主論文の要約

Study on reproductive performance of dairy cows in Afghanistan and on hypothalamic regulator of reproduction in mammals

(ウシの繁殖成績向上に資するアフガニスタンにおける乳牛の現状調査

および哺乳類の視床下部生殖中枢制御因子の探索)

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The reproductive performance is a key trait in cattle, and successful pregnancy, gestation and lactation of cows directly leads to economic return for the farmers. Such information in Afghanistan is extremely limited and scarce. Therefore, the first part of the present study aims to survey the reproductive performance of crossbred cows to address major constraints in their reproduction in Afghanistan. Body condition, reproductive disorders, and feeding scheme were surveyed in dairy farms by trained interviewers together with private veterinarians who regularly serve for these farms. I found that more than 50% dairy cows have reproductive disorders, among them “anestrus” showed the highest percent ratio as a reproductive disorder. I also found that the body condition score (BCS) decrease, the more percentage of the cows showed anestrus in the cows, resulting in a significant difference in the occurrence of anestrus between poor and fair BCS. Further, the cows fed with only straw tended to show lower BCS compared with the animals fed with hay, straw and concentrates. Thus, the present study suggests that lower BCS of cows, which is possibly caused by poor feeding scheme, is closely associated with higher occurrence of anestrus in cows in Afghanistan. The results obtained in the present study may contribute to propose that the improvement of the feeding scheme may increase the reproductive performance of cross-bred cows in Afghanistan.

The hypothalamic kisspeptin neurons are considered to play a critical role in regulating mammalian reproduction and integrating humoral and neuronal inputs to control gonadotropin-releasing hormone (GnRH)/gonadotropin release in mammals. The second part of the study aims to investigate upstream regulator candidates for kisspeptin neurons. *Kiss1* (encoding kisspeptin) expression has been found in two hypothalamic regions: the arcuate nucleus (ARC) and anteroventral periventricular nucleus (AVPV) in rodents. I found that calcitonin receptor (CALCR) gene (*Calcr*) was highly expressed in the data of the next generation sequencing (NGS) analysis in isolated kisspeptin neurons taken from the arcuate

nucleus (ARC) of *Kiss1*-tdTomato female rats. I performed the *in situ* hybridization (ISH) for *Calcr* throughout the whole forebrain of adult ovariectomized Wistar-Imamichi rats implanted with a negative feedback level of estrogen (OVX + low E2 rats). Then, I performed a double ISH for *Calcr* and *Kiss1* in the brain regions, containing either the AVPV or ARC of female rats. I found that *Calcr* was co-expressed in 12% and 22% of *Kiss1*-expressing cells in the ARC and AVPV, respectively. The present result suggests that CALCR signaling may be involved in the regulation of reproductive function through direct control of ARC and/or AVPV kisspeptin neurons, and then GnRH/gonadotropin release in mammals.

In the last part of the present study, I investigated whether a central administration of amylin, a endogenous ligand of CALCR, affect gonadotropin release in female rats. Rat amylin (10pmol/2μl, dissolved in ultra-pure water (UPW)) was injected to the ARC of OVX + low E2 rats. Blood samples (100 μl) were collected every 6 min for 3 h from free-moving conscious rats via the atrial cannula. Plasma luteinizing hormone (LH) concentrations were determined by a radioimmunoassay. Regular LH pulses were found in both amylin-treated and vehicle-treated control rats. Amylin injection tended to increase the mean LH concentration, and the baseline level and amplitude of LH pulses, but the changes were not significant compared with vehicle-treated controls. In summary, the present study suggests that amylin-CALCR signaling may only have moderate role in control of GnRH/gonadotropin release in female rats. Further studies are needed to uncover the roles of amylin-CALCR signaling in the central regulation of reproduction in mammals.