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Identification of uterine proteomic and genomic differences in dairy heifers with different levels of embryo survival rate

Key external stakeholders:

Dairy and beef farmers, advisers and specialists, veterinary surgeons, breeding companies, research laboratories, pharmaceutical industry.

Practical implications for stakeholders:

- Results from these studies quantified the relationship and importance of circulating concentrations of progesterone during the early luteal phase, days 4-7 post insemination, and subsequent pregnancy/ AI (P/AI). These data demonstrated that not only too low a concentration of progesterone but all too high a concentrations of P4 on these days have a deleterious effect on embryo viability. Early luteal P4 were a reasonable predictor of concentrations on day 7 and could be used as a diagnostic tool to identify animals at risk of subsequent embryo loss. Repeatability of concentration of P4 between successive oestrous cycles was low at low at Re= 0.07.
- In this study supplementation with exogenous progesterone using a CIDR device reduced P/AI not withstanding and increase in milk concentrations of progesterone. Based on the results of this study the blanket treatment of lactating dairy cows with an exogenous source of progesterone cannot be recommended. It would appear that the application of exogenous progesterone during the early luteal phase of the cycle has a negative impact on corpus luteum development and results in the premature regression of a proportion of corpus lutea resulting in animal return to oestrus at 10-12 days.
- No evidence was found of a concurrent or carryover of prolonged dietary restriction before insemination on P/AI in beef heifers. The results of this study challenges the frequently proposed hypothesis that low P/AI in dairy cows are caused by NEB and its associated metabolic changes occurring in early lactation.
- Functional analysis of the 34 differentially expressed proteins (including 14 novel to histotroph) revealed distinct biological roles putatively involved in early pregnancy, including remodelling of the uterine environment in preparation for implantation; nutrient metabolism; embryo growth, development and protection; maintenance of uterine health; and maternal immune modulation.

Main results:

- The studies quantified and described the linear and quadratic relationship between progesterone and embryo survival (P/AI and showed both too low and too high a concentrations of P4 on days 4-7 was detrimental to embryo survival. The application of exogenous progesterone during the early luteal phase of the cycle has a negative impact on corpus luteum develop and results in the premature regression of a proportion of corpus luteum resulting in animal return to oestrus at 10-12 days.
- The blanket treatment of lactating dairy cows with an exogenous source of progesterone cannot be recommended.
- No evidence was found of a concurrent or carryover of prolonged dietary restriction before insemination on P/AI in beef heifers.
- Functional analysis of differentially expressed proteins in the histotroph revealed distinct biological roles putatively involved in early pregnancy, including remodelling of the uterine environment in preparation for implantation; nutrient metabolism; embryo growth, development and protection; maintenance of uterine health; and maternal immune modulation.
- This study was the first to report global proteomic characterization of the uterine environment in cattle before implantation and provided novel information on the temporal alterations in histotroph composition during critical stages for early embryo development and uterine function during the early establishment of pregnancy.

Collaborating Institutions: University College Dublin.

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1. Project background:

Efficient pasture-based milk production systems require a compact calving pattern aligned to the onset of the grazing season, a 365-day calving interval and low culling rates for infertility. Achievement of these targets requires high herd reproductive performance. For heifers, beef and moderate yielding dairy cows, fertilisation rate generally lies between 90 and 100%. In high producing dairy cows it would appear that fertilisation rate is lower and more variable. The major component of embryo loss occurs in the first 16 days following breeding (Day 0), with emerging evidence of greater losses before Day 8 in high-producing dairy cows. Progesterone (P4) plays a central role in P/AI as it is involved in a cascade of biochemical, molecular and morphological events in the establishment and maintenance of pregnancy. The timing and/ or strength of the postovulatory rise in P4 may be more important than the final concentration of P4 achieved later in the luteal phase. The overall objective of the experimental studies described in this thesis was to investigate specific endocrine and nutritional factors affecting pregnancy per insemination in cattle (P/AI). Four studies were designed conducted to: (i) determine the relationship between pregnancy per artificial insemination (P/AI) and concentrations of progesterone (P4) in the early luteal phase and establish repeatability estimates for these traits in Holstein- Friesian heifers; (ii) establish the effect of P4 supplementation in the luteal phase on P/AI in dairy cows; (iii) establish the critical timing of supplemental P4 to heifers with induced low P4 in the early luteal phase and to (iv) examine the concurrent and carryover effects of a prolonged period of dietary restriction on P/AI in heifers and (v) establish the effect progesterone on the composition of uterine histotroph.

2. Questions addressed by the project:

- What is the relationship between pregnancy per artificial insemination (P/AI) and early luteal concentrations of progesterone?
- Does progesterone supplementation with progesterone improve P/AI?
- What is the critical time for progesterone supplementation to enhance P/AI?
- Are there carryover effects of prolonged negative energy balance on P/AI?
- What effect has progesterone on the composition of uterine histotroph?

3. The experimental studies:

Study 1 Determination of the relationship between pregnancy per artificial insemination (P/AI) and concentrations of progesterone (P4) in the early luteal phase and establish repeatability estimates for these traits in Holstein- Friesian heifers.
Study 2 Establishment of the effect of P4 supplementation in the luteal phase on P/AI in dairy cows.
Study 3 Establishment of the critical timing of supplemental P4 to heifers with induced low P4 in the early luteal phase.
Study 4 Examination of the concurrent and carryover effects of a prolonged period of dietary restriction on P/AI in heifers.
Study 5 Examination of the effect has progesterone on the composition of uterine histotroph

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4. Main results:

Experiment 1

There was a positive linear and quadratic relationship between pregnancy per AI and concentrations of progesterone on days 4, 5, 6 and 7 post oestrus. Repeatability of concentration of P4 between successive oestrous cycles based on repeated measurements taken from the same heifers, following two different rounds of AI, ranged from 0.05 to 0.20 on days 4 to 7, respectively. These data indicated the importance of P4 in the early luteal phase for pregnancy survival, but also demonstrated that high concentrations of P4 on these days have a deleterious effect on embryo viability. Early luteal P4 were a reasonable predictor of concentrations on day 7 and could be used as a diagnostic tool to identify animals at risk of subsequent embryo loss.

Experiment 2

Following inseminations cows were randomly assigned to 1 of 2 treatments: (1) no subsequent treatment (n =221); (2) insertion of a Controlled Internal Drug Release Device (CIDR) on day 4 and removal on day 9 post oestrus (n =232). P/AI was determined by transrectal ultrasonography at approximately day 30 post oestrus. There was no herd by treatment effect on P/AI. There was a significant negative relationship ($P<0.03$) between P/AI and supplementation of P4 on days 4 to 9. Insertion of a CIDR increased concentrations of milk P4 in supplemented cows by 4.78ng/ml in comparison with control cows. There was no herd by treatment effect on P/AI. There was a significant negative effect ($P<0.03$) of supplementation with P4 on days 4 to 9 on P/AI The results of the study indicate a long period of supplementation of P4 initiated in the early luteal phase had a negative effect on P/AI in dairy cows. Furthermore, it was evident that cows with the greatest concentration of P4 on days 3 and 4 had the highest P/AI. An optimum concentration of 2.5 ng/ml was calculated from the logistic regression curve to achieve a probability of P/AI of 65%. When both treatments groups were entered into the analysis, there was no association ($P>0.05$) between P/AI and concentrations of P4 on day 4. A concentration of 2.5 ng/ml was calculated from the logistic regression curve to achieve a probability of P/AI of 65

Experiment 3

Initiation of supplemental P4 on day 4 had a negative effect on concentrations of P4 and CL cross-sectional area on day 7 in heifers which eventually had shortened luteal phases. Corpus luteum size area was significantly reduced in heifers administered PGP4-7 and PGP4-10 up to day 7, and day 10 (PGP4-10), in comparison to control and LP heifers. Initiation of supplemental P4 on day 7 (PGP7-10) had no effect on CL size area in comparison to LP heifers. Concentrations of P4 were lower in PGP4-7, PGP7-10 and PGP4-10all P4 supplemented groups compared on day 15 in comparison with all other treatment groups. Luteal blood flow (LBF) was lower in heifers administered PGP4-7 and PGP7-10 on day 4 in comparison with controls, but were similar to controls and LP on all other days measured. Heifers administered PGP4-10 had lower LBF compared with all other treatment groups on all days measured. There was no difference in embryo recovery on day 16 in any of the treatment groups. Embryo development size, was greater in PGP4-7 and PGP4-10 in comparison to LP and PGP7-10. It was also evident that P4-10 or the placebo CIDR did not have any detrimental effects on CL and embryo development.

Experiment 4

There was no concurrent or carryover of a 50-day period of dietary restriction before AI insemination on P/AI in beef heifers. Concentrations of NEFA in the ovulatory follicle were significantly greater in dietary RF restricted (RF) heifers compared with control (CF) heifers on day 50. There was no association between concentrations of metabolites in plasma and follicular on day 50 and 93. There was no difference in circulating concentrations of P4 on between both treatment groups on days 4, 5, 6 or 7 in the luteal phase following AI on days 50 and 93. The results indicate that there was no concurrent or carryover of prolonged dietary restriction before insemination on P/AI in beef heifers. The results of this study challenges the frequently proposed hypothesis that low P/AI in dairy cows are caused by NEB and its associated metabolic changes occurring in early lactation

Experiment 5

We identified 300 proteins on Day 7 and 510 proteins on Day 13 including 281 proteins shared between days. Five proteins were more abundant ($P < 0.05$) on Day 7 compared with Day 13 and included novel histotroph proteins cytokeratin 10 and stathmin. Twenty-nine proteins were more abundant ($P < 0.05$) including 13 unique on Day 13 compared with Day 7 and included previously identified legumain, metalloprotease inhibitor-2, and novel histotroph proteins chromogranin A and pyridoxal kinase. Functional analysis of the 34 differentially expressed proteins (including 14 novel to histotroph) revealed distinct biological roles putatively involved in early pregnancy, including remodelling of the uterine environment in preparation for implantation; nutrient metabolism; embryo growth, development and protection; maintenance of uterine health; and maternal immune modulation.

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5. Opportunity/Benefit:

- This research has generated much new knowledge of the relationships between progesterone and embryo survival rate in both heifers and lactating dairy cows.
- The application of progesterone supplementation as a therapy to improve embryo survival is not recommended.
- There was no carryover effects of a 50-day period of negative energy (NEB) yon P/AI not withstanding that blood concentrations of NEFA, BHB, insulin and glucose were all significantly altered by NEB. The results of this study challenges the widespread belief that the reduced P/AI observed in dairy cows, particularly high-yielding dairy cows, is the result of the carryover effect NEB in nearly lactation
- New information on temporal alterations in histotroph composition during critical stages for early embryo development and uterine function during the early establishment of pregnancy were generated.

6. Dissemination:

International conference

XXVI World Buiatrics Congress, Santiago, Chile, 14-Nov-2010,
European Association for Animal Production, Barcelona, 24-Aug-2009,
BCVA Conference Nottiingham 26-June 2012, Nottingham, UK, 26-Jun-2012,
British Society of Animal Science and the Agricultural Research Forum, Belfast, 12-Apr-2010,
British Society of Animal Science conference, University of Nottingham, UK, 04-Apr-2011,
European Society for Domestic Animal Reproduction (ESDAR), UCD, Dublin, Ireland, 01-Sep-2012
Society for the Study of Reproduction, 46th Annual Conference, Montreal, Quebec, Canada, Jul-2013,
American Society of Animal Science Conference, New Orleans, USA, 10-Jul-2011
International Society of Animal Genetics, Edinburgh, Scotland, 26-Jul-2010,

National Conferences

1. Irish Agricultural Research Forum, Tullamore, Co. Offaly, Ireland, 2011 & 2015,

Main publications:

1. Parr MH, Crowe MA, Lonergan P, Evans AC, Fair T, Diskin MG 2015. The concurrent and carry over effects of long term changes in energy intake before insemination on pregnancy per artificial insemination in heifers. Anim Reprod Sci. 157:87-94.
2. Parr MH, Crowe MA, Lonergan P, Evans AC, Rizos D, Diskin MG. 2014. Effect of exogenous progesterone supplementation in the early luteal phase post-insemination on pregnancy per artificial insemination in Holstein-Friesian cows. Animal Reproduction Science, Volume:150 (1-2): 7-14
3. Mullen MP, Bazer FW, Wu G, Parr MH, Evans AC, Crowe MA, Diskin MG (2013) Effects of systemic progesterone during the early luteal phase on the availabilities of amino acids and glucose in the bovine uterine lumen. Reproduction Fertility Development. 26. 282-292

7. Compiled by: Dr Michel G. Diskin

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