

Colony-Forming Potentials of Male Gonad-Derived Cells from Porcine Fetuses and Neonates

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In mammals, male germline stem cells are derived from primordial germ cells and share several characteristics. Despite many efforts to identify stem cells from porcine gonads, there is no study to demonstrate the ability of forming germ cell colony depending on developmental stages of gonads in mammals. In this study, we isolated and characterized porcine germline stem cells from developing fetuses and neonates using stem cell markers that are prevalently expressed in various tissues. Gonadal cells from developing gonad formed distinct colonies and showed similar morphology to mouse ES or EG cell colonies. The colony formation capacity appeared to be dependent on developmental stages of gonad. Colonies were analyzed by AP staining, lectin binding and immunocytochemistry using stem cell markers including SSEA-1, SSEA-3, Sca 1 and nestin. We also elucidated distribution of gonad derived cells showing stem cell markers in developing testis. Many stem cell markers were localized in the cells present in the seminiferous epithelium. To study further cellular identification and differentiation to specific functional cells *in vivo*, we transfected pGFP-LacZ into gonad derived cells. After 8 days, we found expression of GFP throughout the colonies. These results suggest obtained colonies derived from gonad have a pluripotency as a stem cells and its formation is dependent on developing stages. Furthermore, obtained GFP expressing colonies will be useful to study functions of gonad derived stem cells *in vivo*, and may be utilized for germline cell nuclear transplantation.

Key words) *Porcine, Germline stem cells, Colony formation capacity*