Oocyte mRNA and Follicle Androgen Levels Associated with Fertility

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Summary

The environment that the oocyte develops in (follicle) and the mRNA that is produced (mRNA abundance) during development were examined. Androgen levels within the follicle were higher in heifers (≤ 2 years) that never established a pregnancy compared to cows that stayed in the herd at least 3 years and had at least one successful pregnancy. These high androgen levels were associated with increased abundance of several candidate mRNAs in the cumulus-oocyte complex (COC), which includes the oocyte and somatic cells immediately surrounding the oocyte, isolated from the dominant follicle. The data suggest that androgen levels represent a marker for oocyte quality which could be used to select for females to retain in the herd.

Introduction

One factor contributing to early embryonic loss in beef heifers and cows is oocyte quality which is established during growth and maturation of the oocyte. Specifically, DNA content is reduced and mRNAs, proteins, and energy sources are synthesized and stored for use by the developing embryo. These factors determine if the oocyte will be competent for fertilization and the establishment of a successful pregnancy. Somatic cells of the follicle produce androgens and estrogen which regulate growth, maturation, and ovulation of the oocyte. However, the specific role of these hormones on each component of oocyte quality has not been defined. The goal of the current study was to determine the impact of androgen levels on oocyte mRNA abundance.

Procedure

All procedures were approved by the University of Nebraska–Lincoln Institutional Animal Care and Use Committee. Beef cows ranging in age from 1.5 to 11 years were synchronized and ovariectomies performed as previously described (*2011 Nebraska Beef Cattle Report,* pp. 13-15) to obtain follicular fluid and the COC from dominant follicles. The criteria for classification as a dominant follicle were (1) the largest follicle on the ovary and (2) an estrogen-to-progesterone ratio > 1.0. Follicular fluid was assayed for androstenedione levels. Total RNA was collected from individual COCs (Ambion) and subjected to linear amplification (Nugen). Quantitative, real-time polymerase chain reaction (qPCR) was conducted to determine the mRNA abundance of maternal effect genes.

Results

Heifers that do not establish a successful pregnancy (low reproductive longevity, LRL) have fewer antral follicles and reduced ovarian weight than cows that stay in the herd 3-6 years (moderate reproductive longevity, MRL), or greater than 6 years (high reproductive longevity, HRL) (*2010 Nebraska Beef Cattle Report,* pp. 16-18; *2011 Nebraska Beef Cattle Report,* pp. 13-15). To determine differences in ovarian function between LRL, MRL, and HRL heifers and cows, follicular fluid and COCs were collected from the dominant ovarian follicle. Follicular fluid collected from the dominant follicle of LRL heifers had significantly higher levels of androstenedione compared to MRL or HRL cows (Figure 1). Androstenedione is an important precursor of the female sex steroid estrogen. In women, high circulating or follicular levels of androgens are associated with reduced fertility.

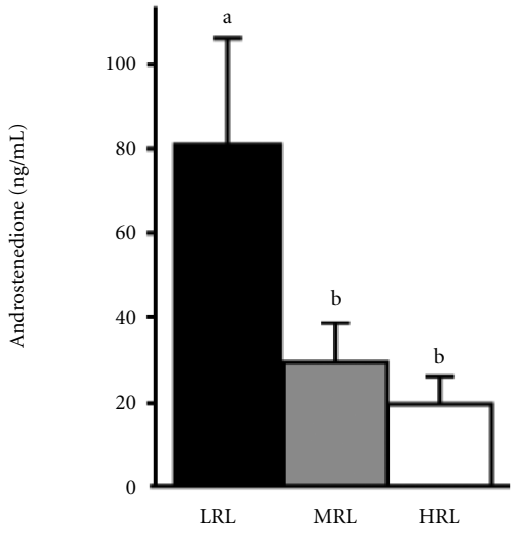

Figure 1. Androstenedione levels were measured in follicular fluid isolated from the dominant follicle on the ovary. Levels were significantly higher in heifers (≤ 2 years; LRL) compared to cows with moderate (>2, <6; MRL) or high (≥ 6 years; HRL) reproductive longevity. Statistical significance ($P < 0.05$) was determined using one-way ANOVA and is indicated by different letters.

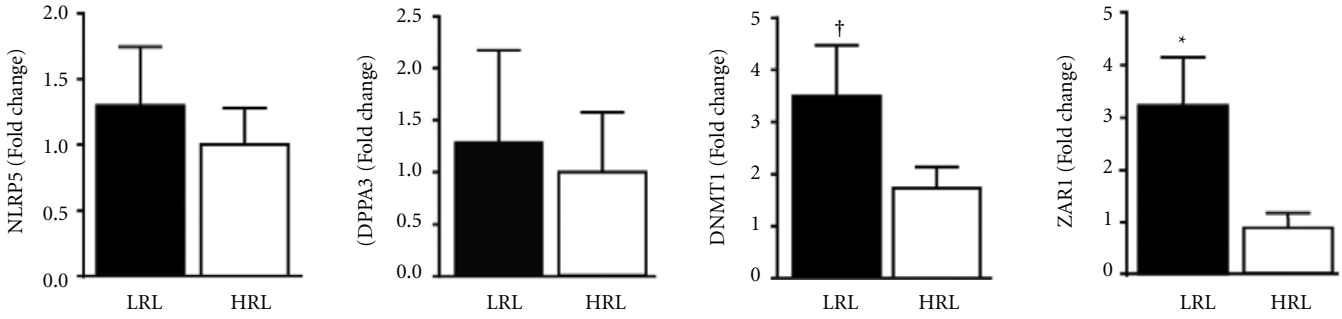

Figure 2. Total RNA from individual COCs was isolated and subjected to linear amplification. The resulting cDNA was used to carry out qPCR using primers directed against maternal effect genes (NLRP5, DPPA3, DNMT1, and ZAR1). The abundance of each specific mRNA was normalized for the housekeeping gene RPL15 and compared to the mean normalized abundance in HRL cows (fold change). Student's t-test was used to identify significant ($P < 0.05$, *) or a trend ($P < 0.1$, †) for differences in mRNA abundance.

Thus, abnormal regulation of androgen production or its conversion to estrogen by the somatic cells of the follicle may contribute to reduced fertility of LRL heifers.

In rodent and human models, high or low levels of specific mRNAs stored in the oocyte are detrimental to the ability of the oocyte to be fertilized or undergo early embryonic development. The abundance of DNMT1 and ZAR1 mRNAs, which are maternal effect genes, was increased in the COCs of LRL compared to HRL cows (Figure 2). Maternal effect genes are stored during oocyte growth and are used during early embryonic development. Thus, these data indicate that mRNA storage in the oocyte may be altered in LRL heifers, which likely results in reduced oocyte quality.

Implications

The data suggest that high follicular androgen levels alter oocyte mRNA abundance and therefore may contribute to poor oocyte quality associated with pregnancy loss. Understanding how androgen levels are regulated and the impact of altered oocyte mRNA accumulation on embryonic development may be used to reverse the negative effects of a poor follicular environment on pregnancy rates in heifers and cows or to select for heifers to retain in the herd.

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