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Plasma IGF-I, INSL3, testosterone, inhibin concentrations and scrotal circumferences surrounding puberty in Japanese Black beef bulls with normal and abnormal semen

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ABSTRACT

The relationships between semen abnormalities and peripheral concentrations of testicular and metabolic hormones in beef bulls are unclear. Here we compared plasma insulin-like growth factor I (IGF-I), insulin-like peptide 3 (INSL3), testosterone, inhibin concentrations, and scrotal circumferences surrounding puberty in Japanese Black beef bulls (n = 66) with normal or abnormal semen. We collected blood samples and measured scrotal circumferences monthly from 4 to 24 months of age. Semen was collected weekly from 12 months until at least 18 months of age. Fresh semen was evaluated for semen volume, sperm motility, concentrations, and morphological defects. The normal fresh semen was frozen by a standard method and examined for post-thaw sperm motility and fertility. Bulls were classified as having either normal post-thaw semen (n = 45) or abnormal semen (n = 21, when at least one of the above test items was abnormal for 6 months). Abnormal semen was classified into abnormal fresh or low-fertility post-thaw which evaluated for rates of transferable embryos. The abnormal fresh was categorized as having sperm morphological defects, low motility, and morphological defects plus low motility. Scrotal circumferences were smaller for the abnormal-semen group vs. the normal-semen group at 20 and 24 months ($p < 0.05$). Plasma IGF-I, INSL3, and inhibin concentrations in the abnormal-semen group were lower than those of the normal-semen group ($p < 0.05$) surrounding puberty (4–6, 8, 18–22, and 24 months for IGF-I; 6, 9, 11–14, 17, and 20–21 months for INSL3; 5, 8–13, 16, 17, 19, and 20 months for inhibin). The plasma testosterone concentrations were lower in the abnormal-semen bulls vs. normal-semen bulls only at 22 months ($p < 0.05$). Analyses of the classified abnormal semen showed lower plasma INSL3 concentrations for morphological defects plus low motility in fresh semen ($p < 0.05$) and lower IGF-I and inhibin concentrations for low-fertility post-thaw semen ($p < 0.05$) compared to the normal semen. Our results suggest that reduced secretions of IGF-I, INSL3, and inhibin surrounding puberty may be associated with semen aberration in beef bulls. Notably, the combined sperm abnormality of morphological defects and low motility in fresh semen could involve lowered INSL3, whereas the low-fertility post-thaw semen might be related to decreases of IGF-I and/or inhibin. Pre-puberty blood IGF-I, INSL3 and inhibin concentrations could be used as indicators to predict aberrant semen in beef bulls.

1. Introduction

Japanese Black is a dominant and native beef breed in Japan that produces highly marbled beef compared to other beef breeds [1,2]. In Japan, most of the beef cattle are being bred by artificial insemination or embryo transfer. A small number of Japanese Black bulls that have shown superior carcass traits in their progeny are

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selected as sires from many young candidates. Occasionally the sire candidates have abnormal semen that is infertile or considered subfertile [3,4]. The causes of the aberrant semen are largely unknown.

It was reported that scrotal circumference is correlated with sperm output in young dairy bulls [5,6] and that at surrounding puberty, early-maturing bulls had a greater scrotal circumference than late-maturing bulls [7]. Other studies indicated that scrotal circumference can also be a predictable reproductive trait for selecting genetically superior bulls [8,9]. However, the association between scrotal circumference and abnormal semen has not been elucidated.

More recently, it was demonstrated that a high plane of nutrition positively affects scrotal circumference and puberty in dairy and beef bulls [10–13]. Those studies also showed higher plasma insulin-like growth factor I (IGF-I) and gonadotropin-releasing hormone (GnRH)-stimulated testosterone concentrations during puberty in bulls with high-plane nutrition compared to those with low-plane nutrition [10,12,13]. Early-maturing bulls have higher luteinizing hormone (LH) peaks in blood at 2–3 months before puberty compared to late-maturing bulls [7]. IGF-I gene null mutants of adult male mice showed small reproductive organs and lower sperm production [14]. Serum IGF-I concentrations in men with abnormal fresh semen were lower than those in men with normal semen [15]. The IGF-I concentrations have not yet been reported in bulls with abnormal semen. IGF-I may have a role in regulating testosterone secretions in bulls at puberty [10], and IGF-I receptors are expressed on bovine spermatozoa [16].

Insulin-like peptide 3 (INSL3) and testosterone secreted from Leydig cells are known to play key roles in testicular descent during fetal development in mice [17,18]. It was recently suggested that INSL3 has another function as a germ cell survival/anti-apoptotic factor in boar testis [19]. We examined changes of blood INSL3 concentrations during pubertal development in normal bulls [20] and male goats [21], and our findings demonstrated a dynamic pattern of INSL3 that differed from that of testosterone. No data are available regarding changes of blood INSL3 and testosterone concentrations in bulls with abnormal semen during pubertal development.

Several studies suggested that serum inhibin B concentrations are a diagnostic marker of men's infertility [22–25]. Serum inhibin concentrations declined in Holstein bulls from birth to 9 months [26]. There has been no report comparing blood inhibin concentrations between bulls with normal and abnormal semen characteristics.

We designed the present study to compare plasma IGF-I, INSL3, testosterone, and inhibin concentrations and scrotal circumferences surrounding puberty in Japanese Black beef bulls with normal and abnormal semen.

2. Materials and methods

2.1. Animals

Japanese Black beef bulls (n = 66) in an experimental beef cattle station at the Northern Center of Agriculture Technology of Hyogo Prefecture, Japan were randomly selected. Scrotal circumferences of the bulls (n = 57) were recorded monthly from 4 to 24 months of age (pre-puberty 4–11 months, puberty 12–17 months, post-puberty 18–24 months). The scrotal circumference was not measured in some bulls, particularly in the early stage of pre-puberty (4–7 months) and at the latter stage of post-puberty (22–24 months). The ranges of the number of bulls of which the scrotal circumference was measured were 13–37, 48–53, and 24–50 in pre-puberty, puberty, and post-puberty, respectively. The body weights of the bulls (n = 59) were recorded monthly from 6 to 24 months of age. The body weight was not measured in some bulls. The ranges of the number of bulls of which the body weight was measured were 10–38, 40–52, and 16–49 in pre-puberty, puberty, and post-puberty, respectively.

The bulls remained normal in appearance and healthy during all experiments. The bulls were kept under natural light in an open shelter covered by a roof and were provided ad libitum hay and concentrate to meet or exceed the Japanese Feeding Standard recommendations for beef bulls. Their diet contained 0.6% mineral-vitamin (as fed), 12% crude protein, 1.59 Mcal/kg net energy of maintenance, and 0.99 Mcal/kg net energy of gain (dry matter basis) [27]. The experiments were approved by the Northern Center of Agriculture Technology of Hyogo Prefecture. The procedures of the animal experiments complied with the guidelines for the Proper Conduct of Animal Experiments at Academic Research Institutions in Japan.

2.2. Blood sampling

Blood samples were obtained monthly from the bulls (n = 66) from 4 to 24 months of age. However, the blood samples were not taken in some bulls, particularly at the early stage of pre-puberty (4–7 months) and at the latter stage of post-puberty (22–24 months). Thus, this was not a complete longitudinal study and the number of blood collections per bull varied from 8 to 20. The ranges of the number of bulls from which blood was taken were 23–56, 59–66, and 30–65 in pre-puberty, puberty, and post-puberty, respectively. The blood was taken between 1:00 and 2:00 p.m. Blood samples were collected by jugular vein into heparinized tubes and immediately placed on ice. The blood was centrifuged at 1700 g for 15 min at 4 °C. The separated plasma was stored (−30 °C) until assay. The plasma concentrations of testosterone and IGF-I were measured by enzyme-immunoassays (EIAs), and the plasma INSL3 and inhibin concentrations were assayed by a time-resolved fluorescence immunoassay (TRFIA).

2.3. Semen analysis

Semen was collected from the all bulls weekly from 12 months until at least 18 months of age, with the use of an artificial vagina. Two ejaculates were collected during one collection with a 5- to 10-min interval. The fresh semen was evaluated for semen volume (normal >3 mL), rate of sperm with highly progressive motility (normal >80%), concentration (normal >500 million/mL), and rate of morphological defects (normal <20%) [28,29]. The morphological examination was done by Hemacolor stain set (Merck, Darmstadt, Germany). For each semen collection, 500 sperm were examined for morphological defects of the head, midpiece, and tail.

The fresh semen classified as normal was frozen by a standard method [30]. To freeze fresh ejaculates, samples were extended in an egg yolk-Tris-citrate extender by a straw method at the Northern Center of Agricultural Technology. A conventional liquid-nitrogen freezer (Simple Quick freezer, Fujihira Industry, Tokyo) was used to freeze semen [31]. The semen was then stored in liquid nitrogen. The frozen semen was thawed in warm water at 38.5 °C for 10 s, and then the rate of sperm with highly progressive motility (normal >40%) was determined.

The fertilizing ability of all bulls was examined by a test of superovulation and artificial insemination (AI) using Japanese Black beef cows (2.2 ± 0.2 cows per bull; mean ± standard error of the mean (SEM)) followed by embryo collection [3]. The fertility examination of post-thaw semen was performed once for each bull between 14 and 18 months of age. Briefly, superovulation treatments were conducted using porcine follicle-stimulating hormone