

学 位 論 文 要 旨

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題 目 : Study on genetic markers in the bovine *IARS* and *FOXP3* genes associated with reproductive performance in cattle

(牛の繁殖成績に関連する牛*IARS*および*FOXP3*遺伝子の遺伝的マーカーに関する研究)

論文要旨 :

Infertility in cows is a significant global concern associated with multiple unidentified factors, including those relating the maternal body, bull semen, nutrition, and the environment. Among the different maternal factors, genetic disorders and detrimental traits linked to inherent gene and chromosomal abnormalities also compromise reproductive efficiency in cattle. Besides, there are some biochemical markers that affect maternal fertility, including anti-Müllerian hormone (AMH), a glycoprotein belonging to the transforming growth factor-beta superfamily and secreted by ovarian granulosa cells primarily from pre-antral and early antral follicles of females, and serum amyloid A (SAA), one of the most reliable acute phase proteins primarily produced by the liver via stimulus of inflammatory cytokines. AMH and SAA are recently demonstrated to be associated with reproductive performance of beef and dairy cows. In this study, bovine isoleucyl-tRNA synthetase (*IARS*) disorder (Chapter 1) and bovine Forkhead Box P3 (*FOXP3*) alteration in combination with AMH and SAA concentrations (Chapter 2) were investigated in order to demonstrate the relationship between these maternal factors and cattle fertility.

Chapter 1: Bovine *IARS* disorder, a major cause of weak calf syndrome, is caused by a homozygous missense (c.235G>C) mutation in the bovine *IARS* gene of JB cattle, which was identified in 2013. However, the extent to which the carrier rate has changed at Kagoshima prefecture, Japan, and whether the carrier status is associated with any clinical or reproductive problems, have yet to be ascertained. In this study, using a real-time polymerase chain reaction-based genotyping assay, we determined the carrier rate in a regional JB cow population at Kagoshima prefecture. Comparative analyses were performed on the metabolic profile test (MPT) results and reproductive performance data obtained for heterozygous carrier and homozygous wild-type cows. In 2009 and 2018, DNA samples were collected from 130 and 462 clinically healthy JB cows, respectively, in Kagoshima

prefecture. Therefore, MPT results and reproductive performance data were evaluated for 62 cows, comprising four heterozygous carriers and 58 wild-type cows. Genotyping revealed that the carrier rate was 6.9% in 2009 and 1.5% in 2018, the difference of which was statistically significant ($P < 0.005$). There were no statistically significant differences between the carrier and wild-type cows with respect to either MPT results or reproductive performance, indicating that the carrier cows have necessary IARS activity to maintain minimal health and reproductive potential.

Chapter 2: Immune adaptation plays an essential role in determining pregnancy, which has been shown to be dependent on sufficient immunological tolerance mediated by FOXP3+ regulatory T cells. Recently, an X-linked maternal single-nucleotide polymorphism (SNP), located 2175 base pairs upstream of the start codon in the bovine *FOXP3* gene (NC_037357.1: g.87298881A>G, rs135720414), was identified in JB cows (*Bos taurus*) in association with recurrent infertility. However, with the exception of JB cows, the frequency of this SNP has yet to be studied in other cow populations. The objective of this study was to evaluate the frequency of this SNP in different cow breeds. Between 2018 and 2021, a total of 809 DNA samples were obtained from 581 JB, 73 Holstein Friesian (HF: *B. taurus*), 125 Korean Hanwoo (KH: *B. taurus coreanae*), and 30 Indonesian Madura (IM: a crossbreed between *B. indicus* and *B. javanicus*) cows, which were genotyped using a TaqMan probe-based real-time polymerase chain reaction assay designed in this study. The G allele frequency was found to be relatively high in local IM (0.700), moderate in dairy HF (0.466), and low in beef JB (0.250) and KH (0.112) cows, with differences in the frequencies between each group being statistically significant ($P < 0.005$) using Fisher's exact test. The results obtained in this study indicate that the G allele frequencies of the identified SNP differ markedly in different breeds of taurine and indicine cattle. Given these findings, it would thus be important to evaluate the relationships between high frequencies of the G allele and infertility in different breeds.

Moreover, in this study using HF cows, AMH concentration in parous cows with SNP A/A was significantly higher ($P < 0.05$) than that in parous cows with A/G and G/G, indicating that A/A parous cows may have a higher fertility than other genotypes of parous cows. There was not a similar observation in HF heifers. In the range of low SAA concentration, A/A parous cows tended to be more abundant than other types of parous cows. Based on these observations, parous HF cows carrying the G allele may be susceptible to infections, leading to subsequent inflammatory conditions. The inflammatory conditions possibly cause decreased concentration of AMH resulting in infertility.

In conclusion, in this study (Chapter 1), genotyping of the JB population in Kagoshima prefecture revealed that there remains a risk of producing both carriers and affected cows for *IARS* gene mutation. There was no statistically significant difference in the MPT results or reproductive performance of carrier and wild-type cows, indicating that the carrier cows have necessary IARS activity to maintain minimal health and reproductive potential. By detecting anomalies such as

underlying ARS deficiencies in cattle populations, these types of analyses could also be beneficially applied to facilitate maintenance of the equality of cow herds. Furthermore, on the basis of the results obtained in this study (Chapter 2), it was established that current frequencies of the detrimental G allele of the SNP in the upstream of the bovine *FOXP3* gene are particularly high in local populations of IM cows compared with those of three other assessed breeds, among which, G allele frequencies are low in KH and JB cows, and moderate in HF dairy cows. Given these findings, it would thus be important to evaluate the relationships between high frequencies of the G allele and infertility in different breeds. Given that the moderate to high G allele frequencies in IM and HF cows are plausibly associated with the infertility of these breeds, it would be interesting to evaluate the relationship between the high frequency of the risk-type G allele and infertility. In this regard, the genotyping assay developed in this study could make a notable contribution to surveying the bovine populations. In addition, on the basis of results regarding AMH and SAA, parous HF cows with wild-type A/A genotype had significantly higher AMH concentration than that in HF cows with other genotypes (G/A and A/A). Inflammatory condition indicated by an increased SAA concentration may potentially decrease AMH concentration particularly in G/A and A/A cows. These results suggest that the G allele may be associated with infertility in HF cows.

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