# Examining the effects of estradiol, trenbolone acetate, or polyamines on bovine satellite cell differentiation

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PSIX-28

## Introduction

- Muscle fiber number in meat animals is predominantly fixed at birth
  - Hypertrophy: The only mechanism of postnatal muscle growth
  - Satellite cells: Provide additional nuclei that facilitate hypertrophy
- Approximately 90% of beef animals in the United States receive at least one anabolic implant to increase the efficiency of muscle growth and economic return
- Bovine satellite cell (BSC) cultures treated with trenbolone acetate (TBA) or estradiol-17$\beta$ (E2) have increased proliferation rates and protein synthesis and decreased protein degradation when compared to control cultures
- Polyamines, natural amino acid derivatives, are beneficial for normal cell growth and differentiation:
  - Putrescine (Put), Spermidine (Spd), Spermine (Spe)
- Major substrates utilized in the polyamine biosynthesis pathway to produce Put, Spd, and Spe are Methionine (Met), Ornithine (Orn), and Arginine
- Recent literature indicates that TBA and/or E2 may help to increase growth of skeletal muscle by impacting the polyamine biosynthetic pathway, however it is currently unknown if this occurs in bovine muscle cells and how these processes impact differentiation.

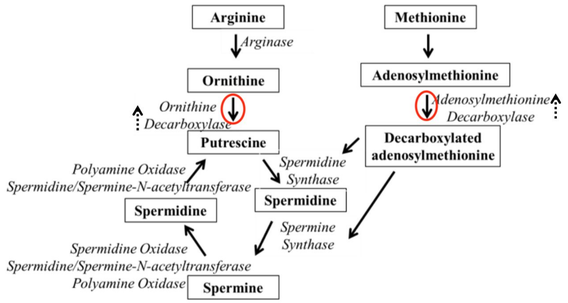


Figure 1: Overview of the polyamine biosynthesis and interconversion pathway. The two rate limiting steps in this pathway are highlighted in red, polyamines and their precursor molecules are shown in bold, and enzymes are shown in italic font.

## Hypotheses

- Biologically relevant levels of polyamines will temporarily enhance differentiation of cultured bovine satellite cells
- Treatment of cells with TBA or E2 will alter the polyamine biosynthetic pathway and impact differentiation

## Objectives

- Determine the effect of trenbolone acetate, estradiol, polyamines and their precursors on differentiation of BSC
- Understand whether trenbolone acetate has an impact on the polyamine biosynthetic pathway to enhance BSC differentiation

## Methods

- Bovine satellite cells were isolated from 3 different steers (Utah State University IACUC #10216)
  - Approximately 12 months of age, weighing approximately 315 kg at harvest
- Cell culture:
  - BSC were plated in wells that were pre-coated with reduced growth factor basement membrane Matrigel and grown to 80% confluency
  - Upon reaching 80% confluency, BSC were treated with TBA, E2, polyamines or their precursors in DMEM containing 3% Horse Serum
- Treatment of BSC cultures:
  - 10 nM TBA, 10 nm E2, 10 nM E2 and 10 nm TBA (Com), 10 mM Met, 8 mM Orn, 2 mM Put, 1.5 mM Spd, or 0.5 mM Spe
- Ribonucleic acid isolation, quantification, and cDNA synthesis:
  - Total RNA was extracted from BSC cultures using the Absolutely RNA Microprep Kit (Agilent Technologies, Cedar Creek, TX) as per the manufacturer's protocol
  - Isolated RNA was quantified using a Take3 plate and synergy H1 multi-mode microplate reader (BioTek)
  - cDNA synthesis was conducted using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA) following the manufacturer's protocol
- An ABI 7500 real-time PCR system (Applied Biosystems) was used to detect relative abundance of ribosomal 18s (18s), ornithine decarboxylase (Odc), S-adenosylmethionine decarboxylase (Amd1), paired box transcription factor 7 (Pax7), Myogenic Factor 5 (Myf5), and Myoblast determination factor 1 (MyoD)
- Statistical analysis:
  - Performed using the MIXED procedure of SAS (version 9.4;SAS Inst. Inc, Cary, NC). All data were analyzed with treatment as a fixed effect and animal and experiment as random effects. All genes were analyzed as repeated measures. Data are presented as the least square mean +/- SEM

## Results

Myf5 mRNA Abundance

Treatments

| | 3% | Com | E2 | Met | Orn | Put | Spd | Spe | TBA | SEM | P-Value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 h | 1 | 8.77 | 5.19 | 2.65 | 4.08 | 13.02 | 13.65 | 3.49 | 0.29 | 6.4 | 0.45 |
| 4 h | 1 | 3.40$^{a}$ | 4.08$^{ab}$ | 3.99$^{ab}$ | 1.6$^{ab}$ | 1.2$^{a}$ | 3.21$^{ab}$ | 4.78$^{ab}$ | 7.20$^{b}$ | 1.59 | 0.02 |
| 8 h | 1 | 1.87 | 6.9 | 0.33 | 5.45 | 0.73 | 0.18 | 1.30 | 4.50 | 2.8 | 0.46 |
| 12 h | 1 | 0.87 | 1.11 | 0.54 | 0.46 | 0.50 | 0.91 | 0.41 | 0.38 | 0.23 | 0.26 |
| 24 h | 1 | 1.33 | 2.55 | 1.50 | 2.96 | 2.45 | 1.48 | 2.37 | 0.59 | 1.22 | 0.38 |
| 48 h | 1 | 0.88 | 1.26 | 1.24 | 1.38 | 0.75 | 1.39 | 0.90 | 1.23 | 1.39 | 0.93 |

[a] Different letters are significantly different (P < 0.05) from each other within rows.

## Results cont.

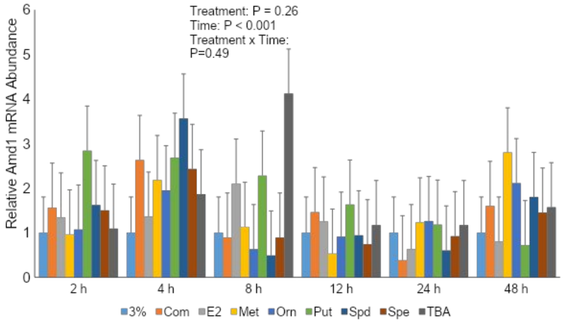


Figure 2: Relative mRNA abundance of Amd1. One of the rate limiting steps in the polyamine biosynthesis pathway. There was a tendency for TBA to increase abundance 8 h post differentiation when compared to a 3% horse serum control (P =1.00), and Spd (P = 0.08).

- Pax7 had a treatment tendency at 48 h post differentiation with Spd increasing abundance compared to 3% horse serum control ( P < 0.10)
- Treatment had no effect on MyoD, MyoG, ODC, or Smox abundance

## Conclusions

- Treatment of BSC with TBA, E2, TBA and E2, polyamines, or polyamine precursors does not appear to enhance abundance of genes associated with bovine satellite cell differentiation at certain time points
- Treatment of BSC with TBA had a tendency (P = 0.10) to increase abundance of Amd1 8 h post treatment compared to control cultures
- Trenbolone acetate increased (P > 0.05) abundance of Myf5 4 h post treatment

## Future Studies

- Analyze the abundance of genes involved in the polyamine biosynthesis pathway and bovine satellite cell differentiation at additional time points
- Determine the fusion index of BSC treated with TBA, E2, TBA and E2, polyamines or polyamine precursors
- Evaluate the effects of polyamines and polyamine precursors on protein synthesis
- Live animal study of cattle supplemented with polyamines

## Acknowledgements

- This work was supported by Hatch Capacity Grant Project no. UTA-01249 from the USDA National Institute of Food and Agriculture

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