Project number: 6626
Funding source: Department of Agriculture, Food and the Marine
Date: January 2020
Project dates: Dec 2013 - Sept 2018

Genetic, Nutritional and Management approaches to improve fertility in lactating dairy cattle

Key external stakeholders:
Dairy farmers
Scientists
Dairy advisors
Dairy nutritionists
Stakeholders in the cattle breeding industry
Veterinarians
Policy makers

Practical implications for stakeholders:

- Long term genetic selection for improved genetic merit for fertility traits will result in favourable fertility phenotypes n dairy cows.
- In cows that are in a state of positive energy balance, strategies to increase circulating glucose may not be beneficial for early embryo development in vivo.
- Progesterone concentrations during the week preceding artificial insemination affected follicle size, estrous behavior and progesterone concentrations during the luteal phase after AI.

Main results:

- Improving genetic merit for fertility traits was associated with earlier postpartum resumption of cyclicity, superior uterine health, and better reproductive performance in lactating dairy cows.
- Provision of supplemental glucose via intravenous infusion retarded early embryo development in vivo.
- Progesterone concentrations during the week preceding artificial insemination affected follicle size, estrous behavior and progesterone concentrations during the luteal phase after AI.

Opportunity / Benefit:

The results highlight the need to continue genetic selection to improve genetic merit for fertility traits. In the long term, this strategy will reduce the incidence of reproductive disorders (anoestrus, endometritis) and associated requirements for treatments.

Collaborating Institutions:

Irish Cattle Breeding Federation
University College Dublin
University of Missouri
University of Wisconsin

Contact Stephen Butler Email: stephen.butler@teagasc.ie

Teagasc project team: Stephen Butler (PI)
Eber Rojas Canadas
Mohanathas Gobikrushanth
Mary Herlihy
Jonathon Kenneally

External collaborators: Francis Kearney (ICBF)
Pat Lonergan (UCD)
Matt Lucy (U. Missouri)
Paul Fricke (U. Wisconsin)

1. Project background:

Pasture-based dairy systems managed for seasonal calving require a 12-mo calving interval to maximize pasture nutrient utilization. Excellent reproductive performance in this production system is necessary to obtain a concentrated calving pattern during late winter and early spring, and calving pattern is a key driver of farm profitability. Improving reproductive performance at farm level requires a combination of strategies to immediately increase submission and conception rates, and longer term strategies to boost the inherent fertility of dairy cattle. This project used a multidisciplinary approach to tackle the problem of poor fertility in dairy cows. This included (i) development of a large database of lactating cow fertility phenotypes and identifying their associations with each other and with phenotypic fertility performance; (ii) genome wide association studies to identify genomic regions putatively associated with fertility phenotypes, with a view to improving the accuracy of genomic selection; (iii) examination of the role of glucose as a key nutrient to improve cow fertility; and (iv) examination of the effect of progesterone concentrations during dominant follicle development on subsequent embryo development after insemination.

2. Questions addressed by the project:

- What is the incidence of delayed resumption of cyclicity and endometritis in Irish dairy cows, and what are the associations between these disorders with each other, with other cow factors (parity, genetic merit for fertility traits, BCS, blood indicators of bioenergetics status) and with phenotypic fertility performance?
- Are there associations between novel fertility phenotypes and phenotypic fertility performance, and can we identify single nucleotide polymorphisms associated with these phenotypes?
- What is the effect of supplementing cows with exogenous glucose on early embryo development?
- Does circulating progesterone concentration during the period of pre-ovulatory follicle development affect the size of the ovulatory follicle, oestrous behavior and luteal phase progesterone concentrations?

3. The experimental studies:

Study 1:

- First- and second-parity spring-calving lactating dairy cows (n = 2,600) from 35 dairy farms in Ireland were enrolled. Farms were visited every 2 weeks; cows that were at wk 3 (range 14 to 27 DIM) and wk 7 (range 42 to 55 DIM) postpartum were examined. Body condition score was measured using a scale of 1 to 5 with 0.25 increments. Transrectal ultrasound examination was performed at wk 3 and 7 postpartum to determine presence or absence of CL and uterine health status (UHS). Blood samples were collected at each visit and the concentrations of glucose, β-hydroxybutyrate (BHB), and fatty acids (FA) were analyzed by using enzymatic colorimetry.
- Fisher's exact test was used to test associations between variables and was supplemented by logistic regression.

Study 2:

- Anogenital distance (AGD) was measured using digital calipers in 1,180 Holstein cows (mean ± standard deviation: 225 ± 79 d in milk) from 10 dairy herds located in Munster, Ireland. In addition, age (yr), weight (kg), height at hip (cm), and body condition score (BCS) at the time of AGD measurement were determined in a subset of 281 cows. Genotype information available from 908 cows was subsequently imputed to the Illumina Bovine High Density BeadChip (Illumina Inc., San Diego, CA) for genome-wide association analysis of phenotypic variation in AGD.
- Plasma AMH concentration was determined from a blood sample collected (mean ± standard deviation) 10 ± 2 d after first insemination in 2,628 first- and second-parity Irish dairy cows. Of the 2,628 cows with AMH information, 1,725 cows that were classified as ≥75% Holstein-Friesian

Contact Stephen Butler Email: stephen.butler@teagasc.ie

(hereafter referred to as Holstein) also had genotype information available.

Study 3:

- On d 7 after a synchronized estrus, cows were randomly assigned to receive an intravenous infusion of either 750 g/d of exogenous glucose (GLUC; 78 mL/h of 40% glucose wt/vol) or saline (CTRL; 78 mL/h of 0.9% saline solution). The infusion period lasted 7 d and cows were confined to metabolism stalls for the duration of the study. Coincident with the commencement of the infusion on d 7 after estrus, 15 in vitro-produced grade 1 blastocysts were transferred into the uterine horn ipsilateral to the corpus luteum. All animals were slaughtered on d 14 to recover conceptuses, uterine fluid, and endometrial tissue.

Study 4:

- Holstein-Friesian dairy cows managed in a seasonal-calving, pasture-based production system were randomly assigned to 2 treatments to manipulate progesterone before timed AI (TAI) during growth of the preovulatory follicle (i.e., high or low progesterone). The impact on follicle development, oestrous behavior and and subsequent luteal progesterone production was examined.

4. Main results:

Study 1

- We found associations between UHS (wk 3 and 7), BCS (wk 3 and 7), parity (wk 3 and 7) metabolic status (wk 3), and predicted transmitting ability for calving interval (PTA for CIV; wk 3) and CL status. Cows that had abnormal UHS, low BCS, primiparity, and poor metabolic status, and were in the quartile with the greatest PTA for CIV were less likely to have had CL present at wk 3 and 7 postpartum. We also found associations between CL status (wk 3 and 7), BCS (wk 3 and 7), parity (wk 3 and 7), and PTA for CIV (wk 3) and UHS. Cows that did not have a CL present had low BCS, primiparity, and that were in the quartile with greatest PTA for CIV, had a greater risk of abnormal UHS at wk 3 and 7 postpartum.
- More cows with a CL at wk 7 were served during the first 21 d of the breeding period compared with cows without a CL. Cows classified as having poor UHS at wk 3 and 7 had lower odds of pregnancy establishment during the breeding period compared with animals with good UHS. Animals with low BCS at wk 7 had lower odds of pregnancy establishment than cows with a target BCS. Cows classified as having good metabolic status at both wk 3 and wk 7 had greater odds of pregnancy establishment during the first 21 d of the breeding season than those classified as having poor metabolic status. Animals in the quartiles with the best predicted transmitting ability for survival and calving interval had better reproductive performance compared with animals in the other quartiles.
- Cows that had better genetic merit for fertility traits and good metabolic status, achieved target BCS, and had a favorable UHS and a CL present at wk 7 postpartum had superior reproductive performance.

Study 2

- Overall, AGD had a normal distribution and high variability (mean ± standard deviation; 119.2 ± 11.6 mm). The estimated heritability for AGD was 0.37. Six SNP of suggestive significance were identified on Bos taurus autosomes 6, 15, 20, and 26; however, none of these SNP were related to previously identified candidate genes for fertility. Cows were categorized into quartiles based on AGD and the association with reproductive outcomes examined (21-d submission rate, pregnancy to first AI, pregnancy rate within 21, 42 and 84-d after the farm mating start date). None of the reproductive variables differed significantly between AGD categories.
- Overall, plasma AMH had a positively skewed distribution with mean (± standard deviation), median, minimum, and maximum concentrations of 326 ± 231, 268, 15, and 2,863 pg/mL, respectively. Plasma AMH was greatest for Jersey, followed by Holstein × Jersey, Holstein × Norwegian Red, and Holstein cows (410, 332, 284, and 257 pg/mL, respectively). Cows with high and intermediate plasma AMH had 1.42- and 1.51-times-greater odds of becoming pregnant within 84 d after the MSD than those with low plasma AMH (90.3 and 90.8 vs. 86.8%, respectively); however, P/AI and pregnancy rate within 21 and 42 d after the MSD did not differ among AMH categories. Plasma AMH was moderately heritable (pedigree heritability of 0.40 ± 0.06 and genomic heritability of 0.45 ± 0.05), and 68 SNP across Bos taurus autosomes 7 and 11 were associated with phenotypic variation in plasma AMH.

Study 3

- Glucose infusion increased circulating glucose concentrations (4.70 ± 0.12 vs. 4.15 ± 0.12 mmol/L) but did not affect milk production or dry matter intake. Plasma progesterone, and insulin concentrations were unaffected by treatment. Treatment did not affect either uterine lumen fluid

Contact Stephen Butler Email: stephen.butler@teagasc.ie

glucose concentration or the mRNA abundance of specific glucose transporters in the endometrium. Mean conceptus length, width, and area on d 14 were reduced in the GLUC treatment compared with the CTRL treatment. A greater proportion of embryos in the CTRL group had elongated to all length cut-off measurements between 11 and 20 mm (measured in 1-mm increments) compared with the GLUC treatment. In conclusion, infusion of glucose into lactating dairy cows from d 7 to d 14 post-estrus during the critical period of conceptus elongation had an adverse impact on early embryonic development.

Study 4

- Unexpectedly, 37% of Low progesterone cows were detected in estrus ~24 h before scheduled TAI and were inseminated ~16 h before scheduled TAI.
- High progesterone cows had the smallest mean ovulatory follicle diameter, whereas Low progesterone cows with no estrus before TAI had intermediate mean follicle ovulatory diameter, and Low progesterone cows with estrus before TAI had the largest mean ovulatory follicle diameter. Low progesterone cows with estrus before TAI had larger corpora lutea 15 d after TAI than Low progesterone cows without estrus before TAI or High progesterone cows. In accordance with corpus luteum size on d 15, High progesterone cows and Low progesterone cows without estrus before TAI had lower progesterone from 4 to 46 d after TAI than Low progesterone cows with estrus before TAI.

5. Opportunity/Benefit:

- The results underpin the critical role of good animal husbandry and genetics to achieve high levels of fertility performance in seasonal calving systems.

6. Dissemination:

International conferences

Presented at American Dairy Science Association Annual meeting (2017, 2018, 2019)

National Conferences and seminars

Presented at the Teagasc Moorepark Open Day (2017).

Main publications:

- Canadas, E. R., Herlihy, M. M., Kenneally, J., Grant, J., Kearney, F., Lonergan, P., & Butler, S. T. (2020). Associations between postpartum fertility phenotypes and genetic traits in seasonal-calving, pasture-based lactating dairy cows. Journal of dairy science, 103(1), 1002-1015.
- Canadas, E. R., Herlihy, M. M., Kenneally, J., Grant, J., Kearney, F., Lonergan, P., & Butler, S. T. (2020). Associations between postpartum phenotypes, cow factors, genetic traits, and reproductive performance in seasonal-calving, pasture-based lactating dairy cows. Journal of dairy science, 103(1), 1016-1030.
- Leane, S., Herlihy, M.M., Curran, F., Kenneally, J., Forde, N., Simintiras, C.A., Sturmey, R.G., Lucy, M.C., Lonergan, P. & Butler, S.T., (2018). The effect of exogenous glucose infusion on early embryonic development in lactating dairy cows. Journal of dairy science, 101(12), 11285-11296.

Popular publications:

- Canadas, E.R., Herlihy, M.M., Kenneally, J.K., & Butler, S. T. (2018). Oestrous cyclicity and uterine health: is there a link? TResearch Volume 13(2): 20-21.
- Canadas, E.R., Herlihy, M.M., Kenneally, J.K., & Butler, S. T. (2017). Body condition score, resumption of cyclicity and uterine health status in dairy cows. Moorepark '17 Open Day Booklet, pages 94 to 95.

7. Compiled by: Dr. Stephen Butler

Contact Stephen Butler Email: stephen.butler@teagasc.ie