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# Plasma insulin-like peptide 3 concentrations are acutely regulated by luteinizing hormone in pubertal Japanese Black beef bulls

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ABSTRACT

Insulin-like peptide 3 (INSL3) is a major secretory product of testicular Leydig cells. The mechanism of acute regulation of INSL3 secretion is still unknown. The present study was undertaken in pubertal beef bulls to (1) determine the temporal relationship of pulsatile secretion among LH, INSL3, and testosterone and (2) monitor acute regulation of INSL3 secretion by LH using GnRH analogue and hCG. Blood samples were collected from Japanese Black beef bulls (N = 6) at 15-minute intervals for 8 hours. Moreover, blood samples were collected at −0.5, 0, 1, 2, 3, 4, 5, and 6 hours after GnRH treatment and −0.5, 0, 2, 4, and 8 hours on the day of treatment (Day 0), and Days 1, 2, 4, 8, and 12 after hCG treatment. Concentrations of LH, INSL3, and testosterone determined by EIAs indicated that secretion in the general circulation was pulsatile. The frequency of LH, INSL3, and testosterone pulses was 4.7 ± 0.9, 3.8 ± 0.2, and 1.0 ± 0.0, respectively, during the 8-hour period. Seventy percent of these INSL3 pulses peaked within 1 hour after a peak of an LH pulse had occurred. The mean increase (peak per basal concentration) of testosterone pulses was higher ($P < 0.001$) than that of INSL3 pulses. After GnRH treatment, LH concentrations increased ($P < 0.01$) dramatically 1 hour after treatment and remained high ($P < 0.05$) until the end of sampling, whereas an elevated ($P < 0.05$) INSL3 concentration occurred at 1, 2, 5, and 6 hours after treatment. Testosterone concentrations increased ($P < 0.01$) 1 hour after the treatment and remained high until the end of sampling. After hCG treatment, an increase of INSL3 concentration occurred at 2 and 4 hours, and Days 2, 4, and 8 after treatment ($P < 0.05$), whereas in case of testosterone, concentrations remained high ($P < 0.01$) until Day 8 after treatment. The increase (maximum per pretreatment concentration) of INSL3 concentrations after injecting GnRH or hCG was much lower ($P < 0.001$) than that of testosterone. In conclusion, secretion of INSL3 in blood of bulls occurred in a pulsatile manner. We inferred an acute regulation of INSL3 by LH in bulls because INSL3 concentrations increased immediately after endogenous and exogenous LH stimulation. The increase of INSL3 concentrations by LH was much lower than that of testosterone in bulls.


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## 1. Introduction

Insulin-like peptide 3 (INSL3) is a major secretory product of testicular Leydig cells in all mammalian species examined so far [1,2]. The two main known functions of INSL3 in the male are the endocrine regulatory effect involved in completing the transabdominal phase of testicular descent in mice [3,4] and a paracrine function exhibiting an antiapoptotic effect to protect male germ cells in rats [5]. According to studies on humans, secretion of INSL3 is related to the differentiation status of testicular Leydig cells and is stimulated by the long-term trophic effects of LH [1,6–9]. However, the process of acute regulation of INSL3 secretion is mostly unknown. Detection of INSL3 in the peripheral blood of humans [7,10,11], dogs [12], and cattle [13] indicates that INSL3 may have additional endocrine effects in mammalian males. According to recent studies in our laboratory, dynamics of secretory patterns of INSL3 and testosterone in peripheral plasma are different during sexual development in male dogs [12] and beef bulls [13], although both hormones are secreted from the unique source of testicular Leydig cells. It was well documented that in many species including cattle [14,15], secretion of LH occurred in a pulsatile manner stimulating testicular Leydig cells to produce pulsatile secretion of testosterone. However, under physiological conditions, the pulsatile secretory pattern of INSL3 and its relation with LH have not been elucidated.

Endogenous LH increased by GnRH or hCG (which possesses LH activity) caused a significant increase of testosterone in the general circulation of bulls [16–19], male goats [20], and rams [21]. In men, testosterone concentrations in peripheral blood taken daily for 8 days increased after hCG treatment, whereas INSL3 concentrations did not change [22]. It remains unknown whether endogenous and exogenous LH can acutely regulate the secretion of INSL3 in domestic animals.

The objectives of this article were (1) to determine the temporal relationship of pulsatile secretion among LH, INSL3, and testosterone and (2) to monitor acute regulation of INSL3 secretion by LH using GnRH analogue and hCG in pubertal beef bulls. Our hypothesis was that LH would acutely stimulate INSL3 secretion from testicular Leydig cells in bulls.

## 2. Materials and methods

The following experiments using bulls were performed as a part of study on evaluation of fertility in Japanese Black beef bulls which was approved by a committee of General Technological Center of Hyogo Prefecture for Agriculture. The procedures of animal experiments complied with guidelines for Proper Conduct of Animal Experiment in Academic Research Institutions in Japan.

### 2.1. Animals

Japanese Black beef bulls (N = 6) raised in an experimental beef cattle station in the Northern Center of Agriculture Technology of Hyogo Prefecture in Japan were used for the present study. The selected beef bulls had no apparent abnormalities of the reproductive status, and testicular presence was checked manually to confirm the presence of both testes inside the scrotum. Body weight and scrotal circumference of the bulls were recorded monthly from 6 to 23 months of age. These bulls remained normal in appearance and health during all experiments. The bulls were kept under natural light in an open shelter covered by a roof and were maintained by *ad libitum* hay and concentrate to meet or exceed Japanese Feeding Standard recommendations for the beef bulls.

### 2.2. Experiment 1

Experiment 1 was done to determine the temporal relationship among INSL3, LH, and testosterone at 15-minute intervals of sampling for an 8-hour session in beef bulls (aged 10–11 months; N = 6). Blood sampling for all bulls was started at 10 AM and ended at 6 PM. An indwelling jugular venous catheter (Argyle; Covidien Ltd., Dublin, Ireland) was inserted about 1 hour before the beginning of sampling. No sedation was performed before inserting the intravenous catheter and during sampling. Head restraint by either a stanchion or a halter was not used, except during insertion of the intravenous catheter. The bulls were given access to water and hay at every 2 to 3 hours during collection of the samples. Blood samples were collected into heparinized tubes and immediately placed in ice before centrifuging (1700 × *g* for 15 minutes at 4 °C). The plasma was decanted and stored (−30 °C) until assay.

### 2.3. Experiment 2

A single injection of GnRH analogue (fertirelin acetate, Conceral; Intervet, Tokyo) was given intramuscularly at a dose of 0.5 μg/kg (aged 11–12 months; N = 6). The same beef bulls that were used in experiment 1 were used for experiment 2, which took place at least 1 week after completion of experiment 1. The blood samples for assaying INSL3, LH, and testosterone were collected at −0.5, 0, 1, 2, 3, 4, 5, and 6 hours after treatment. The treatment was given immediately after the 0-hour sample was drawn. Thus, blood samplings taken at −0.5 and 0 hours are pretreatment samples. Blood samples were collected into heparinized vacutainers by jugular venipuncture and processed as previously mentioned in experiment 1.

### 2.4. Experiment 3

Six beef bulls that were used for experiments 1 and 2 were also used for experiment 3. This experiment was conducted about 6 months after completion of experiment 2 (aged 18–19 months). A single dose of hCG (5 IU/kg, intramuscular; Veterinary Puberogen, Novartis Animal Health, Tokyo) was administered. Two pretreatment blood samples were taken at −0.5 hour and immediately before the hCG treatment (0 hour). The sampling was then continued at 2, 4, and 8 hours on the day of treatment (Day 0) and Days 1, 2, 4, 8, and 12 after treatment. Blood collection and processing of plasma were done as mentioned previously in experiment 2.