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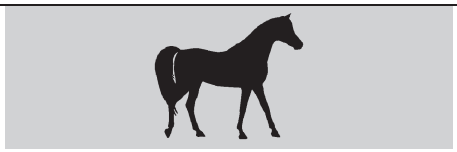

Blood plasma concentrations of testosterone, luteinizing hormone, and estrone sulfate in stallions following hemicastration

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ABSTRACT

Hemicastration is a veterinary surgical procedure in stallions and may be needed for the removal of a diseased or damaged testicle. The effects of hemicastration on the neuroendocrine system and the hormonal response of the remaining testicle is unclear. Therefore, blood plasma concentrations of testosterone, luteinizing hormone, and estrone sulfate were assessed following hemicastration. Miniature stallions (n = 8) were used, and blood was drawn 7 d before hemicastration and 12 h, 48 h, 14 d, 30 d, and 90 d after hemicastration. Blood samples from all stallions were drawn every 15 min (T0, T15, T30, T45, T60 min) for 60 min each sampling period. Plasma luteinizing-hormone concentrations at 12 h, 48 h, 14 d, and 90 d were greater (P < 0.05) than presurgical concentrations. Compared with prehemicastration values, plasma testosterone concentrations were less (P < 0.05) 12 h, 14 d, and 30 d after but were greater (P < 0.05) than prehemicastration values 90 d after surgery. Compared with prehemicastration values, plasma concentrations of estrone sulfate were reduced (P < 0.05) at all time periods but tended to increase up to 90 d (P < 0.05). After 60 d, stallions were housed together rather than individually, creating a harem group. Interestingly, testosterone and luteinizing-hormone concentrations increased dramatically compared with previous time periods following the housing modification (P < 0.05). These results provide insight to better understand the hormonal profiles and compensatory response of the remaining testicle following hemicastration.

Key words: estrone sulfate, hemicastration, luteinizing hormone, stallion, testosterone

INTRODUCTION

Subfertility in stallions can be associated with testicular degeneration that may be linked to genetics, aging, effects of long-term medications, or environmental effects, such as scrotal trauma, heat, or fever (Varner et al., 2008). To minimize any potential negative effects of a damaged testicle and delay the onset of infertility due to a diseased testicle, hemicastration may be an elected procedure (Papa et al., 1990; Zhang et al., 1990). Compensatory hypertrophy occurs in the remaining testis following hemicastration. In a companion study, testis size increased following hemicastration (McCormick et al., 2012). Compensatory hypertrophy provides an attractive model to study changes in hormone levels associated with an increase in testis size and sperm number. Potentially, understanding this hormonal milieu could lead to treatments for stallions that are undergoing testicular degeneration. Recent research investigated the response of the remaining testicle by evaluating seminal quality after hemicastration (McCormick et al., 2012). In this study, mean testis volume and weight of the remaining testicle were found to be significantly greater than presurgical values (McCormick et al., 2012). The effect of hemicastration on

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the hypothalamic-pituitary-testicular axis is unclear. Though stallion fertility and return to successful breeding following hemicastration are of upmost concern to veterinarians, stallion owners, and breeding managers, research investigating the hormonal response of the remaining testicle will provide insight for researchers and provide sound, comprehensive understanding of stallion reproductive efficiency. The objective of this study was to measure blood plasma concentrations of testosterone, luteinizing hormone (**LH**), and estrone sulfate to assess the response of the remaining testicle following hemicastration.

MATERIALS AND METHODS

The Texas A&M University Institutional Agricultural Animal Care and Use Committee provided project approval using guidelines set forth by the Federation of Animal Science Societies (2010).

Stallions, Housing, and Diets

Eight Miniature Horse stallions (4 to 15 yr, 72.1 to 110.7 kg) were used in a 14-wk study conducted from November 2011 through February 2012. Stallions were housed, before hemicastration and up until 60 d after hemicastration, individually at the Texas A&M University Horse Center in stalls (1.8 × 1.8 m), allowed visual access to other horses, and allowed free exercise every other day in individual dry-paddock runs (7.3 × 1.8 m). Upon completion of semen collection 60 d after hemicastration, stallions were housed in a harem group from 60 to 90 d after hemicastration. While housed in this harem group, stallions had visual access to mares located in an adjacent pasture through a mutual fence line. Each stallion was fed 0.5% of BW in concentrate per day and 1.5% of BW in coastal bermudagrass hay per day, adjusted accordingly to maintain a BCS of 5 (Henneke et al., 1983) throughout the study. Ad libitum access to water was provided. The stallions were hemicastrated under hospital conditions by a surgeon using a closed orchiectomy technique (McCormick et al., 2012; Schumacher, 2012).

Blood Sampling

Blood samples were collected for evaluation of plasma concentrations of testosterone, LH, and estrone sulfate before and after hemicastration. Blood (10 mL) was collected, via jugular catheterization with a 7.62-cm 20-gauge i.v. catheter, 7 d before and 12 h, 48 h, 14 d, 30 d, and 90 d after hemicastration. At each time point, 5 blood samples were collected at 15-min intervals (T0, T15, T30, T45, T60). Upon completion of each blood draw, catheters were flushed with heparinized saline, and the blood collection tubes were placed on ice until they were centrifuged.

Following each sampling period, blood samples were refrigerated until time of processing (within 2 h of collection). Blood samples were centrifuged for 20 min at 1,225 × g in a refrigerated centrifuge at 15°C. Blood plasma was then aspirated with disposable pipettes, placed in micro centrifuge tubes (1.8 mL), and stored at −20°C until assayed for testosterone, LH, and estrone sulfate concentrations.

RIA Procedures

Plasma testosterone and estrone sulfate were analyzed with an RIA Kit (Diagnostic Systems Laboratories Inc., Webster, TX) previously validated for horse samples (Johnson et al., 2003; Villani et al., 2006). The measurement range of the RIA quantifying testosterone concentrations was 0.18 to 100.0 pg/mL, and kit sensitivity was 0.18 pg/mL. The intraassay coefficient of variation for testosterone was 2%. The measurement range of the RIA quantifying estrone sulfate concentrations was 2.0 to 320.0 ng/mL with a sensitivity of 0.01 ng/mL. The intraassay and interassay coefficients of variation for estrone sulfate were 6 and 3% (n = 3 assays), respectively. Plasma LH concentrations were measured by RIA as described and validated for the horse (Williams et al., 1982). The intraassay coefficient of variation for LH was 7%, respectively. All samples were counted using Perkin-Elmer Packard Cobra II Auto-Gamma counter (Packard Systems, Meridan, CT).

Statistical Analysis

Mixed-model repeated-measures ANOVA was used to analyze data (SAS v 9.2; SAS Institute Inc., Cary, NC). Hormone (testosterone, LH, estrone sulfate) was considered a fixed effect, stallion was considered a random effect, and time was the repeated measure. Differences in hormone levels between time periods were determined using the method of least squares means. Data were considered to be significantly different at $P \leq 0.05$.

RESULTS AND DISCUSSION

Mean plasma concentrations for each stallion at each sampling time were used to calculate results (Table 1). Prehemicastration testosterone values (1.69 ng/mL ± 0.18) were greater ($P < 0.05$) than 12-h (0.67 ng/mL ± 0.05), 14-d (1.27 ng/mL ± 0.09), and 30-d (1.00 ng/mL ± 0.12) but similar to 48-h concentrations (Figure 1). Plasma testosterone concentrations at 90 d (3.92 ng/mL ± 0.41) after hemicastration were greater ($P < 0.0001$) than all previous time periods.

Prehemicastration plasma LH concentration (0.30 ng/mL ± 0.03) was less ($P < 0.05$) than 12 h (0.61 ng/mL ± 0.07), 48 h (0.65 ng/mL ± 0.07), and 14 d (0.51 ng/mL ± 0.06) after hemicastration but similar to the 30-d concentration (Figure 2). The 90-d LH concentration (1.61 ng/mL ± 0.21) was greater ($P < 0.0001$) than all other time periods.

In general, estrone sulfate concentrations decreased from before hemicastration to 48 h and then increased to 90 d. Prehemicastration blood plasma estrone sulfate concentrations (53.87 ng/mL ± 3.58) was greater ($P < 0.05$) than all other time points,