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学位論文内容の要旨
Abstract of the dissertation

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Effect of seminal plasma proteins on the normalization of endometrial epidermal growth factor profile and fertility in repeat breeder dairy cows

（精漿タンパク質がリピートブリーダー牛の子宮内膜における
上皮成長因子濃度変化および受胎性の正常化に及ぼす効果）

In dairy herds, repeat breeding is a major source of infertility. Incidence of repeat breeding is estimated between 10% and 24% in dairy herds. In normal cows, endometrial EGF concentrations peak twice, on Days 2-4 and 13-14 during the estrous cycle. Loss of EGF peaks has been found in about 70% of Holstein repeat breeder cows and this has been linked to infertility in repeat breeder cows. In an earlier study, hormonal treatment with an increased dose of estrogen normalized the EGF profile and restored fertility in repeat breeder cows. However, the efficacy of the treatment to normalize the EGF profile varied among herds from about 30% to 85% in the field. In addition, use of estrogen products in food animals has been restricted in many countries. Thus, it is necessary to develop another treatment option. The objective of this study was to examine the effect of seminal plasma (SP) or SP proteins on fertility of repeat breeder cows by evaluating the capacity of SP to normalize the endometrial EGF concentrations on Day 3 (≥ 4.7 ng/g tissue weight), an established indicator of recovery of fertility.

In Chapter I, the effect of SP to normalize the EGF profile was examined. Firstly, the effect of deposition sites (the vagina and uterus) of SP on the EGF concentrations on Day 3 in repeat breeder cows was examined. SP infusion into the vagina, but not uterus, at estrus normalized the EGF concentrations on Day 3. Then, the effect of volume of SP (0.5 ml or 10 ml of SP and 0.5 ml of SP diluted to 10 ml) on the EGF concentrations was examined. All groups with SP infusion increased the EGF concentrations and 10 ml of SP and 0.5 ml of SP diluted to 10 ml increased the EGF concentrations to the highest levels. Finally, the effect of SP infusion on fertility was examined. SP infusion normalized the two peaks of the EGF concentrations in about 60% of repeat breeder cows and resulted in higher pregnancy rates than controls (44.4 vs. 19.4%).

In Chapter II, SP protein with an activity to normalize the EGF profile was identified.

Firstly, SP protein was separated by gel filtration and 2-dimensional electrophoresis. A protein fraction with molecular weight range of 16-29 kDa and isoelectric point range of 5.8-7.0 was found to have the activity. Proteins in this area was identified using liquid chromatography with tandem mass spectrometry. Twelve protein spots that include 3 putative fertility-associated proteins: osteopontins (OPNs), transforming growth factor-β_1 and lipocalin–type prostaglandin D synthase were identified. Protein extracts of one of the OPN spots normalized the EGF concentrations on Day 3 in 26 out of 62 (41.9%) repeat breeder cows.

It is concluded that SP proteins could improve fertility in repeat breeder dairy cows by normalizing the endometrial receptivity that can be measured by the EGF profile. A form of OPN seems responsible for the activity of SP, although the present study could not provide direct evidence for OPN to improve fertility.