Formation of Bovine Uterine Gland-Like Structures in a 3D Culture System.

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Embryonic mortality is one of the major reasons for the decrease in the conception rate of cattle. A high frequency of embryonic loss occurs between 8-16 days after insemination. During this period, the bovine conceptus rapidly elongates due to a variety of secretions from the uterine glands. Uterine gland knock-out ewes have been reported to show a conceptus elongation failure due to the lack of uterine gland secretions; therefore, the secretory function of uterine glands is important for the establishment of pregnancy. However, the secretory functions of uterine glands are not fully understood. Three-dimensional (3D) culture systems are widely used to maintain the morphology and function of epithelial cells *in vitro*. It has been reported that salivary and mammary glands elongate by Wnt proteins and growth factors in the 3D culture system, and show the structures resemble *in vivo* counterparts. The aim of this study was to investigate the effects of Wnt proteins and a growth factor on the morphology of 3D-cultured bovine uterine glands.

Bovine uteri were transported from a local abattoir to the laboratory after exsanguination, and uteri at 11-17 days after ovulation were used. The intercaruncular region of the endometrium was collected and, uterine gland fragments were isolated by enzymatic digestion. The uterine gland fragments were embedded in Matrigel for the 3D culture system and treated with or without Wnt proteins (Wnt3a, Wnt5a, and Wnt7a) and epidermal growth factor (EGF) as described below: (1) without Wnt proteins and EGF (control), (2) with Wnt proteins and EGF, (3) only with Wnt proteins, and (4) only with EGF. During 5 days of 3D culture, the morphology of uterine glands was observed in each culture condition. Furthermore, uterine glands before and after 3D culture were stained with a proliferation marker (Ki67) and an apico-basal polarity marker (ZO-1), and analyzed gene expression levels of secretory proteins from the uterine glands (*SERPINA14*, *MEP1B*, and *SPP1*) by qRT-PCR.

In the control, the fragments formed spherical structures with cavity (cysts) after the 3-5 days of 3D culture. On the other hand, in the presence of Wnt proteins and EGF, the cells of the fragments lost lumen and formed aggregates after 1-2 days of culture, and the aggregates showed the structures which resemble *in vivo* counterparts (uterine gland-like structures) accompanying elongation with twisting after the 3-5 days of culture. The presence of Wnt proteins was not important for the formation of uterine gland-like structures. During the elongation, proliferating cells were detected in

the whole area of uterine gland-like structures. Although uterine glands isolated from endometria consisted of only simple layers, uterine gland-like structures contained both simple and stratified layers. Meanwhile, ZO-1 was detected at the apical side of uterine gland-like structures same as uterine glands isolated from endometria. Compared to the cysts, the gene expression level of *SERPINA14* was significantly lower in uterine gland-like structures and *in vivo* counterparts. On the other hand, gene expression levels of *MEP1B* and *SPP1* did not significantly change among these structures. In summary, only EGF but not Wnt proteins contributed to the stratification and elongation of bovine uterine glands in the 3D culture system.