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論　文　名	Blood concentrations of testicular and metabolic hormones and biochemical parameters differed in Japanese Black beef bulls with normal versus abnormal semen （正常および異常精液を示す黒毛和種雄ウシの性成熟過程における血中精巣・代謝ホルモンと生化学パラメータ濃度は異なる）
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論文要旨

Introduction

Early detection of high-performance sires has always been important for beef breeders to reduce high costs of feeding and shorten generation intervals to hasten genetic gains. Japanese Black, a dominant beef breed in Japan, have a unique fat deposition pattern characterized by substantial marbling. Most Japanese Black cattle are propagated by artificial insemination or embryo transfer. Japanese Black bulls siring progeny with superior carcass traits are selected for breeding. Some of these sire candidates have abnormal semen that is infertile or subfertile, although the underlying cause is usually unknown.

Scrotal circumference (SC) can be useful for identifying genetically superior bulls, as it is strongly correlated with sperm production and bulls with large SC generally have better semen quality and they have sisters and daughters that reach puberty earlier. Insulin-like peptide 3 (INSL3) and testosterone are secreted from Leydig cells. The former hormone has key roles in testicular descent during fetal development in mice and it has anti-apoptotic properties that support germ cell survival. Testosterone is secreted into peripheral blood in high-amplitude pulses in response to LH pulses in bulls and has a critical role in supporting germ cell development. However, LH and testosterone responses to GnRH challenges in beef bulls with abnormal semen have not yet been elucidated. Furthermore, there are apparently no reports regarding changes in

plasma INSL3 and testosterone concentrations and scrotal circumference in peripubertal beef bulls with abnormal semen.

Metabolic hormones such as insulin-like growth factor I (IGF-I) and insulin may have direct effects on testes. For example, adult male mice with IGF-I gene null mutants had small reproductive organs and lower sperm production. Moreover, serum IGF-I concentrations were lower in men with abnormal fresh semen than those in men with normal semen. Serum biochemical analysis is a valuable diagnostic tool to evaluate metabolic and health status of animals. However, there are apparently no reports comparing blood testicular, metabolic hormones and biochemical parameters between bulls with normal versus abnormal semen.

A series of studies were performed to characterize SC, testicular and metabolic hormones and blood biochemical parameters of young Japanese Black beef bulls with normal versus abnormal semen.

Chapter 1: Plasma testicular hormone concentrations and scrotal circumference in Japanese Black beef bulls with normal versus abnormal semen

Objectives were to determine changes in plasma concentrations of INSL3, testosterone, estradiol-17β and inhibin, as well as scrotal circumference in young Japanese Black beef bulls with normal versus abnormal semen. Collection of blood samples and measurement of scrotal circumference was done monthly from 4 to 24 months of age. Semen was collected weekly (artificial vagina) from 12 to >18 months of age. Fresh semen that met minimum standards (ejaculate volume >3 ml with >500 x 106 sperm/ml, >80% motile and >80% morphologically normal) were cryopreserved in an egg yolk-Tris-citrate extender. Minimum acceptable post-thaw sperm motility was >40%. Furthermore, frozen-thawed semen was used to inseminate super stimulated cattle, with nonsurgical embryo recovery 7.5 days after estrus; minimum acceptable fertility was >40% of embryos being of transferable quality. Bulls were classified as having abnormal semen when they consistently failed to meet minimum standards for fresh or frozen-thawed semen for >6 months.

Scrotal circumference was consistently greater in bulls with normal versus abnormal semen from 7 to 24 months, with differences ($P<0.05$) at 20 and 24 months. Bulls classified as having abnormal semen due to high morphological defects and low motility had lower ($P<0.05$) plasma INSL3 concentrations than bulls with normal semen at 4 and 6-22 months of age. Bulls with normal semen had greater ($P<0.05$) plasma inhibin concentrations than those with abnormal fresh semen at 8-13, 16 and 19-20 months and those with low fertility post-thaw semen at 10-12 and 16-21 months. Plasma testosterone concentrations were higher ($P<0.05$) in bulls with normal versus abnormal semen only at 22 months. Plasma estradiol-17β concentrations were higher in bulls in the normal post-thaw semen group compared to low-fertility post-thaw semen group at 17-21 months of age. We inferred that reduced plasma concentrations of INSL3 and inhibin in young bulls contributed to poor semen quality. Reduced INSL3 would result in lower

anti-apoptotic properties and poorer germ cell survival. Peripheral inhibin concentrations were significantly lower in infertile men; perhaps lower inhibin concentrations in bulls results in increased FSH concentrations, with deleterious effects on Sertoli cell function.

Chapter 2: LH and testosterone concentrations in response to GnRH challenge in Japanese Black beef bulls with normal versus abnormal semen

Plasma testosterone concentrations only differed between bulls with normal and abnormal semen at 22 months of age (Chapter 1). We previously demonstrated that plasma concentrations of LH and testosterone increase with high amplitude after GnRH challenge in beef bulls. Therefore, our objective was to determine plasma LH and testosterone concentrations in response to a GnRH analogue (fertirelin acetate, 50 μg/100 kg) at 10 and 20 months of age in Japanese Black beef bulls, with blood sampling before treatment and at hourly intervals from 1 to 6 hours post treatment. Plasma LH concentrations increased more than 5-fold (effect of time, $P<0.001$) and peaked at 2 hours; there was a main effect of age ($P<0.05$), and although 20-month old bulls had higher LH at 3 to 5 hours, differences were not significant at any time point. Plasma testosterone increased ($P<0.001$) in response to GnRH and from 1 to 6 h after treatment, had plateaus of ~8 versus 10 ng/ml in 10 versus 20 months old bulls, respectively (age effect, $P<0.0001$). There were no significant differences between bulls with normal semen versus those with poor morphology or motility for GnRH-induced concentrations of LH or testosterone at 20 months of age. In conclusion, although plasma testosterone concentrations before and after GnRH treatment was augmented in post-pubertal (20 months) bulls, there was no significant effect of semen quality on post-GnRH concentrations of either LH or testosterone.

Chapter 3: Plasma metabolic hormone concentrations and serum biochemical parameters in Japanese Black beef bulls with normal versus abnormal semen

The objective was to compare peripheral concentrations of IGF-I, insulin and cortisol, as well as other common biochemical end points, between bulls with normal versus abnormal semen. Blood samples were collected monthly from 4 to 24 months of age for plasma concentrations of metabolic hormones and every 3 months from 6 to 21 months of age for serum biochemical end points. Semen was collected weekly from 12 to >18 months of age. In bulls with low-fertility post-thaw semen compared to those with normal fertility, there were reductions ($P<0.05$) in plasma IGF-I concentrations at 8-9, 11 and 15-21 months of age and in plasma insulin concentrations at 11 and 20 months. Plasma cortisol concentrations were higher ($P<0.05$) bulls with abnormal fresh semen compared to those with normal at 14 and 24 months. Serum concentrations of magnesium and of aspartate aminotransferase in bulls with abnormal fresh semen were higher ($P<0.05$) than those with normal semen at 6 and 9 months and at 9, 12 and 15 months, respectively. Serum creatinine concentrations were higher ($P<0.05$) in bulls with sperm morphological defects only compared to those with normal semen at 6-12 and 15 months. In the

present study, plasma concentrations of IGF-I were decreased in young bulls with low-fertility post-thaw semen. The presence of IGF-I receptors on sperm and IGF-I in semen, plus ability of IGF-I to stimulate sperm motility have been reported in beef bulls. Therefore, reduced plasma IGF-I concentrations in bulls with low-fertility post-thaw semen may have contributed to reduced fertilizing capacity.

Overall conclusions in Japanese Black bulls

1. Post-pubertal bulls with abnormal semen had significantly smaller SC than those with normal semen.
2. Bulls with abnormal semen had significantly lower plasma concentrations of INSL3 and inhibin than those with normal semen; perhaps those differences contributed to lower anti-apoptotic properties and poorer germ cell survival and increased FSH concentrations, with deleterious effects on Sertoli cell function.
3. Responses of LH and of testosterone secretions to GnRH challenge were not clearly altered in pubertal beef bulls with morphological defects plus low motility.
4. Plasma IGF-I concentrations were decreased in bulls with abnormal versus normal semen; perhaps lower IGF-I reduced fertilizing capacity.
5. Plasma INSL3, inhibin and IGF-I concentrations pre-puberty may be used to predict aberrant semen in pubertal beef bulls.

審査結果の要旨

日本の主要な肉用品種である黒毛和種牛は脂肪交雑が入りやすい遺伝的形質を保有している。その形質を維持・改良するために大多数の黒毛和種牛は人工授精または胚移植で繁殖が行われている。黒毛和種牛の精液を提供する種雄牛の価値は高く、その候補牛を子ウシの時期から飼育して優良な個体を種雄牛として選抜していく方法が採用されている。それら種雄候補牛の中には、性成熟後に不良な精液を示す個体がみられるが、その原因は不明となっている。精子の形成と成熟はテストステロン、インスリン様ペプチド 3（INSL3）およびインヒビン等の精巣ホルモンやインスリン様成長因子-I（IGF-I）といった栄養・代謝関連のホルモンによって促進されるが、過去に正常および異常な精液を示す黒毛和種牛の精巣・代謝ホルモンの血中濃度を比較・解析した報告は皆無である。さらに黒毛和種雄ウシにおいて栄養状態の指標となる血液生化学値を調査して、正常精液と異常精液で比較した報告も我々の知る限りみられない。本研究では、そのような背景から、正常および異常な精液を示す黒毛和種雄ウシの性成熟過程の血中精巣・代謝ホルモンと一般生化学項目の濃度の比較を行い、それらの因子と精液性状の関連性を検討している。

第 1 章では、正常および異常精液を示す黒毛和種雄ウシの血中精巣ホルモンと陰嚢周囲長を解析した。その結果、異常な新鮮精液を示す雄ウシの陰嚢周囲長は、性成熟後の限られた月齢でのみ、正常精液のウシよりも小さいことが示された。異常な新鮮精液（低運動性＋高奇形率）を示すウシの性成熟前から性成熟後までの血中 INSL3 濃度は正常精液のウシよりも顕著に低いことが判明した。また新鮮精液が異常なウシと凍結融解後精液が低受胎のウシの性成熟前から性成熟後までの血中インヒビン濃度は正常精液のウシよりも低いことが明らかにされた。

第 2 章では、正常および異常精液を示す黒毛和種雄ウシに性腺刺激ホルモン放出ホルモン（GnRH）剤を投与して血中黄体形成ホルモン（LH）とテストステロン濃度の上昇程度を調べた。その結果、異常な新鮮精液（低運動性＋高奇形率）を示す雄ウシの性成熟前後における GnRH に対する LH とテストステロンの上昇反応は正常精液のウシに比べて明瞭な差はみられないことがわかった。

第 3 章では、正常および異常精液を示す黒毛和種雄ウシの血中代謝ホルモンと一般生化学項目の濃度を調べた。その結果、凍結融解後精液が低受胎のウシの性成熟前から性成熟後までの IGF-I 濃度は正常精液のウシに比較して低下することが判明した。異常な新鮮精液を示すウシの性成熟前の血中マグネシウム濃度は正常精液のウシに比べて高く、精子の低運動性を示すウシの性成熟前の血中クレアチニン濃度は正常精液のウシに比較して高い結果がえられた。

以上の研究により、異常な精液を示す黒毛和種雄ウシの性成熟前後における INSL3、インヒビンおよび IGF-I の分泌は低下していることが示唆された。さらに性成熟前のそれらのホルモン濃度の低下から性成熟以降の精液不良を予知できる可能性が示された。これらの研究成果は、黒毛和種牛の精液不良の原因解明に寄与するとともに、優良な種雄牛の早期選抜技術の開発につながることから、獣医繁殖学領域における生殖内分泌学の発展に大きく貢献するものであり、本論文の審査ならびに最終試験の結果と併せて、博士（獣医学）の学位を授与することを適当と認める。