alamo MM, Reilas T, Kindahl H, Katila T. Mechanisms behind intrauterine device-induced luteal persistence in mares. Anim Reprod Sci 2008; 107:94-106.

124. Thie M, Rospel R, Dettmann W, Benoit M, Ludwig M, Gaub HE, Denker HW. Interactions between trophoblast and uterine epithelium: monitoring of adhesive forces. Hum Reprod 1998; 13:3211-3219.

125. Burghardt RC, Burghardt JR, Taylor JD, 2nd, Reeder AT, Nguen BT, Spencer TE, Bayless KJ, Johnson GA. Enhanced focal adhesion assembly reflects increased mechanosensation and mechanotransduction at maternal-conceptus interface and uterine wall during ovine pregnancy. Reproduction 2009; 137:567-582.

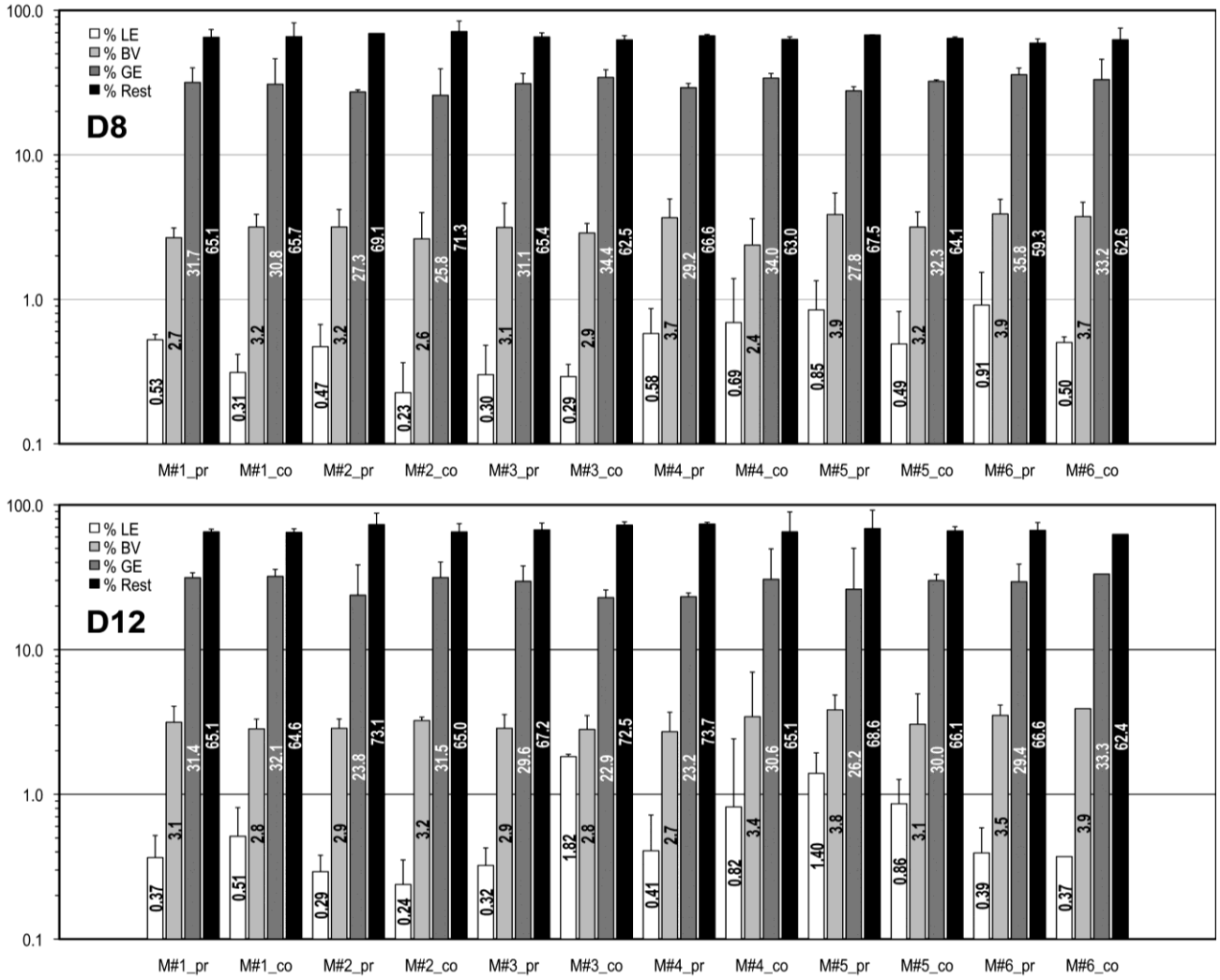
8 Appendix

Supplemental Figure 1: Section of a day 12 endometrial biopsy sample (H&E)



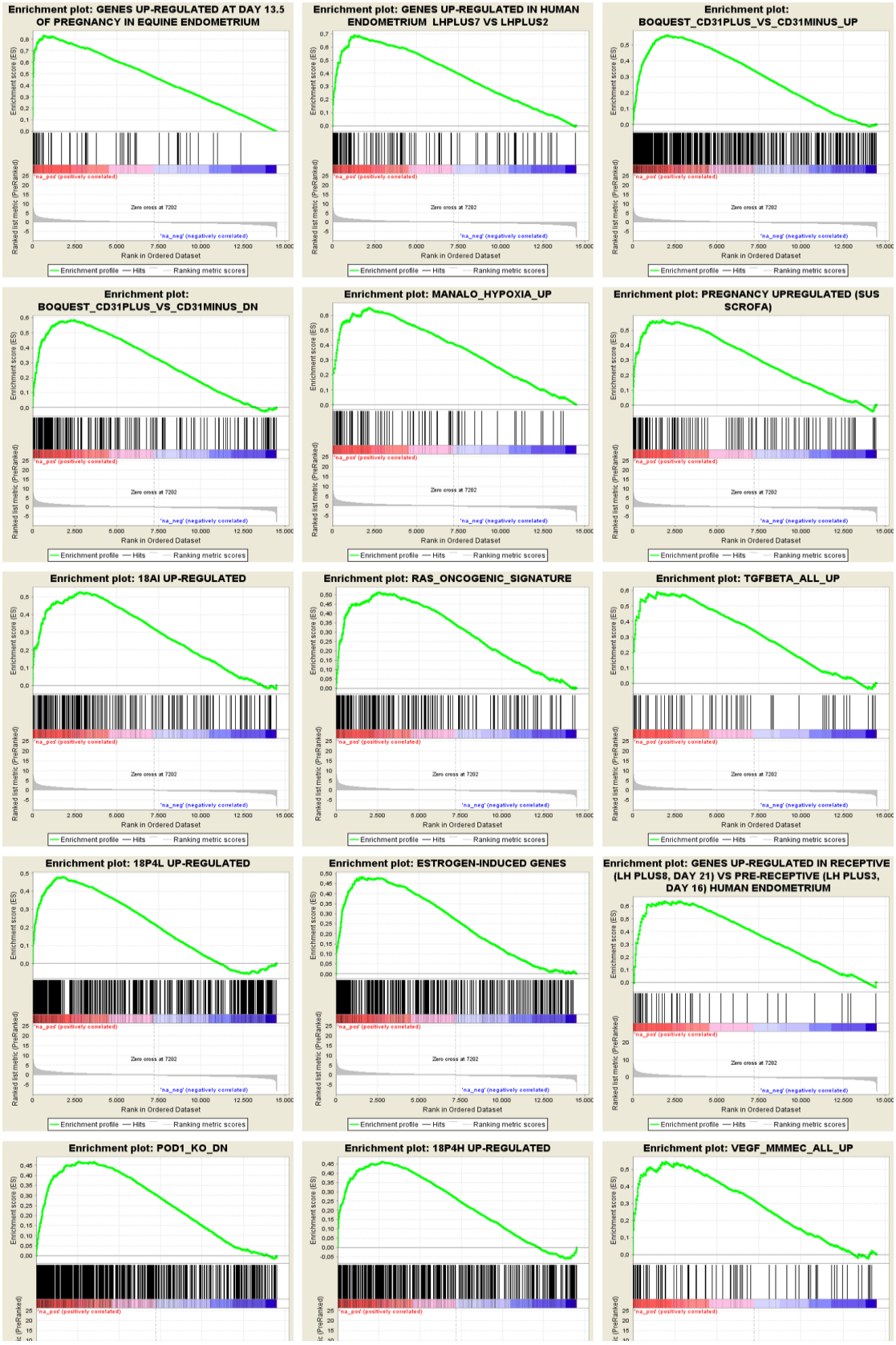
LE: luminal epithelium, BV: blood vessels, GE: glandular epithelium, REST: remaining tissue, Bar = 50µm.

Supplemental Figure 2: Quantitative stereological analysis of endometrial biopsy samples.



The volume fraction of luminal epithelium (LE), blood vessels (BV), glandular epithelium (GE) and the remaining tissue (Rest) was determined in biopsy samples collected at Day 8 and Day 12 of pregnancy (pr) and the corresponding days of the estrous cycle (co), respectively. M#1. Mare no. 1.

Supplemental Figure 3: Enrichment plots obtained by the Gene Set Enrichment Analysis (GSEA) for the gene sets shown in Table 3.



Supplemental Table 1: Primer sequences for quantitative real-time RT-PCR

Gene	Forward primer [5´-... -3´]	Reverse primer [5´-... -3´]	AT [°C]	FA [°C]	MP [°C]
H3F3A	*AGATCCAGGATAAGGAAGGCAT*	*GCTCCACCTCCAGGGTGAT*	*60*	*80*	*87*
UBQ3	*AGATCCAGGATAAGGAAGGCAT*	*GCTCCACCTCCAGGGTGAT*	*60*	*83*	*88*
18S rRNA	*AAGTCTTTGGGTTCCGGG*	*GGACATCTAAGGGCATCACA*	*60*	*84*	*89*
CTSL1	*ACGGCGTTTTGGTGGTTGGCT*	*ATGCCCCAATCTTCACCCCAGC*	*64*	*77*	*82*
ERRFI1	*TGCAAGCACCCAAATCCAGCCA*	*TTACGCTTCACATGGCCGCCT*	*60*	*79*	*84*
FGF9	*ACGTCAGCTCCACTGTTGCCAAA*	*AAGCAAGTGGGCACAGGCAGT*	*64*	*80*	*86*
HHIP	*TCCGGCTGGATGTGGACACAGA*	*AGCACATCTGCCTGGATCGTGGA*	*60*	*85*	*89*
KDR	*ATCCGCCCAGGCTCAGCATACA*	*TTGGGCCAGAGCCAGTCCAAGT*	*60*	*79*	*83*
KLF9	*AGGTGAGGCCGCCTATTTCCGA*	*TGCCAGGCAACCCCAAACTCCT*	*60*	*82*	*87*
OXTR	*TGCAGATGTGGAGCGTCTGGGA*	*TGGAAGAGGTGGCCCGTGAACA*	*60*	*85*	*89*
PAQR5	*CGCACGTGCAGATGGAAGCCATA*	*CCGAGGCTGAAGACAAGGCACA*	*60*	*81*	*87*
PTGER4	*TGTCTGGCCACTCTCGCTCCTT*	*GCCAGGCACACCTGGAAGCAAA*	*60*	*82*	*87*
PTGER1	*TCGGAAGAATTGCCATATGTGGGGC*	*AGTTCTCTAGTTGGTGCTGGGGGAA*	*64*	*76*	*82*
SFRP1	*ATTCTCACGGGCAGGTTGGGGA*	*AACCACTGCGGTTCCAGGAGGT*	*64*	*85*	*88*
SLC36A2	*ATGAAGGATGCCCGCCGCTT*	*TCCAAACCGCAGGTAGCCCAGA*	*60*	*85*	*87*
SLC36A2	*ACCATCCCAGTTGAACCCCGTCT*	*TCAGCTTGACTGGAGCTGGGTCT*	*60*	*80*	*86*
SLCO2A1	*CGGCCAAACTGCCATGAGACTGA*	*TGAAACTGGCCCCTGAGGTTGC*	*60*	*80*	*84*

Supplemental Table 2: Differentially expressed genes in endometrial tissue samples of day 12 pregnant vs. day 12 control mares

Systematic Name	Eca Ensembl Gene ID	Eca Entrez Gene ID	Eca Gene symbol	Eca Gene name	Hsa Entrez Gene ID	Hsa Gene symbol	Hsa Gene name	Mean FC D12 Pr/Co	SAM Score (d)	SAM q-value	M#3 FC Pr/Co	Mean FC/ M#3 FC	FC Co D12/ D8	SAM q-value	FC Pr D12/D8	SAM q-value
ENSECAT00000011605	ENSECAG00000010765	100056656	*LOC100056656*	similar to Acyl-CoA synthetase long-chain family member 3	2181	*ACSL3*	acyl-CoA synthetase long-chain family member 3	-1.6	-5.22	0.0069	1.5	2.1	-1.03	0.2695	-1.59	0.0384
ENSECAT00000025063	ENSECAG00000023388	100069420	*AP3S2,LOC100069420*	AP-3 complex subunit sigma-2	10239	*AP3S2*	AP-3 complex subunit sigma-2	-1.6	-5.61	0.0030	1.3	1.2	-1.03	0.4743	-1.36	0.1179
AJ555456, NM_001114607	ENSECAG00000008500	100136906	*AQP5*	aquaporin 5	362	*AQP5*	aquaporin 5	-2.0	-4.29	0.0119	4.5	2.2	1.25	0.2400	-1.88	0.0112
ENSECAT00000012243	ENSECAG00000011608	100054827	*LOC100054827*	similar to alveolar soft part sarcoma chromosome region, candidate 1	79058	*ASPSCR1*	alveolar soft part sarcoma chromosome region, candidate 1	-1.5	-3.85	0.0130	1.3	1.8	-1.20	0.2825	-1.85	0.0019
ENSECAT00000013979	ENSECAG00000012083	100072684	*LOC100072684*	similar to ATPase, Ca++ transporting, ubiquitous	489	*ATP2A3*	ATPase, Ca++ transporting, ubiquitous	-2.0	-6.06	0.0012	2.7	3.6	1.44	0.1094	-1.51	0.0520
ENSECAT00000018675	ENSECAG00000017552	100062307	*CREB3L4*	cAMP responsive element binding protein 3-like 4	148327	*CREB3L4*	cAMP responsive element binding protein 3-like 4	-1.8	-5.84	0.0020	1.8	1.7	-1.12	0.3655	-2.10	0.0000
ENSECAT00000007808	ENSECAG00000007097	100060874	*CRTAC1*	cartilage acidic protein1	55118	*CRTAC1*	cartilage acidic protein 1	-2.4	-3.22	0.0317	1.1	3.6	1.63	0.1382	-1.89	0.0046
ENSECAT00000007651	ENSECAG00000007210	100061532	*LOC100061532*	similar to cathepsin L	1514	*CTSL1*	cathepsin L1	-2.2	-4.35	0.0115	3.1	1.8	-2.73	0.0103	-6.99	0.0000
ENSECAT00000019801	ENSECAG00000018550	100052258	*LOC100052258*	similar to docking protein 4	55715	*DOK4*	docking protein 4	-1.7	-4.22	0.0115	1.2	2.8	-1.27	0.2277	-2.25	0.0008
DN508969					80303	*EFHD1*	EF-hand domain family, member D1	-1.6	-3.95	0.0130	1.7	1.8	1.10	0.3937	-1.56	0.0404
ENSECAT00000000916	ENSECAG00000000794	100064199	*LOC100064199*	similar to Embigin homolog (mouse)	133418	*EMB*	embigin homolog (mouse)	-1.5	-4.16	0.0126	1.6	3.5	-1.41	0.1147	-2.08	0.0004

DN510735		100050067	*FXYD4*	FXYD domain containing ion transport regulator 4	53828	*FXYD4*	FXYD domain containing ion transport regulator 4	-3.4	-3.83	0.0167	-6.7	-1.1	-2.27	0.0454	-6.99	0.0000
ENSECAT00000017503	ENSECAG00000016546	100063861	*GALNT12*	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 12 (GalNAc-T12)	79695	*GALNT12*	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 12 (GalNAc-T12)	-1.6	-4.75	0.0092	1.5	1.7	1.03	0.5191	-1.77	0.0019
ENSECAT00000009900	ENSECAG00000009689	100068840	*LOC100068840*	similar to Guanidinoacetate N-methyltransferase	2593	*GAMT*	guanidinoacetate N-methyltransferase	-1.6	-4.36	0.0115	1.3	1.0	1.05	0.4916	-1.54	0.0046
ENSECAT00000021302	ENSECAG00000019938	100061157	*GFPT1*	Glucosamine--fructose-6-phosphate aminotransferase 1	2673	*GFPT1*	glutamine-fructose-6-phosphate transaminase 1	-1.6	-5.15	0.0063	1.5	3.2	-1.19	0.2831	-2.02	0.0007
ENSECAT00000020943	ENSECAG00000019684	100054883	*LOC100054883*	similar to Glucosamine-phosphate N-acetyltransferase 1	64841	*GNPNAT1*	glucosamine-phosphate N-acetyltransferase 1	-1.7	-3.82	0.0169	1.4	2.3	-1.31	0.2119	-2.39	0.0007
ENSECAT00000025114	ENSECAG00000023427	100059540	*LOC100059540*	similar to hairy and enhancer of split 2 (Drosophila)	54626	*HES2*	hairy and enhancer of split 2 (Drosophila)	-1.8	-8.49	0.0000	1.6	-1.3	-1.02	0.5191	-1.95	0.0006
CX604860	ENSECAG00000024485	100062868	*HHIP*	Hedgehog-interacting protein	64399	*HHIP*	Hedgehog-interacting protein	-1.7	-6.14	0.0012	-1.6	1.2	1.07	0.4350	-1.32	0.1374
ENSECAT00000019537	ENSECAG00000018234	100061857	*KRT4*	keratin 4	3851	*KRT4*	keratin 4	-2.8	-4.42	0.0113	8.1	17.2	1.34	0.1382	-2.96	0.0008
XM_001491714		100058910	*LOC100058910*	similar to Kinesin-Like Protein family member (klp-6)	1E+08	*LOC100130097*	hypothetical LOC100130097	-2.0	-3.84	0.0169	4.3	2.0	-2.83	0.0029	-5.60	0.0000
ENSECAT00000014270	ENSECAG00000013518	100072699	*LOC100072699*	similar to Methyltransferase 11 domain containing 1	64745	*METT11D1*	methyltransferase 11 domain containing 1	-1.5	-4.08	0.0126	1.4	1.0	1.09	0.4567	-1.43	0.0971
ENSECAT00000015995	ENSECAG00000015275		*MT1B_HORSE*	Metallothionein-1B	4502	*MT2A*	metallothionein 2A	-1.5	-4.18	0.0126	-1.0	-9.0	-1.30	0.0403	-1.85	0.0012

ENSECAT00000010294	ENSECAG00000009820	100034193	*LOC100034193*	BLGI	138159	*PAEP*	beta-lactoglobulin pseudogene) (Pregnancy-associated endometrial alpha-2 globulin)(PAEG)(PEG)(Placental protein 14)(PP14)(Progesterone-associated endometrial protein)(Progestagen-associated endometrial protein)	-1.7	-5.15	0.0052	2.2	2.3	-1.23	0.1976	-2.25	0.0000
ENSECAT00000023958	ENSECAG00000022301	100050911	*LOC100050911*	similar to pyridoxal kinase	8566	*PDXK*	pyridoxal (pyridoxine, vitamin B6) kinase	-1.5	-4.54	0.0113	1.9	-1.1	1.02	0.5397	-1.39	0.0520
ENSECAT00000026783	ENSECAG00000024845	100053848	*PI16*	Peptidase inhibitor 16 Precursor	221476	*PI16*	peptidase inhibitor 16	-1.5	-4.22	0.0115	-1.4	3.2	1.23	0.1681	-1.49	0.0062
ENSECAT00000022054	ENSECAG00000020058	100052355	*LOC100052355*	similar to Polyribonucleotide nucleotidyltransferase 1, mitochondrial precursor (PNPase 1) (Polynucleotide phosphorylase-like protein) (PNPase old-35) (3-5 RNA exonuclease OLD35)	87178	*PNPT1*	polyribonucleotide nucleotidyltransferase 1	-1.7	-4.89	0.0092	1.9	-2.2	-1.59	0.0216	-2.39	0.0000

ENSECAT00000014219	ENSECAG00000013456	100058914	*LOC100058914*	similar to Phosphoribosyl pyrophosphate synthetase-associated protein 1 (PRPP synthetase-associated protein 1) (39 kDa phosphoribosypyrophosphate synthetase-associated protein) (PAP39)	5635	*PRPSAP1*	phosphoribosyl pyrophosphate synthetase-associated protein 1	-1.8	-3.86	0.0130	2.1	1.7	-1.18	0.2828	-2.08	0.0002
ENSECAT00000007691	ENSECAG00000007172	100069969	*A6P3D2_HORSE*	Pleckstrin and Sec7 domain protein Fragment	5662	*PSD*	pleckstrin and Sec7 domain containing	-1.6	-4.09	0.0126	1.4	-7.0	-1.45	0.0216	-2.41	0.0000
ENSECAT00000024588	ENSECAG00000022970	100065954	*LOC100065954*	similar to RAB32	10981	*RAB32*	RAB32, member RAS oncogene family	-1.5	-4.67	0.0092	1.7	1.6	1.08	0.3566	-1.52	0.0232
ENSECAT00000021347	ENSECAG00000020085	100061373	*LOC100061373*	hypothetical protein LOC100061373	285613	*RELL2*	RELT-like 2	-2.2	-6.39	0.0000	1.0	-1.9	-1.91	0.0159	-5.10	0.0000
ENSECAT00000025222	ENSECAG00000023535	100069409	*LOC100069409*	hypothetical protein LOC100069409	91461	*SGK493*	protein kinase-like protein SgK493	-2.1	-5.30	0.0052	-2.5	1.1	-1.05	0.5191	-2.07	0.0006
ENSECAT00000021490	ENSECAG00000020175	100070338	*SLC12A8*	solute carrier family 12 (potassium/chloride transporters), member 8	84561	*SLC12A8*	solute carrier family 12 (potassium/chloride transporters), member 8	-1.5	-4.82	0.0092	1.4	35.0	1.42	0.0430	-1.22	0.2312
ENSECAT00000017320	ENSECAG00000015404	100034163	*LOC100034163*	chloride anion exchanger solute carrier family 26 member 3-like protein	1811	*SLC26A3*	solute carrier family 26, member 3	-1.6	-4.63	0.0090	2.9	2.7	1.63	0.1529	1.20	0.1207
ENSECAT00000005957	ENSECAG00000005266	100070575	*LOC100070575*	hypothetical LOC100070575	6652	*SORD*	sorbitol dehydrogenase	-1.6	-3.67	0.0208	1.2	2.7	-1.27	0.2029	-2.02	0.0000
ENSECAT00000023110	ENSECAG00000021717	100053106	*SPDEF*	similar to SAM pointed domain containing ets transcription factor	25803	*SPDEF*	SAM pointed domain containing ets transcriptionfact.	-1.9	-5.11	0.0052	2.3	22.9	1.43	0.0430	1.06	0.3671

ENSECAT00000022839	ENSECAG00000021481	100052744	*LOC100052744*	similar to Somatostatin receptor type 2 (SS2R) (SRIF-1)	6752	*SSTR2*	somatostatin receptor 2	-1.6	-4.59	0.0113	-2.9	3.6	-1.26	0.1407	-1.90	0.0058
ENSECAT00000018256	ENSECAG00000016969	100051799	*LOC100051799*	similar to stimulated by retinoic acid gene 6 homolog	64220	*STRA6*	stimulated by retinoic acid gene 6 homolog (mouse)	-1.7	-4.32	0.0115	-1.0	1.9	1.11	0.3389	-1.62	0.0041
ENSECAT00000026750	ENSECAG00000024798	100057098	*LOC100057098*	similar to KIAA0984 protein	23329	*TBC1D30*	TBC1 domain family, member 30	-1.6	-5.09	0.0052	1.8	1.4	1.12	0.3905	-1.42	0.0645
ENSECAT00000026301	ENSECAG00000024314	100059793	*LOC100059793*	hypothetical LOC100059793	7089	*TLE2*	transducin-like enhancer of split 2 (E(sp1) homolog, Drosophila)	-1.6	-5.20	0.0052	1.3	3.1	-1.24	0.2551	-2.06	0.0019
ENSECAT00000017141	ENSECAG00000016225	100061910	*LOC100061910*	similar to Transmembrane protein 144	55314	*TMEM144*	transmembrane protein 144	-1.7	-7.26	0.0000	2.0	4.0	-1.39	0.1025	-2.39	0.0003
ENSECAT00000007637	ENSECAG00000007522	100065101	*LOC100065101*	similar to Thioredoxin domain containing 13	56255	*TMX4*	thioredoxin-related transmembrane protein 4	-1.5	-4.81	0.0092	1.1	1.4	1.13	0.3905	-1.48	0.0520
ENSECAT00000008296	ENSECAG00000008224	100056069	*LOC100056069*	hypothetical LOC100056069	79755	*ZNF750*	zinc finger protein 750	-1.5	-3.76	0.0177	1.4	-2.4	1.60	0.0430	1.05	0.3478
CD464985								1.5	3.32	0.0113	3.3	1.7	1.26	0.3389	2.44	0.0019
BI961011								1.5	3.12	0.0126	1.9	-2.6	1.91	0.0430	3.67	0.0000
ENSECAT00000005098	ENSECAG00000005194							1.6	2.94	0.0130	-1.8	16.9	-1.45	0.0536	-1.44	0.0645
ENSECAT00000026333	ENSECAG00000024481	100146176	*LOC100146176*	similar to AHNAK nucleoprotein 2				1.6	2.83	0.0177	1.2	5.1	1.41	0.0850	2.35	0.0000
DQ125451								1.6	3.49	0.0082	-2.7	5.2	-1.64	0.1094	-1.19	0.2312
ENSECAT00000015293	ENSECAG00000014674							1.6	2.79	0.0208	-2.9	5.8	-1.55	0.1094	-1.24	0.1603
CX602835								1.6	3.61	0.0046	-1.4	2.1	-1.06	0.4134	1.44	0.0397
DN508071								1.6	2.84	0.0173	1.4	2.2	-1.03	0.5064	1.52	0.0100
ENSECAT00000006270	ENSECAG00000006311							1.7	3.03	0.0130	1.0	1.5	-1.13	0.3905	1.61	0.0282
CX594010								1.7	2.74	0.0208	-1.1	1.3	1.04	0.5064	2.16	0.0031
AY246829								1.7	3.22	0.0115	1.9	1.5	-1.34	0.3655	2.00	0.0019
ENSECAT00000005125	ENSECAG00000005231							1.8	3.28	0.0113	-2.0	-2.3	-2.01	0.0430	-1.56	0.0209

ENSECAT00000005381	ENSECAG00000005480							2.0	2.90	0.0177	-3.8	17.5	-1.86	0.0536	-1.25	0.2070
L07563, L07564; L07569								2.0	3.07	0.0161	-3.7	2.4	-1.59	0.1976	-1.29	0.1165
ENSECAT00000017827	ENSECAG00000016970	100072855	*LOC100072855*	similar to hCG2043240				2.0	4.00	0.0020	-3.7	-1.6	-2.06	0.0430	-1.37	0.0645
L07571		100147255	*LOC100147255*	similar to lambda-immunoglobulin				2.1	3.11	0.0145	-3.0	4.4	-1.82	0.1382	-1.25	0.2312
CX602982								2.1	3.20	0.0115	-1.1	7.4	1.64	0.0668	2.87	0.0000
ENSECAT00000011591	ENSECAG00000011261							2.2	3.03	0.0145	1.2	1.6	1.11	0.3389	2.29	0.0012
BM780446								2.4	3.11	0.0126	-3.2	1.7	1.08	0.4567	3.12	0.0015
XM_001501228		100066131	*LOC100066131*	hypothetical protein LOC100066131				2.5	5.56	0.0000	-1.4	1.9	1.18	0.4134	3.56	0.0008
ENSECAT00000009965	ENSECAG00000009441							2.6	6.02	0.0000	-3.0	3.3	-1.25	0.1382	2.49	0.0000
DN508758								3.1	4.14	0.0012	2.4	26.3	1.86	0.0536	6.16	0.0000
BM780317								3.7	3.40	0.0092	-1.8	3.7	-1.46	0.1094	2.72	0.0031
ENSECAT00000025397, EU810388, EU810390, EU810391, EU810392, EU810393, EU810394	ENSECAG00000023696	100188974	*LOC100188974*	uterine serpin				4.0	4.13	0.0023	3.0	1.2	-1.25	0.4350	4.97	0.0000
ENSECAT00000007376	ENSECAG00000007258							4.0	2.75	0.0208	-6.5	1.9	-1.64	0.2551	1.59	0.1838
ENSECAT00000008402	ENSECAG00000008204	100056564	*LOC100056564*	hypothetical LOC100056564				4.4	12.51	0.0000	-2.2	2.1	1.31	0.2825	8.13	0.0000
ENSECAT00000021235	ENSECAG00000018992	100062560	*ABCA8*	ATP-binding cassette sub-family A member 8	10351	*ABCA8*	ATP-binding cassette, sub-family A (ABC1), member 8	1.8	3.73	0.0046	-1.3	-2.5	1.61	0.0216	2.25	0.0077
ENSECAT00000020403	ENSECAG00000017842	100034074	*ABCB1*	ATP-binding cassette, sub-family B (MDR/TAP), member 1	5243	*ABCB1*	ATP-binding cassette, sub-family B (MDR/TAP), member 1	1.6	3.75	0.0044	-1.0	2.4	1.17	0.2479	1.70	0.0040
NM_001081763		791240	*ABCC1*	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	4363	*ABCC1*	ATP-binding cassette, sub-family C (CFTR/MRP), member 1	1.7	3.79	0.0046	1.3	2.6	1.37	0.1775	2.12	0.0143

DQ825759		100034164	*ABCG2*	ATP-binding cassette, sub-family G (WHITE), member 2	9429	*ABCG2*	ATP-binding cassette, sub-family G (WHITE), member 2	1.8	4.49	0.0000	1.0	6.4	1.28	0.0850	2.02	0.0008
ENSECAT00000009606	ENSECAG00000009385	100066699	*LOC100066699*	similar to C14ORF29	145447	*ABHD12B*	abhydrolase domain containing 12B	1.7	3.58	0.0046	-1.2	7.6	1.45	0.0668	2.39	0.0007
ENSECAT00000002458	ENSECAG00000000430	100055952	*ACE2*	Angiotensin-converting enzyme 2 Precursor	59272	*ACE2*	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	1.6	4.45	0.0009	1.1	1.5	1.01	0.3738	1.28	0.0919
ENSECAT00000016072	ENSECAG00000015145	100062175	*ACTA2*	actin, alpha 2, smooth muscle, aorta	59	*ACTA2*	actin, alpha 2, smooth muscle, aorta	2.6	2.80	0.0208	1.7	-2.2	-1.70	0.0430	1.12	0.3257
ENSECAT00000018746	ENSECAG00000017366	100061064	*ADSSL1*	adenylosuccinate synthase like 1	122622	*ADSSL1*	adenylosuccinate synthase like 1	1.8	3.15	0.0115	1.7	5.0	-1.06	0.4350	1.63	0.0209
ENSECAT00000012711	ENSECAG00000012283	100066130	*AGR3*	Anterior gradient protein 3 homolog Precursor	155465	*AGR3*	anterior gradient homolog 3 (Xenopus laevis)	1.8	2.87	0.0172	1.6	-1.5	-2.13	0.0022	1.09	0.3096
ENSECAT00000012399	ENSECAG00000010841	100070501	*LOC100070501*	similar to prostaglandin F synthase	340811	*AKR1CL1*	aldo-keto reductase family 1, member C-like 1	2.3	2.79	0.0208	1.3	4.3	1.87	0.1000	2.35	0.0474
ENSECAT00000022771	ENSECAG00000021292	100070491	*LOC100070491*	similar to prostaglandin F synthase	340811	*AKR1CL1*	aldo-keto reductase family 1, member C-like 1	2.3	3.02	0.0130	1.3	5.1	1.87	0.1000	2.35	0.0474
ENSECAT00000008116	ENSECAG00000007330	100065123	*ALS2CL*	ALS2 C-terminal-like protein	259173	*ALS2CL*	ALS2 C-terminal like	1.5	5.76	0.0000	-1.6	1.1	1.07	0.4567	1.58	0.0100
ENSECAT00000011822	ENSECAG00000011198	100072355	*LOC100072355*	similar to S-adenosylmethionine decarboxylase proenzyme (AdoMetDC) (SamDC)	262	*AMD1*	S-adenosylmethionine decarboxylase proenzyme Precursor (AdoMetDC)	1.5	3.11	0.0126	-1.7	3.9	1.05	0.1031	1.62	0.1103
ENSECAT00000002468	ENSECAG00000000816	100055812	*LOC100055812*	similar to AMP deaminase	272	*AMPD3*	adenosine monophosphate deaminase (isoform E)	2.3	4.51	0.0000	-1.6	1.6	1.05	0.3282	2.31	0.0176
ENSECAT00000017044	ENSECAG00000015894	100051890	*ANGPT2*	angiopoietin 2	285	*ANGPT2*	angiopoietin 2	1.6	3.27	0.0113	1.1	2.4	1.20	0.2277	1.87	0.0007

XM_001501670	ENSECAG00000016234	100067146	*LOC100067146*	similar to angiopoietin-related protein-2	23452	*ANGPTL2*	angiopoietin-like 2	1.7	4.75	0.0000	1.8	2.5	-1.24	0.1701	1.27	0.1095
ENSECAT00000009786	ENSECAG00000009211	100067036	*ANGPT4*	Angiopoietin-4	51129	*ANGPTL4*	angiopoietin-like 4	2.5	2.58	0.0298	1.1	1.2	-1.18	0.4350	2.38	0.0050
ENSECAT00000023736	ENSECAG00000022239	100071652	*LOC100071652*	similar to ankyrin repeat domain 22	118932	*ANKRD22*	ankyrin repeat domain 22	1.9	4.34	0.0000	-1.1	-1.1	1.02	0.5029	1.76	0.0062
ENSECAT00000022026	ENSECAG00000020314	100061836	*ANO1*	Anoctamin-1	55107	*ANO1*	anoctamin 1, calcium activated chloride channel	1.9	4.96	0.0000	-4.1	1.4	1.11	0.3869	1.75	0.0697
ENSECAT00000021769	ENSECAG00000020129	100052045	*LOC100052045*	similar to Annexin A8 (Annexin VIII) (Vascular anticoagulant-beta) (VAC-beta)	244	*ANXA8L2*	annexin A8-like 2	6.5	8.28	0.0000	1.3	2.8	-1.09	0.1661	6.84	0.0000
ENSECAT00000008977	ENSECAG00000008739	100065767	*LOC100065767*	similar to copper monamine oxidase	8639	*AOC3*	amine oxidase, copper containing 3 (vascular adhesion protein 1)	2.1	3.16	0.0115	1.1	1.5	1.03	0.4238	1.32	0.1197
ENSECAT00000026929	ENSECAG00000024701	100067782	*LOC100067782*	similar to aldehyde oxidase 2	344454	*AOX2P*	aldehyde oxidase 2 pseudogene	1.6	3.72	0.0054	1.3	-4.5	-1.47	0.0243	1.55	0.0246
ENSECAT00000022036	ENSECAG00000020719	100054890	*LOC100054890*	similar to Amyloid beta A4 precursor protein-binding family B member 2 (Fe65-like protein)	323	*APBB2*	amyloid beta (A4) precursor protein-binding, family B, member 2	1.6	2.76	0.0208	-1.3	3.2	1.04	0.5064	1.60	0.0077
ENSECAT00000012256	ENSECAG00000011774	100055678	*LOC100055678*	similar to adenomatosis polyposis coli down-regulated 1	147495	*APCDD1*	adenomatosis polyposis coli down-regulated 1	1.5	3.49	0.0073	1.3	7.8	1.33	0.1094	1.75	0.0145
ENSECAT00000009311	ENSECAG00000008600	100071824	*LOC100071824*	similar to apolipoprotein B-100	338	*APOB*	apolipoprotein B (including Ag(x) antigen)	1.5	4.00	0.0029	-1.2	1.5	-1.14	0.3761	1.41	0.0663
CX603769					358	*AQP1*	aquaporin 1 (Colton blood group)	2.6	3.54	0.0066	1.1	2.1	1.18	0.2828	2.56	0.0616

ENSECAT00000024083	ENSECAG00000022525	100050528	*LOC100050528*	similar to Amphiregulin precursor (AR) (Colorectum cell-derived growth factor) (CRDGF)	374	*AREG*	amphiregulin	2.6	2.39	0.0317	2.4	-1.2	1.00	0.4929	1.17	0.2947
ENSECAT00000017770	ENSECAG00000016534	100034051	*LOC100034051*	arginase type II	384	*ARG2*	arginase, type II	2.1	5.75	0.0000	-1.8	-2.1	-1.58	0.0301	1.84	0.0182
ENSECAT00000010265	ENSECAG00000009393	100051002	*LOC100051002*	similar to Rho GTPase activating protein 29	9411	*ARHGAP29*	Rho GTPase activating protein 29	1.8	3.55	0.0073	-1.8	3.7	1.31	0.1148	1.76	0.0077
ENSECAT00000021030	ENSECAG00000019506	100065030	*ARRDC3*	arrestin domain containing 3	57561	*ARRDC3*	arrestin domain containing 3	1.6	2.84	0.0177	1.2	2.0	1.29	0.3150	1.58	0.1507
ENSECAT00000007366	ENSECAG00000007047	100054817	*ASPN*	asporin	54829	*ASPN*	asporin	1.6	3.32	0.0113	-1.3	1.7	-1.13	0.3337	1.23	0.2183
ENSECAT00000021520	ENSECAG00000017460	100052912	*LOC100052912*	similar to Potassium-transporting ATPase alpha chain 2 (Proton pump) (Non-gastric H(+)/K(+) ATPase subunit alpha)	479	*ATP12A*	ATPase, H+/K+ transporting, nongastric, alpha polypeptide	1.5	3.17	0.0115	1.1	2.1	1.11	0.3389	1.65	0.0007
ENSECAT00000018643	ENSECAG00000016114	100064797	*ATP6V0A4*	V-type proton ATPase 116 kDa subunit a isoform 4	50617	*ATP6V0A4*	ATPase, H+ transporting, lysosomal V0 subunit a4	7.8	9.82	0.0000	2.0	2.9	1.16	0.4041	9.36	0.0000
ENSECAT00000011182	ENSECAG00000010555	100057005	*ATP6V1C2*	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C2	245973	*ATP6V1C2*	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C2	1.6	3.03	0.0130	2.3	23.1	1.41	0.0536	2.03	0.0007
ENSECAT00000006093	ENSECAG00000005305		*BACE2*	Beta-secretase 2 Precursor	25825	*BACE2*	beta-site APP-cleaving enzyme 2	1.9	3.25	0.0122	1.0	1.2	-1.03	0.4879	1.98	0.0003
CX604253					10974	*C10orf116*	chromosome 10 open reading frame 116	2.0	4.01	0.0023	-1.6	2.4	1.27	0.1822	2.61	0.0000
ENSECAT00000019253	ENSECAG00000018133		*C10orf54*	Platelet receptor Gi24 Precurso	64115	*C10orf54*	chromosome 10 open reading frame 54	1.6	4.14	0.0020	1.3	1.5	-1.08	0.3660	1.41	0.0855
ENSECAT00000016036	ENSECAG00000015337	100050889	*LOC100050889*	hypothetical LOC100050889	343990	*C2orf55*	chromosome 2 open reading frame 55	2.1	3.78	0.0045	-3.1	2.1	1.09	0.2892	2.61	0.0005

ENSECAT00000023752	ENSECAG00000022187	100058920	*LOC100058920*	similar to Uncharacterized protein C3orf32	51066	*C3orf32*	chromosome 3 open reading frame 32	2.6	2.75	0.0209	3.0	-2.9	2.35	0.0359	4.19	0.0007
ENSECAT00000004680	ENSECAG00000004811	100059938	*LOC100059938*	similar to Chromosome 3 open reading frame 59	151963	*C3orf59*	chromosome 3 open reading frame 59	1.7	5.18	0.0031	-2.0	2.4	1.16	0.1765	2.02	0.0165
ENSECAT00000014288	ENSECAG00000013486	100056222	*LOC100056222*	similar to C4b-binding protein alpha chain precursor (C4bp) (Proline-rich protein) (PRP)	722	*C4BPA*	complement component 4 binding protein, alpha	1.7	2.72	0.0208	-3.5	1.8	-1.13	0.3119	1.51	0.0520
ENSECAT00000017369	ENSECAG00000016569	100064702	*LOC100064702*	hypothetical protein LOC100064702	90355	*C5orf30*	chromosome 5 open reading frame 30	3.2	3.44	0.0082	-1.9	1.2	-1.12	0.4227	3.10	0.0006
ENSECAT00000026456	ENSECAG00000024559	100059002	*LOC100059002*	hypothetical protein LOC100059002	153222	*C5orf41*	chromosome 5 open reading frame 41	1.6	2.90	0.0153	1.5	2.1	1.31	0.2407	1.99	0.0019
ENSECAT00000008515	ENSECAG00000008425	100052009	*LOC100052009*	hypothetical protein LOC100052009	286343	*C9orf150*	chromosome 9 open reading frame 150	2.0	3.31	0.0106	-1.4	1.4	-1.14	0.3881	1.60	0.0197
ENSECAT00000019210	ENSECAG00000018004	100052678	*LOC100052678*	similar to carbonic anhydrase VIII	767	*CA8*	carbonic anhydrase VIII	1.7	3.70	0.0063	-1.7	1.7	1.11	0.3149	1.94	0.0002
ENSECAT00000007157	ENSECAG00000006844	100068106	*CALD1*	Caldesmon1	800	*CALD1*	caldesmon 1	1.6	3.00	0.0130	-1.4	1.7	1.18	0.3578	1.79	0.0201
XM_001498122	ENSECAG00000016034	100068258	*LOC100068258*	similar to Lice2 beta cysteine protease	840	*CASP7*	caspase 7, apoptosis-related cysteine peptidase	1.5	2.83	0.0177	-2.0	2.8	1.05	0.1532	1.31	0.1701
ENSECAT00000018992	ENSECAG00000017925	100071509	*CD200*	CD200 antigen	4345	*CD200*	CD200 molecule	1.9	5.80	0.0000	-1.0	2.6	1.55	0.1707	2.74	0.0002
ENSECAT00000011095	ENSECAG00000010267	100034221	*LOC100034221*	lymphocyte surface antigen precursor CD44	960	*CD44*	CD44 molecule (Indian blood group)	1.7	4.20	0.0023	-1.5	2.3	-1.13	0.3110	1.67	0.0052
ENSECAT00000022486	ENSECAG00000021162	100055760	*CDH13*	cadherin 13	1012	*CDH13*	cadherin 13, H-cadherin (heart)	2.4	6.28	0.0000	1.2	1.4	1.20	0.3790	2.44	0.0002
ENSECAT00000013286	ENSECAG00000012826		*CDO1*	Cysteine dioxygenase type 1	1036	*CDO1*	cysteine dioxygenase, type I	1.6	3.00	0.0130	-1.5	-1.9	1.00	0.5580	1.56	0.0520
ENSECAT00000004474	ENSECAG00000004572	100062138	*LOC100062138*	hypothetical protein LOC100062138	9023	*CH25H*	cholesterol 25-hydroxylase	1.6	4.62	0.0021	1.2	-4.5	-1.46	0.0423	1.05	0.4093

ENSECAT00000020510	ENSECAG00000019194	100033828	*CHGA*	chromogranin A (parathyroid secretory protein 1)	1113	*CHGA*	chromogranin A (parathyroid secretory protein 1)	4.1	3.64	0.0055	22.5	3.9	2.44	0.1218	11.2	0.0000
ENSECAT00000010183	ENSECAG00000009680	100058853	*LOC100058853*	similar to Chloride intracellular channel protein 1 (Nuclear chloride ion channel 27) (NCC27) (Chloride channel ABP) (Regulatory nuclear chloride ion channel protein) (hRNCC)	1192	*CLIC1*	chloride intracellular channel 1	1.6	2.96	0.0130	-1.0	2.9	-1.23	0.1546	1.23	0.1608
ENSECAT00000007460	ENSECAG00000007010	100034172	*LOC100034172*	clusterin	1191	*CLU*	clusterin	1.7	2.89	0.0177	-1.4	1.1	1.11	0.4350	1.67	0.0031
XM_001491941	ENSECAG00000000149	100059301	*CNKSR2*	connector enhancer of kinase suppressor of Ras 2	22866	*CNKSR2*	connector enhancer of kinase suppressor of Ras 2	1.9	3.62	0.0046	-2.0	-1.3	-1.02	0.5397	1.54	0.2102
ENSECAT00000023540	ENSECAG00000021944	100055742	*COCH*	coagulation factor C homolog, cochlin (Limulus polyphemus)	1690	*COCH*	coagulation factor C homolog, cochlin (Limulus polyphemus)	4.9	2.96	0.0130	-4.7	3.8	1.55	0.1976	7.36	0.0000
ENSECAT00000019794	ENSECAG00000018359	100072695	*A6P3B6_HORSE*	Collagen, type XIII, alpha 1 Fragment	1305	*COL13A1*	collagen, type XIII, alpha 1	1.6	4.02	0.0037	-1.5	3.5	-1.30	0.1260	1.31	0.1802
ENSECAT00000014457	ENSECAG00000013598	100063901	*LOC100063901*	similar to collagen, type XXVIII	340267	*COL28A1*	collagen, type XXVIII, alpha 1	1.6	2.79	0.0208	-1.0	1.8	-1.09	0.3807	1.37	0.0724
ENSECAT00000022446	ENSECAG00000019838	100066148	*LOC100066148*	similar to alpha-1 type IV collagen	1282	*COL4A1*	collagen, type IV, alpha 1	1.6	2.86	0.0177	2.0	1.2	-1.05	0.4316	1.19	0.1054
ENSECAT00000020647	ENSECAG00000019508	100062187	*LOC100062187*	similar to Collagen, type VIII, alpha 1	1295	*COL8A1*	collagen, type VIII, alpha 1	5.4	2.21	0.0456	1.5	1.3	-1.30	0.4134	2.73	0.0005
ENSECAT00000013229	ENSECAG00000012064	100058573	*LOC100058573*	similar to Ceruloplasmin precursor (Ferroxidase)	1356	*CP*	ceruloplasmin (ferroxidase)	2.0	3.24	0.0111	-17.5	-2.2	-1.73	0.0383	1.46	0.0466
ENSECAT00000010984	ENSECAG00000010700		*CREG2*	Protein CREG2 Precursor	200407	*CREG2*	cellular repressor of E1A-stimulated genes 2	1.8	3.79	0.0053	1.1	22.8	1.48	0.0343	2.52	0.0000
ENSECAT00000008715	ENSECAG00000008409	100056249	*CRTAP*	Cartilage-associated protein	10491	*CRTAP*	cartilage associated	1.6	2.93	0.0153	-1.2	2.2	-1.02	0.3003	1.13	0.1620

							protein									
ENSECAT00000012936	ENSECAG00000012507	100061921	*LOC100061921*	similar to Alpha crystallin B chain (Alpha(B)-crystallin)	1410	*CRYAB*	crystallin, alpha B	2.2	3.91	0.0033	-2.4	3.5	1.04	0.4736	2.08	0.0024
ENSECAT00000009215	ENSECAG00000008566	100055161	*CTSE*	cathepsin E	1510	*CTSE*	cathepsin E	4.5	3.83	0.0039	-6.0	7.5	-1.80	0.0568	2.11	0.0043
ENSECAT00000020386	ENSECAG00000019087	100054991	*LOC100054991*	similar to cathepsin K	1513	*CTSK*	cathepsin K	1.6	4.28	0.0000	-1.3	1.1	1.06	0.4567	1.76	0.0031
ENSECAT00000011206	ENSECAG00000010817	100059014	*CTTNBP2NL*	CTTNBP2 N-terminal like	55917	*CTTNBP2 NL*	CTTNBP2 N-terminal like	1.5	3.09	0.0126	-1.1	1.8	1.15	0.2825	1.78	0.0007
ENSECAT00000019475	ENSECAG00000018406	100061442	*LOC100061442*	similar to SR-PSOX	58191	*CXCL16*	chemokine (C-X-C motif) ligand 16	1.7	2.98	0.0156	-3.3	2.5	-1.26	0.1948	1.61	0.0177
ENSECAT00000000643	ENSECAG00000000790		*CXCL17*	VEGF co-regulated chemokine 1 Precursor	284340	*CXCL17*	chemokine (C-X-C motif) ligand 17	2.4	4.95	0.0000	-3.6	2.0	-1.16	0.4106	2.79	0.0006
ENSECAT00000003720	ENSECAG00000003837	100050974	*LOC100050974*	similar to chemokine (C-X-C motif) receptor 4	7852	*CXCR4*	chemokine (C-X-C motif) receptor 4	1.6	3.17	0.0131	-1.3	3.9	-1.11	0.4340	1.33	0.1767
ENSECAT00000018424	ENSECAG00000017457	100059499	*PSCDBP*	Cytohesin-interacting protein / LOC100059499 similar to Pleckstrin homology, Sec7 and coiled-coil domains, binding protein	9595	*CYTIP*	cytohesin 1 interacting protein	1.8	3.24	0.0114	-1.4	3.5	-1.35	0.2057	1.06	0.2009
ENSECAT00000010145	ENSECAG00000009450	100052557	*LOC100052557*	similar to Aromatic-L-amino-acid decarboxylase (AADC) (DOPA decarboxylase) (DDC)	1644	*DDC*	dopa decarboxylase (aromatic L-amino acid decarboxylase)	1.7	2.88	0.0177	1.4	2.0	-1.15	0.2825	1.34	0.0645
ENSECAT00000026051	ENSECAG00000024167	100067586	*LOC100067586*	hypothetical LOC100067586	55601	*DDX60*	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60	1.8	3.94	0.0029	-1.8	1.3	1.14	0.4018	2.40	0.0003
ENSECAT00000024453	ENSECAG00000022804	100055937	*DKK3*	Dickkopf-related 3	27122	*DKK3*	dickkopf homolog 3 (Xenopus laevis)	1.8	5.59	0.0000	-1.2	2.1	-1.01	0.2698	1.39	0.0724
ENSECAT00000015171	ENSECAG00000014357	100052876	*LOC100052876*	similar to delta-like 1	28514	*DLL1*	delta-like 1 (Drosophila)	1.9	3.81	0.0057	-1.0	3.3	1.25	0.1512	2.14	0.0004

ENSECAT00000005542	ENSECAG00000005588				1755	*DMBT1*	deleted in malignant brain tumors 1	2.1	3.64	0.0046	-7.3	2.0	-1.25	0.3119	2.42	0.0041
ENSECAT00000010863	ENSECAG00000009336	100061058	*DOCK9*	Dedicator of cytokinesis protein 9 (Cdc42 guanine nucleotide exchange factor zizimin-1)	23348	*DOCK9*	dedicator of cytokinesis 9	1.5	4.65	0.0000	-2.3	2.3	1.21	0.1965	1.70	0.0042
ENSECAT00000024598	ENSECAG00000022735	100051829	*DOPEY2*	Protein dopey-2	9980	*DOPEY2*	dopey family member 2	2.0	4.39	0.0000	-1.4	2.2	-1.18	0.1976	2.02	0.0007
ENSECAT00000018689	ENSECAG00000017697	100072509	*LOC100072509*	similar to RIKEN cDNA 1110006O17	641700	*ECSCR*	endothelial cell-specific chemotaxis regulator	1.7	2.95	0.0130	-1.4	26.8	1.57	0.0536	2.38	0.0005
ENSECAT00000012106	ENSECAG00000011618	100034060	*LOC100034060*	preproendothelin 1	1906	*EDN1*	endothelin 1	1.7	3.79	0.0042	1.9	2.4	1.23	0.1958	2.44	0.0011
ENSECAT00000011070	ENSECAG00000010447	100066175	*LOC100066175*	hypothetical LOC100066175	2202	*EFEMP1*	EGF-containing fibulin-like extracellular matrix protein 1	1.6	2.78	0.0208	-1.3	2.2	-1.21	0.2277	1.07	0.3854
ENSECAT00000005524	ENSECAG00000005086		*EGLN3*	Egl nine homolog 3	112399	*EGLN3*	egl nine homolog 3 (C. elegans)	1.5	3.70	0.0046	-1.5	2.0	-1.22	0.2750	2.03	0.0089
ENSECAT00000001357	ENSECAG00000000752	100059218	*LOC100059218*	similar to Ets homologous factor	26298	*EHF*	ets homologous factor	1.6	3.52	0.0073	-3.1	1.2	1.08	0.4236	1.97	0.0000
ENSECAT00000022944	ENSECAG00000021497	100063668	*EMP-1*	Epithelial membrane protein 1	2012	*EMP1*	epithelial membrane protein 1	2.4	4.07	0.0020	2.1	1.3	-1.06	0.4326	1.37	0.2639
ENSECAT00000006533	ENSECAG00000005896	100052983	*LOC100052983*	similar to EGF, latrophilin and seven transmembrane domain-containing protein 1 precursor (EGF-TM7-latrophilin-related protein) (ETL protein)	2015	*EMR1*	egf-like module containing, mucin-like, hormone receptor-like 1	1.6	3.60	0.0069	1.1	2.2	-1.01	0.4973	1.51	0.0206
ENSECAT00000022487	ENSECAG00000021102	100067044	*LOC100067044*	similar to empty spiracles homolog 2	2018	*EMX2*	empty spiracles homeobox 2	1.6	2.94	0.0164	-2.3	2.6	1.32	0.1910	2.35	0.0001
ENSECAT00000018614	ENSECAG00000017667	100067528	*ENDOD1*	Endonuclease domain-containing 1 protein Precursor - XP_001497597.2	23052	*ENDOD1*	endonuclease domain containing 1	2.1	6.97	0.0000	-1.4	-3.0	2.12	0.0268	4.77	0.0000

ENSECAT00000013992	ENSECAG00000013035		*ENPP1*	Ectonucleotide pyrophosphatase/phosphodiesterase family member 1	5167	*ENPP1*	ectonucleotide pyrophosphatase/phosphodiesterase 1	1.7	4.04	0.0028	-2.0	-1.9	-1.86	0.0269	-1.00	0.1504
ENSECAT00000012066	ENSECAG00000011627	100058368	*ENPP6*	ectonucleotide pyrophosphatase/phosphodiesterase 6	133121	*ENPP6*	ectonucleotide pyrophosphatase/phosphodiesterase 6	2.4	5.53	0.0000	-1.7	1.8	1.27	0.2825	3.04	0.0000
CX603777		100051563	*ERG*	v-ets erythroblastosis virus E26 oncogene homolog (avian)	2078	*ERG*	v-ets erythroblastosis virus E26 oncogene homolog (avian)	1.7	3.17	0.0130	1.1	1.7	-1.02	0.4171	1.50	0.0272
ENSECAT00000017985	ENSECAG00000017104	100052062	*ERRFI1*	ERBB receptor feedback inhibitor 1	54206	*ERRFI1*	ERBB receptor feedback inhibitor 1	2.6	3.45	0.0086	2.0	3.7	1.54	0.1478	3.64	0.0001
ENSECAT00000016104	ENSECAG00000015044	100063026	*LOC100063026*	similar to factor VIII	2157	*F8*	coagulation factor VIII, procoagulant component	1.7	3.94	0.0035	-1.1	1.3	1.10	0.4134	1.94	0.0008
CX603294	ENSECAG00000007040	100061276	*LOC100061276*	hypothetical protein LOC100061276	144347	*FAM101A*	family with sequence similarity 101, member A	3.7	6.80	0.0000	2.1	1.4	-1.20	0.3848	2.31	0.0703
ENSECAT00000016368	ENSECAG00000015653	100067399	*LOC100067399*	similar to hCG26607	58489	*FAM108C1*	family with sequence similarity 108, member C1	1.7	3.06	0.0136	-1.1	2.8	1.36	0.1540	2.21	0.0056
ENSECAT00000007658	ENSECAG00000007385	100055982	*FAM129A*	family with sequence similarity 129, member A	116496	*FAM129A*	family with sequence similarity 129, member A	1.9	3.32	0.0113	-1.9	-8.7	-1.42	0.0270	1.25	0.0830
ENSECAT00000020704	ENSECAG00000019173	100063998	*FAM13A*	family with sequence similarity 13, member A	10144	*FAM13A*	family with sequence similarity 13, member A	2.0	3.72	0.0046	1.2	2.4	1.10	0.2254	1.63	0.0150
ENSECAT00000000067	ENSECAG00000000046	100062666	*LOC100062666*	similar to Family with sequence similarity 13, member C1	220965	*FAM13C*	family with sequence similarity 13, member C	1.8	3.00	0.0130	-1.1	1.7	1.06	0.3535	1.46	0.0152

ENSECAT00000023093	ENSECAG00000021708	100072672	*LOC100072672*	hypothetical LOC100072672	441168	*FAM26F*	family with sequence similarity 26, member F	2.5	3.78	0.0041	1.4	-1.1	-1.07	0.4990	2.63	0.0000
CX597239					131583	*FAM43A*	family with sequence similarity 43, member A	1.8	4.11	0.0028	1.2	1.6	-1.15	0.3154	1.55	0.0623
ENSECAT00000012287	ENSECAG00000011553	100067716	*LOC100067716*	similar to chromosome 6 open reading frame 32	9750	*FAM65B*	family with sequence similarity 65, member B	1.6	2.74	0.0208	1.6	2.1	1.22	0.2814	1.68	0.0911
ENSECAT00000009437	ENSECAG00000009038	100055277	*LOC100055277*	similar to family with sequence similarity 70, member A	55026	*FAM70A*	family with sequence similarity 70, member A	1.6	3.87	0.0058	-1.2	3.4	-1.25	0.1116	1.37	0.1068
XM_001502870		100063659	*FAT4*	FAT tumor suppressor homolog 4 (Drosophila)	79633	*FAT4*	FAT tumor suppressor homolog 4 (Drosophila)	1.8	3.72	0.0046	1.0	1.4	-1.07	0.3905	1.42	0.0645
ENSECAT00000026223	ENSECAG00000024399	100059907	*LOC100059907*	similar to fibroblast growth factor homologous factor 1	2257	*FGF12*	fibroblast growth factor 12	1.5	2.85	0.0177	1.5	2.8	1.21	0.2250	1.62	0.0094
ENSECAT00000019234	ENSECAG00000018011		*FGF13*	Fibroblast growth factor 13	2258	*FGF13*	fibroblast growth factor 13	1.6	3.75	0.0046	-1.5	3.7	-1.16	0.1094	1.34	0.0397
ENSECAT00000019888	ENSECAG00000018716	100050353	*LOC100050353*	similar to fibroblast growth factor 9	2254	*FGF9*	fibroblast growth factor 9 (glia-activating factor)	8.8	5.57	0.0009	-2.0	-2.1	-1.56	0.0187	5.89	0.0005
CD535938	ENSECAG00000018716as	100050353-as	*FGF9-as*	antisense of similar to fibroblast growth factor 9	2254	*FGF9-as*	Fibroblast growth factor 9	8.3	6.20	0.0000	-1.6	-2.0	-1.75	0.0312	4.65	0.0006
ENSECAT00000007435	ENSECAG00000007171	100054096	*LOC100054096*	hypothetical protein LOC100054096	2267	*FGL1*	fibrinogen-like 1	3.3	3.27	0.0113	-2.4	-2.5	1.78	0.0270	3.95	0.0000
ENSECAT00000027018	ENSECAG00000025020	100056943	*FHL-1*	Four and a half LIM domains protein 1	2273	*FHL1*	four and a half LIM domains 1	2.0	7.04	0.0000	1.1	-2.5	1.57	0.0216	2.59	0.0007
ENSECAT00000018723	ENSECAG00000017657	100071081	*FOSL2*	FOS-like antigen 2	2355	*FOSL2*	FOS-like antigen 2	1.6	6.24	0.0000	1.3	1.8	1.23	0.3149	1.64	0.1229
ENSECAT00000008260	ENSECAG00000007878	100054067	*FRMD3*	FERM domain containing 3	257019	*FRMD3*	FERM domain containing 3	1.5	3.10	0.0126	-1.2	3.6	1.26	0.1094	1.93	0.0019
ENSECAT00000003375	ENSECAG00000003535	100056475	*LOC100056475*	similar to Putative lymphocyte G0/G1 switch protein 2	50486	*G0S2*	G0/G1switch 2	2.3	2.70	0.0208	2.2	6.3	1.24	0.1976	2.10	0.0031

ENSECAT00000002578	ENSECAG00000002702	100063207	*GALNT4*	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 4 (GalNAc-T4)	8693	*GALNT4*	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 4 (GalNAc-T4)	1.6	4.69	0.0000	-1.0	-2.1	-1.01	0.5531	1.88	0.0062
ENSECAT00000017720	ENSECAG00000016679	100052358	*LOC100052358*	similar to Polypeptide N-acetylgalactosaminyltransferase-like protein 2 (Protein-UDP acetylgalactosaminyltransferase-like protein 2) (UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase-like protein 2) (Polypeptide GalNAc transferase-like prot	117248	*GALNTL2*	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 2	3.2	5.73	0.0000	-1.1	2.0	1.39	0.2549	6.08	0.0000
ENSECAT00000023055	ENSECAG00000021157	100070564	*LOC100070564*	similar to GTPase activating Rap/RanGAP domain-like 3	84253	*GARNL3*	GTPase activating Rap/RanGAP domain-like 3	1.7	8.19	0.0000	-1.6	1.1	1.03	0.4912	1.72	0.0249
ENSECAT00000005099	ENSECAG00000005150	100055573	*LOC100055573*	similar to connexin31	2707	*GJB3*	gap junction protein, beta 3, 31kDa	2.5	2.84	0.0177	-1.3	1.3	-1.01	0.5397	2.60	0.0031
ENSECAT00000021914	ENSECAG00000020587	100034082	*LOC100034082*	GM2 activator protein precursor	2760	*GM2A*	GM2 ganglioside activator	4.4	7.43	0.0000	2.0	-3.4	2.96	0.0058	10.9	0.0000
ENSECAT00000021781	ENSECAG00000020518	100063170	*LOC100063170*	similar to guanine nucleotide-binding protein alpha 14	9630	*GNA14*	guanine nucleotide binding protein (G protein), alpha 14	1.6	3.01	0.0151	1.1	2.1	1.19	0.2547	1.89	0.0006

ENSECAT00000000229	ENSECAG00000000229	100055673	*LOC100055673*	similar to Chain A, Crystal Structure Of The Heterodimeric Complex Of Human Rgs1 And Activated Gi Alpha 1	2770	*GNAI1*	guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 1	1.6	3.11	0.0126	-1.8	1.8	-1.08	0.3264	1.13	0.2092
ENSECAT00000010298	ENSECAG00000010025	100054222	*LOC100054222*	similar to glypican-3 splice	2719	*GPC3*	glypican 3	1.6	2.91	0.0177	-1.4	1.8	-1.05	0.4707	1.16	0.2311
ENSECAT00000000189	ENSECAG00000000234	100058693	*GPC6*	Glypican-6 Precursor	10082	*GPC6*	glypican 6	1.7	3.64	0.0046	-1.4	5.5	-1.20	0.1532	1.18	0.2032
ENSECAT00000017831	ENSECAG00000016756	100051492	*LOC100051492*	similar to glycoprotein M6A	2823	*GPM6A*	glycoprotein M6A	1.8	4.80	0.0000	-2.9	1.8	-1.13	0.3119	1.69	0.0054
ENSECAT00000001905	ENSECAG00000000658	100067870	*LOC100067870*	similar to Glycoprotein (transmembrane) nmb	10457	*GPNMB*	glycoprotein (transmembrane) nmb	2.5	3.03	0.0130	1.2	-1.5	-1.04	0.5302	2.52	0.0015
ENSECAT00000008678	ENSECAG00000008565	100071585	*LOC100071585*	hypothetical LOC100071585	2861	*GPR37*	G protein-coupled receptor 37 (endothelin receptor type B-like)	1.7	3.68	0.0046	-1.2	1.1	-1.01	0.5302	1.64	0.0089
ENSECAT00000010702	ENSECAG00000010419	100069454	*GPRASP2*	G protein-coupled receptor associated sorting protein 2	114928	*GPRASP2*	G protein-coupled receptor associated sorting protein 2	1.5	2.84	0.0177	-2.1	2.4	1.17	0.2825	1.92	0.0019
ENSECAT00000025701	ENSECAG00000023764	100065919	*LOC100065919*	similar to G protein-coupled receptor kinase	2869	*GRK5*	G protein-coupled receptor kinase 5	1.5	2.99	0.0130	1.1	2.2	1.26	0.2091	1.98	0.0005
NM_001081953		100034186	*LOC100034186*	gelsolin	2934	*GSN*	gelsolin (amyloidosis, Finnish type)	1.5	4.51	0.0000	-1.3	-3.1	2.26	0.0070	2.24	0.0028
ENSECAT00000008834	ENSECAG00000008606	100052126	*LOC100052126*	similar to Granzyme K precursor (Granzyme-3) (NK-tryptase-2) (NK-TRYP-2)	3001	*GZMA*	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	1.5	2.80	0.0177	-2.0	2.2	-1.05	0.2334	1.20	0.1814
ENSECAT00000000640	ENSECAG00000000674	100069760	*HAPLN3*	Hyaluronan and proteoglycan link protein 3	145864	*HAPLN3*	Hyaluronan and proteoglycan link protein 3	2.0	3.65	0.0065	-2.1	1.9	-1.31	0.2572	1.24	0.0427
ENSECAT00000003577	ENSECAG00000002905	100055240	*LOC100055240*	similar to Histone deacetylase 11	79885	*HDAC11*	histone deacetylase 11	1.6	2.85	0.0177	1.3	-2.4	1.57	0.0216	2.20	0.0000

ENSECAT00000020769	ENSECAG00000019411	100063706	*HERC6*	hect domain and RLD 6	55008	*HERC6*	hect domain and RLD 6	2.8	3.83	0.0035	-1.1	1.6	-1.18	0.3389	2.64	0.0000
ENSECAT00000014779	ENSECAG00000014094	100073020	*LOC100073020*	similar to hairy/enhancer-of-split related with YRPW motif 2	23493	*HEY2*	hairy/enhancer-of-split related with YRPW motif 2	1.5	5.19	0.0003	1.1	1.7	-1.09	0.4075	1.47	0.0296
ENSECAT00000005150	ENSECAG00000005199	100071280	*HHEX*	similar to hematopoietically expressed homeobox	3087	*HHEX*	hematopoietically expressed homeobox	1.5	3.68	0.0065	1.2	1.5	1.09	0.3535	1.73	0.0024
ENSECAT00000024819	ENSECAG00000023226	100050651	*LOC100050651*	hypothetical protein LOC100050651	51751	*HIGD1B*	HIG1 hypoxia inducible domain family, member 1B	1.7	4.38	0.0000	1.3	1.0	-1.04	0.4916	1.46	0.0062
ENSECAT00000018358	ENSECAG00000017324	100050473	*LOC100050473*	similar to MHC class I antigen	3105	*HLA-A*	major histocompatibility complex, class I, A	1.6	6.32	0.0000	-1.5	2.0	1.28	0.2523	1.22	0.2031
ENSECAT00000002405	ENSECAG00000002570	100056091	*LOC100056091*	hypothetical protein LOC100056091	9957	*HS3ST1*	heparan sulfate (glucosamine) 3-O-sulfotransferase 1	2.1	3.31	0.0113	-1.0	2.3	-1.36	0.2277	1.84	0.0041
ENSECAT00000027183	ENSECAG00000025171	100073112	*LOC100073112*	similar to heparan sulfate D-glucosaminyl 3-O-sulfotransferase 3A1	9955	*HS3ST3A1*	heparan sulfate (glucosamine) 3-O-sulfotransferase 3A1	2.1	2.89	0.0177	-1.0	1.4	1.14	0.3655	1.79	0.0282
ENSECAT00000020548	ENSECAG00000019243	100056429	*LOC100056429*	similar to 11-beta-hydroxysteroid dehydrogenase type 1	3290	*HSD11B1*	hydroxysteroid (11-beta) dehydrogenase 1	2.9	3.33	0.0113	6.2	-9.8	nd		3.76	0.0015
ENSECAT00000013201	ENSECAG00000012754	100061956	*LOC100061956*	similar to HSPB2	3316	*HSPB2*	heat shock 27kDa protein 2	1.6	2.79	0.0208	-1.7	1.4	-1.04	0.4756	1.50	0.0100

ENSECAT00000020461	ENSECAG00000019345	100050779	*LOC100050779*	similar to Heat shock protein beta-8 (HspB8) (Alpha-crystallin C chain) (Small stress protein-like protein HSP22) (E2-induced gene 1 protein) (Protein kinase H11)	26353	*HSPB8*	heat shock 22kDa protein 8	5.0	3.94	0.0035	1.3	-22.3	1.39	0.0270	5.60	0.0000
ENSECAT00000018086	ENSECAG00000017157	100055430	*LOC100055430*	similar to immediate early response 3	8870	*IER3*	immediate early response 3	4.4	3.75	0.0046	3.9	7.6	1.55	0.0668	5.90	0.0000
ENSECAT00000018190	ENSECAG00000017172	100064838	*ISG12(A)*	ISG12(a) protein-like	83982	*IFI27L2*	interferon, alpha-inducible protein 27-like 2	1.6	3.54	0.0073	-1.1	-3.8	-1.49	0.0187	-1.94	0.0008
XM_001496475		100066067	*IFIT1L*	interferon-induced protein with tetratricopeptide repeats 1-like	439996	*IFIT1L*	interferon-induced protein with tetratricopeptide repeats 1-like	1.7	3.65	0.0046	2.0	2.5	-1.05	0.4002	2.00	0.0144
ENSECAT00000015626	ENSECAG00000014889	100034154	*IGFBP-1*	insulin-like growth factor binding protein-1	3484	*IGFBP1*	insulin-like growth factor binding protein 1	5.8	3.82	0.0036	11.2	1.5	nd		5.19	0.0008
ENSECAT00000012491	ENSECAG00000012058	100034061	*IGFBP-2*	insulin-like growth factor binding protein-2	3485	*IGFBP2*	insulin-like growth factor binding protein 2, 36kDa	1.9	4.02	0.0020	-1.9	1.3	1.12	0.4350	1.89	0.0062
ENSECAT00000019151	ENSECAG00000018104	100034155	*IGFBP-3*	insulin-like growth factor binding protein-3	3486	*IGFBP3*	insulin-like growth factor binding protein 3	3.5	5.89	0.0000	-3.9	-2.8	1.63	0.0270	5.60	0.0000
ENSECAT00000013593	ENSECAG00000013087	100033844	*AGM*	angiomodulin	3490	*IGFBP7*	insulin-like growth factor binding protein 7	1.7	3.85	0.0038	-1.2	1.1	1.03	0.5025	1.46	0.0210
ENSECAT00000009745	ENSECAG00000009556	100066058	*IGHC1*	immunogobulin gamma 1 heavy chain constant region	3500	*IGHG1*	immunoglobulin heavy constant gamma 1 (G1m marker)	3.3	2.49	0.0298	-5.3	6.6	-1.63	0.0850	-1.27	0.2070
ENSECAT00000003731	ENSECAG00000003774	100066058	*IGHC1*	immunogobulin gamma 1 heavy chain constant region	3500	*IGHG1*	immunoglobulin heavy constant gamma 1 (G1m marker)	3.5	3.37	0.0094	-4.9	13.3	-1.74	0.0640	-1.12	0.3322

ENSECAT00000006097	ENSECAG00000006095		*IGHG3*	Immunoglobulin gamma 3 heavy chain constant region (IGHG3 gene), exon 1-4		*IGHG3*		3.5	3.01	0.0130	-4.4	1.2	-2.67	0.0333	1.22	0.3025
ENSECAT00000015122	ENSECAG00000014509	100052564	*LOC100052564*	similar to hCG2043214		*IGLV1-40*	immunoglobulin lambda variable 1-40	2.4	2.49	0.0298	-2.4	-2.8	-1.95	0.0430	-1.12	0.3478
ENSECAT00000015778	ENSECAG00000015109	100060365	*LOC100060365*	similar to lambda-immunoglobulin	28809	*IGLV3-1*	Ig lambda chain V-IV region	2.3	2.65	0.0236	-3.0	3.5	-1.79	0.1413	-1.28	0.1962
XM_001499705		100065894	*LOC100065894*	similar to interleukin 32	9235	*IL32*	interleukin 32	1.6	3.30	0.0113	1.1	-5.5	-1.00	0.5580	1.39	0.0397
ENSECAT00000003156	ENSECAG00000003315	100146249	*IRS2*	Insulin receptor substrate 2	8660	*IRS2*	insulin receptor substrate 2	1.8	5.51	0.0000	1.0	2.2	1.36	0.2253	2.57	0.0021
ENSECAT00000018839	ENSECAG00000017386	100063434	*ITGA1*	integrin, alpha 1	3672	*ITGA1*	integrin, alpha 1	1.6	2.97	0.0130	-1.1	2.5	1.06	0.4383	1.65	0.0197
ENSECAT00000020530	ENSECAG00000019215	100053462	*LOC100053462*	similar to integrin beta-8	3696	*ITGB8*	integrin, beta 8	1.5	3.41	0.0092	1.3	-3.2	2.06	0.0137	3.13	0.0000
ENSECAT00000023419	ENSECAG00000020933	100052808	*ITPR1*	Inositol 1,4,5-trisphosphate receptor type 2	3708	*ITPR1*	inositol 1,4,5-triphosphate receptor, type 1	4.1	4.42	0.0000	1.9	4.0	-1.02	0.5029	3.02	0.0006
ENSECAT00000014473	ENSECAG00000012993	100064289	*LOC100064289*	similar to Jagged 1	182	*JAG1*	jagged 1 (Alagille syndrome)	1.5	2.84	0.0177	-1.8	2.3	1.31	0.2031	2.01	0.0017
ENSECAT00000015802	ENSECAG00000014992	100053905	*LOC100053905*	similar to C21ORF43	58494	*JAM2*	junctional adhesion molecule 2	1.7	3.45	0.0092	-1.2	9.7	1.30	0.1094	2.06	0.0000
ENSECAT00000021324	ENSECAG00000020082	100064342	*LOC100064342*	hypothetical LOC100064342	3781	*KCNN2*	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2	6.5	3.77	0.0046	2.0	1.6	-1.18	0.4134	4.79	0.0005
ENSECAT00000016198	ENSECAG00000015488	100147493	*LOC100147493*	similar to intermediate-conductance calcium-activated potassium channel	3783	*KCNN4*	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4	2.2	3.67	0.0046	1.6	2.3	1.09	0.3905	1.37	0.2224

ENSECAT00000021639	ENSECAG00000019429	100033959	*KDR*	kinase insert domain receptor	3791	*KDR*	kinase insert domain receptor (a type III receptor tyrosine kinase)	1.7	3.23	0.0115	1.5	1.3	1.07	0.4119	1.55	0.0599
ENSECAT00000010895	ENSECAG00000010546	100052058	*LOC100052058*	similar to kruppel-like factor 5	688	*KLF5*	Kruppel-like factor 5 (intestinal)	1.6	4.69	0.0056	1.8	1.2	1.08	0.4582	1.71	0.0089
CX596677	ENSECAG00000024925	100050300	*KLF9*	Krueppel-like factor 9	687	*KLF9*	Kruppel-like factor 9	1.5	2.82	0.0177	1.2	2.0	1.14	0.2722	1.51	0.2290
ENSECAT00000015505	ENSECAG00000014646	100068133	*KNG1*	kininogen 1	3827	*KNG1*	kininogen 1	4.3	4.74	0.0000	-1.5	15.4	2.08	0.0517	7.21	0.0000
NM_001081768	ENSECAG00000021903	791245	*LAMC2*	laminin, gamma 2	3918	*LAMC2*	laminin, gamma 2	2.6	5.89	0.0000	-1.7	2.2	-1.18	0.2825	2.86	0.0000
ENSECAT00000022362	ENSECAG00000020957	100070310	*LOC100070310*	similar to lipocalin 2 (oncogene 24p3)	3934	*LCN2*	lipocalin 2	2.8	4.43	0.0000	-3.5	1.9	-1.46	0.3389	3.89	0.0000
ENSECAT00000011488	ENSECAG00000010840	100071626	*LIPA*	lipase A, lysosomal acid, cholesterol esterase	3988	*LIPA*	lipase A, lysosomal acid, cholesterol esterase	1.6	3.39	0.0092	1.1	-2.5	1.69	0.0187	2.27	0.0007
XM_001492772		100060540	*LOC100060540*	similar to lambda-immunoglobulin	1E+08	*LOC100290481*	similar to immunoglobulin lambda locus	2.1	2.86	0.0177	-3.0	-5.4	-1.47	0.0347	-1.38	0.0853
DN508620					645638	*LOC645638*	similar to WDNM1-like protein	1.8	4.37	0.0006	1.8	1.2	-1.02	0.5122	1.53	0.0227
ENSECAT00000015502	ENSECAG00000014771	100064016	*LOX*	Protein-lysine 6-oxidase Precursor	4015	*LOX*	lysyl oxidase	1.6	3.09	0.0126	-1.2	1.6	-1.00	0.3881	1.32	0.1022
ENSECAT00000006621	ENSECAG00000005573	100070637	*LOXL4*	Lysyl oxidase homolog 4 Precursor	84171	*LOXL4*	lysyl oxidase-like 4	1.6	3.96	0.0040	1.1	1.4	-1.06	0.4047	1.67	0.0020
ENSECAT00000026820	ENSECAG00000024824	100052932	*XP_001497658.2*	similar to latrophilin 2	23266	*LPHN2*	latrophilin 2	1.6	3.15	0.0115	-1.4	1.2	1.03	0.5064	1.56	0.0077
ENSECAT00000006441	ENSECAG00000006476	100061270	*LRRC8D*	leucine rich repeat containing 8 family, member D	23507	*LRRC8B*	leucine rich repeat containing 8 family, member B	1.6	3.61	0.0046	-1.4	2.0	-1.17	0.2677	-1.12	0.1511
ENSECAT00000021853	ENSECAG00000019691	100070522	*LTBP1*	latent transforming growth factor beta binding protein 1	4052	*LTBP1*	latent transforming growth factor beta binding protein 1	2.6	6.01	0.0000	-1.5	1.7	1.11	0.3851	2.74	0.0002
XM_001505010		100066253	*LOC100066253*	hypothetical protein LOC100066253	4062	*LY6H*	lymphocyte antigen 6 complex, locus H	1.7	4.51	0.0000	-4.4	1.9	-1.12	0.3333	1.31	0.2318

ENSECAT00000020208	ENSECAG00000019109	100063854	*LOC100063854*	hypothetical protein LOC100063854	7851	*MALL*	mal, T-cell differentiation protein-like	1.6	4.26	0.0000	-1.7	4.1	1.23	0.1681	1.93	0.0012
ENSECAT00000024822	ENSECAG00000022893	100064575	*MAN2A1*	mannosidase, alpha, class 2A, member 1	4124	*MAN2A1*	mannosidase, alpha, class 2A, member 1	1.8	5.28	0.0000	-1.0	4.0	1.30	0.1532	2.50	0.0000
ENSECAT00000026898	ENSECAG00000024860	100073186	*MAP3K5*	mitogen-activated protein kinase kinase kinase 5	4217	*MAP3K5*	mitogen-activated protein kinase kinase kinase 5	1.6	3.15	0.0115	-1.5	1.8	-1.18	0.3389	1.49	0.0520
ENSECAT00000007639	ENSECAG00000007518	100063655	*LOC100063655*	hypothetical protein LOC100063655	115123	*MARCH3*	membrane-associated ring finger (C3HC4) 3	1.7	3.49	0.0073	-1.5	1.1	-1.06	0.4567	1.50	0.0282
ENSECAT00000008696	ENSECAG00000007798	100061008	*LOC100061008*	similar to malic enzyme 3, NADP(+)-dependent, mitochondrial	10873	*ME3*	malic enzyme 3, NADP(+)-dependent, mitochondrial	1.6	3.72	0.0046	-2.3	2.6	-1.35	0.1729	-1.05	0.0260
ENSECAT00000025391	ENSECAG00000023488	100055637	*MED13L*	mediator complex subunit 13-like	23389	*MED13L*	mediator complex subunit 13-like	1.6	2.86	0.0177	-1.2	2.0	1.17	0.3946	1.61	0.1839
ENSECAT00000011493	ENSECAG00000010385	100056013	*MET*	met proto-oncogene (hepatocyte growth factor receptor)	4233	*MET*	met proto-oncogene (hepatocyte growth factor receptor)	1.7	3.55	0.0073	-1.2	2.5	1.48	0.1772	2.63	0.0033
ENSECAT00000007866	ENSECAG00000007658	100053785	*LOC100053785*	similar to lysophospholipase homolog	11343	*MGLL*	monoglyceride lipase	1.9	3.49	0.0074	-1.1	5.5	1.44	0.1124	2.64	0.0006
ENSECAT00000011027	ENSECAG00000010721	100063934	*LOC100063934*	similar to matrix Gla protein	4256	*MGP*	matrix Gla protein	3.0	4.82	0.0000	-1.3	1.3	1.14	0.4236	3.36	0.0000
XM_001498375		100033918	*LOC100033918*	microphthalmia transcription factor	4286	*MITF*	microphthalmia-associated transcription factor	1.7	3.39	0.0092	1.1	2.5	1.50	0.1694	2.51	0.0033
ENSECAT00000012186	ENSECAG00000011719	100053317	*MMRN1*	multimerin 1	22915	*MMRN1*	multimerin 1	2.2	2.91	0.0153	-2.0	1.0	1.00	0.4836	1.86	0.0018
ENSECAT00000026782	ENSECAG00000024812	100062492	*MST1R*	macrophage stimulating 1 receptor (c-met-related tyrosine kinase)	4486	*MST1R*	macrophage stimulating 1 receptor (c-met-related tyrosine kinase)	1.9	5.66	0.0000	-1.4	8.7	1.31	0.0668	2.34	0.0005
CD465149					85027	*MSTP150*	putative small membrane protein	2.9	3.73	0.0041	1.2	1.8	1.16	0.3772	3.28	0.0011

ENSECAT00000008458	ENSECAG00000007931	100070060	*LOC100070060*	similar to mucin 4	4585	*MUC4*	mucin 4, cell surface associated	2.5	3.14	0.0126	-9.2	2.6	-1.37	0.1681	2.22	0.0012
ENSECAT00000002008	ENSECAG00000002106	100058571	*LOC100058571*	similar to N-acetyltransferase 8B	9027	*NAT8*	N-acetyltransferase 8 (GCN5-related, putative)	1.6	3.28	0.0113	1.0	3.0	1.39	0.1382	1.64	0.0397
XM_001488410	ENSECAG00000018997	100052657	*LOC100052657*	similar to nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	4773	*NFATC2*	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2	1.5	3.32	0.0113	1.1	1.9	1.07	0.3046	1.52	0.0083
ENSECAT00000021581	ENSECAG00000020093	100068418	*LOC100068418*	similar to type C atrial natriuretic peptide receptor	4883	*NPR3*	natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C)	2.3	4.32	0.0015	-1.1	2.9	1.55	0.1584	2.96	0.0019
ENSECAT00000008512	ENSECAG00000008381	100146166	*LOC100146166*	similar to COUP transcription factor 2 (COUP-TF2) (COUP-TF II) (Nuclear receptor subfamily 2 group F member 2) (Apolipoprotein AI regulatory protein 1) (ARP-1)	7026	*NR2F2*	nuclear receptor subfamily 2, group F, member 2	1.5	3.64	0.0046	1.1	-1.0	-1.03	0.5191	1.47	0.0645
ENSECAT00000018899	ENSECAG00000016824	100066305	*NRP2*	Neuropilin-2	8828	*NRP2*	Neuropilin-2	2.0	2.89	0.0177	-3.8	5.1	-1.39	0.1382	1.59	0.0282
ENSECAT00000026487	ENSECAG00000024611	100051839	*LOC100051839*	similar to neurotrophin 3	4908	*NTF3*	neurotrophin 3	1.7	4.19	0.0036	-1.4	3.0	-1.12	0.1668	1.56	0.0946
ENSECAT00000002536	ENSECAG00000002145	100053752	*NUAK1*	NUAK family, SNF1-like kinase, 1	9891	*NUAK1*	NUAK family, SNF1-like kinase, 1	1.7	4.46	0.0000	-1.1	-2.1	1.01	0.5475	1.75	0.0031
NM_001081773	ENSECAG00000014422	791250	*OAS2*	2'-5'-oligoadenylate synthetase 2, 69/71kDa	4939	*OAS2*	2'-5'-oligoadenylate synthetase 2, 69/71kDa	1.5	2.83	0.0169	-1.5	3.6	1.39	0.1682	2.58	0.0001
ENSECAT00000010293	ENSECAG00000009637	100034107	*LOC100034107*	OCA2	4948	*OCA2*	oculocutaneous albinism II	1.9	4.48	0.0000	1.2	5.7	1.55	0.0693	3.13	0.0000

ENSECAT00000009190	ENSECAG00000008038	100062728	*ODZ4*	odz, odd Oz/ten-m homolog 4 (Drosophila)	26011	*ODZ4*	odz, odd Oz/ten-m homolog 4 (Drosophila)	1.9	3.50	0.0073	-1.9	1.9	1.17	0.3655	2.54	0.0008
BM734727					220213	*OTUD1*	OTU domain containing 1	1.5	4.03	0.0020	1.0	2.2	1.19	0.3119	1.77	0.0019
ENSECAT00000018873	ENSECAG00000017844	100058848	*LOC100058848*	similar to oxytocin receptor	5021	*OXTR*	oxytocin receptor	1.6	2.86	0.0177	-1.0	1.6	1.24	0.3389	1.93	0.0062
ENSECAT00000008438	ENSECAG00000008154	100064749	*LOC100064749*	similar to progestin and adipoQ receptor family member V	54852	*PAQR5*	progestin and adipoQ receptor family member V	2.0	4.15	0.0021	3.0	-1.2	1.03	0.5115	2.25	0.0068
NM_001101655		100064309	*PECAM1*	platelet/endothelial cell adhesion molecule	5175	*PECAM1*	platelet/endothelial cell adhesion molecule	1.6	3.10	0.0122	-1.3	7.6	1.40	0.0731	2.07	0.0008
ENSECAT00000013827	ENSECAG00000013131	100054894	*LOC100054894*	similar to HPDHase	55825	*PECR*	peroxisomal trans-2-enoyl-CoA reductase	2.5	8.07	0.0000	-1.1	1.8	1.21	0.2825	2.81	0.0015
ENSECAT00000025907	ENSECAG00000024110	100067723	*LOC100067723*	similar to Proenkephalin A precursor	5179	*PENK*	proenkephalin	2.1	2.78	0.0208	4.4	-1.7	-1.98	0.0225	1.00	0.3743
ENSECAT00000014506	ENSECAG00000013247	100034167	*PER2*	period homolog 2 (Drosophila)	8864	*PER2*	period homolog 2 (Drosophila)	1.6	3.79	0.0049	-1.0	2.7	1.26	0.1991	2.34	0.0013
ENSECAT00000006309	ENSECAG00000005228	100059940	*LOC100059940*	similar to Phosphoglycerate dehydrogenase	26227	*PHGDH*	phosphoglycerate dehydrogenase	2.0	3.84	0.0035	1.1	18.7	1.47	0.0187	2.82	0.0000
ENSECAT00000017734	ENSECAG00000016613	100071564	*LOC100071564*	similar to LL5 beta protein	90102	*PHLDB2*	pleckstrin homology-like domain, family B, member 2	1.9	4.01	0.0020	-1.4	6.8	1.31	0.0850	2.48	0.0000
ENSECAT00000017184	ENSECAG00000016196	100055765	*PIGR*	Polymeric immunoglobulin receptor Precursor (Poly-Ig receptor)	5284	*PIGR*	polymeric immunoglobulin receptor	1.9	3.26	0.0113	-8.2	-1.5	1.02	0.5397	1.99	0.0015
ENSECAT00000021930	ENSECAG00000020563	100053898	*PIM1*	pim-1 oncogene	5292	*PIM1*	pim-1 oncogene	1.6	3.42	0.0092	-1.6	1.5	1.11	0.4134	1.53	0.0046
ENSECAT00000014292	ENSECAG00000013700	100050951	*LOC100050951*	similar to Pirin	8544	*PIR*	pirin (iron-binding nuclear protein)	1.5	4.87	0.0000	-2.6	1.5	1.08	0.3808	1.50	0.0214
ENSECAT00000026856	ENSECAG00000024810	100033889	*PLA2G1B*	phospholipase A2, group IB (pancreas)	5321	*PLA2G4A*	phospholipase A2, group IVA (cytosolic, calcium-dependent)	1.6	3.62	0.0063	-1.6	1.7	-1.20	0.2966	1.54	0.0109

ENSECAT00000008239	ENSECAG00000007571	100050239	*PLAT*	plasminogen activator, tissue	5327	*PLAT*	plasminogen activator, tissue	2.5	6.43	0.0000	-1.4	5.3	1.51	0.0943	2.84	0.0007
ENSECAT00000025706	ENSECAG00000023703	100054233	*PLCD1*	phospholipase C, delta 1	5333	*PLCD1*	phospholipase C, delta 1	2.3	3.80	0.0046	-1.8	1.2	1.06	0.4350	2.74	0.0000
ENSECAT00000018697	ENSECAG00000017520	100055892	*LOC100055892*	hypothetical protein LOC100055892	84898	*PLXDC2*	plexin domain containing 2	2.4	2.41	0.0317	1.3	3.0	1.63	0.1152	3.97	0.0000
ENSECAT00000005858	ENSECAG00000001716	100051646	*PLXNA2*	Plexin-A2 Precursor	5362	*PLXNA2*	plexin A2	1.6	4.02	0.0028	-1.5	1.5	-1.02	0.4910	1.59	0.0172
ENSECAT00000009314	ENSECAG00000009044	100071967	*PRDM1*	PR domain containing 1, with ZNF domain	639	*PRDM1*	PR domain containing 1, with ZNF domain	1.6	3.10	0.0126	1.4	2.2	1.19	0.3119	1.88	0.0008
ENSECAT00000010056	ENSECAG00000009483	100053793	*LOC100053793*	similar to prolactin receptor	5618	*PRLR*	prolactin receptor	2.0	2.61	0.0264	-1.1	3.4	1.31	0.2277	2.24	0.0000
ENSECAT00000004895, XM_001495172	ENSECAG00000004897	100065904	*PRNP*	prion protein	5621	*PRNP*	prion protein	1.8	5.05	0.0000	-2.9	2.7	1.25	0.3066	2.77	0.0000
ENSECAT00000026279	ENSECAG00000024428	100069445	*LOC100069445*	similar to endothelial cell protein C/APC receptor	10544	*PROCR*	protein C receptor, endothelial (EPCR)	1.6	2.84	0.0177	-1.3	1.5	1.11	0.4211	1.66	0.0046
ENSECAT00000005511	ENSECAG00000005563	100060937	*LOC100060937*	similar to putative serine protease 23	11098	*PRSS23*	protease, serine, 23	2.6	5.64	0.0000	-1.9	1.4	1.13	0.3780	2.81	0.0000
ENSECAT00000018732	ENSECAG00000017483	100051830	*PSD3*	pleckstrin and Sec7 domain containing 3	23362	*PSD3*	pleckstrin and Sec7 domain containing 3	1.5	3.28	0.0113	-1.5	3.2	1.38	0.1094	2.16	0.0019
ENSECAT00000014888	ENSECAG00000014239	100053557	*LOC100053557*	similar to protaglandin receptor EP3E	5733	*PTGER3*	prostaglandin E receptor 3 (subtype EP3)	1.8	2.97	0.0157	-1.3	2.1	1.17	0.3367	2.05	0.0036
ENSECAT00000011519	ENSECAG00000011145	100053208	*LOC100053208*	similar to prostaglandin E2 receptor EP4 subtype	5734	*PTGER4*	prostaglandin E receptor 4 (subtype EP4)	2.0	7.04	0.0000	-1.3	1.7	1.13	0.3589	2.37	0.0001
ENSECAT00000005057	ENSECAG00000004698	100058059	*PTGR1*	Prostaglandin reductase 1	22949	*PTGR1*	prostaglandin reductase 1	2.7	2.80	0.0177	-2.1	15.4	1.66	0.0536	3.91	0.0000
ENSECAT00000012202, XM_001501199	ENSECAG00000011293	100071439	*R-PTP-zeta,PTPRZ1*	Receptor-type tyrosine-protein phosphatase zeta Precursor, protein tyrosine phosphatase, receptor-type, Z polypeptide 1	5793	*PTPRG*	protein tyrosine phosphatase, receptor type, G	1.8	4.70	0.0010	-2.0	2.1	-1.02	0.3774	1.49	0.0437

ENSECAT00000016660	ENSECAG00000015669	100062293	*LOC100062293*	similar to Ras-related protein Rab-3B (SMG P25B)	5865	*RAB3B*	RAB3B, member RAS oncogene family	3.5	3.54	0.0073	1.5	-1.3	-1.05	0.5064	3.67	0.0007
ENSECAT00000005983	ENSECAG00000006060	100051458	*RAI2*	retinoic acid induced 2	10742	*RAI2*	retinoic acid induced 2	1.5	5.81	0.0000	-2.0	1.1	1.07	0.4541	1.48	0.0323
ENSECAT00000023599	ENSECAG00000022153	100050263	*LOC100050263*	similar to RALY RNA binding protein-like	138046	*RALYL*	RALY RNA binding protein-like	1.5	3.80	0.0046	1.3	12.9	1.63	0.1754	1.84	0.0406
XM_001494115		100051546	*RARB*	retinoic acid receptor, beta	5914	*RARA*	retinoic acid receptor, alpha	1.5	3.22	0.0115	-1.4	7.6	-1.54	0.0653	-1.31	0.0877
ENSECAT00000015490	ENSECAG00000014799	100063047	*LOC100063047*	hypothetical LOC100063047	5919	*RARRES2*	retinoic acid receptor responder (tazarotene induced) 2	1.7	2.93	0.0130	-1.4	2.5	1.39	0.1681	2.19	0.0000
ENSECAT00000024212	ENSECAG00000022648	100064529	*LOC100064529*	similar to carcinoma associated protein HOJ-1	11228	*RASSF8*	Ras association (RalGDS/AF-6) domain family (N-terminal) member 8	1.7	3.24	0.0119	-1.5	1.1	-1.01	0.5441	1.79	0.0027
ENSECAT00000003068	ENSECAG00000003124	100056851	*RCSD1*	Capz-interacting protein	92241	*RCSD1*	RCSD domain containing 1	1.5	3.42	0.0092	-1.0	-1.1	1.06	0.4916	1.66	0.0145
ENSECAT00000012594	ENSECAG00000012179	100051067	*RGS2*	regulator of G-protein signaling 2, 24kDa	5997	*RGS2*	regulator of G-protein signaling 2, 24kDa	1.8	2.74	0.0208	-1.1	2.5	1.24	0.1976	2.49	0.0000
ENSECAT00000025401	ENSECAG00000023668	100059437	*LOC100059437*	similar to regulator of G-protein signalling 5	8490	*RGS5*	regulator of G-protein signaling 5	1.9	2.69	0.0208	1.2	2.0	1.24	0.2518	2.19	0.0031
ENSECAT00000003662	ENSECAG00000003386	100051825	*LOC100051825*	hypothetical protein LOC100051825	57381	*RHOJ*	ras homolog gene family, member J	1.7	2.90	0.0177	1.0	2.0	1.09	0.4235	1.39	0.0826
ENSECAT00000016226	ENSECAG00000015381	100061415	*LOC100061415*	hypothetical LOC100061415	54453	*RIN2*	Ras and Rab interactor 2	1.6	3.07	0.0146	1.2	2.2	1.42	0.1963	2.07	0.0390
ENSECAT00000012082	ENSECAG00000011727	100072691	*Rnase5*	Ribonuclease 4 Precursor	6038	*RNASE4*	ribonuclease, RNase A family, 4	2.3	2.99	0.0130	-1.6	2.5	-1.00	0.3078	1.88	0.0130
ENSECAT00000022372	ENSECAG00000020971	100058747	*LOC100058747*	similar to Rho-related GTP-binding protein Rho6 precursor (Rho family GTPase 1) (Rnd1)	27289	*RND1*	Rho family GTPase 1	2.0	2.89	0.0167	2.6	7.5	-1.28	0.0593	1.38	0.0783

XM_001488213		100050088	*LOC100050088*	similar to Rho-related GTP-binding protein RhoE precursor (Rho family GTPase 3) (Rnd3) (Rho8) (MemB protein)	390	*RND3*	Rho family GTPase 3	1.7	7.07	0.0000	1.5	3.5	-1.08	0.2866	1.58	0.0333
ENSECAT00000004699	ENSECAG00000003462	100051950	*RUNX1*	Runt-related transcription factor 1	861	*RUNX1*	Runt-related transcription factor 1	2.1	3.30	0.0098	-1.2	6.6	1.46	0.1464	3.03	0.0696
ENSECAT00000023493	ENSECAG00000021962	100053446	*SCHIP1*	Schwannomin-interacting protein 1	29970	*SCHIP1*	schwannomin interacting protein 1	1.8	5.36	0.0000	-1.1	1.6	1.09	0.4134	1.80	0.0031
ENSECAT00000022129	ENSECAG00000020842	100054790	*LOC100054790*	similar to serum deprivation response	8436	*SDPR*	serum deprivation response (phosphatidylserine binding protein)	1.9	3.24	0.0115	-1.0	6.3	1.37	0.0850	2.16	0.0005
ENSECAT00000015356, NM_001114533	ENSECAG00000011847	100065158	*SPI2*	alpha-1-antitrypsin	5265	*SERPINA 1*	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1	3.1	3.80	0.0043	1.5	2.3	-1.17	0.3898	3.62	0.0011
ENSECAT00000022717	ENSECAG00000021166	100057505	*LOC100057505*	similar to SCCA2/SCCA1 fusion protein	6318	*SERPINB 4*	serpin peptidase inhibitor, clade B (ovalbumin), member 4	3.4	5.15	0.0000	1.2	-2.0	-1.92	0.0159	1.88	0.0209
ENSECAT00000021200	ENSECAG00000019781	100033931	*PAI-1*	Plasminogen activator inhibitor-1 Fragment	5054	*SERPINE 1*	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	3.1	2.69	0.0238	7.2	1.9	-1.24	0.3203	1.69	0.1503
ENSECAT00000012633	ENSECAG00000011753	100067450	*LOC100067450*	similar to SEC14 and spectrin domains 1	91404	*SESTD1*	SEC14 and spectrin domains 1	1.6	3.55	0.0070	-1.1	1.8	-1.00	0.4291	1.68	0.0029
ENSECAT00000022770	ENSECAG00000021358	100055845	*LOC100055845*	similar to Secreted frizzled-related sequence protein 1	6422	*SFRP1*	secreted frizzled-related protein 1	1.7	3.31	0.0119	-2.1	3.8	1.22	0.2454	1.89	0.0136

ENSECAT00000023082	ENSECAG00000021576	100050428	*LOC100050428*	similar to gamma-sarcoglycan	6445	*SGCG*	sarcoglycan, gamma (35kDa dystrophin-associated glycoprotein)	1.9	2.88	0.0177	1.3	-2.1	1.52	0.0216	2.40	0.0005
ENSECAT00000025491	ENSECAG00000023754		*SHE*	SH2 domain-containing adapter protein E Source: UniProtKB/Swiss-Prot Q5VZ18	126669	*SHE*	Src homology 2 domain containing E	1.6	3.17	0.0125	-1.2	2.6	1.13	0.2515	1.59	0.0245
ENSECAT00000010617	ENSECAG00000009334	100060961	*LOC100060961*	hypothetical protein LOC100060961	6564	*SLC15A1*	solute carrier family 15 (oligopeptide transporter), member 1	2.8	2.44	0.0317	1.5	4.1	-1.45	0.1094	1.90	0.0111
ENSECAT00000010474	ENSECAG00000010094	100063698	*LOC100063698*	similar to Solute carrier family 25, member 36"	55186	*SLC25A36*	solute carrier family 25, member 36	1.6	6.74	0.0000	-1.6	2.2	1.00	0.2686	1.63	0.0306
ENSECAT00000000516	ENSECAG00000000303	100034080	*SLC2A1*	solute carrier family 2 (facilitated glucose transporter), member 1	6513	*SLC2A1*	solute carrier family 2 (facilitated glucose transporter), member 1	2.0	4.89	0.0011	-1.1	2.2	1.35	0.2361	2.62	0.0015
XM_001500484		100146160	*LOC100146160*	similar to Proton myo-inositol cotransporter (H(+)-myo-inositol cotransporter) (Hmit) (H(+)-myo-inositol symporter)	114134	*SLC2A13*	solute carrier family 2 (facilitated glucose transporter), member 13	1.7	2.95	0.0130	-1.1	10.0	1.56	0.0668	2.51	0.0000
DN508408	ENSECAG00000011961	100071541	*LOC100071541 3'-UTR*	similar to Proton-coupled amino acid transporter 2 (Proton/amino acid transporter 2) (Tramdorin-1) (Solute carrier family 36 member 2)	153201	*SLC36A2*	solute carrier family 36 (proton/amino acid symporter), member 2	84.3	11.54	0.0000	55.7	-3.7	3.57	0.0116	198	0.0000

ENSECAT00000012868	ENSECAG00000011961	100071541	*LOC100071541 ORF*	similar to Proton-coupled amino acid transporter 2 (Proton/amino acid transporter 2) (Tramdorin-1) (Solute carrier family 36 member 2)	153201	*SLC36A2-ORF*	solute carrier family 36 (proton/amino acid symporter), member 2	2.5	5.51	0.0000	2.0	1.8	-1.12	0.3119	2.07	0.0012
ENSECAT00000026975	ENSECAG00000024948	100065438	*SLCO2A1*	Solute carrier organic anion transporter family member 2A1	6578	*SLCO2A1*	solute carrier organic anion transporter family, member 2A1	2.0	2.79	0.0206	1.9	1.4	1.08	0.3839	2.05	0.0263
ENSECAT00000024172	ENSECAG00000022407	100068338	*Slit-2*	Slit homolog 2 protein Precursor / LOC100068338 similar to Slit-2 protein	9353	*SLIT2*	slit homolog 2 (Drosophila)	1.5	3.75	0.0058	-2.6	1.5	1.16	0.3516	1.46	0.0701
ENSECAT00000013524	ENSECAG00000012686	100063985	*LOC100063985*	similar to synuclein alpha interacting protein	9627	*SNCAIP*	synuclein, alpha interacting protein	1.9	3.98	0.0028	-1.2	2.2	1.24	0.2253	2.16	0.0071
ENSECAT00000026551	ENSECAG00000024612	100067569	*SNED1*	sushi, nidogen and EGF-like domains 1 [	25992	*SNED1*	sushi, nidogen and EGF-like domains 1	1.7	2.91	0.0161	-1.7	2.4	1.03	0.2038	1.41	0.0846
ENSECAT00000023902	ENSECAG00000022428		*SPINK7*	Serine protease inhibitor Kazal-type 7	84651	*SPINK7*	serine peptidase inhibitor, Kazal type 7 (putative)	2.3	3.14	0.0121	22.7	-1.1	-2.67	0.0177	-1.19	0.3626
ENSECAT00000014299	ENSECAG00000013746	100063739	*LOC100063739*	similar to sprouty homolog 1, antagonist of FGF signaling	10252	*SPRY1*	sprouty homolog 1, antagonist of FGF signaling (Drosophila)	1.6	3.25	0.0115	-1.0	1.8	1.07	0.4479	1.59	0.0852
ENSECAT00000015653	ENSECAG00000014728	100057264	*LOC100057264*	similar to sushi-repeat protein	27286	*SRPX2*	sushi-repeat-containing protein, X-linked 2	1.7	3.27	0.0113	-1.5	3.4	-1.03	0.4916	1.77	0.0041
ENSECAT00000025335	ENSECAG00000023170	100054693	*STAT4*	signal transducer and activator of transcription 4	6775	*STAT4*	signal transducer and activator of transcription 4	2.0	3.02	0.0130	1.6	1.0	-1.03	0.5302	2.17	0.0000
ENSECAT00000014465	ENSECAG00000013731	100054071	*LOC100054071*	similar to stanniocalcin	6781	*STC1*	stanniocalcin 1	3.1	5.55	0.0000	-1.8	15.8	2.05	0.0513	6.26	0.0000

ENSECAT00000024982	ENSECAG00000023308	100060223	*LOC100060223*	hypothetical protein LOC100060223	26872	*STEAP1*	six transmembrane epithelial antigen of the prostate 1	1.8	3.47	0.0073	1.1	7.7	-1.42	0.0850	1.38	0.0520
ENSECAT00000000416; XR_036417	ENSECAG00000000520	100054971	*LOC100054971*	similar to death-associated protein kinase-related apoptosis inducing protein kinase	9262	*STK17B*	serine/threonine kinase 17b	1.6	3.56	0.0096	-1.1	1.2	1.08	0.4169	1.89	0.0036
ENSECAT00000023783	ENSECAG00000021653	100053852	*SVEP1*	Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1	79987	*SVEP1*	sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1	1.9	2.90	0.0167	1.1	2.1	1.27	0.2401	2.19	0.0016
ENSECAT00000007290	ENSECAG00000006803	100050854	*SYTL2*	Synaptotagmin-like 2	54843	*SYTL2*	synaptotagmin-like 2	1.7	5.42	0.0000	-1.1	1.8	-1.14	0.3034	1.35	0.1415
ENSECAT00000005124	ENSECAG00000004961	100067576	*LOC100067576*	hypothetical protein LOC100067576	4070	*TACSTD2*	tumor-associated calcium signal transducer 2	3.2	2.32	0.0378	-1.9	1.8	-1.11	0.4350	1.91	0.0100
NM_001110134	ENSECAG00000011268	100062690	*TAGLN*	transgelin	6876	*TAGLN*	transgelin	2.3	2.71	0.0228	1.1	2.3	1.13	0.4577	1.92	0.0055
ENSECAT00000010210	ENSECAG00000009793	100061511	*LOC100061511*	similar to neuronal protein	29114	*TAGLN3*	transgelin 3	2.0	3.28	0.0113	2.1	4.5	-1.32	0.1601	1.22	0.1513
ENSECAT00000024938	ENSECAG00000023297		*TDRD10*	Tudor domain-containing protein 10	126668	*TDRD10*	tudor domain containing 10	1.6	3.98	0.0020	-1.1	1.3	1.05	0.4747	1.52	0.0325
ENSECAT00000018137	ENSECAG00000016566	100066963	*LOC100066963*	similar to receptor tyrosine kinase	7010	*TEK*	TEK tyrosine kinase, endothelial	1.5	3.93	0.0035	1.2	7.5	1.36	0.0536	1.92	0.0000
BM780537	ENSECAG00000006290	100070046	*TGM2*	Protein-glutamine gamma-glutamyltransferase 2	7052	*TGM2*	transglutaminase 2 (C polypeptide, protein-glutamine-gamma-glutamyltransferase)	1.8	4.54	0.0005	1.8	1.4	1.00	0.4927	1.61	0.0531
ENSECAT00000009707	ENSECAG00000008923	100057478	*THBS1*	thrombospondin 1	7057	*THBS1*	thrombospondin 1	1.7	5.72	0.0000	2.2	2.8	-1.59	0.2325	-1.02	0.0808
ENSECAT00000023244	ENSECAG00000021122	100050044	*THBS2*	thrombospondin 2	7058	*THBS2*	thrombospondin 2	2.5	4.65	0.0000	2.4	2.0	-1.30	0.2894	2.06	0.0121
ENSECAT00000019031, XM_001487840	ENSECAG00000018029	100146573	*LOC100146573*	similar to thrombospondin type I domain-containing 1	55901	*THSD1*	thrombospondin, type I, domain containing 1	1.6	4.42	0.0000	-1.3	1.8	1.12	0.2831	1.60	0.0603

ENSECAT00000014937	ENSECAG00000014259	100034220	*TIMP-1*	tissue inhibitor of metalloproteinase-1	7076	*TIMP1*	TIMP metallopeptidase inhibitor 1	1.8	3.75	0.0050	-1.0	1.4	-1.09	0.4086	1.51	0.0245
XM_001494169		100062680	*LOC100062680*	hypothetical protein LOC100062680	7090	*TLE3*	transducin-like enhancer of split 3 (E(sp1) homolog, Drosophila)	1.5	3.45	0.0092	-1.1	1.6	1.04	0.3238	1.29	0.1559
ENSECAT00000019751	ENSECAG00000018664	100058490	*LOC100058490*	similar to transmembrane 4 L six family member 18	116441	*TM4SF18*	transmembrane 4 L six family member 18	1.6	2.84	0.0177	-1.1	2.3	1.18	0.2435	1.67	0.0206
ENSECAT00000003310	ENSECAG00000003418	100065089	*TMEM140*	Transmembrane protein 140	55281	*TMEM140*	transmembrane protein 140	1.6	3.05	0.0122	1.1	3.3	1.43	0.1448	1.80	0.0089
ENSECAT00000011766	ENSECAG00000011418	100066723	*LOC100066723*	similar to LOC155006 protein	155006	*TMEM213*	transmembrane protein 213	6.0	10.91	0.0000	1.8	6.1	1.78	0.0668	11.0	0.0000
ENSECAT00000020075	ENSECAG00000018940	100064015	*LOC100064015*	similar to LOC124446 protein	124446	*TMEM219*	transmembrane protein 219	1.5	6.28	0.0000	-2.9	1.3	1.02	0.5309	1.50	0.0166
ENSECAT00000023935	ENSECAG00000022424	100146755	*TNFRSF12A*	Tumor necrosis factor receptor superfamily member 12A	51330	*TNFRSF12A*	tumor necrosis factor receptor superfamily, member 12A	2.0	3.48	0.0073	1.6	-2.3	1.01	0.5531	2.13	0.0145
ENSECAT00000022137	ENSECAG00000020810	100066195	*LOC100066195*	similar to glucocorticoid-induced TNFR-related protein	8784	*TNFRSF18*	tumor necrosis factor receptor superfamily, member 18	2.0	2.93	0.0130	1.2	2.1	1.05	0.4567	2.31	0.0012
CX604004, ENSECAT00000018293	ENSECAG00000017269	100068460	*LOC100068460*	similar to TNFR-related death receptor-6	27242	*TNFRSF21*	tumor necrosis factor receptor superfamily, member 21	2.3	3.67	0.0067	1.2	1.6	1.09	0.3655	2.51	0.0010
ENSECAT00000020632	ENSECAG00000019391	100064377	*LOC100064377*	similar to TNF-related apoptosis-inducing ligand	8743	*TNFSF10*	tumor necrosis factor (ligand) superfamily, member 10	2.0	4.17	0.0017	-1.1	6.9	1.73	0.0640	3.31	0.0000
ENSECAT00000011803	ENSECAG00000011429	100059910	*LOC100059910*	similar to hCG1639853	27324	*TOX3*	TOX high mobility group box family member 3	1.6	2.81	0.0177	-11.5	3.7	-1.34	0.1382	1.50	0.0645
L38383		100056867	*LOC100056867*	similar to This CDS feature is included to show the translation of the corresponding C_region	6955	*TRA@*	T cell receptor alpha locus	1.5	3.16	0.0115	-2.4	1.6	-1.06	0.3166	1.28	0.2066

ENSECAT00000005859	ENSECAG00000004594	100034071	*LOC100034071*	epithelial calcium channel 1	56302	*TRPV5*	transient receptor potential cation channel, subfamily V, member 5	1.7	3.62	0.0046	1.3	1.8	-1.12	0.3119	1.58	0.0282
ENSECAT00000024399	ENSECAG00000022404	100055509	*TRPV6*	transient receptor potential cation channel, subfamily V, member 6	55503	*TRPV6*	transient receptor potential cation channel, subfamily V, member 6	2.3	5.62	0.0000	1.1	3.8	1.43	0.1681	3.33	0.0000
ENSECAT00000017616	ENSECAG00000016573	100055873	*LOC100055873*	hypothetical protein LOC100055873	7102	*TSPAN7*	tetraspanin 7	1.7	3.17	0.0115	-4.1	4.8	1.33	0.0850	2.22	0.0015
XM_001495057		100064038	*LOC100064038*	similar to Tspan8 protein	7103	*TSPAN8*	tetraspanin 8	1.7	3.45	0.0092	-4.4	1.1	1.06	0.4892	2.17	0.0005
NM_001081820		100033838	*UCHL1*	ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)	7345	*UCHL1*	ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase)	1.6	3.03	0.0135	-2.1	1.7	1.14	0.2832	1.86	0.0020
ENSECAT00000009010	ENSECAG00000008764	100050109	*UNC93A*	unc-93 homolog A (C. elegans)	54346	*UNC93A*	unc-93 homolog A (C. elegans)	2.5	3.21	0.0115	1.4	-2.2	1.02	0.5397	3.49	0.0000
ENSECAT00000020357	ENSECAG00000019042	100071097	*UP1b*	Uroplakin-1b / LOC100071097	7348	*UPK1B*	uroplakin 1B	1.7	4.24	0.0010	-13.1	1.3	-1.01	0.4166	1.24	0.2029
ENSECAT00000009366	ENSECAG00000009125	100052016	*LOC100052016*	similar to uroplakin III	7380	*UPK3A*	uroplakin 3A	1.5	6.19	0.0000	1.0	-1.1	-1.05	0.4916	1.64	0.0100
ENSECAT00000004661	ENSECAG00000003318	100070306	*VIT*	vitrin	5212	*VIT*	vitrin	2.5	4.46	0.0006	1.9	4.4	1.54	0.0934	2.99	0.0002
ENSECAT00000024225	ENSECAG00000021859	100050907	*VLDLR*	Very low-density lipoprotein receptor Precursor	7436	*VLDLR*	very low density lipoprotein receptor	3.5	10.58	0.0000	1.4	6.3	1.40	0.1382	4.35	0.0000
ENSECAT00000016918	ENSECAG00000015984	100064973	*LOC100064973*	similar to WAS/WASL interacting protein family, member 1	7456	*WIPF1*	WAS/WASL interacting protein family, member 1	1.7	2.81	0.0193	-1.3	3.1	1.36	0.1302	2.00	0.0270
ENSECAT00000014004	ENSECAG00000013347	100072430	*WISP3*	WNT1-inducible-signaling pathway protein 3 Precursor (WISP-3) Source: UniProtKB/Swiss-Prot O95389	8838	*WISP3*	WNT1 inducible signaling pathway protein 3	2.0	2.62	0.0264	1.7	1.8	1.29	0.3119	1.72	0.0266

ENSECAT00000023038	ENSECAG00000021613	100050661	*WWTR1*	WW domain containing transcription regulator 1	25937	*WWTR1*	WW domain containing transcription regulator 1	1.5	3.89	0.0029	1.1	2.2	1.30	0.2253	1.76	0.0329
ENSECAT00000011930	ENSECAG00000011576	100066936	*LOC100066936*	hypothetical protein LOC100066936	219539	*YPEL4*	yippee-like 4 (Drosophila)	1.6	4.17	0.0012	-1.2	6.1	1.30	0.1398	1.45	0.0630
ENSECAT00000006598	ENSECAG00000006546	100063937	*ZNF521*	zinc finger protein 521	25925	*ZNF521*	zinc finger protein 521	1.5	2.98	0.0130	1.2	2.5	-1.22	0.1976	1.06	0.3854

Mean FC D12 Pr/Co	Mean fold change day 12 pregnant vs. control for mares #1, 2, 4, 5, 6
M#3 FC Pr/Co	Fold change day 12 pregnant vs. control for mare #3
Mean FC/M#3 FC	Ratio of day 12 mean fold change and fold change mare #3
FC Pr D12/D8	Fold change pregnant samples day 12 vs. Day 8
FC Co D12/D8	Fold change control samples day 12 vs. Day 8

Supplemental Table 3: Additional information for the gene sets and the genes overlapping with the top 500 of the day 12 preranked gene list

Gene set	Desination in Supplemental Table 4	Size	ES	NES	Nom p-val	FDR q-val	FWER p-val	Rank at Max	genes top 500	%	genes top 250	%	Genes overlapping with top 500
Up-regulated at day 13.5 of pregnancy in equine endometrium	D13.5 of pregnancy up (Eca)	63	0.833	3.18	0.0000	0.0000	0.0000	634	24	38.1	21	33.3	*SLC36A2 ATP6V0A4 TMEM213 GM2A STC1 FGF9 PRSS23 DOPEY2 ABCG2 IGFBP1 IFIT1 HSPB8 ITPR1 COCH CRYAB TACSTD2 TIMP1 RASSF8 GJB5 SLC37A1 IRF7 ANGPTL2 S100A2 SLC4A11*
Up-regulated in human endometrium LH+7 vs. LH+2	Window of implantation up (Hsa)	122	0.692	2.90	0.0000	0.0000	0.0000	1283	29	23.8	17	13.9	*IGFBP3 STC1 LCN2 THBS2 IGFBP1 IER3 TNFSF10 CRYAB TEK IL15 STK17B CP ACTA2 MAP3K5 G0S2 RNASE4 SLC15A1 CLU TAGLN C4BPA TSPAN8 SLPI C10orf10 AGR2 BCL6 ARID5B HTATIP2 GPRC5B PCDH17*
Boquest_CD31^{+}_vs_CD31^{-}_up	Boquest CD31+ vs CD31- up	540	0.562	2.69	0.0000	0.0000	0.0000	2046	75	13.9	37	6.9	*STC1 MGP PTGER4 SCHIP1 CTSK DOCK9 RAI2 TPST2 NPR3 TNFSF10 CD44 COCH SLC7A11 TEK ANXA3 CD74 PER2 IL15 GPNMB MET JAM2 SDPR HHEX SMAD1 SRPX2 TSPAN7 ANGPT2 RND1 G0S2 HLA-DMA RNASE4 SERPINE2 UCHL1 CDO1 COL4A5 FLI1 MFAP4 MATN2 ME1 GPC3 SPRY1 OXTR PECAM1 LOX KLF6 RGS2 KDR ERG RGS5 TSPAN8 PLTP PPL ANGPTL2 GSN TPM4 PRKD1 SERPINE1 MITF THBS1 MMRN2 FRZB ANGPTL4 DPT SERPINA5 CD14 TM4SF1 VWF ZBTB10 NPY1R DUSP6 TFPI DCN PCDH17 BST2 DFNA5*
Boquest_CD31^{+}_vs_CD31^{-}_dn	Boquest CD31+ vs CD31- dn	215	0.585	2.63	0.0000	0.0000	0.0000	2465	38	17.7	20	9.3	*IGFBP3 MGP THBS2 IRS2 CTSK HSPB8 CD44 CRYAB ABCA8 TIMP1 STEAP1 GPNMB PLA2G4A MME SVEP1 RNASE4 SERPINE2 SLIT2 CDO1 MFAP4 GPC3 LOX EFEMP1 TSPAN8 PLTP PPL PTPN13 GSN NT5E MITF DPT SERPINA5 TNXB SHOX2 NPY1R RHOBTB3 COL6A3 DCN*
Manalo_hypoxia_up	Manalo hypoxia up	84	0.651	2.60	0.0000	0.0000	0.0000	2169	16	19.0	8	9.5	*VLDLR IGFBP3 STC1 ENPP1 COL4A1 RGS3 EDN1 CXCR4 LOX EGLN3 GRK5 ITPR2 ANGPTL4 SHOX2 DUSP6 BCL6*
Genes up-regulated at day 14 of pregnancy in porcine endometrium	D14 of pregnancy up (Ssc)	131	0.568	2.40	0.0000	0.0000	0.0000	1746	23	17.6	14	10.7	*STC1 FGF9 TRPV6 IRS2 SLC2A1 PAQR5 IGFBP2 MUC4 ENPP1 UNC93A STEAP1 PTGR1 GJB3 PSD3 PLXDC2 FAM105A GULP1 UBD FOSL2 CD14 VWF BCL6 GPRC5B*

Genes up-regulated at day 18 of pregnancy in bovine endometrium	D18 of pregnancy up (Bta)	226	0.528	2.37	0.0000	0.0001	0.0010	2815	25	11.1	12	5.3	*ATP6V0A4 AMPD3 ARG2 MST1R IFIT1 CRYM HERC6 IGFBP2 CRYAB TACSTD2 SPINK7 IRF7 XAF1 AREG CKMT1B TMEM140 PARP14 RTP4 UBD UBE2L6 TM4SF1 AGR2 TFPI OAS1 BST2*
RAS_oncogenic_signature	RAS oncogenic signature	200	0.515	2.30	0.0000	0.0003	0.0080	2601	25	12.5	14	7.0	*ARG2 MALL IER3 KLF5 PLXNA2 TIMP1 DLL1 TNFRSF12A EFNA5 PRNP GJB3 PIM1 G0S2 GJB5 KLF6 DKK3 NT5E ATP2B1 ANGPTL4 EPHA2 DUSP6 BCL6 LRIG3 ARHGAP25 DUSP4*
TGFbeta_all_up	TGFbeta all up	73	0.590	2.30	0.0000	0.0003	0.0090	1447	16	21.9	10	13.7	*IGFBP3 THBS2 PLAT IGFBP2 CD44 TIMP1 ITGB8 EFNA5 COL8A1 RND3 SERPINE1 THBS1 NID1 NEO1 EPHA2 COL6A3*
Genes up-regulated at estrus in bovine endometrium	Estrus up (Bta)	462	0.480	2.29	0.0000	0.0004	0.0100	1815	58	12.6	34	7.4	*KNG1 IGFBP3 STC1 PRSS23 THBS2 CDH13 ARG2 PTGER4 SCHIP1 IER3 NPR3 SERPINA1 IGFBP2 CD44 SLC7A11 CRYAB CH25H FXYD5 NR4A3 HSD11B1 LOXL4 TIMP1 TNFRSF12A UNC93A COL4A1 CP PIM1 SRPX2 ACTA2 G0S2 ACTG2 SLIT2 RND3 UCK2 GNA14 ME1 OXTR LOX TAGLN PROCR C4BPA PRLR GRK5 AREG SLCO2A1 TPM4 SERPINE1 THBS1 C1QTNF5 MMD NOV TRIB2 SNAI2 GHR COL6A3 TFPI IL1R1 P2RY14*
Estrogen-induced genes	Estrogen-induced	400	0.483	2.29	0.0000	0.0004	0.0100	1575	47	11.8	21	5.3	*KNG1 IGFBP3 STC1 PRSS23 THBS2 CDH13 SCHIP1 IER3 PHLDB2 WWTR1 IGFBP7 GPC6 EFNA5 COL4A1 PTRF STAT4 ANGPT2 SERPINE2 VIM LDB2 MFAP4 MATN2 PECAM1 KLF6 KDR MFGE8 CYR61 RGS5 GRK5 STAB2 SFRP1 SLPI SLCO2A1 PTPN13 SLC7A3 ECE1 NT5E KCNJ8 MMRN2 FRZB DPT UBD CD19 PPP2R2B PLXND1 EDNRA DCN*
Up-regulated in receptive (LH+8, day 21) vs. pre-receptive (LH+3, day 16) human endometrium	Window of implantation up (Hsa)	44	0.638	2.27	0.0000	0.0000	0.0000	2778	11	25.0	6	13.6	*CD44 CRYAB IL15 COL4A1 ACTA2 MAP3K5 MFGE8 SLPI UBE2L6 NID1 ARID5B*
Pod1_KO_dn	POD1 (TCF21) KO down	592	0.468	2.23	0.0000	0.0005	0.0180	2576	53	9.0	27	4.6	*PLAT ENDOD1 PTGER4 SCHIP1 HEY2 NPR3 RAPGEF2 CRYAB SNCAIP FAM43A WWTR1 TEK ABCB1 PER2 PTPRJ MGLL KLHL5 LIPA SDPR HHEX HIP1 PRDM1 CXCR4 VIM COL4A5 FLI1 MYO1B RGS2 ERG RIN2 GRK5 APBB2 TSPAN8 SLCO2A1 HS3ST6 RANBP9 PARP14 GSN GULP1 NT5E THBS1 MMRN2 C1QTNF7 PLSCR4 MMD TM4SF1 NID1 TRIB2 DUSP6 ARID5B CRIM1 PCDH17 CABLES1*

Genes up-regulated at diestrus in bovine endometrium	Diestrus up (Bta)	466	0.462	2.19	0.0000	0.0009	0.0400	2731	44	9.4	25	5.4	*ATP6V0A4 GM2A VLDLR MGP FGF9 PECR ENPP6 ALS2CL IGFBP1 KCNN2 SLC2A1 CHGA TNFSF10 RAB3B PHLDB2 ENPP1 PYGL COL13A1 GPNMB MET SESTD1 PIGR TSPAN7 CXCR4 MFAP4 RGS2 EGLN3 EFEMP1 PENK C10orf10 PLTP TC2N KCNJ8 UBD UBE2L6 FGF12 KCNMB2 S100A13 KIAA0408 KIAA0922 ARID5B CYP39A1 NR3C2 GPRC5B*
VEGF_MMMEC_all_up	VEGF MMMEC all up	84	0.544	2.17	0.0000	0.0011	0.0550	1949	14	16.7	7	8.3	*IGFBP3 MGP PIR TNFSF10 EMR1 PIM1 ANGPT2 OXTR KDR RGS5 EFEMP1 UBD VWF COL6A3*
Up-regulated in ovine endometrium between days 9 and 12 of pregnancy	D12 vs. D9 of pregnancy up (Oar)	358	0.440	2.07	0.0000	0.0004	0.0010	2947	27	7.5	12	3.4	*PLAT PTGER4 IGFBP1 CHGA PHLDB2 PLXNA2 HSD11B1 SPINK7 MET SLIT2 PCDH18 UCK2 MATN2 GNA14 EMX2 PECAM1 LOX SOAT1 RGS5 ZFPM2 CADM1 ECE1 KCNJ8 VWF ZBTB10 AGR2 OAS1*
PGE2 up-regulated genes in human monocyte-derived dendritic cells	PGE2 up in human monocyte-derived DCs	121	0.459	1.92	0.0000	0.0004	0.0060	2439	16	13.2	9	7.4	*TIMP1 CD74 MET STAT4 G0S2 RNASE4 PRDM1 CXCR4 TGFBI RGS2 CXCL16 AREG THBS1 BTG1 ARID5B CABLES1*

Supplemental Table 4: A list of genes and their frequencies in the gene sets

Genes	Fold change D12 Pr/Co	q-value	Rank in gene list for GSEA	Frequency in all gene sets	Frequency in pregnancy and P4 up	Frequency in pregnancy up	Frequency in E2 up	D13.5 of pregnancy up (Eca)	Window of implantation up (Hsa)	D14 of pregnancy up (Ssc)	D18 of pregnancy up (Bta)	D12 vs. D9 of pregnancy up (Oar)	Diestrus up (Bta)	Estrus up (Bta)	Estrogen-induced	Boquest CD31+ vs CD31- up	Boquest CD31+ vs CD31- dn	Manalo hypoxia up	VEGF MMMEC all up	RAS oncogenic signature	TGFbeta all up	POD1 (TCF21) KO down	PGE2 up
UBD	1.38	0.0250	395	5	3	2	1			x	x		x		x				x				
AGR2	1.62	0.0348	433	3	3	2	0		x		x	x											
GPRC5B	1.13	0.0264	480	3	3	2	0		x	x			x										
UBE2L6	1.17	0.0208	401	3	3	2	0		x		x		x										
STC1	3.14	0.0001	10	7	2	2	2	x	x	x				x	x	x			x				
CRYAB	2.17	0.0033	85	6	2	2	1	x	x		x			x			x						x	
IGFBP2	1.91	0.0020	74	4	2	2	1			x	x			x								x		
BCL6	1.68	0.0387	453	4	2	2	0		x	x									x		x			
IGFBP1	5.84	0.0036	50	4	3	1	0	x	x			x	x											
ATP6V0A4	7.81	0.0001	2	3	2	1	0	x			x		x											
FGF9	10.23	0.0035	13	3	2	1	0	x		x			x											
ARID5B	1.41	0.0316	454	4	2	1	0		x				x										x	x
TNFSF10	2.02	0.0018	72	4	2	1	0		x				x			x			x					
VWF	1.54	0.0317	419	4	2	1	0			x		x				x			x					
ENPP1	1.66	0.0029	102	3	2	1	0			x			x					x						
C10orf10	1.95	0.0298	332	2	2	1	0		x				x											
OAS1	1.27	0.0300	477	2	2	1	0				x	x												
SLC2A1	1.98	0.0007	65	2	2	1	0			x			x											
SPINK7	2.31	0.0121	151	2	2	1	0				x	x												
IGFBP3	3.46	0.0001	8	7	1	1	2		x					x	x		x	x	x			x		
THBS2	2.47	0.0001	17	5	1	1	2		x					x	x		x					x		
COL4A1	1.39	0.0059	160	4	1	1	2		x					x	x			x						
IER3	4.45	0.0046	64	4	1	1	2		x					x	x					x				
ACTA2	2.55	0.0208	191	2	1	1	1		x					x										
SLPI	1.80	0.0264	328	2	1	1	1		x						x									
CD44	1.71	0.0016	75	5	1	1	1		x					x		x	x					x		
G0S2	2.33	0.0208	204	5	1	1	1		x					x		x				x				x
AREG	2.48	0.0382	320	3	1	1	1				x			x										x
ARG2	2.07	0.0001	25	3	1	1	1				x			x						x				
TFPI	1.57	0.0378	472	3	1	1	1				x			x		x								

Genes	Fold change D12 Pr/Co	q-value	Rank in gene list for GSEA	Frequency in all gene sets	Frequency in pregnancy and P4 up	Frequency in pregnancy up	Frequency in E2 up	D13.5 of pregnancy up (Eca)	Window of implantation up (Hsa)	D14 of pregnancy up (Ssc)	D18 of pregnancy up (Bta)	D12 vs. D9 of pregnancy up (Oar)	Diestrus up (Bta)	Estrus up (Bta)	Estrogen-induced	Boquest CD31+ vs CD31- up	Boquest CD31+ vs CD31- dn	Manalo hypoxia up	VEGF MMMEC all up	RAS oncogenic signature	TGFbeta all up	POD1 (TCF21) KO down	PGE2 up
C4BPA	1.72	0.0208	293	2	1	1	1		x					x									
CP	2.00	0.0111	164	2	1	1	1		x					x									
MFGE8	1.39	0.0157	295	2	1	1	1		x						x								
TAGLN	2.25	0.0274	273	2	1	1	1		x					x									
UNC93A	2.52	0.0115	136	2	1	1	1			x				x									
IFIT1	1.47	0.0001	55	2	1	1	0	x			x												
IRF7	1.43	0.0126	246	2	1	1	0	x			x												
IL15	1.41	0.0046	144	2	1	1	0		x							x							
RNASE4	2.07	0.0186	213	4	1	1	0		x							x	x						x
TSPAN8	1.64	0.0224	318	4	1	1	0		x							x	x					x	
NID1	1.32	0.0264	420	3	1	1	0		x												x	x	
PCDH17	1.30	0.0317	485	3	1	1	0		x							x						x	
TEK	1.50	0.0035	118	3	1	1	0		x							x						x	
BST2	1.45	0.0378	498	2	1	1	0				x					x							
CD14	1.55	0.0317	415	2	1	1	0			x						x							
GJB3	2.51	0.0177	174	2	1	1	0			x										x			
GULP1	1.60	0.0264	361	2	1	1	0			x												x	
IRS2	1.78	0.0001	37	2	1	1	0			x							x						
PARP14	1.30	0.0195	353	2	1	1	0				x											x	
STEAP1	1.84	0.0073	143	2	1	1	0			x							x						
TACSTD2	3.21	0.0378	101	2	1	1	0	x			x												
AMPD3	2.33	0.0001	23	1	1	1	0				x												
CKMT1B	2.29	0.0378	337	1	1	1	0				x												
CRYM	1.36	0.0001	59	1	1	1	0				x												
FAM105A	1.39	0.0208	348	1	1	1	0			x													
FOSL2	4.56	0.0960	409	1	1	1	0			x													
HERC6	2.85	0.0035	70	1	1	1	0				x												
MST1R	1.87	0.0001	33	1	1	1	0				x												
MUC4	2.48	0.0126	91	1	1	1	0			x													
PAQR5	2.03	0.0021	73	1	1	1	0			x													
PLXDC2	2.44	0.0354	306	1	1	1	0			x													
PSD3	1.54	0.0113	205	1	1	1	0			x													
PTGR1	2.67	0.0177	166	1	1	1	0			x													
RTP4	1.32	0.0208	364	1	1	1	0				x												

Genes	Fold change D12 Pr/Co	q-value	Rank in gene list for GSEA	Frequency in all gene sets	Frequency in pregnancy and P4 up	Frequency in pregnancy up	Frequency in E2 up	D13.5 of pregnancy up (Eca)	Window of implantation up (Hsa)	D14 of pregnancy up (Ssc)	D18 of pregnancy up (Bta)	D12 vs. D9 of pregnancy up (Oar)	Diestrus up (Bta)	Estrus up (Bta)	Estrogen-induced	Boquest CD31+ vs CD31- up	Boquest CD31+ vs CD31- dn	Manalo hypoxia up	VEGF MMMEC all up	RAS oncogenic signature	TGFbeta all up	POD1 (TCF21) KO down	PGE2 up
TMEM140	1.46	0.0227	352	1	1	1	0				x												
TRPV6	2.28	0.0001	24	1	1	1	0			x													
XAF1	1.33	0.0130	267	1	1	1	0				x												
HTATIP2	1.19	0.0274	474	1	1	1	0		x														
LCN2	2.81	0.0001	12	1	1	1	0		x														
MAP3K5	1.62	0.0115	197	1	1	1	0		x														
SLC15A1	2.76	0.0317	250	1	1	1	0		x														
STK17B	1.58	0.0075	163	1	1	1	0		x														
TM4SF1	1.65	0.0344	417	3	1	1	0				x					x						x	
CLU	1.65	0.0177	268	1	1	1	0		x														
KCNJ8	1.75	0.0308	369	3	2	0	1					x	x		x								
PHLDB2	1.87	0.0020	77	3	2	0	1					x	x		x								
MET	1.70	0.0073	152	4	2	0	0					x	x			x							x
CHGA	4.05	0.0055	68	2	2	0	0					x	x										
LOX	1.48	0.0153	272	5	1	0	1					x		x		x	x	x					
MFAP4	1.46	0.0130	245	4	1	0	1						x		x	x	x						
PTGER4	2.04	0.0001	28	4	1	0	1					x		x		x						x	
RGS5	1.88	0.0253	307	4	1	0	1					x			x	x			x				
PECAM1	1.59	0.0170	270	3	1	0	1					x			x	x							
ECE1	1.33	0.0208	359	2	1	0	1					x			x								
GNA14	1.56	0.0151	257	2	1	0	1					x		x									
HSD11B1	2.93	0.0113	115	2	1	0	1					x		x									
UCK2	1.39	0.0113	233	2	1	0	1					x		x									
MATN2	1.42	0.0130	254	3	1	0	1					x			x	x							
SLIT2	1.51	0.0117	216	3	1	0	1					x		x			x						
GM2A	5.36	0.0001	4	2	1	0	0	x					x										
CXCR4	1.61	0.0131	218	4	1	0	0						x					x				x	x
MGP	2.97	0.0001	11	4	1	0	0						x			x	x		x				
RGS2	1.81	0.0208	281	4	1	0	0						x			x						x	x
EFEMP1	1.60	0.0208	311	3	1	0	0						x				x		x				
GPNMB	2.47	0.0130	148	3	1	0	0						x			x	x						
PLAT	2.47	0.0001	18	3	1	0	0					x									x	x	
PLTP	1.45	0.0208	335	3	1	0	0						x			x	x						
EGLN3	2.57	0.0371	305	2	1	0	0						x					x					

Genes	Fold change D12 Pr/Co	q-value	Rank in gene list for GSEA	Frequency in all gene sets	Frequency in pregnancy and P4 up	Frequency in pregnancy up	Frequency in E2 up	D13.5 of pregnancy up (Eca)	Window of implantation up (Hsa)	D14 of pregnancy up (Ssc)	D18 of pregnancy up (Bta)	D12 vs. D9 of pregnancy up (Oar)	Diestrus up (Bta)	Estrus up (Bta)	Estrogen-induced	Boquest CD31+ vs CD31- up	Boquest CD31+ vs CD31- dn	Manalo hypoxia up	VEGF MMMEC all up	RAS oncogenic signature	TGFbeta all up	POD1 (TCF21) KO down	PGE2 up
PLXNA2	1.57	0.0028	105	2	1	0	0					x								x			
TSPAN7	1.70	0.0115	192	2	1	0	0						x			x							
VLDLR	3.51	0.0001	7	2	1	0	0						x					x					
ZBTB10	1.71	0.0360	421	2	1	0	0					x				x							
ALS2CL	1.53	0.0001	49	1	1	0	0						x										
COL13A1	1.58	0.0044	130	1	1	0	0						x										
CYP39A1	1.40	0.0316	455	1	1	0	0						x										
ENPP6	2.41	0.0001	21	1	1	0	0						x										
FGF12	1.40	0.0270	406	1	1	0	0						x										
KCNMB2	1.46	0.0291	410	1	1	0	0						x										
KCNN2	6.49	0.0046	53	1	1	0	0						x										
KIAA0408	1.30	0.0264	430	1	1	0	0						x										
NR3C2	1.27	0.0298	476	1	1	0	0						x										
PECR	2.46	0.0001	19	1	1	0	0						x										
PENK	2.00	0.0304	329	1	1	0	0						x										
PIGR	1.93	0.0113	171	1	1	0	0						x										
PYGL	1.49	0.0035	121	1	1	0	0						x										
RAB3B	3.51	0.0073	76	1	1	0	0						x										
S100A13	1.31	0.0264	422	1	1	0	0						x										
SESTD1	1.58	0.0070	157	1	1	0	0						x										
TC2N	1.40	0.0212	350	1	1	0	0						x										
CADM1	1.35	0.0187	333	1	1	0	0					x											
EMX2	1.61	0.0164	261	1	1	0	0					x											
PCDH18	1.43	0.0115	226	1	1	0	0					x											
SOAT1	1.43	0.0150	282	1	1	0	0					x											
ZFPM2	1.55	0.0208	319	1	1	0	0					x											
KIAA0922	1.23	0.0264	451	1	1	0	0						x										
PRSS23	2.60	0.0001	15	3	0	0	2	x						x	x								
GRK5	1.49	0.0189	310	4	0	0	2							x	x			x				x	
SCHIP1	1.81	0.0001	34	4	0	0	2							x	x	x						x	
SLCO2A1	1.84	0.0283	334	3	0	0	2							x	x							x	
CDH13	2.36	0.0001	22	2	0	0	2							x	x								
KNG1	4.32	0.0001	6	2	0	0	2							x	x								
TIMP1	1.77	0.0050	125	6	0	0	1	x						x			x			x	x		x

Genes	Fold change D12 Pr/Co	q-value	Rank in gene list for GSEA	Frequency in all gene sets	Frequency in pregnancy and P4 up	Frequency in pregnancy up	Frequency in E2 up	D13.5 of pregnancy up (Eca)	Window of implantation up (Hsa)	D14 of pregnancy up (Ssc)	D18 of pregnancy up (Bta)	D12 vs. D9 of pregnancy up (Oar)	Diestrus up (Bta)	Estrus up (Bta)	Estrogen-induced	Boquest CD31+ vs CD31- up	Boquest CD31+ vs CD31- dn	Manalo hypoxia up	VEGF MMMEC all up	RAS oncogenic signature	TGFbeta all up	POD1 (TCF21) KO down	PGE2 up
THBS1	1.68	0.0291	368	5	0	0	1							x		x					x	x	x
COL6A3	1.34	0.0308	464	4	0	0	1							x			x		x		x		
NT5E	1.43	0.0231	362	4	0	0	1								x		x			x		x	
ANGPT2	1.63	0.0113	194	3	0	0	1								x	x			x				
DCN	1.31	0.0321	484	3	0	0	1								x	x	x						
DPT	1.70	0.0320	393	3	0	0	1								x	x	x						
EFNA5	1.29	0.0046	155	3	0	0	1								x					x	x		
KDR	1.58	0.0185	291	3	0	0	1								x	x			x				
KLF6	1.42	0.0145	274	3	0	0	1								x	x				x			
MMRN2	1.52	0.0264	375	3	0	0	1								x	x						x	
NPR3	2.30	0.0016	67	3	0	0	1							x		x						x	
OXTR	1.64	0.0177	269	3	0	0	1							x		x			x				
PIM1	1.57	0.0092	184	3	0	0	1							x					x	x			
SERPINE1	2.05	0.0358	360	3	0	0	1							x		x					x		
SERPINE2	1.30	0.0092	214	3	0	0	1								x	x	x						
FRZB	1.33	0.0231	381	2	0	0	1								x	x							
ME1	1.36	0.0130	262	2	0	0	1							x		x							
PTPN13	1.24	0.0177	345	2	0	0	1								x		x						
RND3	1.70	0.0150	228	2	0	0	1							x							x		
SLC7A11	1.34	0.0012	84	2	0	0	1							x		x							
SRPX2	1.73	0.0113	189	2	0	0	1							x		x							
STAT4	1.99	0.0130	181	2	0	0	1								x								x
TNFRSF12A	2.03	0.0073	134	2	0	0	1							x						x			
TPM4	1.49	0.0236	356	2	0	0	1							x		x							
TRIB2	1.39	0.0286	429	2	0	0	1							x								x	
VIM	1.38	0.0112	235	2	0	0	1								x							x	
WWTR1	1.52	0.0029	109	2	0	0	1								x							x	
ACTG2	5.80	0.0655	210	1	0	0	1							x									
C1QTNF5	1.70	0.0298	372	1	0	0	1							x									
CD19	1.28	0.0264	436	1	0	0	1								x								
CH25H	1.64	0.0021	92	1	0	0	1							x									
CYR61	5.22	0.0782	300	1	0	0	1								x								
EDNRA	1.30	0.0298	465	1	0	0	1								x								
FXYD5	1.39	0.0020	103	1	0	0	1							x									

Genes	Fold change D12 Pr/Co	q-value	Rank in gene list for GSEA	Frequency in all gene sets	Frequency in pregnancy and P4 up	Frequency in pregnancy up	Frequency in E2 up	D13.5 of pregnancy up (Eca)	Window of implantation up (Hsa)	D14 of pregnancy up (Ssc)	D18 of pregnancy up (Bta)	D12 vs. D9 of pregnancy up (Oar)	Diestrus up (Bta)	Estrus up (Bta)	Estrogen-induced	Boquest CD31+ vs CD31- up	Boquest CD31+ vs CD31- dn	Manalo hypoxia up	VEGF MMMEC all up	RAS oncogenic signature	TGFbeta all up	POD1 (TCF21) KO down	PGE2 up
NOV	1.60	0.0317	407	1	0	0	1							x									
GHR	1.77	0.0420	461	1	0	0	1							x									
GPC6	1.59	0.0046	133	1	0	0	1								x								
IGFBP7	1.75	0.0038	110	1	0	0	1								x								
LDB2	1.40	0.0119	242	1	0	0	1								x								
LOXL4	1.58	0.0040	122	1	0	0	1							x									
NR4A3	16.14	0.0786	108	1	0	0	1							x									
P2RY14	2.80	0.0740	491	1	0	0	1							x									
PLXND1	1.39	0.0317	459	1	0	0	1								x								
PPP2R2B	1.26	0.0264	443	1	0	0	1								x								
PRLR	2.04	0.0264	296	1	0	0	1							x									
PROCR	1.62	0.0177	278	1	0	0	1							x									
PTRF	1.39	0.0060	162	1	0	0	1								x								
SERPINA1	3.09	0.0043	71	1	0	0	1							x									
SFRP1	1.62	0.0229	325	1	0	0	1								x								
SLC7A3	1.44	0.0221	349	1	0	0	1								x								
SNAI2	1.48	0.0340	457	1	0	0	1							x									
STAB2	1.52	0.0208	322	1	0	0	1								x								
MMD	1.65	0.0317	399	2	0	0	1							x								x	
IL1R1	1.28	0.0317	488	1	0	0	1							x									
DUSP6	1.37	0.0297	447	4	0	0	0									x		x		x		x	
ANGPTL4	2.08	0.0406	391	3	0	0	0									x		x		x			
GSN	1.46	0.0228	354	3	0	0	0									x	x					x	
ANGPTL2	1.55	0.0233	340	2	0	0	0	x								x							
COCH	4.90	0.0130	78	2	0	0	0	x								x							
GJB5	1.49	0.0115	217	2	0	0	0	x												x			
HSPB8	5.02	0.0035	61	2	0	0	0	x									x						
CABLES1	1.47	0.0378	495	2	0	0	0															x	x
CD74	1.43	0.0035	127	2	0	0	0									x							x
CDO1	1.58	0.0130	222	2	0	0	0									x	x						
COL4A5	1.46	0.0126	239	2	0	0	0									x						x	
CTSK	1.60	0.0001	45	2	0	0	0									x	x						
EPHA2	1.41	0.0298	438	2	0	0	0													x	x		
ERG	1.69	0.0206	297	2	0	0	0									x						x	
FLI1	1.34	0.0113	243	2	0	0	0									x						x	

Genes	Fold change D12 Pr/Co	q-value	Rank in gene list for GSEA	Frequency in all gene sets	Frequency in pregnancy and P4 up	Frequency in pregnancy up	Frequency in E2 up	D13.5 of pregnancy up (Eca)	Window of implantation up (Hsa)	D14 of pregnancy up (Ssc)	D18 of pregnancy up (Bta)	D12 vs. D9 of pregnancy up (Oar)	Diestrus up (Bta)	Estrus up (Bta)	Estrogen-induced	Boquest CD31+ vs CD31- up	Boquest CD31+ vs CD31- dn	Manalo hypoxia up	VEGF MMMEC all up	RAS oncogenic signature	TGFbeta all up	POD1 (TCF21) KO down	PGE2 up
MITF	1.70	0.0288	366	2	0	0	0									x	x						
GPC3	1.52	0.0153	263	2	0	0	0									x	x						
HHEX	1.52	0.0087	183	2	0	0	0									x						x	
NPY1R	1.52	0.0317	427	2	0	0	0									x	x						
PER2	1.56	0.0043	131	2	0	0	0									x						x	
PPL	1.28	0.0177	336	2	0	0	0									x	x						
PRDM1	1.59	0.0126	215	2	0	0	0															x	x
SDPR	1.86	0.0115	178	2	0	0	0									x						x	
SERPINA5	2.30	0.0474	402	2	0	0	0									x	x						
SHOX2	2.00	0.0423	412	2	0	0	0										x	x					
ABCG2	1.80	0.0001	36	1	0	0	0	x															
DOPEY2	2.04	0.0001	27	1	0	0	0	x															
ITPR1	3.21	0.0044	69	1	0	0	0	x															
RASSF8	1.74	0.0119	193	1	0	0	0	x															
S100A2	2.98	0.0577	374	1	0	0	0	x															
SLC36A2	70.68	0.0001	1	1	0	0	0	x															
SLC37A1	1.37	0.0115	241	1	0	0	0	x															
SLC4A11	1.33	0.0264	416	1	0	0	0	x															
TMEM213	6.05	0.0001	3	1	0	0	0	x															
ABCA8	1.84	0.0046	112	1	0	0	0										x						
ABCB1	1.60	0.0044	128	1	0	0	0															x	
APBB2	1.55	0.0208	317	1	0	0	0															x	
ARHGAP25	1.28	0.0298	475	1	0	0	0													x			
ATP2B1	1.28	0.0201	365	1	0	0	0													x			
BTG1	1.33	0.0273	432	1	0	0	0																x
C1QTNF7	1.56	0.0281	382	1	0	0	0															x	
COL8A1	5.36	0.0456	170	1	0	0	0														x		
CRIM1	1.38	0.0326	473	1	0	0	0															x	
CXCL16	1.68	0.0200	289	1	0	0	0																x
DFNA5	1.45	0.0381	500	1	0	0	0									x							
DKK3	1.50	0.0228	346	1	0	0	0													x			
DLL1	1.88	0.0057	126	1	0	0	0													x			
DOCK9	1.52	0.0001	51	1	0	0	0									x							
EDN1	1.69	0.0137	212	1	0	0	0											x					

Genes	Fold change D12 Pr/Co	q-value	Rank in gene list for GSEA	Frequency in all gene sets	Frequency in pregnancy and P4 up	Frequency in pregnancy up	Frequency in E2 up	D13.5 of pregnancy up (Eca)	Window of implantation up (Hsa)	D14 of pregnancy up (Ssc)	D18 of pregnancy up (Bta)	D12 vs. D9 of pregnancy up (Oar)	Diestrus up (Bta)	Estrus up (Bta)	Estrogen-induced	Boquest CD31+ vs CD31- up	Boquest CD31+ vs CD31- dn	Manalo hypoxia up	VEGF MMMEC all up	RAS oncogenic signature	TGFbeta all up	POD1 (TCF21) KO down	PGE2 up
EMR1	1.65	0.0069	153	1	0	0	0												x				
ENDOD1	2.06	0.0001	26	1	0	0	0															x	
FAM43A	1.83	0.0028	94	1	0	0	0															x	
HEY2	1.54	0.0003	63	1	0	0	0															x	
HIP1	1.45	0.0104	206	1	0	0	0															x	
HLA-DMA	1.34	0.0092	208	1	0	0	0									x							
HS3ST6	1.87	0.0298	341	1	0	0	0															x	
ITGB8	1.52	0.0056	147	1	0	0	0														x		
ITPR2	1.42	0.0189	321	1	0	0	0											x					
JAM2	1.70	0.0092	173	1	0	0	0									x							
KLF5	1.57	0.0020	93	1	0	0	0													x			
KLHL5	1.40	0.0046	145	1	0	0	0															x	
LIPA	1.62	0.0092	177	1	0	0	0															x	
LRIG3	1.38	0.0317	462	1	0	0	0													x			
MALL	1.64	0.0001	43	1	0	0	0													x			
MGLL	1.90	0.0074	140	1	0	0	0															x	
MME	1.43	0.0066	169	1	0	0	0										x						
NEO1	1.36	0.0278	428	1	0	0	0														x		
PIR	1.50	0.0001	52	1	0	0	0												x				
PLA2G4A	1.56	0.0063	154	1	0	0	0										x						
PLSCR4	1.46	0.0272	398	1	0	0	0															x	
PRKD1	1.31	0.0202	358	1	0	0	0									x							
PRNP	2.89	0.0194	161	1	0	0	0													x			
PTPRJ	1.35	0.0033	132	1	0	0	0															x	
RAI2	1.45	0.0001	56	1	0	0	0									x							
RANBP9	1.37	0.0208	351	1	0	0	0															x	
RAPGEF2	272.53	0.2315	83	1	0	0	0															x	
RGS3	1.29	0.0055	168	1	0	0	0											x					
RHOBTB3	1.40	0.0295	437	1	0	0	0										x						
RIN2	1.58	0.0197	304	1	0	0	0															x	
RND1	2.04	0.0167	199	1	0	0	0									x							
SMAD1	1.44	0.0080	185	1	0	0	0									x							

Genes	Fold change D12 Pr/Co	q-value	Rank in gene list for GSEA	Frequency in all gene sets	Frequency in pregnancy and P4 up	Frequency in pregnancy up	Frequency in E2 up	D13.5 of pregnancy up (Eca)	Window of implantation up (Hsa)	D14 of pregnancy up (Ssc)	D18 of pregnancy up (Bta)	D12 vs. D9 of pregnancy up (Oar)	Diestrus up (Bta)	Estrus up (Bta)	Estrogen-induced	Boquest CD31+ vs CD31- up	Boquest CD31+ vs CD31- dn	Manalo hypoxia up	VEGF MMMEC all up	RAS oncogenic signature	TGFbeta all up	POD1 (TCF21) KO down	PGE2 up
SNCAIP	2.07	0.0033	87	1	0	0	0															x	
SPRY1	1.57	0.0162	265	1	0	0	0									x							
SVEP1	1.93	0.0167	211	1	0	0	0										x						
TGFBI	1.43	0.0126	244	1	0	0	0																x
TNXB	1.49	0.0298	411	1	0	0	0										x						
TPST2	1.32	0.0001	60	1	0	0	0									x							
UCHL1	1.63	0.0135	221	1	0	0	0									x							
ANXA3	1.46	0.0035	123	1	0	0	0									x							
DUSP4	1.45	0.0378	499	1	0	0	0													x			
MYO1B	1.34	0.0115	249	1	0	0	0															x	

Supplemental Table 5: Results of Functional Annotation Clustering of genes up-regulated at day 12 of pregnancy

Functional Annotation Cluster Description[1]	Enrichm. Score[2]	Genes [3]
Glycoprotein (129, 2.3); signal peptide (93, 2.1); secreted (61, 2.9); extracellular region (57, 2.8); disulfide bond (83, 2.0)	14.52	150
Developmental process (99, 2.1); cell differentiation (55, 2.0)	10.57	121
Anatomical structure morphogenesis (49, 2.9); blood vessel development (17, 6.1); angiogenesis (15, 7.1)	8.34	50
Egf-like, type 3 (18, 5.8); EGF-like calcium-binding (10, 6.4); calcium ion binding (29, 2.1)	5.54	37
Glycoprotein (129, 2.3); membrane (112, 1.4); plasma membrane (79, 1.6)	5.38	178
Carbohydrate binding (15, 3.2); glycosaminoglycan binding (10, 6.8)	4.24	15
Response to external stimulus (27, 2.9); response to stress (27, 1.7); blood coagulation (9, 6.2); wound healing (10, 5.3)	3.94	38
Cell differentiation (55, 2.0); apoptosis (25, 2.2); regulation of apoptosis (19, 2.4); neg. regulation of apoptosis (13, 3.9)	3.76	63
Anatomical structure formation (16, 6.1); cell motility (16, 2.6); cell migration (12, 3.0)	3.63	28
Tissue development (13, 2.6); tissue remodeling (8, 4.7); bone remodeling (7, 4.5)	2.38	15
Nervous system development (24, 2.1); cell morphogenesis (17, 2.3); neurogenesis (12, 2.6)	2.01	34
Cell morphogenesis (17, 2.3); cell growth (8, 2.8)	1.91	18
Signal transducer activity (54, 1.5); receptor activity (42, 1.4); transmembrane receptor activity (22, 1.1)	1.91	80
Cell proliferation (24, 2.1); regulation of cell proliferation (14, 1.9); positive regulation of cell proliferation (9, 2.6)	1.79	38
Regulation of apoptosis (19, 2.4); positive regulation of apoptosis (7, 1.9)	1.76	31
Cytoplasmic vesicle (14, 2.2); cytoplasmic membrane-bound vesicle (11, 2.1)	1.69	14
Chemical homeostasis (10, 2.5); di-, tri-valent inorganic cation homeostasis (8, 3.4)	1.59	11
Enzyme regulator activity (22, 1.9); endopeptidase inhibitor activity (7, 3.0)	1.54	22

[1]Based on the most meaningful terms; [2]geometric mean (in -log10 scale) of member's p-values of the corresponding annotation cluster; [3]total number of different genes in a functional annotation cluster; in brackets: number of genes and fold enrichment of the functional term.

Supplemental Table 6: Results of text mining using CoPub

Keyword	p-value	# Genes
Angiogenesis	2.33E-09	41
Vasculogenesis	3.52E-04	11
Response to hypoxia	2.85E-04	10
Wound healing	3.20E-09	26
Blood coagulation	1.70E-03	8
Glycosylation	1.28E-07	38
N-glycosylation	6.90E-04	16
Cell proliferation	2.80E-07	61
Cell differentiation	6.36E-07	49
Epithelial cell differentiation, proliferation	7.29E-06	17
Endothelial cell differentiation, activation	1.62E-04	12
Cell growth, cell growth and/or maintenance	8.89E-06	51
Apoptosis, cell death	3.86E-07	66
Induction of apoptosis	5.87E-04	21
Cell migration	6.09E-07	41
Cell motility	2.00E-05	25
Chemotaxis	3.24E-03	19
Inflammation	5.85E-06	33
Cell adhesion	2.26E-06	44
Cell invasion	1.82E-05	21
Cell-matrix recognition, cell-matrix adhesion	5.33E-03	8
Cytoskeleton	9.11E-03	30
Bone remodeling	1.76E-05	13
Osteoblast differentiation	7.42E-04	13
Menstrual cycle	9.82E-05	11
Embryonic development	1.36E-04	31
Ovulation	3.51E-04	10
Luteinization	3.92E-04	8
Luteolysis	4.54E-04	8
Decidualization	9.41E-04	10
Prostaglandin metabolism, biosynthesis, transport	1.09E-03	6
Secretion, secretory pathway	1.43E-04	34
Ion transport	3.36E-03	9
Endocytosis	5.15E-03	24

Supplemental Table 7 Expression of genes involved in prostaglandin signaling and metabolism

Eca Gene symbol	Eca Gene name	Eca Entrez Gene ID	Hsa Gene symbol	Hsa Gene name	Hsa Entrez Gene ID	FC Pr/Co	q-value
PTGER2	prostaglandin E receptor 2 (subtype EP2), 53kDa	100067279	*PTGER2*	prostaglandin E receptor 2 (subtype EP2), 53kDa	5732	-1.12	0.272
LOC100053557	similar to protaglandin receptor EP3E	100053557	*PTGER3*	prostaglandin E receptor 3 (subtype EP3)	5733	**1.76**	**0.016**
LOC100053208	similar to prostaglandin E2 receptor EP4 subtype	100053208	*PTGER4*	prostaglandin E receptor 4 (subtype EP4)	5734	**2.04**	**<0.001**
PTGFR	prostaglandin F receptor (FP)	100009714	*PTGFR*	prostaglandin F receptor (FP)	5737	-1.35	0.084
LOC100146680	similar to KIAA1436 protein	100146680	*PTGFRN*	prostaglandin F2 receptor negative regulator	5738	-1.02	0.554
LOC100067254	hypothetical protein LOC100067254	100067254	*PTGDR*	prostaglandin D2 receptor (DP)	5729	1.22	0.090
LOC100071157	similar to prostacyclin receptor	100071157	*PTGIR*	prostaglandin I2 (prostacyclin) receptor (IP)	5739	-1.03	0.510
LOC100034143	prostaglandin E synthase	100034143	*PTGES*	prostaglandin E synthase	9536	-1.09	0.360
LOC100070332	hypothetical protein LOC100070332	100070332	*PTGES2*	prostaglandin E synthase 2	80142	-1.09	0.392
LOC100059858	similar to p23	100059858	*PTGES3*	prostaglandin E synthase 3 (cytosolic)	10728	1.14	0.083
LOC100065145	hypothetical protein LOC100065145	100065145	*AKR1B1*	aldo-keto reductase family 1, member B1 (aldose reductase)	231	-1.01	0.645
PGFS	prostaglandin F synthase	100034026	*AKR1C1*	aldo-keto reductase family 1, member C1	1645	1.35	0.030
LOC100057251	similar to prostaglandin F synthase	100057251	*AKR1C4*	aldo-keto reductase family 1, member C4	1109	1.26	0.065
LOC100070616	similar to prostaglandin F synthase	100070616	*AKR1C4*	aldo-keto reductase family 1, member C4	1109	1.33	0.046
LOC100057212	similar to prostaglandin F synthase	100057212	*AKR1CL1*	aldo-keto reductase family 1, member C-like 1	340811	1.67	0.078
LOC100070491	similar to prostaglandin F synthase	100070491	*AKR1CL1*	aldo-keto reductase family 1, member C-like 1	340811	**2.32**	**0.013**
LOC100070501	similar to prostaglandin F synthase	100070501	*AKR1CL1*	aldo-keto reductase family 1, member C-like 1	340811	**2.25**	**0.021**
PTGDS	prostaglandin D2 synthase 21kDa (brain)	100067921	*PTGDS*	prostaglandin D2 synthase 21kDa (brain)	5730	-1.24	0.041
LOC100053460	similar to glutathione-requiring prostaglandin D synthase	100053460	*PGDS*	prostaglandin D2 synthase, hematopoietic	27306	1.24	0.392
LOC100071412	similar to prostacyclin synthase	100071412	*PTGIS*	prostaglandin I2 (prostacyclin) synthase	5740	-1.00	0.592
PLA2G1B	phospholipase A2, group IB (pancreas)	100033889	*PLA2G4A*	phospholipase A2, group IVA (cytosolic, calcium-dependent)	5321	**1.56**	**0.006**
PTGS1	prostaglandin-endoperoxide synthase 1	100034087	*PTGS1*	prostaglandin-endoperoxide synthase 1 (COX1)	5742	1.14	0.306
PGHS2	prostaglandin G/H synthase-2	791253	*PTGS2*	prostaglandin-endoperoxide synthase 2 (COX2)	5743	1.22	0.063
LOC100065438	hypothetical LOC100065438	100065438	*SLCO2A1*	solute carrier organic anion transporter family, member 2A1 (Prostaglandin transporter)	6578	**2.00**	**0.021**
HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)	100009687	*HPGD*	hydroxyprostaglandin dehydrogenase 15-(NAD)	3248	1.13	0.592
LOC100061690	similar to NADP+ dependent prostaglandin dehydrogenase	100061690	*CBR1*	carbonyl reductase 1	873	-1.21	0.289
LOC100061787	hypothetical LOC100061787	100061787	*CBR1*	carbonyl reductase 1	873	1.15	0.083
	ENSECAG00000004698		*PTGR1*	prostaglandin reductase 1	22949	**2.67**	**0.018**
	ENSECAG00000013284		*PTGR2*	prostaglandin reductase 2	145482	1.02	0.629

Acknowledgements

Ich bedanke mich ganz herzlich bei meinem Doktorvater Herrn Prof. Dr. Johannes Handler für die Bereitstellung, Planung und Betreuung dieses großartigen Projekts. Vielen Dank für die Organisation und die tatkräftige Unterstützung bei der Durchführung des praktischen Teils. Vielen Dank für Dein stets offenes Ohr, Deine Gabe zu begeistern und zu motivieren, und dafür dass ich diese Arbeit, die mich sowohl fachlich als auch persönlich bereichert hat, ausführen durfte.

Ein besonderer Dank gebührt auch meinem Betreuer und Ansprechpartner im Genzentrum, Dr. Stefan Bauersachs, ohne dessen Fachwissen, Hilfe und Engagement mir die Umsetzung dieser Arbeit so nicht möglich gewesen wäre. Lieber Stefan, danke Dir für Deine Unterstützung und Dein unermüdliches Engagement bei der Auswertung der Daten, beim Verfassen des Papers, bei sämtlichen Vorträgen und bei der Stipendiensuche.

Ich danke Herrn Dr. Senckenberg vom bayerischen Haupt- und Landgestüt Schwaiganger, der uns die Stuten für die Probennahme zur Verfügung gestellt hat. Vielen Dank an Dr. Hans-Peter Remler und Julia für die freundliche Aufnahme und die Mitbenützung des Untersuchungsraums während dieser Zeit. Danke auch an die Mitarbeiter des Schulstalls, insbesondere Herrn Wallner für seine Hilfsbereitschaft.

Tausend Dank an das spitzen „Spülteam“, insbesondere Frau Dr. Christiane Otzdorff, Elke Fink und Dr. Stefanie Neuhauser, die mir jederzeit bei den Embryospülungen tatkräftig zur Seite standen, auch wenn dafür wieder einmal ein Wochenende / Feiertag draufgegangen ist! Besonderer Dank gebührt dabei Dr. Christiane Otzdorff für ihre fachliche Kompetenz, ihre unglaubliche Geduld und Hilfsbereitschaft. Danke auch an Sophie, Sasu und Verena, die eingesprungen sind, wenn mal Not am Mann war. Danke Euch allen für die Hilfe, aber auch für Eure Freundschaft und den Zuspruch den Ihr mir entgegengebracht habt.

Lieben Dank auch an Dr. Birgit Kolberg und Dr. Sandra Hake-Paulus für ihr stets offenes Ohr, an Sandy, Tobi und das ehemalige Team vom OWF für ihre regelmäßige Hilfestellung, und an Sophia Wiebe für die Vorarbeit mit den Stuten aus Schwaiganger.

Ebenfalls möchte ich der gesamten AG Blum für ihre freundschaftliche Aufnahme in ihr Team danken. Ich danke insbesondere unserer Junior Group, vorneweg Karin Groß, die mich in die Grundlagen der Labortätigkeit einführte und mir die Arbeit mit Gewebeproben, RNAs und den verschiedenen Kits und Gerätschaften näherbrachte. Danke auch an meine Mit-Doktoranden Stasi, Mone und Alex für ihr stets offenes Ohr und die kurzweiligen Kaffee- und Mittagspausen.

Herzlichen Dank auch an Prof. Dr. Eckard Wolf für seine freundliche Unterstützung in allen Belangen.

Vielen Dank an Prof. Dr. Rüdiger Wanke und Frau Dr. Nadja Herbach des Tierpathologischen Instituts für die Planung der quantitativ stereologischen Untersuchungen und für das zur Verfügung stellen des Morphomaten. Danke Nadja für Deine Unterstützung und sämtliche Erklärungen in Sachen Morphometrie! Danke auch an Dr. Andreas Blutke für seine Hilfe.

Vielen lieben Dank an Frau Dr. Susanne Ulbrich von der Physiologie der TU München in Weihenstephan und ihren Mitarbeitern für Ihre Kooperation und die Durchführung der qPCR Analysen die mir eine maßgebliche Hilfe waren.

Ich danke dem ehemaligen Graduiertenkolleg 1029 „Funktionale Genomforschung in der Tiermedizin“ für meine Aufnahme, die Ausbildung, die vielen produktiven Diskussionen und die finanzielle Unterstützung des Projekts während der ersten zwei Jahre.

Des Weiteren danke ich der H. Wilhelm Schaumann Stiftung für mein Stipendium während des letzten halben Jahres.

Ich möchte meinem Freund Ulli danken, der mich zu dieser Doktorarbeit ermutigt hat und immer für mich da war. Außerdem danke ich meinen Freunden für ihren Rückhalt, durch den mir alles so viel leichter von der Hand ging.

Zum Schluss möchte ich von Herzen meinen Eltern danken. Danke dafür, dass ihr mich immer und in allen Belangen unterstützt habt, dass ihr mir das Studium und die 2 ½ Jahre Doktorarbeit ermöglicht habt und immer für mich da ward. Ohne Euch hätte ich das alles nicht geschafft! 1000 Dank für alles, Ihr seid die tollsten Eltern, die ich mir vorstellen und wünschen kann.

This study has been supported by the German Ministry for Education and Research (BMBF, FUGATO-plus, COMPENDIUM), the German Research Foundation (DFG, Research Training Unit 1029 Functional Genome Research in Veterinary Medicine), and the H. Wilhelm Schaumann Foundation.