Reproductive success in ruminants:
a complex interaction among endocrine, metabolic,
and environmental factors

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Central role of the hypothalamus in regulating reproduction
GnRH

LH

T

2
Metabolic links?
Puberty

The process in which an animal gains the ability to reproduce successfully

Similar to embryogenesis, sequence of events leading to puberty is critical to reproductive performance in adult animal
Factors that influence the maturation of hypothalamic GnRH neurons

- Genetics
- Environmental Cues
- Plane of Nutrition
Threshold level of fat and body condition causes levels of metabolic factors to change

Sensitivity of hypothalamic neurons to these factors influences release of GnRH

Neurons in hypothalamus monitor blood levels of glucose and fatty acids

Fig 6-5
  Injection of ovx prepubertal ewes with substances that influence glucose and fatty acid availability
Injection of 2-deoxyglucose and methyypalmoxirrate into ovarectomized ewe lambs

Blocks glucose and fatty acid metabolism

Reduced frequency and amplitude of LH pulses

Injection of GnRH indicates it is the hypothalamus that monitors the levels of glucose and fatty acids in the blood
Leptin (Greek *leptos*, meaning thin)—hormone produced by adipocytes (fat cells) and level in blood is directly related to amount of fat in body.

Receptors for leptin are present in cells in anterior pituitary and hypothalamus.

Hypothesized to have a direct effect on GnRH secretion and onset of LH pulses from pituitary.

Summary of influence of metabolic signals upon GnRH neurons – Fig 6-7
Does puberty begin with a Kiss?

Kisspeptin (kiss1) – protein first identified by cancer researchers in Hershey, PA

Kiss1 – high expression in hypothalamus

Mutations of its receptor (GPCR54) in humans and mice results in hypogonadotrophic hypogonadism
Possible role of kisspeptin in onset of puberty and maintaining reproductive function
Kisspeptin neurons may act as central processors for relaying signals from the periphery to GnRH neurons.
Metabolic Signals Influence GnRH neuron function

Blood Glucose

Leptin

Adipocytes (Fat)

Blood Fatty Acids

Glucose sensing neurons

Kisspeptin neurons

Neuropeptide Y neurons

Kisspeptin neurons

GnRH neurons

Fatty Acid sensing neurons

GnRH
Times of stress?
Seasonal transitions?
Gonadotropin-inhibitory hormone (GnIH)

- Inhibitor of gonadotropin synthesis and release
- Acts through GPR147


- Clarke IJ, Bartolini D, Conductier G, Henry BA. Stress increases gonadotropin inhibitory hormone cell activity and input to gonadotropin releasing hormone cells in ewes. Endocrinology. 2016 Sep 9;en20161513.


Recall the relationship between ADG vs puberty in heifers.
Elevated body weight gain during the juvenile period alters neuropeptide Y-gonadotropin-releasing hormone circuitry in prepubertal heifers.

Alves BR¹, Cardoso RC², Prezotto LD², Thorson JE², Bedenbaugh M¹, Sharpton SM¹, Caraty A³, Keisler DH⁴, Tedeschi LO¹, Williams GL⁵, Amstalden M¹.

Abstract
Increased body weight (BW) gain during the juvenile period leads to early maturation of the reproductive neuroendocrine system. We investigated whether a nutritional regimen that advances the onset of puberty leads to alterations in the hypothalamic neuropeptide Y (NPY) circuitry that are permissive for enhanced gonadotropin-releasing hormone (GnRH) secretion. It was hypothesized that NPY mRNA and NPY projections to GnRH and kisspeptin neurons are reduced in heifers that gain BW at an accelerated rate, compared with a lower one, during the juvenile period. Heifers were weaned at approximately 4 mo of age and fed diets to promote relatively low (0.5 kg/day; low gain [LG]) or high (1.0 kg/day; high gain [HG]) rates of BW gain until 8.5 mo of age. Heifers that gained BW at a higher rate exhibited greater circulating concentrations of leptin and reduced overall NPY expression in the arcuate nucleus. The proportion of GnRH neurons in close apposition to NPY fibers and the magnitude of NPY projections to GnRH neurons located in the mediobasal hypothalamus were reduced in HG heifers. However, no differences in NPY projections to kisspeptin neurons in the arcuate nucleus were detected between HG and LG heifers. Results indicate that a reduction in NPY innervation of GnRH neurons, particularly at the level of the mediobasal hypothalamus, occurs in response to elevated BW gain during the juvenile period. This functional plasticity may facilitate early onset of puberty in heifers.
Genetic selection for RFI

Neuropeptide-Y (64\% lower)
relaxin-3 (59\% lower)
melanocortin 4 receptor (58\% lower)
GnRH (86\% lower)
GnIH: 198\% higher
POMC: 350\% higher

Low RFI
N=48

High RFI
N=48

Appetite :: Reproduction

Fat
Leptin: 245\% higher
Stress and Male Reproduction

• Cortisol (DEX) has negative impacts on germ cell development

• Cortisol (DEX) has negative impacts on testicular steroidogenesis
Experiment #1 Design

Focused on cellular changes from 0 to 120 hrs, revised protocol and extended the viable period of tissue and cells for two weeks *ex-vivo*

Effect of various factors on biological activity of SSCs; typically added every 2 days with media change

*The Berry College Animal Care and Use Committee approved all animal procedures*
Effect of explant culture on the SSC niche microenvironment. Germ and somatic cells remain in intimate association during the culture period. Scale bars, 40 μm.
Conclusions

• Here we demonstrate successful culture of bovine spermatogonial stem cells, albeit with time limitations.

• Our results and experimental approach provides a useful *ex vivo* model for investigating spermatogenesis.

• Morphological and TUNEL analyses suggest that our approach is effective in maintaining undifferentiated germ cell proliferation and viability for up to 120 hours.
The Berry College Animal Care and Use Committee approved all animal procedures.
Conclusions

• DEX and BOLD have direct and or indirect effects on cell signaling associated with SSC differentiation, self-renewal and survival events.
  – Stimulates proliferation of undifferentiated spermatogonia
  – Stimulates differentiation of spermatogonia and apoptosis of differentiating germ cells

• Future directions
**Phospho-NRP1**
- T916

**Phospho-KDR**
- Y1054

**Phospho-FLT1**
- Y1333

Control
- 24HR
- 48HR
- 96HR

Endogenous VEGFR signaling

- premigratory gonocytes
- B-spermatogonia
- spermatocytes

- proliferating gonocytes
- spermatogonia
- spermatocytes

- gonocytes
- undifferentiated spermatogonia

Scale bars, 50 μm
Proper development of bulls
Common Microflora Found in the Reproductive Tracts of Dairy Cows

Noëlle Mouton & Cassie Fiebiger
Background

• Infection of the uterus has a significant impact on the profitability of the dairy industry [1-3]:
  – lowered reproductive efficiency
  – decreased milk production
  – increased costs associated with treatment
  – culling of animals due to infertility

• Uterine infections in dairy cows are associated with predisposing factors [4,5]:
  – calving difficulty
  – retained placenta
  – compromised immune status
  – overgrowth of pathogenic microorganisms
Background

- In humans, lactobacilli are the most common vaginal microflora [6]
  - Similar to the bacteria in yogurt
- The microbial ecology of the vagina may confer disease resistance or susceptibility in host human females [7]:
  - outcompeting pathogens for real estate
  - stimulation of the host immune system
  - production of antibacterial compounds
    - acetic and lactic acids
    - hydrogen peroxide
    - antimicrobial peptides
Background

• In cows, it appears that either Streptococci or Staphylococci are the most common vaginal flora [6], in contrast to reports in humans

• At this point, little is known in cows:
  – conflicting reports
  – poor documentation
  – wide differences in environments

• Potential differences due to stage of production and inflammatory state of the uterus
  • metritis and endometritis
  • age and season of sampling
  • genetics of host immune response

Endometritis
http://www.nadis.org.uk
Objective and Hypothesis

• The aim of our study is to characterize the vaginal microbiota of healthy dairy cows in Northwest Georgia at Berry College

• We hypothesize that several unique patterns and profiles of vaginal microbial populations exist in dairy cows
  • We tested that hypothesis by using culture-dependent analysis
Materials and Methods

• Obtained samples of vaginal microflora using a sterile swab technique
  – Supervised by Dr. Martin Goldberg and Dr. Kyle Caires

• Sampled twenty, healthy dairy cows at various ages and stages of production
  – No mucopurulent discharge

• Cultured dependent analysis
  – We created custom quad plates using selective and differential media
Materials and Methods

- Quad plates (Agar used: Blood, Eosin Methylene Blue (EMB), MRS, TSI, Brilliant Green, Brain Heart Infusion (BHI), MacConkey, Nutrient agar)
- Triple Sugar Iron (TSI) slants
- Gram Staining
  - Cell wall and morphological characteristics
- Controls
  - *Serratia marcescens*
  - *Escherichia coli*
  - *Staphylococcus aureus*
Procedure

• Mix, autoclave, and pour selective agar:
  – Quadrant 1: Blood—differentiates alpha, beta, gamma hemolysis (+ control)
  – Quadrant 2: EMB—differentiates bacteria that ferment lactose from those that do not
  – Quadrant 3: MRS—Lactobacilli
  – Quadrant 4: TSI—differentiates based on ability to ferment sugars and produce hydrogen sulfide
  – Quadrant 5: Brilliant Green—Salmonella
  – Quadrant 6: BHI—fastidious pathogenic bacteria, yeasts, molds
  – Quadrant 7: MacConkey—Gram-negative bacteria
  – Full plate: Nutrient agar—general purpose growth (+ control)
Procedure

• Obtain and plate samples from 20 dairy cows
Procedure

• Plates incubated at 37°C and photographed at 24, 48, and 72 hours
• Observations recorded at 48 hour mark

Cow 1211 plate 1

Quad 1 shows beta-hemolysis

Quad 2 shows lactose fermenting bacteria (metallic green)

Quad 3 show Lactobacillus growth

Quad 4 shows non-fermenting bacteria
Procedure

- TSI slants and nutrient broth plates were also poured and inoculated
Procedure

• Created centrifuge tubes of each different colony present in each quadrant for each cow
  – 87 different colonies for morphological analysis
Procedure

• Gram staining and microscope observation
Procedure

• TSI slants after 24 hours

• 1303 exhibits bacteria that ferment sugars whereas 1133 does not (compare to control groups)
Results

- Data chart displaying which cows exhibited growth on each agar

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<th>3-MRS</th>
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Of these cows, only 1 (1211) showed positive for lactose fermenting bacteria on EMB—*E. coli*
Results

• Characteristic iridescent green metallic color of *E. coli* on 1211

• 1211 was also the cow who had calved most recently of the 20 cows tested. She had only been milking for 15 days at the time of testing.
Results

• 1301 is the only cow with high somatic cell count at the time of testing (mastitis) and the only cow with quadrant 7 displaying the same characteristics as *E. coli*.
• *E. coli* infection is one of the primary causes of mastitis.
Results

- 40% No Lactobacillus
- 55% Lactobacillus only
- 5% Lactobacillus + quad 6 growth

Image showing growth patterns in different sections.
Discussion

- *Strep vs Staph vs Lactobacillus*
  - More *Lactobacillus* than previously reported [1,3,5]
  - Probiotic effects? [6,7]
- Quadrant 6: *Strep-, Staph- & Enterococci; Fungus*
  - Need to take a closer look
- Do heifers get their vaginal flora from their mothers?
  - Inoculation from passage through birth canal?
  - Or is it environmentally acquired throughout development?
- Heritability of host immune response?
  - Sire & Dam
Future Directions

• Association analysis of vaginal microflora and their relationship on reproductive and production characteristics to determine their impact on the dairy industry
• Investigate the populations of vaginal microflora in beef cows
  – Environments?

• Suggestions?
• Questions?
References


