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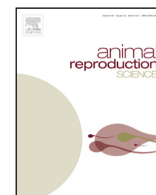

Dexamethasone acutely regulates endocrine parameters in stallions and subsequently affects gene expression in testicular germ cells

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ABSTRACT

Testicular steroidogenesis and spermatogenesis are negatively impacted by stress-related hormones such as glucocorticoids. The effects of two injections of a therapeutic dose of dexamethasone (a synthetic glucocorticoid, 0.1 mg/kg; i.v.) given 24 h apart to each of three stallions were investigated and compared to three saline-injected control stallions. Dexamethasone decreased circulating concentrations of cortisol by 50% at 24 h after the initial injection. Serum testosterone decreased by a maximum of 94% from 4 to 20 h after the initial injection of dexamethasone. Semen parameters of the dexamethasone-treated stallions were unchanged in the subsequent two weeks. Two weeks after treatment, stallions were castrated. Functional genomic analyses of the testes revealed that, of eight gene products analyzed, dexamethasone depressed concentrations of heat shock protein DNAJC4 and sperm-specific calcium channel CATSPER1 mRNAs by more than 60%. Both genes are expressed in germ cells during spermiogenesis and have been related to male fertility in other species, including humans. This is the first report of decreased DNAJC4 and CATSPER1 mRNA concentrations in testes weeks after dexamethasone treatment. Concentrations of these mRNAs in sperm may be useful as novel markers of fertility in stallions.

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1. Introduction

Unlike males of other large animal species, stallions are generally chosen for breeding based on attributes that do not include fertility. In two surveys, 36–43% of prospective breeding stallions failed a fertility evaluation (Blanchard and Varner, 1997; Pickett et al., 1988). Some stallions pass a fertility evaluation but still are subfertile (Varner, 2008), similar to the 10–15% of men with idiopathic subfertility (Attia and Al-Inany, 2009). Because of this, stallion fertility is a major factor that limits horse production and the rate of genetic improvement (Varner et al., 1991). Therefore, there is a need to understand the molecular basis of male fertility more completely and develop better diagnostic tests to detect underlying causes and thus potential therapies.

The spermatogenic efficiency of the testes is affected by many factors, including genetics, maturity, and health. Treatment with synthetic glucocorticoid drugs, such

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as dexamethasone, negatively impact sperm production and fertility in men and animals [reviewed in (Cooke et al., 2004) and (Hardy et al., 2005)]. Spermatogenesis requires an intratesticular environment with a very high concentration of testosterone, which is provided by androgen-binding protein capture of testosterone produced by the neighboring steroidogenic Leydig cells (Roser, 2001; Steinberger, 1971). Treatment with glucocorticoids acutely reduces circulating concentrations of testosterone by 60% or more in rams, boars, bulls, baboons and men (Barth and Bowman, 1994; Cumming et al., 1983; Gaon and Liptrap, 1989; Juniewicz et al., 1987; Sapolsky, 1985). In the weeks following dexamethasone treatment in bulls and rams, semen quality is impaired, with increased numbers of sperm with abnormal morphology and biochemical parameters (Barth and Bowman, 1994; Tsantarliotou et al., 2002).

Horses with inflammatory diseases are commonly treated with dexamethasone. However, little information is available on the effects of glucocorticoids on stallion fertility. It is known that administration of dexamethasone to normal stallions reduces serum concentrations of testosterone (Danek, 2004; Juhasz et al., 1999; Pozor et al., 2010). Additionally, in the weeks following dexamethasone treatment, semen collected from treated stallions contained a greater proportion of spermatozoa having abnormal morphology (Danek, 2004; Pozor et al., 2010).

Glucocorticoids, and the related suppression of testosterone secretion, may affect the expression of genes important to sperm function and male fertility. To the best of our knowledge, there is no information available on the effect of glucocorticoids on the expression of fertility-related genes in the stallion. The present study was conducted to examine the effects of acute dexamethasone treatment (two doses, 24 h apart) on serum testosterone concentration, sperm morphology and motility parameters, and expression of testicular genes that could relate to the expected decline in sperm function in the near future. The mRNAs selected for analyses have been demonstrated to occur at different levels in sperm from fertile and subfertile men (Steger et al., 2008; Garrido et al., 2009; Platts et al., 2007; Li et al., 2007). The mRNA concentrations measured included those encoding protamines PRM1 and PRM2, heat shock proteins A8 (HSPA8) and DNAJC4, gamma-glutamyltranspeptidase 1 (GGT1), ornithine decarboxylase antizyme 3 (OAZ3) and the sperm-specific cation channel, CATSPER1. Tristetraprolin (ZFP36) was also examined as a glucocorticoid-induced gene in other model systems (Smoak and Cidlowski, 2006).

2. Materials and methods

2.1. Stallions and in vivo treatments

The study was performed with six adult Quarter Horse stallions that were 3–12 years of age (average age of 6 years) and weighed 452 ± 15 kg. All experimental procedures were performed according to the United States Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training and were approved by the Laboratory Animal Care Committee at Texas A&M University. The study design is depicted in Fig. 1. Stallions were subjected to blood and semen collection once a day, at 8 A.M., for 10 days to determine semen parameters at baseline (daily sperm output). Semen parameters measured included volume, sperm concentration, morphology and motility (Blanchard et al., 2003; Kenney et al., 1983). Sperm motility was assessed by computer-assisted sperm motion analysis (HTM IVOS; version 10.8; Hamilton Thorne Research, Beverly, MA, USA) as previously described (Waite et al., 2008). Beginning at 3 P.M. on Day 11, blood was drawn from a jugular venous catheter every 15 min. At 9 P.M. on Days 11 and 12, 0.10 mg/kg dexamethasone (RX Veterinary Products; Grapevine, TX, USA) was administered i.v. to three stallions while three control stallions received an equivalent volume of saline i.v. After the second injection of dexamethasone or saline blood was collected every 3 h through Day 14. On Days 15–24, daily blood and semen collections resumed. At 10 A.M. on Day 25, fourteen days after the first injection of dexamethasone, the stallions were castrated and parenchymal tissue was minced and snap frozen in liquid nitrogen for hormone and RNA analysis. Testis tissue sections were also fixed in paraformaldehyde for histological analyses.

2.2. Radioimmunoassays of serum cortisol and testosterone and testis testosterone and total estrogens (estradiol and estrone)

Serum was harvested by centrifugation of the clotted blood samples at $1500 \times g$ at 4 °C followed by transfer of the supernatant to 12×75-mm polypropylene test tubes for storage at −80 °C until concentrations of testosterone and cortisol were determined by radioimmunoassay (RIA).

Concentrations of cortisol were determined in duplicate 25 μl aliquots of daily serum samples by a single-antibody RIA that utilized polypropylene tubes coated with cortisol antiserum [MP Biomedicals, Orangeburg, NY, USA (Gold et al., 2012)]. The radiolabeled tracer was ^{125}I cortisol. The cortisol antiserum cross-reactivity with steroids was aldosterone, 0.03%; corticosterone, 0.94%; deoxycorticosterone, 0.26%; progesterone, 0.02%; and estradiol, 0.01%. The minimum detectable concentration was 1.2 ng/ml and the intra-assay and inter-assay coefficients of variation were 3.8% and 4.7%, respectively.

Concentrations of testosterone were determined in duplicate 100-μl aliquots of selected serum samples by a single-antibody RIA that used testosterone antiserum [GDN S-250; Dr. Gordon D. Niswender, Colorado State University, Ft. Collins, CO, USA; (Welsh and Johnson, 1981)]. The radiolabeled tracer was ^{3}H testosterone (Perkin–Elmer, New England Nuclear, Boston, MA, USA). The cross-reactivity of the antiserum is approximately 0.01%, 0.01%, 0.11% and 1.7% with pregnenolone, progesterone, estradiol and androstenedione, respectively. The testosterone was purchased from Steraloids (Wilton, NH) and standards were made in concentrations ranging from 3.9 pg/ml to 16,000 pg/ml. Serum samples were ether-extracted with an extraction efficiency of 88.1%. The minimum detectable concentration was 15 pg/ml and the intra-assay

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