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Fetal gender prediction based on maternal plasma testosterone and insulin-like peptide 3 concentrations at midgestation and late gestation in cattle

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ABSTRACT

We compared maternal plasma testosterone and insulin-like peptide 3 (INSL3) concentrations between dams carrying a male versus female fetus from early to late gestation and examined the application of maternal hormonal concentrations to fetal gender prediction in dairy and beef cattle. Blood samples were collected from Holstein cows or heifers (N = 31) and Japanese Black beef cows (N = 33) at 1-month intervals at 2 to 8 months of gestation. Fetal gender was confirmed by visual observation of external genitalia of calves just after birth. Plasma testosterone and INSL3 concentrations were determined by enzyme-immunoassay. Fetal genders were judged based on cutoff values of maternal testosterone and INSL3 concentrations (male, if it was ≥ cutoff value; female, if < cutoff value), which we set for each hormone at each gestational month using receiver operating characteristic curves. Plasma testosterone concentrations were higher for dams with a male fetus than those with a female at 4, 5, 7, and 8 months for the dairy cattle ($P < 0.05$) and at 4, 5, 6, and 8 months for the beef cows ($P < 0.05$). Plasma INSL3 concentrations were higher for dams with a male fetus than those with a female at 2 and 6 months for the dairy cattle ($P < 0.05$) and at 4 to 8 months for the beef cows ($P < 0.05$). The predictive values and detection rates for fetal gender prediction based on maternal testosterone concentrations were 75.8% to 79.3% for dairy cattle at 5 and 7 months and for beef cows at 5 and 6 months, whereas those values by maternal INSL3 concentrations were 71.0% to 72.4% for the dairy cattle at 6 months and beef cows at 4 and 8 months. When multiple time points of testosterone and INSL3 concentrations at several midgestation and late gestation months were considered for fetal gender prediction, predictive values were 89.3% (5–7 months) and 85.7% to 88.0% (4–6, 8 months) for the dairy and beef breeds, respectively. Maternal testosterone and INSL3 concentrations in dams carrying a male fetus were higher than those carrying a female at midgestation and/or late gestation in Holstein and Japanese Black beef cattle. Nearly, 80% accuracy was obtained for fetal gender prediction by a single time point of maternal plasma testosterone concentrations at

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midgestation. Nearly 90% accuracy for the prediction was obtained when multiple time points of testosterone and INSL3 concentrations from midgestation to late gestation were considered.

1. Introduction

Newborn calf genders have a substantial impact on dairy- and beef-farming operations. On dairy farms, female calves are needed as future replacement heifers and are thus more valuable than males. In Japanese Black beef production, male calves are more valuable than females because of the males' higher body weights and daily gains. Knowing the bovine fetal gender during pregnancy offers an opportunity to perform more profitable reproductive management [1,2]. For example, on dairy farms, after an adequate number of female fetuses is secured for heifer replacement, the farmers can take a more profitable breeding approach by the insemination of Japanese Black beef semen or the transfer of their embryos to Holstein cows or heifers to produce more valuable beef-breed calves. Gender prediction also provides valuable information for making decisions regarding the buying and selling of pregnant cattle [1,3] and the necessity of monitoring and assisting calving.

Ultrasound monitoring of fetal external genitalia in the first trimester of pregnancy is being used for gender prediction in cattle [1,3]. The best periods for fetal gender prediction by ultrasonography were reported to be 55 to 70 days of pregnancy for larger breeds and older cows and 60 to 80 days of pregnancy for smaller breeds and younger cows [4]. Beyond the first trimester, the gravid horn descends ventrally into the abdominal cavity, and the monitoring of fetal external genitalia by ultrasonography becomes very difficult or impossible. Thus, bovine fetal genders can be predicted accurately by ultrasonography only within a short period of the first trimester.

The detection of male-specific DNA or androgens in fetal fluid was also reported as an alternative method to predict bovine fetal gender [2,5,6]. The fetal fluid was aspirated from pregnant cows by transvaginal ultrasound-guided amniocentesis [2,6]. However, the amniocentesis had drawbacks of a high risk of abortion and aspiration failure of fetal fluid from the pregnant uterus in some cases [2,6].

As safer and easier methods for fetal gender prediction, measurements of testicular hormones such as androgens and insulin-like peptide 3 (INSL3) — which are secreted from testicular Leydig cells — in peripheral blood from pregnant cows may have potential. Serum testosterone concentrations were higher in pregnant Holstein heifers carrying a male fetus than those carrying a female fetus [7]. Serum INSL3 concentrations were also greater in pregnant beef heifers carrying a male fetus than those carrying a female fetus at midpregnancy and late pregnancy [8]. However, applications of maternal blood testosterone and INSL3 concentrations to fetal gender prediction in cattle have not been reported.

Our objectives in the present study were to (1) compare maternal plasma testosterone and INSL3 concentrations between dams carrying a male fetus and those carrying a female fetus from early to late pregnancy and (2) examine the application of the maternal testicular hormonal concentrations to fetal gender prediction in dairy and beef cattle.

2. Materials and methods

2.1. Animals

Pregnant Holstein cattle (N = 31; age 4.9 ± 0.5 years, mean ± standard error of the mean) and Japanese Black beef cattle (N = 33; age 4.3 ± 0.4 years) were used. Artificial inseminations were conducted for the Holstein cattle at various seasons throughout the year and for the Japanese Black cattle in April or May. The Holstein cattle (cows N = 24; heifers N = 7) were raised at the Research Institute of Environment, Agriculture and Fisheries, Osaka Prefectural Government. The Japanese Black beef cows were kept at the Northern Center of Agriculture Technology of Hyogo Prefecture (N = 30) and the Hyogo Prefecture College of Agriculture, General Technological Center of Hyogo Prefecture for Agriculture (N = 3). They were kept in cattle sheds and provided ad libitum hay and concentrate to meet or exceed the Japanese Feeding Standard recommendations for dairy cows or heifers [9] and beef cows [10]. The following experiments were approved by those research institutes. The procedures of the animal experiments complied with the guidelines for The Proper Conduct of Animal Experiments in Academic Research Institutions in Japan.

2.2. Experiment

Blood samples were collected from the Holstein cows or heifers and from the Japanese Black cows every month from 2 to 8 months of gestation. The blood collection was performed at a fixed date of every month for all cattle at each experimental farm. In the Holstein cattle, the blood collection was started at 3 to 6 months of gestation and ended at 7 months for some animals. In the Japanese Black beef cows, the blood collection was started at 3 or 4 months of gestation and ended at 7 months for some animals. The experiment was thus not a complete longitudinal study.

The pregnancy was confirmed by the presence of a fetus at 40 to 50 days after artificial insemination or embryo transfer, using ultrasonography (Tringa Linear, Medical Task Force, Osaka, Japan). The blood samples were taken from 11 AM to 12 PM in the dairy cattle and from 1 30 PM to 2 30 PM in the beef cows. The blood samples were collected from the coccygeal vein or artery of the dairy cows or heifers and from the jugular vein of the beef cows into heparinized tubes and immediately placed in ice before centrifuging (1700 × *g* for 15 minutes at 4 °C). The iced blood was centrifuged within 10 minutes after the collection of all samples. The plasma was decanted and stored