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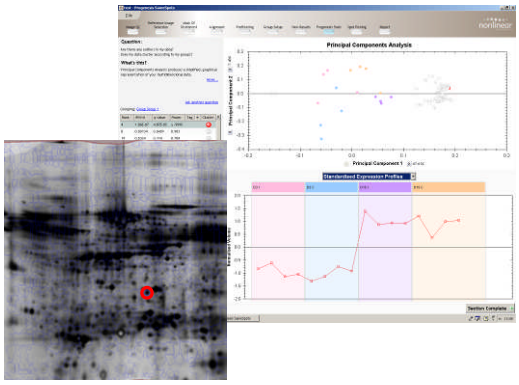
The effect of stage of cycle and steroid environment on the uterine proteome of the cow and differences from plasma



Key external stakeholders:

Dairy and beef cattle breeders, ICBF, veterinary diagnostic companies, scientific community.

Practical implications for stakeholders:

The main findings from this research show that the uterus is a dynamic organ capable of regulating the proteomic composition of the histotroph or uterine milieu and that this is in turn affected by the systemic concentration of progesterone the key hormone of reproduction.

- The protein composition of uterine fluid changes throughout the oestrous cycle consistent with the demands of the rapidly growing embryo.
- The concentrations of many uterine proteins 7 to 15 days after oestrus (the period of greatest embryonic loss) are directly related to the concentration of systemic progesterone as early as 3 days after oestrus.
- Adequate progesterone very early in the oestrous cycle is indicated for a uterine proteomic environment conductive to optimal embryo growth and subsequent survival.
- The concentrations of many uterine proteins are very different from plasma a further indication of the dynamic nature of the uterine proteome.

Main results:

- The protein composition of uterine fluid changes throughout the cycle consistent with the demands of the rapidly growing embryo.
- The concentrations of many uterine proteins 7 to 15 days after oestrus (the period of greatest embryonic loss) are directly related to the concentration or changes in the concentration of systemic progesterone as early as 3 days after oestrus and the response is greatest to changes in progesterone on day 3.
- This study indicates that the uterus is responsive to changes in progesterone very early in the oestrous cycle and that an adequate supply of progesterone is indicated for a uterine environment conducive to optimal embryo growth and survival.
- The uterine proteome is in some cases very different to that of plasma and this has implications for the composition of synthetic *in vitro* culture media in particular those using serum as an additive.

Opportunity / Benefit:

Knowledge gained from this study will guide researchers in defining the critical window for adequate progesterone and the development of strategies designed to increase the concentration of systemic and uterine concentrations of progesterone, particularly in dairy cows where systemic progesterone concentrations during the early part of the cycle are known to be inadequate in a high proportion of cows.

Collaborating Institutions:

UCD

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1. Project background:

Embryo loss is the single greatest factor affecting fertility in both the dairy and beef herds with over 40% of embryos lost before term. Early embryo loss occurring in the first two weeks after fertilization accounts for over 70% of that loss. Although there are many potential factors that contribute to embryo death, it is the physiology of the maternal reproductive system and principally the uterus, however, that has the greatest potential to affect embryo viability until implantation. Prior to implantation the embryo is metabolically most active and grows at an exponential rate between days 8-16 after fertilization a period which also coincides with the time of greatest embryo loss. The embryo maintains a free floating existence in the uterus from day 4 to commencement of implantation at around day 20 and the role of the uterus is to supply nutrients and growth factors necessary for development. Therefore inadequacies in the regulation of this environment are detrimental to a successful pregnancy. The hormonal mediated regulation of uterine secretions can indirectly influence the growth of the embryo and an increase in embryonic loss is observed when the physiological regulation of uterine function is sub-optimal. For example, low circulating concentrations of progesterone in the first few days after AI is associated with a low probability of embryo survival in dairy cows and heifers. Furthermore, uterine gene expression is sensitive to changes in systemic concentrations of progesterone. Many of these genes are essential for normal embryo growth, survival and implantation.

The objectives of this study were to identify bovine uterine proteins that are sensitive to changes in the systemic concentration of progesterone and how this varies throughout the oestrous cycle. The ultimate aim was to improve our understanding of the dynamics of uterine protein expression and identify the critical period affecting embryo growth and development.

2. Questions addressed by the project:

- Does the uterine proteome differ from plasma or is it essentially a transudate of plasma?
- Does the uterine proteome change throughout the oestrous cycle?
- Is the uterine proteome affected by changes in the concentration of systemic progesterone?

3. The experimental studies:

Sample collection was carried out under license in accordance with European Community Directive 86/609/EEC. Uterine flushings (UF) were collected non-surgically from the ipsi- and contralateral uterine horns of cross-bred beef heifers on day 7 or day 15 of the oestrous cycle. The protein was fractionated and subjected to strong cation exchange chromatography followed by mass spectrometric analysis. Bioinformatics was used to identify and classify proteins and to assign biological functions.

4. Main results:

The findings of this study indicate that there are many proteins in uterine fluid whose concentrations differ significantly from plasma. Many proteins were found to be present at much higher (or lower) concentrations in uterine fluid when compared to plasma indicating the dynamic nature of the uterus in maintaining an environment different to that of the peripheral circulation and this is the first study to demonstrate this.

The proteome of the uterus changes significantly between the two stages of the cycle examined and this is consistent with an exponential increase in embryo size during this period and with the period of greatest embryo loss.

In addition, this is the first study to demonstrate a linear positive relationship between the concentrations of uterine proteins and the concentration or the rate of increase in the concentration of systemic progesterone in the days prior to uterine fluid collection. Changes in the concentrations of systemic progesterone as early as day 3 after oestrus were seen to affect the subsequent concentrations of uterine proteins recovered on day 7 or day 15 after oestrus. The magnitude of the effect of progesterone was also greatest on day 3 and decreased with increasing days after oestrus.

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The progesterone regulated proteins identified in this study are involved in a variety of functions critical to ensuring embryo survival including energy substrate availability, regulation of prostaglandin synthesis and moderation of immune response and reduction of oxidative stress. The concentrations of these proteins appear to be regulated by the uterine endometrium through either selective mechanisms or synthesis and are likely influenced by environmental factors including the local steroidal milieu.

5. Opportunity/Benefit:

This study has led to an increased understanding of the dynamic nature of the uterus and its proteome. The results indicate that changes in the composition of the uterine proteome are consistent with the requirements of the embryo and with the provision of a receptive environment for the embryo.

The most significant and unexpected finding was that the uterine proteome in the mid to late luteal phase (day 7 to day 15) of the cycle is responsive to changes in the steroidal environment in the early luteal phase (as early as day 3) of the cycle and that the effect of progesterone is more pronounced at this early stage.

This study changes the way we now think about the relationship between systemic concentrations of progesterone and embryo loss and ways in which we might develop strategies to reduce the embryonic loss associated with inadequate progesterone in the first week after AI in dairy and beef cow herds.

6. Dissemination:

The outputs from this research have been and continue to be disseminated to stakeholders through seminars, research forums and presentations at national and international scientific meeting including an EU COST action FA1002 on Farm Animal Proteomics. To date one peer reviewed publication has been published, one accepted for publication and one is in preparation for submission to the journal *Proteomics*. A review of uterine proteomic and technological advances is also in preparation.

Main publications:

Faulkner, S., Elia, G., Hillard, M., O'Boyle, P., Dunn, M. and Morris, D. (2011) 'Immunodepletion of albumin and immunoglobulin G from bovine plasma'. *Proteomics* 11: 2329–2335 doi: 10.1002/pmic.201000364.

Faulkner, S., Elia, G., Mullen, M.P., O'Boyle, P., Dunn, M. and Morris, D. (2012) 'A comparison of the bovine uterine and plasma proteome using ITRAQ proteomics'. *Proteomics* 12, 2014–2023.

Faulkner, S., Elia, G., Hillard, M., O'Boyle, P., Dunn, M. and Morris, D. (2011) 'An efficient and reproducible method for the depletion of albumin and immunoglobulin G from bovine plasma'. *EU COST ACTION FA1002; Farm Animal Proteomics* Spring Meeting, Glasgow, p65.

Popular publications:

Faulkner, S., Dunn, M. and Morris, D.G. (2009) 'Evaluation of an immunospecific technique for the depletion of albumin from bovine plasma'. *Agricultural Research Forum*, Tullamore, Offaly, p62.

Faulkner, S., Elia, G., Mullen, M., O'Boyle, P., Dunn, M. and Morris, D. (2011) 'The effect of stage of cycle on the bovine uterine proteome'. *4th International Symposium on Animal Functional Genomics*, Dublin, p255.

Faulkner, S., Elia, G., Mullen, M., O'Boyle, P., Dunn, M. and Morris, D. (2011) 'Differences in the bovine uterine and plasma proteome'. *BSPR-EBI* Meeting, Cambridge, p49.

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