Reproductive immunology and endocrinology of pregnant mare

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Abstract

Pregnancy is a distinct event that induces a unique relationship between immune and endocrine systems. In humans, there is growing evidence that pregnancy is regulated by both immune-immune and immune-endocrine interactions, and that these are constantly shifting throughout pregnancy. Pregnancy-related hormones (progesterone, estrogen, and human chorionic gonadotropin) are critically involved in successful establishment, maintenance, and termination of pregnancy. In humans, these hormones suppress detrimental maternal alloresponses to semi-allogeneic fetus while promoting tolerance pathways. Although there is a better understanding of endocrinology of equine pregnancy, minimal inferences are made understanding immune-endocrine interactions. Our current understanding of endocrinology and immunology of equine pregnancy is reviewed and advancements needed to properly identify interactions between these 2 systems are provided.

Keywords: Mare, pregnancy, immunology, endocrinology, fetus

Reproductive endocrinology of pregnant mare

Progestins

Progesterone is necessary for maintenance of pregnancy in mammals, but mare is somewhat unique, as source and type of progestin varies during pregnancy.[1] This aspect of endocrinology of equine pregnancy has been known since mid-1950s when Short demonstrated that progesterone could not be measured in blood of mares after mid-pregnancy.[2] Advent of mass spectrometry coupled with chromatography provided a more specific analysis of individual progestins in blood required to further characterize progestins present in circulation during latter half of equine pregnancy.[3] This study identified 5_{α} dihydroprogesterone (DHP) and its metabolites as major pregnanes present in circulation of mares during second half of pregnancy. Relative bioactivity of DHP in mare remained speculative until studies demonstrated DHP alone could maintain pregnancy in mares in progesterone absence and that DHP and progesterone had equal potency in binding to equine progesterone receptor (PR).[4] Interestingly, equine PR has a mutation in progesterone binding region similar to elephant, another species that utilizes DHP for pregnancy maintenance.[5]

Initial source of progesterone for pregnancy maintenance in mares is corpus luteum. In addition to progesterone, DHP is present during early pregnancy (also in nonpregnant luteal phase) in concentrations ~ 50% of that of progesterone in circulation.[4,6] Source of DHP during early luteal phase is uncertain; however, it appears that luteal progesterone is rapidly metabolized to DHP through activity of 5_{α} reductase type II,[6] which is highly expressed in skin.[7] Beginning near days 35 - 37 of pregnancy, secretion of equine chorionic gonadotropin (eCG) by endometrial cups initiates an increase in circulating progestins (both DHP and P_4) from primary corpus luteum (CL) and from newly formed secondary CLs in pregnant mare. As chorioallantois develops more fully, placental synthesis of progestins increases after day 70 of pregnancy, and concentrations of DHP exceed those of P_4 ~ 105 days of pregnancy.[8,9] This transition has been termed luteal-placental shift in progestin synthesis,[10] characterized by inputs from both fetus and placenta for progestin synthesis.[3, 9,11] Feto-placental synthesis of progestins involves synthesis of pregnenolone (a universal precursor in steroidogenesis) that is subsequently converted to DHP by

5_α reductase (Figure 1) expressed in chorioallantois.[9] A number of metabolites of DHP are detected in circulation of pregnant mares after mid-pregnancy, some of which are present in very high concentrations as gestation progresses.[3,8] Biological function of these DHP metabolites remains uncertain, although some may have a role in activating PR, because despite their low affinity for PR, they are present in such high concentrations.[12]

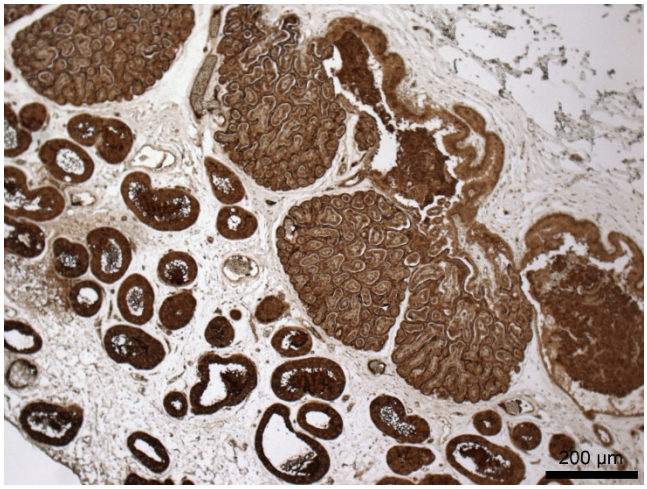

Figure 1. Immunohistochemical labeling of 5_α reductase in equine placenta at day 300 of pregnancy. Both chorionic and endometrial epithelial cells have distinct (brown) labeling.

Withdrawal of progestogenic support at end of pregnancy is an important mechanism in parturition initiation in most species studied. Data related to a reduction of progestins prior to parturition in mares have been conflicted, with some but not all studies identifying changes detected prior to foaling.[1,10] More recent research has identified a decline in DHP and its associated metabolites 3 days preceding parturition in mares.[8,12] This decline in 5_α reduced progestins in days preceding foaling is associated with marked reduction in expression and enzymatic activity of 5_α reductase in chorioallantois.[13] Although mechanism regulating this downregulation of 5_α reductase is unknown, this appears to be a key event in preparing the endocrine environment for onset of parturition.

Monitoring progesterone concentrations (and its 5_α reduced metabolites) has been used for many years to evaluate well-being of equine pregnancies in both experimental and field studies.[14-18] Interpretation of these studies is sometimes confusing and is hindered by use of immunoassays for measurement of progesterone concentrations, with variable cross-reactivities to pregnanes, some of which may be present in very high concentrations.[19] Application of mass spectrometry has been helpful to understand changes in pregnanes in both normal and abnormal equine pregnancy.[3,18,20] More chronic pathology of placenta (e.g. ascending placentitis) of late pregnancy is associated with a marked increase (3 - 4 fold; Figure 2) in 5_α dihydroprogesterone (DHP), along with several of its metabolites.[18, 20] Although concentrations of P_4 also change based upon liquid chromatography-mass spectrometry (LC-MS), P_4 concentrations remain relatively low; therefore, most studies measuring changes in progestins with immunoassays were likely detecting changes in 5_α reduced progestins (present in vastly higher concentrations). Interpretation of maternal serum pregnane concentrations becomes more difficult after day 300 of pregnancy, due to normal prepartum rise in pregnanes that occurs at this time[8] and because pregnane concentrations become more variable as parturition approaches.

In contrast to chronic placental disease, more acute placental infections appear to be associated with a rapid decline in maternal serum pregnane concentrations that precede abortion by ~ 3 days.[18] At endometrium and chorioallantois, tissue concentrations of 5α DHP and its metabolites are decreased in acute inflammation.[21] This reduction in local concentrations of pregnanes is associated with a downregulation of key steroidogenic enzymes (5α reductase) which is likely responsible for both tissue and systemic decrease in pregnanes in these acute placental lesions.

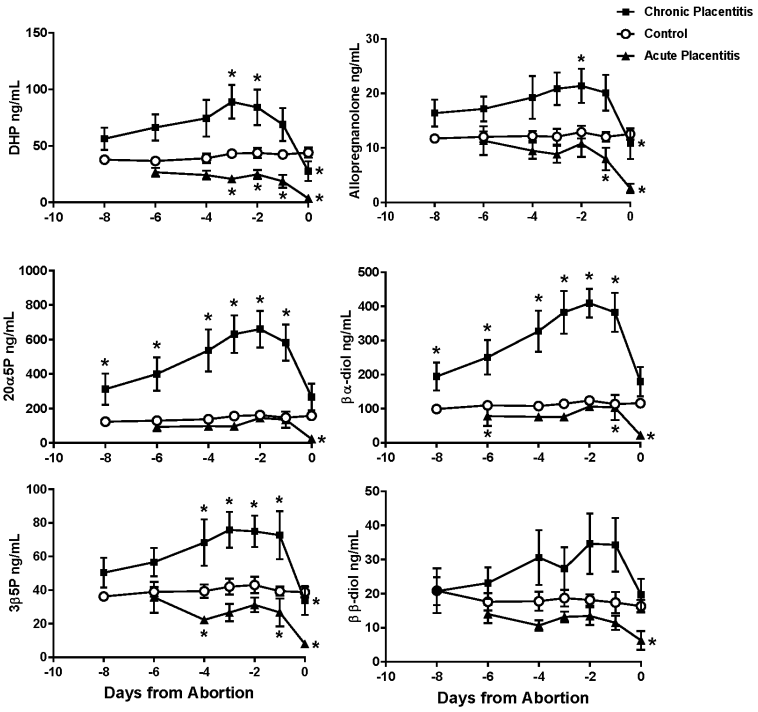

Figure 2. Plasma concentrations of pregnanes (5α dihydroprogesterone (DHP), allopregnanolone, 20α hydroxy 5α pregnan 3 one (20α5P), 5α pregnan 3β,20α diol (βα diol), and 3β hydroxy 5α pregnan 20 one (3β5P), and 5αpregnan 3β, 20β diol (ββ diol)) measured by liquid chromatography tandem mass spectrometry LC-MS/MS. Reprinted (Theriogenology 2018;122:130-136.) with permission.

Estrogens

Elevated estrogen concentrations in peripheral circulation are a hallmark of equine pregnancy and have been described since the 1930s.[22] Increased estrogens concentrations may be detected in early pregnancy from corpus luteum, associated with eCG secretion.[23-25]Again, as feto-placental unit becomes more active in steroidogenesis, placenta takes over estrogen secretions. In this case, secretion of androgens (dehydroepiandrosterone, DHEA) by fetal gonads serve as a precursor for aromatization by placenta, through aromatase action (Figure 3), which is highly expressed in chorioallantois.[1] Circulating maternal estrogens peak at ~ 7 months, which corresponds to a marked hypertrophy of fetal gonads (both ovary and testis) associated with growth of interstitial tissue.

Exact function of elevated estrogens during equine pregnancy is not fully understood. Inhibition of estrogen synthesis by aromatase during months 2 - 4 reduced fetal growth, reduced expression of angiogenic genes and reduced vascular development in endometrium (Haneda and Ball, unpublished). Inhibition of aromatase activity by administration of letrozole during last trimester of equine pregnancy decreased maternal concentrations of 17β estradiol and estrone sulfate by 90% compared to controls.[26] Uterine blood flow (measured in uterine artery by Doppler ultrasonography) was not affected by

decreased maternal estrogens and pregnancy length and neonatal viability was similarly not different when estrogens were markedly suppressed.[26] Birthweights were reduced by ~ 15% in estrogen-suppressed mares, and this effect may have been due to effects of uteroplacental microvasculature that were not detected as changes in uterine artery blood flow.

Elevations in maternal estrogens are classically associated with initiation of parturition in domestic ruminants. Although there is no prepartum increase in circulating estrogens in peripartum mares, there is a sustained estrogen secretion and aromatase activity in chorioallantois remains elevated through parturition in mare.[13]

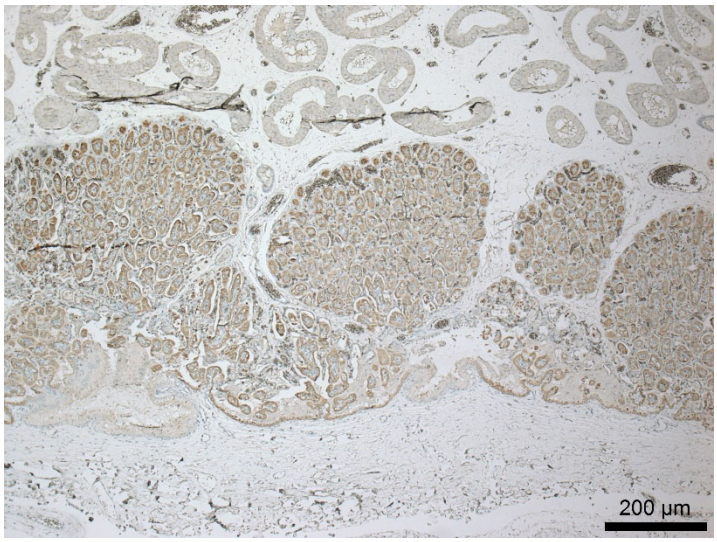

Figure 3. Immunolabelling of aromatase in day 300 equine placenta. Aromatase is expressed primarily in chorion epithelial cells and chorionic villi.

Maternal concentrations of estrogens have also been used to assess fetal wellbeing.[17,27-29] Reductions in 17β estradiol appear to be more closely indicative of feto-placental endocrine function than changes in estrone sulfate,[29] possibly because of relatively longer half-life of sulfo-conjugated estrone. In experimentally induced ascending placentitis, 17β estradiol concentrations declined ~ 6 days preceding abortion.[29] Similarly, mares with fetal loss had a lower 17β estradiol concentration than mares that produced a healthy foal in a field study of 459 pregnant mares.[17] These changes in peripheral estrogen concentrations in mares with placental compromise appeared to be associated with a decreased expression of aromatase and a reduced tissue concentration of estrogens in mares with placental infection[21].

Activins and inhibins

Although inhibins and activins are most commonly thought of as gonadal hormones, elevations in inhibins and activins have been reported during pregnancy in women;[30] however, studies on changes in these hormones during equine pregnancy are contradictory. Higher concentrations of immunoreactive inhibin in circulation of mares during later pregnancy originated from fetal gonads.[31] This immunoreactive inhibin was not biologically active and assay did not distinguish different forms of inhibin. Specific immunoassays detected decreased inhibin A and inhibin B at 60 days after ovulation and lower amounts until end of pregnancy.[32] Molecular characterization of inhibin/activin subunits from the endometrial and fetal placenta suggested that inhibin/activin A was primary isoform present.[33] This suggested that activins rather than inhibins were predominant form in pregnant mare uterus.

Activins belong to transforming growth factor β superfamily. Activins have a common β subunit and are homodimers of β with t3 isoforms (A, B, and AB). Induction of experimental placentitis in mares ~ 280 days of pregnancy resulted in an increase in activin A in maternal circulation, along with an increase in activin A in allantoic fluid of mares with placentitis compared to gestationally age matched control mares.[34] Activin A has been described as an acute mediator of inflammation in horses and other species. Although source of increased activin A in mares with induced placentitis is unknown, it is likely derived from the uteroplacental unit. Future studies should address the utility of activin A determination in clinical cases of placentitis.

Reproductive immunology of pregnant mare

Field of reproductive immunology in humans has advanced considerably in the 70 years since Sir Peter Medawar stated his initial hypotheses for how the maternal immune system supports a semi-allogeneic fetus. In 1953, Father of Transplantation stated ‘‘The immunological problem of pregnancy may be formulated thus: how does pregnant mother contrive to nourish within itself, for many weeks or months, a foetus that is an antigenically foreign body?’’ Yet through inventive research on his part alongside others, it is now understood that pregnancy is controlled by both innate and adaptive system, and that fetus develops under a state of active tolerance. Additionally, pregnancy cannot be considered a single event, and different stages of pregnancy require immensely different immunophenotypes. Recent research indicates that this accomplished through a series of immune-immune interactions and immune-endocrine interactions that create a complex network of immune regulation to ensure fetal survival. Various hormones that are upregulated in pregnancy have profound effects on the immune system; this includes progestins, estrogens, and human chorionic gonadotropin (hCG). Equine pregnancy differs from humans' in a multitude of ways, including placentation, implantation, and endocrinology and therefore inferences are difficult to make. Here we review our current knowledge of this process in the horse.

Immediately after deposition of foreign and allogeneic semen into uterine lumen, innate immune system is activated through a series of proinflammatory mediators, leading to the chemotaxis of neutrophils, macrophages, and dendritic cells.[35,36] Neutrophils are present within 30 minutes following breeding, are at the highest numbers at 12 hours, and undetected by 24 hours after breeding in the normal horse and assist in the clearance and digestion of excess spermatozoa and contaminants.[37,38] Anti-inflammatory cytokines IL10 and IL1RN are elevated < 6 hours postbreeding, signaling for innate response resolution and persistent inflammation suppression.[35] In other species, maternal uterine dendritic cells bind to paternal antigens present in seminal fluid before migrating to lymph nodes to activate adaptive immune system to recognize seminal antigens as self, and this is specifically performed by T cells.[39-43] This has not been confirmed in mare.[44] T cells are key regulators of adaptive immune response to pregnancy and can be divided into numerous subsets, including helper T cells (Th1, Th2, Th9, Th17, Th22), regulatory T cells (Tregs), and cytotoxic T cells (CTL).[45]

Both CD4+ cells, Th1 and Th2 cells develop following antigen presentation through a series of interactions with specific cytokines, transcription factors, and hormones. Th1, or IFNγ secreting cells, are proinflammatory and are required for response to intracellular pathogens. In contrast, Th2, or IL4-secreting cells, are anti-inflammatory and necessary for response to extracellular pathogens. In humans, estrogen receptor (ER) is found on both CD4+ and CD8+ cells, and estrogen has been shown to decrease both Th1 and Th2 cytokines TNF and IL4.[46] In contrast, progesterone inhibits Th1 secreted IFNγ,[47] whereas also promoting Th2 secreted IL4.[48] Studies from our lab identified in diestrus mare decreased endometrial expression of proinflammatory IFNγ, IL1β, and IL8 in comparison to day of ovulation, indicating an anti-inflammatory effect of progesterone in mare.[49] Due to progesterone-dependency of

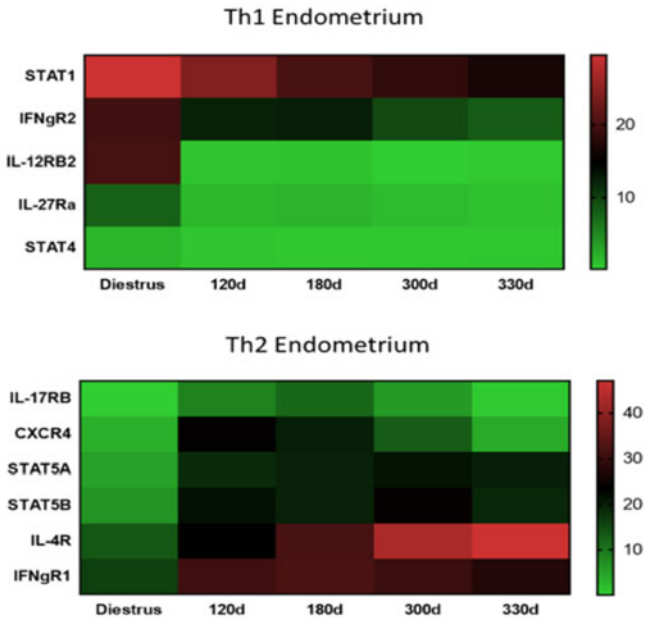

Figure 4. Heat map of Th1 and Th2 related transcripts altering during equine pregnancy. Red indicates higher transcript expression levels; green indicates lower transcript expression levels

Th2 cells,[48] lymphocyte subset was initially believed to regulate pregnancy maintenance and prevent rejection of the semiallogeneic tissues (both fetal and chorion).[50-56] It is now understood that the ratio of Th1:Th2 is in constant fluctuation throughout pregnancy, with implantation and parturition being primarily Th1 events, whereas mid-pregnancy is shifted towards a Th2 environment. In mare, this relationship was similar to that previously described in humans. Th1 related transcripts at the feto-maternal interface were highest in diestrus endometrium and decreased as pregnancy advanced. In contrast, Th2 transcripts increased in endometrium to mid-pregnancy and declined prepartum (Figure 4).[57] Interestingly, no Th1 related transcripts were altered during equine pregnancy in chorioallantois, whereas Th2 transcripts followed a similar profile to that noted in the endometrium.

Recently, immunology of pregnancy was determined to be less controlled by the Th1:Th2 ratio, and more so by regulatory T cells (Tregs). Paternal antigens are present within semen,[42] and signal expansion of Tregs to identify this antigen as self and not requiring attack.[58] Tregs are believed to be the key regulator of active state of tolerance during pregnancy. Estrogen causes a dose-dependent increase in Treg secreted IL10 and Foxp3+ Tregs that are increased in estrus in comparison to diestrus, indicating estrogen dependency.[59] Interestingly, recent research in a murine model identified activin induced production of Foxp3 gene and IL10 secreting Tregs.[60] These cells are highly immunosuppressive and inhibit production of Th2 cell types. Additionally, inhibin-null mice (Inh$\alpha^{-/-}$) had increased production of Tregs in periphery, indicating endocrine involvement in Treg development.[61] In humans, Tregs are lowest during implantation, increase towards mid-pregnancy and decline prepartum, following a similar profile to that of estrogen production during pregnancy.[60-62] In mare, a similar profile was noted in Treg-related transcripts at fetal maternal interface.[57] Treg-related transcripts were higher in pregnancy compared to the nonpregnant endometrium; this profile was also noted in the chorioallantois. Additionally, transcript was expressed lowest in early pregnancy, increased towards mid-pregnancy and declined prepartum, also following estrogen profile production during equine pregnancy (Figure 5).

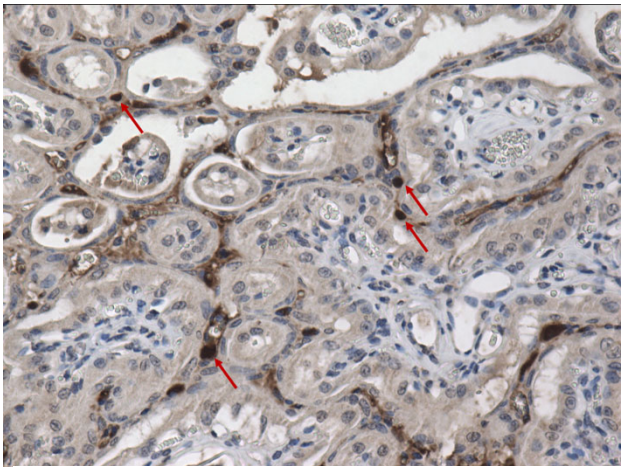

Figure 5. Foxp3+ Tregs in the 300d intact placenta. Arrows indicate positive immunolabeling (brown). Reprinted from (Placenta 2020;89:78-87) with permission.

It should be noted that comparative physiology between equine and human pregnancy indicates many differences, and therefore inferences are difficult. Human implantation occurs on day 9 following fertilization, whereas equine pregnancy does not have a true implantation event until day 35 when the eCG-secreting endometrial cups develop from the chorionic girdle.[62-64] Invasion of the trophoblast into the endometrium leads to a rapid influx of maternal lymphocytes, including CD4+ and CD8+ T cells, but their activity remains suppressed until ~ 80 days of gestation, at which point they actively attack and degrade the cups.[65,66] Ratio of lymphocytes within the endometrial cup is predominantly Th1, implying that a heightened Th1 response may be necessary for their degradation, although the signaling required is unknown.[67] The regression of the cups leads to a decrease in eCG production, and a subsequent decrease in ovarian-produced progesterone.[8] This coincides with an increase in the fetal placental progestins, including 5_{α} DHP and 20_{α} 5P. Similar to eCG, early human pregnancy has high hCG concentrations. Little is known about the role of eCG on the immune system, but it was recently reported that hCG affects the immune response in a variety of ways, including proliferation of uterine natural killer cells,[68] increased monocytic function,[69] inhibition of Th1 development,[70] and recruitment and activation of Tregs.[71] Although effects of eCG on immune function in mare has not been

well investigated, it is known that placental explants containing eCG were immunosuppressive and inhibited proliferation of lymphocytes (specific cell type remains to be determined).[72]

Pregnancy complications can lead to alterations in normal endocrine-immune response. As noted, fetal compromise is associated with shifting endocrine profiles, including a decrease in estrogens and an alteration in progestin concentration dependent on disease progression nature. Immunologically, abortion-prone women tend to experience an increase in Th1 cell populations in circulation, in addition to having increased concentrations of Th1-secreted IL2 and TNF, although Th2 immunophenotypes have also been noted in women suffering recurrent miscarriage.[51,54] Interestingly, a deletion of either cell type does not lead to abortion or infertility, indicating lack of reliance for pregnancy maintenance. In contrast, a deletion of Tregs leads to both abortion and implantation failure,[73] whereas a decrease in estrogen Tregs is associated with a variety of pregnancy complications, including pre-eclampsia, chorioamnionitis, repeat miscarriage, and preterm labor.[58,74-77] In the horse, a decrease in Tregs is associated with early embryonic loss, although less is understood in late gestation.[78] Data from our lab (unpublished) suggests that ascending placentitis is associated with an increase in Th1-related transcripts and dysregulation of Th2 and Treg-related transcripts at the feto-maternal interface, but less is known in the periphery. Additionally, it appears that the chorioallantois is the primary responder to induced infection and undergoes upregulation of Th1-related transcripts following infection. It is unknown if this alteration in Tregs-related transcripts is associated with the systematic decline in estrogens noted during fetal compromise in the horse, but this warrants additional research.

Conclusion

Pregnancy is a distinct event that induces a unique relationship between immune and endocrine systems. How maternal immune system tolerates the semi-allogeneic fetus has fascinated researchers for centuries. Recent research indicates that this is accomplished through a series of immune-immune interactions and immune-endocrine interactions that create a complex network of immune regulation to ensure fetal survival. Various hormones that are upregulated in pregnancy had profound effects on immune system; this includes progesterone, estradiol, and human chorionic gonadotropin, although few of these interactions have been confirmed in mare. It is imperative that we improve our understanding of this relationship in normal equine pregnancy before making inferences into alterations of the endocrine-immune interaction of the abnormal.

Acknowledgement

Supported by Albert G. Clay Endowment of University of Kentucky.

Conflict of interest

None to report.

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