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Identification of bovine prolactin in seminal fluid, and expression and localization of the prolactin receptor and prolactin-inducible protein in the testis and epididymis of bulls exposed to ergot alkaloids

S.L. Pratt [a,*], S.M. Calcatera [a], H.M. Stowe [a], M.A. Dimmick [a], F.N. Schrick [b], S.K. Duckett [a], J.G. Andrae [a]

[a] Department of Animal and Veterinary Sciences, Clemson University, Clemson, South Carolina, USA
[b] Department of Animal Science, University of Tennessee, Knoxville, Tennessee, USA

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ABSTRACT

The objectives of this study were to determine (1) the presence and expression levels of bovine prolactin receptor (PRLR) and prolactin-inducible protein (PIP) in bovine testis and epididymis, and (2) the presence and concentrations of prolactin (PRL) present in seminiferous fluid in bulls consuming diets with (E+) or without (E−) ergot alkaloids. Bulls ($n = 8$) were sacrificed after 126 days (group A) of E+ or E− treatment or 60 days after all bulls ($n = 6$) were switched to the E− ration (group B). End point and real-time quantitative reverse transcription–polymerase chain reaction and immunohistochemistry were conducted on testis and epididymis samples to establish the presence and relative expression of PRLR and PIP. Seminal fluid samples obtained from bulls consuming E− and E+ diets were subjected to RIA for PRL. Both PIP and PRLR were present in testis and epididymis as determined by reverse transcription–polymerase chain reaction and immunohistochemistry. Prolactin-inducible protein mRNA abundance was affected by time of slaughter in testis and epididymis head, respectively ($P < 0.05$). Prolactin receptor mRNA expression was affected by time of slaughter in the epididymis ($P < 0.05$) and differed in testis samples because of treatment ($P < 0.05$). Radioimmunoassay establishes the presence of PRL in seminal fluid; however, differences in the concentration of PRL over two separate studies were inconsistent, possibly because of differences in diet. The presence and localization of the PRLR are consistent with expression data reported for other species, and the presence of PIP and PRL in seminal fluid is consistent with data generated in humans.

1. Introduction

Studies conducted in multiple species using detection assays such as reverse transcription–polymerase chain reaction (RT-PCR), radio-receptor assays, immunohistochemistry (IHC), or *in situ* hybridization have identified the prolactin receptor (PRLR) to be present in the testis [1–8] and accessory glands [5,7]. Furthermore, the PRLR has been shown to exist in Leydig cells [1,5,8,9], Sertoli cells [4,9,10], and spermatogonia [5,9] of several species by using IHC or *in situ* hybridization. Literature examining the location, expression level, and variant of the PRLR in bull reproductive tissues does not currently exist [11,12].

Prolactin is present in human seminal plasma [13,14] and is postulated to have a role in male fertility. Physiological concentrations of PRL stimulated metabolism in human

* Corresponding author. Tel.: +1 864 656 3135; fax: +1 864 656 3131.
E-mail address: scottp@clemson.edu (S.L. Pratt).

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spermatozoa *in vitro* [15] and exhibited putative effects on motility [16,17]. Prolactin has been reported to exert a pro-survival effect on sperm cells [18] as pharmacologic PRL concentrations of 500 ng/mL or greater significantly maintained sperm motility for 19 hours posttreatment as assessed by computer assisted semen analysis. The presence of PRL in bovine seminal fluid has not been examined.

Prolactin can stimulate the expression and release of proteins that may be involved in reproduction such as the prolactin-inducible protein (PIP) [19–21]. The PIP is expressed in multiple exocrine glands such as the prostate and seminal vesicles and excreted into seminal plasma [22,23]. The PIP gene is present in the bovine genome and its expression has been shown in the submaxillary gland [23]. Prolactin-inducible protein has a strong binding affinity for the Fc region of immunoglobulin G (IgG) molecules [24] and may be involved in the neutralization of immunosuppressive factors present in human semen [25] such as antisperm antigens [24,26]. The PIP excreted in seminal fluid appears to bind to the postacrosomal region of the sperm head [27] suggesting a possible role in fertilization events. Further investigation is required to establish PIP's role (if any) in male fertility; however, being regulated by PRL and being present and expressed in the bovine exocrine glands examined to date make PIP a logical choice to investigate in bovine males suffering from fescue toxicosis and possibly explain fertility issues.

Tall fescue toxicosis drastically decreases circulating PRL concentrations [28] because of the consumption of ergot alkaloids present in the plant [29] and may serve as a model to assess the effects of PRL, if any, on bovine male reproduction. These studies were conducted to (1) more thoroughly examine PRLR expression in the bovine testis and epididymis, (2) determine the presence and expression of PIP in the bovine testis and epididymis, (3) determine the presence or absence of PRL in bull seminal fluid, and (4) evaluate expression levels of PRLR, PIP, and PRL in response to fescue toxicosis.

2. Materials and methods

2.1. Experimental design

All animal research was approved by the Clemson University Institutional Animal Care and Use Committee (IACUC protocol #ARC2010–68). All reagents were purchased from Sigma Scientific (St. Louis, Missouri, USA) unless stated otherwise. Two separate studies were conducted for more than a 2-year period to assess effects of ergot alkaloid consumption on semen characteristics [30,31]. A pen experiment with either toxic or nontoxic seed fed as the treatment [31], and a grazing experiment conducted to make comparisons between bulls grazing either toxic or nontoxic endophyte-infected tall fescue pastures [30].

2.2. Treatment

In the pen-fed experiment, 14 bulls between the age of 13 and 16 months exhibiting greater than 32 cm scrotal circumference were assigned to one of two dietary treatments accounting for body condition score (BCS), weight, and their ability to pass a breeding soundness examination. Animals were fed a ration calculated to give a 1.0 kg per day gain in the presence or absence of tall fescue seed lacking ergot alkaloids or containing ergot alkaloid seed at a fixed concentration (0.8 μg of ergovaline and erovalanine per gram of dry matter [DM]). The total concentrations of ergovaline and erovalanine for the E+ and E− seed were 2.1 mg/kg DM and 0 mg/kg DM, respectively, as assayed by United States Department of Agriculture-Agricultural Research Service-Forage-Animal Production Research Unit using high performance lipid chromatography procedures as described in Johnson et al., 2012 [32]. The diet (including seed) was formulated to meet energy, crude protein, and mineral requirements (85% DM; 68% total digestible nutrients; 12.7% crude protein on DM basis) of the bulls and fed to provide adequate nutrients for 1.0 kg/day body weight gain [33]. All bulls were adjusted to the concentrate diet before initiation of the study. During this 2-week time period, the diet contained only E− seed. At the start of the test, bulls were weighed, BCS was evaluated, were subjected to a breeding soundness exam, and then allotted to diets and remained on E+ or E− diets for the remainder of the 126-day study (April 2011 to August 2011). Blood and semen samples were collected from the bulls at the start of the study and every 21 days and processed into serum and seminal fluid samples, respectively. Seminal fluid and serum were stored at −80 °C until used in analysis.

At the end of the 126-day study, bulls were sacrificed and testicles and epididymis collected either immediately at the end of the study (group A with five and three bulls on E− and E+ diet, respectively) or 60 days after removal from treatment (group B with three bulls from each treatment). In the grazing experiment, the E+ and E− treatments consisted of grazing the ergot alkaloid–producing Kentucky 31 or a non–ergot alkaloid containing tall fescue possessing a novel endophyte, respectively. Enzyme-linked immunosorbent assay for the presence of ergot alkaloids in the forage (Agrinostics, LTC. Co., Watkinsville, Georgia, USA) was conducted on 50 tillers per pasture and the Kentucky 31 pasture exhibited a 98% infection rate. Two weeks before the start of the study, all bulls ($n = 11$ and 10, for E+ and E− treatments, respectively) were adjusted to a forage diet by grazing nontoxic pasture. At the start of the test (Day 0), bulls were weighed, BCS was evaluated, were subjected to a breeding soundness exam, and were allotted to E− and E+ treatments and remained on treatment for 155 days (April 2012 to August 2012). Ejaculates were collected on Day 0 and every 28 days to Day 140, separated into cell and fluid components for both experiments, frozen, and stored at −80 °C until used for analysis.

2.3. Radioimmunoassay

To determine the presence and concentration of PRL in seminal fluid PRL RIA assays were performed by the F. Neal Schrick laboratory as previously described [34] for serum with mean inter- and intra-assay coefficients of variation of 9.7% and 6.0%, respectively.

2.4. Immunohistochemistry

Formalin-fixed samples were processed and IHC performed as described by Calcatera et al. [35]. Briefly, samples were processed overnight in a Leica paraffin processor