Early pregnancy in the mare: information to interpret, concepts to accrue

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Knowledge of how the mare and her conceptus have to interact during their first month together if pregnancy is to be maintained is growing rapidly. That growth is owed to enormous advances in genomic and proteomic technology coupled to the need to combat the economically important problem of early embryonic loss.

The first part of this presentation will describe and discuss some of our recent findings on how certain proteins and cytokines change at the interface between the mare and her conceptus; these we have studied in biopsy samples of endometrium, the uterine environment (as reflected in flush fluid), and three components of the conceptus (the capsule and the bi- and trilaminar yolk-sac wall) before and after conceptus fixation, and in response to experimental treatment of the mare with the prostaglandin (PG) cloprostenol to induce luteolysis and embryonic death. Proteomic profiles of flush and yolk-sac fluids and of capsule-associated proteins were established from trypsin digested peptides by LC MS/MS with Scaffold-2 interpretation, or by SDS-PAGE and immunoblots. Transcriptomes for endometrium and trilaminar trophoblast were determined by Agilent 44K expression microarrays.

Prominent amongst the temporal changes during normal pregnancy are limited proteolysis of capsule-associated β-2 microglobulin, the disappearance of secretory phopholipase-A2 (sPLA2) from the capsule, cessation of the passage of uterocalin into the yolk sac, and diminishing trophoblastic expression of GM2-activator protein (probably involved in lipid and/or phospholipid transportation) — all occurring around the time of fixation.

Comparisons of the same sets of tissues and fluids from normal (control) pregnancies and from pregnancies failing after treatment of mares with PG have revealed important differences subsequent to the luteolysis. In the endometrium and uterine flush fluid, expression and/or concentrations of both uteroglobin and sPLA2 are increased by PG treatment and sPLA2 increases in the capsule and yolk-sac fluid. Proteins corresponding to SERPINA14, ANXA1, MUC6, and B2M were also increased in uterine flush fluids after PG treatment, with SERPINA14 being simultaneously increased in yolk-sac fluid. By comparison, VNN2, VNN3, GZMB, CD109 and A2M were consistently reduced in uterine flush fluid from treated mares.

Microarray analyses of endometrial gene expression have revealed that PG treatment results in 10- to 100-fold increases in expression of many genes, most notably six proteins involved in signalling, four related to lipid metabolism (including sPLA2 and uteroglobin), two associated with extracellular matrix modification, and one with lipid transport. Conversely, significant >2-fold reductions in endometrial expression of various genes were also associated with PG treatment. In contrast, gene expression in trophoblasts from control and treated mares were more similar; only 14 significant elevations (in the 2- to 5-fold range) resulted from treatment. These included increases in serpins B2 and B10.

These studies have provided data that complement the findings of other investigators who have made similar comparisons of expression of proteins and cytokines during the estrous cycle and early pregnancy. They have revealed several alterations in the endometrium and conceptus that might be harmful effectors, or biomarkers of adverse uterine conditions, useful for clarifying the mechanisms of pregnancy establishment and pregnancy failure in mares.

The second part of the talk will discuss the advantages and limitations of applying 'cutting-edge' techniques to investigations of early pregnancy. The advantages include unparalleled opportunities to obtain a 'global view' of concomitant events; limitations include the temptations to associate magnitudes of genomic and proteomic changes with biological significance.

The third section of the presentation will be a reminder of some of the many deficits in our knowledge of mare-conceptus interactions that remain to be investigated by techniques old and new, of genetic predispositions to early embryo loss, and of behavioral factors that may affect the progress of pregnancy. All must be integrated if we are to understand "… what a tangled web [mares] weave, when first [they] practice to [conceive]" [apologies to Sir Walter Scott].