HOKKAIDO UNIVERSITY

Title	Isolation methods of bovine primary follicles and their developmental capacity
Author(s)	YAMAMOTO, Naoto
Citation	Japanese Journal of Veterinary Research, 48(1), 83-83
Issue Date	2000-05-31
Doc URL	http://hdl.handle.net/2115/2839
Type	bulletin (article)
File Information	KJ00003408173.pdf

Instructions for use

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Isolation methods of bovine primary follicles and their developmental capacity

Naoto YAMAMOTO

Laboratory of Theriogenology,
Department of Veterinary Clinical Sciences,
School of Veterinary Medicine,
Hokkaido University, Sapporo, 060-0818, Japan

Several researches on the collection and culture methods of bovine primary follicles have been carried out to clarify the mechanisms of follicular growth and to produce embryos from immature follicles *in vitro*. However, there is no report showing that primary follicles can be successfully grown to antral stage in cattle. It is necessary for the *in vitro* production of antral follicles from primary follicles to establish the methods of follicle isolation and to define a criteria of follicle selection.

In experiment 1, primary follicles were collected using grater (method A), stainless strainer (method B), or handmade tissue chopper (method C). The efficiency of these methods was evaluated according to the number of primary follicles collected and the viability of the oocytes stained with Hoechst 33258. Follicles were morphologically classified as normal when they had a clear and compact granulosa cell layer and a lucid oocyte, and as abnormal when granulosa cell layer had vacuole and/or a dark condensed oocyte were present. Although the number of follicles isolated from a ovary was smaller in the method B than in A and C, the viability of oocytes was the highest in the method B. The total number of follicles with viable oocytes in the method B was highest. The viability of the oocytes of morphologically normal follicles (77.8%) was higher than that of abnormal follicles (<10.0%, $p<0.05$) regardless the collection method. In experiment 2, morphologically normal primary follicles were cultured for 8 days to evaluate the morphology of follicles and the viability of oocytes at the end of the culture period. Forty-five percent of cultured follicles maintained their normal morphology and oocytes viability. Morphologically normal cultured follicles had a comparable number of the granulosa cells to *in vivo* grown follicles of the similar size. The number of the granulosa cells of the morphologically abnormal cultured follicles was smaller than that of *in vivo* grown follicles of similar size.

In conclusion, the present study demonstrated that the number of primary follicles and the viability of oocytes depend on collection methods. Results also suggested that primary follicles with normal developmantal capacity could be the selected according to their morphologies in the granulosa cells and oocytes. In addition to the follicular size, the total number of the granulosa cells could be used as a good criterion to assess the follicular growth *in vitro*.