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Effects of a systemic high urea concentration on the endometrial and embryonic transcriptome of mares

Efeitos de concentrações sistêmicas elevadas de ureia no transcriptoma do endométrio e embriões de éguas

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Abstract

It has been shown in ruminants that increased dietary protein leading to elevated blood urea nitrogen concentrations (BUN) can be a factor in decreased survival of early embryos. This work is a review of the effects of elevated BUN on endometrium and embryos from mares. An experimental model was used to elevate BUN with intravenous urea infusion, acute treatment, or oral urea, chronic treatment. After the acute urea treatment there was a decrease in uterine pH and changes in genes related to cell pH and ion homeostasis. After the chronic urea treatment there was no difference in uterine pH but genes related to necrosis and cellular movement had a different expression. The effect of high BUN was also evaluated on equine embryo transcriptome, with a positive correlation between plasma BUN and blastocoele fluid urea nitrogen concentration. Additionally, the expression of genes related to survival of organism and adhesion were different. Lastly, using mares from private farms, lower pregnancy rate was seen when embryos were collected from mares with higher BUN concentrations. In conclusion, these novel results show that high BUN results in endometrial and embryonic alterations, suggesting that it might lead to decreased fertility.

keywords: Equine, High protein diet, uterus, embryo, RNA sequencing, fertility.

Introduction

Early embryonic loss is an important cause of infertility, and it remains as an important cause of economic loss to the equine industry. In ruminants, it has been shown that elevated crude protein intake results in high blood urea nitrogen (BUN) systemic concentrations leading to decreased oocyte and embryo quality and lower pregnancy rates (Butler, 2000; Elrod and Butler, 1993; McEvoy et al., 1997). Recently, we investigated the effects of high BUN on reproductive parameters in mares and its transcriptomic signature in the oocyte, embryo, and endometrium. The current review aims to summarize our findings and the transcriptomic changes in the aforementioned cells and tissues in mares subjected to high BUN.

Donor embryo mares with high BUN and relationship with embryo survival in recipient mares

Our objective was to determine if there was a relationship between the BUN concentrations in mares that donate embryos and the pregnancy rates in recipient mares (Boakari et al., 2021). Embryo donor mares had embryos recovered through uterine lavages at D7 or D8. Immediately before the embryo collections, blood samples were taken for BUN evaluation. Embryos were transferred to recipient mares and pregnancy diagnosis was done using ultrasound exams at D14.

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The BUN concentrations were not different from mares that yielded embryos during lavages and those that did not. Donor mares that had embryos that resulted in pregnancies had lower BUN than donor mares that did not result in pregnancies. These results suggest that higher BUN might have negative effects on early embryonic development. However, a specific concentration of BUN that is deleterious to early pregnancy in mares was not determined and requires further studies.

Effects of high protein models on the endometrial transcriptome of mares

Experimental models of intravenous urea infusion or oral urea administration were developed to elevate blood urea nitrogen (BUN) in mares and evaluate this effect on the endometrial transcriptome in two different experiments. Mares received an intravenous urea treatment at day 7 of diestrus (D7) over 6 hours or an oral urea treatment at the day of ovulation (D0) and continuing for 7 days (Boakari et al., 2019; Boakari et al., 2020).

Both urea treatments caused an increase in the BUN concentrations after the urea treatments. However, only the intravenous urea treatment caused a decrease in uterine pH while the oral urea treatment did not result in a difference between the control and urea groups. We hypothesize that with the oral urea treatment, a more chronic urea treatment, there was sufficient time for the uterine environment to regain its normal homeostasis and return to its physiological uterine pH.

Additionally, there was a long list of differentially expressed genes (DEGs) in the different treatments, thus some examples are shown here. Functional genomics of the DEGs between the urea and control groups evaluated with RNA sequencing from the intravenous urea treatment showed genes related to cell pH (epidermal growth factor and pyruvate dehydrogenase kinase 4), ion homeostasis (carbonic anhydrase 2, aquaporin 5), and solute carriers (potassium voltage-gated channel subfamily A member 3). After the oral urea treatment, the DEGs were related to necrosis (serum and glucocorticoid-regulated kinase 1 and early growth response 3) and concentration of lipids (phospholipase A1 member A). Genes related to abnormal growth and cell migration were found to be DEGs (kinesin family member 5C and SERPINA 14) in the endometrial transcriptome after both treatments. These differences in function of DEGs between the acute and chronic urea treatments likely are due to the additional time that the uterus had to regain homeostasis in the oral urea treatment.

In conclusion, changes in gene expression after urea treatments that increased the BUN resulted in alterations in endometrial function that could have detrimental effects on fertility of mares and adversely affect the embryonic transcriptome.

Effects of a high protein model on the equine embryo transcriptome

The oral urea treatment, which is a more physiological method to elevate BUN, was used to determine if this elevated BUN would affect embryo parameters and transcriptome during early pregnancy in mares (Boakari et al., 2020). Mares that were artificially inseminated received an oral urea treatment. The embryonic vesicle height and width were measured daily from D11 to D14 (D0 was considered as the day of ovulation) to calculate the ellipse area. Embryo collections were performed on D14, and RNA sequencing was performed on the entire conceptus.

There was no difference in the size of the embryo between the urea and control groups. There was a tendency for blastocoele fluid urea nitrogen concentrations to be higher in embryos from the urea group with a strong positive correlation between plasma BUN and blastocoele fluid urea nitrogen concentrations. Furthermore, the DEGs were related to nervous system development (G-protein-coupled receptor 155, neurofascin, and serpin family G member 1, endothelin development (apelin receptor), detoxification (glutathione S-transferase 1), and adhesion (fibrinogen gamma chain). Overall, these results show that higher BUN in mares during early pregnancy results in transcriptomic changes in their embryos. However, further studies are needed to evaluate if these effects would affect embryonic survival.

Conclusions

The above-mentioned studies were the first that evaluated the effects and relationship of high BUN on reproductive parameters in mares. In conclusion, the higher BUN resulted in molecular changes to the endometrium and embryos and lower pregnancy rates with embryos recovered from mares with

higher BUN. Overall, these results suggest that higher BUN concentrations will have deleterious effects on early pregnancy in mares (Fig. 1).

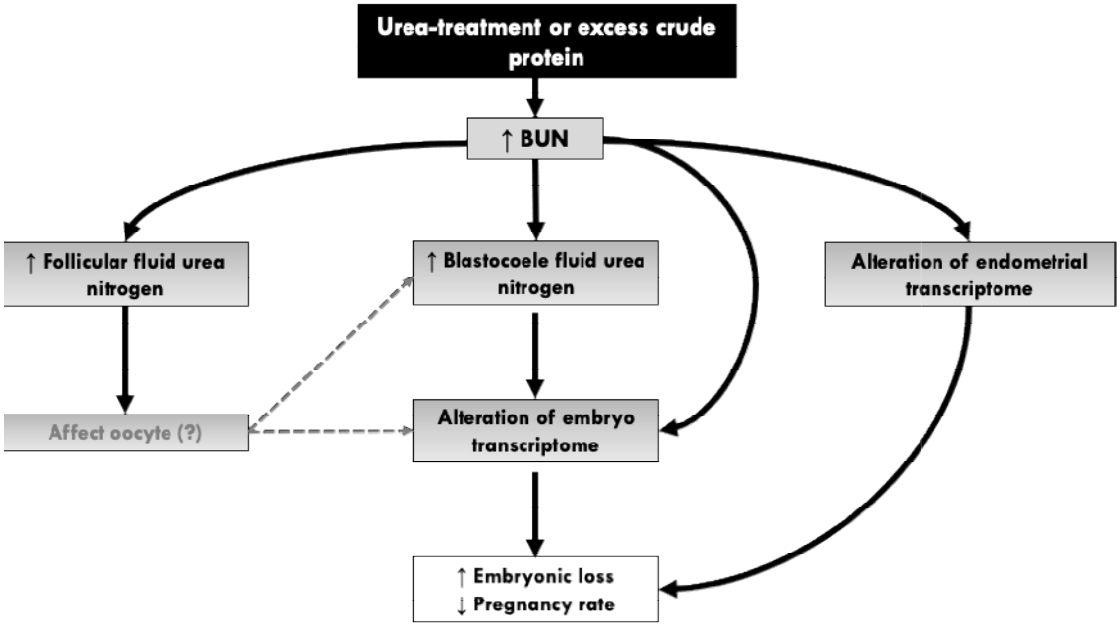

Figure 1: Diagram representing the effects of high blood urea nitrogen (BUN) in the reproductive system and embryos of mares.

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