Theriogenology 79 (2013) 766–777

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Associations of adiponectin and fertility estimates in Holstein bulls

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ARTICLE INFO

Article history:
Received 22 May 2012
Received in revised form 25 November 2012
Accepted 2 December 2012

Keywords:
Dairy bull
Sperm capacitation
Adiponectin
Sire conception rate

ABSTRACT

Adiponectin is a pleiotropic regulator of numerous biological functions, including gonadal steroidogenesis and might play a role in sperm structures and functions. The objectives were to: (1) determine associations among serum concentrations of adiponectin, testosterone, and prolactin, and the sperm DNA fragmentation index; (2) associate sperm adiponectin mRNA abundance with estimates of fertility (sire conception rate); and (3) determine sperm protein expression of adiponectin and its receptor in pre- and postcapacitated sperm from Holstein bulls. In experiment 1, biweekly serum concentrations of adiponectin, prolactin, and testosterone were greater ($P < 0.05$) for high fertility bulls compared with average and low fertility bulls. Furthermore, sperm DNA fragmentation index was greater ($P < 0.05$) for low fertility compared with both average and high fertility bulls. In experiment 2, samples of sperm from a single collection from commercial Holstein bulls ($N = 34$) were used to evaluate relative sperm mRNA expression of adiponectin and its receptors, AdipoR1 and AdipoR2, and protein levels of adiponectin and its receptors, AdipoR1 and AdipoR2, in pre- and postcapacitation sperm. The mRNA abundance of adiponectin and its receptors, AdipoR1 and AdipoR2, were greater for high fertility bulls (>2 to ≤4 sire conception rate) compared with average (≥2 to ≤2) and low (>−2 to ≤−4) fertility bulls. Based on the sperm capacitation assay, average fertility bulls had a greater percentage of acrosome-reacted sperm at 5 hours than high and low fertility bulls, whereas high fertility bulls had a greater percentage of acrosome-reacted sperm than low fertility bulls. After capacitation, levels of adiponectin protein were lower in average fertility bulls, AdipoR1 was lower in all fertility groups, and AdipoR2 was lower in average and high fertility bulls. In conclusion, adiponectin and its receptors had vital roles in sperm structural and functional traits and consequently they were associated with fertility. In addition to its role in steroidogenesis and sperm capacitation, adiponectin might be involved in sperm-egg fusion and fertilization.

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1. Introduction

Fertility in male mammals is regulated by two adenohypophyseal gonadotropic hormones, LH and FSH, which modulate testosterone synthesis in Leydig cells and its aromatization to estradiol in Sertoli cells, respectively. The hypothalamo-hypophyseal-gonadal axis regulates release of hypothalamic GnRH, which alters secretion of gonadotropins and testosterone that are essential for spermatogenesis, sperm maturation, and reproductive behavior [1–3].

Prolactin, a 23 KDa hormone, is synthesized in the adenohypophyseal lactotrophs. Although no clear function was initially ascribed to prolactin in male mammals, including humans [4,5], it is now apparent that prolactin enhances several aspects of testicular function [4,5]. Thus,

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0093-691X/$ – see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.theriogenology.2012.12.001

prolactin has been implicated in maintenance of spermatogenic cell morphology [6], upregulation of LH receptor number in Leydig cells [7,8], and stimulation of steroidogenesis including androgen production [8–10]. In contrast, prolactin might also inhibit aromatase activity [11]. Prolactin increased FSH receptor number in Sertoli cells *in vitro* [12] and is involved in morphogenesis of spermatocytes to spermatids [13]. Moreover, several *in vitro* effects of prolactin in sperm have been reported, including increased calcium binding and/or transport of ejaculated and epididymal sperm [13], increased energy metabolism [14], maintenance of mobility and attachment to the oocyte [15], and reduced capacitation time [15]. It is noteworthy that acute hyperprolactinemia suppressed testosterone synthesis and male fertility by inducing hypersecretion of adrenal corticoids or by inhibiting secretion of GnRH through prolactin receptors on hypothalamic dopaminergic neurons [16,17].

Emergence of the metabolic hormone, adiponectin, as a key endocrine signal, has been a major development, not only in energy balance, but more generally in areas including reproduction, inflammation, and immunology [18–20]. Adiponectin is a pleiotropic regulator of numerous biological functions, including gonadal steroidogenesis. Adiponectin secretion was suppressed by prolactin and growth hormone [21]. A significant, positive relationship between plasma adiponectin and high-density lipoprotein cholesterol previously reported in men [22]. This might contribute to increased testosterone production. In contrast, incubation of Leydig cells with recombinant adiponectin decreased testosterone production [23]. It is plausible that this recombinant adiponectin would have been ineffective or contended with the native adiponectin. We hypothesize that increased sperm adiponectin mRNA abundance might improve male fertility by improving sperm structure and function. The objectives were to: (1) determine associations among serum concentrations of adiponectin, testosterone, and prolactin, and the sperm DNA fragmentation index (% DFI; sperm chromatin structure assay); (2) associate sperm adiponectin mRNA abundance with estimates of fertility (sire conception rate; SCR); and (3) determine sperm protein expression of adiponectin in pre- and postcapacitated sperm obtained from Holstein bulls.

2. Materials and methods

2.1. Experiment 1: associations of adiponectin, prolactin, testosterone, and DNA fragmentation index

2.1.1. Bulls and sample collections

Holstein bulls (N = 10; age, 16.9 ± 0.42 [14–18] months old) housed in a commercial bull stud center were selected for use in this study. There were four, four, and two bulls in the low, average, and high fertility sire groups, respectively, representing SCRs scores of ≥ -4 to < -2, ≥ -2 to ≤ 2, and > 2 to ≥ 4, respectively. Blood samples were collected (nine times, 2 weeks apart) by jugular venipuncture using serum separator Vacutainer tubes (Becton Dickinson and Co., Franklin Lakes, NJ, USA) between 8:00 AM and 10:00 AM. Samples were allowed to clot, centrifuged (800× *g* for 15 minutes), and then frozen pending subsequent analysis of serum concentrations of testosterone, prolactin, and adiponectin. On each blood collection day, for each sire, two ejaculates were collected via artificial vagina, pooled, extended, loaded in 0.5 mL French straws, cryopreserved, and stored in liquid nitrogen. For each sire, 10 straws of frozen semen were subsequently randomly selected, thawed, and evaluated.

2.1.2. Determination of serum testosterone and prolactin concentrations

Serum testosterone concentrations were determined by RIA (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA, USA) according to the manufacturer's instructions. The manufacturer reports a sensitivity of 4 ng/dL with a interassay CV of 4% to 18% and intra-assay CV of 6% to 11%. Prolactin was determined using a previously validated RIA [24] with a primary antibody (bPRL; A.F. Parlow) diluted to 1:200,000. Sensitivity averaged 0.3 ng/mL, and intra- and interassay CVs (six assays) averaged 6.8% and 6.3%, respectively.

2.1.3. Determination of adiponectin in serum samples

Serum adiponectin concentrations were estimated by direct ELISA, as described [25]. Briefly, 100 μL of goat-Acrp30- antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) were added to 96-well plates that were precoated with standards and samples, and kept at 4 °C for at least 24 hours. After washing with buffer, 100 μL of secondary antibody, donkey anti-goat IgG-HRP (Santa Cruz Biotechnology Inc.) were added to each well. After washing with buffer, 200 μL of reagent containing the substrate (acetyl cholinesterase; Thermo Scientific Inc., Waltham, MA, USA) were added, followed by 50 μL of stop solution (Thermo Scientific Inc.) in 5 to 10 minutes, depending on color development. Plates were read at 450 nm using a Glomax-Multi Detection System (Promega Corporation, Madison, WI, USA) and serum adiponectin concentrations were calculated from the standard curve. Intra- and interassay CVs were 6.2% and 10.4%, respectively.

2.1.4. Determination of sperm DNA fragmentation index

The sperm chromatin structure assay was performed as described [26]. It is based on the metachromatic properties of acridine orange to distinguish denatured from intact native DNA in sperm. The semen sample was thawed and diluted (2×10^6 sperm/mL) using TNE buffer (0.01 mol/L TRIS-HCl, 0.15 mol/L NaCl, and 1 mmol/L EDTA, pH 7.4). Acid-induced denaturation of DNA *in situ* was attained by adding 400 μL of an acid-detergent solution (0.1% [wt/vol] Triton X-100, 0.15 mol/L NaCl, and 0.08 N HCl; pH 1.2) to 200 μL of semen. After 30 seconds, sperm were stained by adding 1.2 mL of a solution containing 6 μg purified acridine orange (Polysciences, Warrington, PA, USA) per mL of buffer (0.1 mol/L citric acid, 0.2 mol/L Na_2HPO_4, 1 mmol/L EDTA, 0.15 mol/L NaCl; pH 6.0). All steps were performed at room temperature. At 3 minutes after acid-induced denaturation, samples were analyzed using a Coulter EPICS XL-MCL (Beckman Coulter, Fullerton, CA, USA) flow cytometer with 480 nm argon laser and 15 mW. Data corresponding to the red (>630 nm as detected by the FL2 detector) and green (530 nm as detected by the FL1 detector) fluorescence of