Growth Activity of Bovine Herpesvirus 1 in Bovine Follicular Oocytes with Cumulus Cells
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ABSTRACT. Bovine follicular oocytes collected from bovine ovaries were exposed to bovine herpesvirus 1 (BHV-1). After washings, these oocytes were cultured to mature. As a result BHV-1 could not be removed from the oocytes and could replicate in the oocytes with cumulus cells, but not in the oocytes without the cells. Moreover, the specific fluorescence for BHV-1 was detected in the cumulus cells by an indirect immunofluorescent technique. Therefore these findings suggested BHV-1 could be absorbed in the oocytes but the replication of BHV-1 was done in the cumulus cells.—KEY WORDS: bovid herpesvirus 1, bovine follicular oocyte, cumulus cell.


Bovine embryo transfer is an important technique in animal husbandry and is useful not only for the production of twins but also for the improvement of genetic disposition. Many studies have been made to examine the transmission of infectious agents to embryos. Especially, Bowen [4] reviewed the viral infection of preimplantation embryos. Eaglesome et al. [6] and Singh [11] have reported on the infectivity of embryos after virus exposure. Recently, embryos used for the transfer to a recipient have been collected not only from a superovulated donor, but also from the produced in vitro fertilization (IVF) of a cow. Bovine follicular oocytes with cumulus cells are needed for the IVF process. However, there has been little information about the susceptibility of bovine follicular oocytes with cumulus cells after virus infection. It is well known that bovid herpesvirus 1 (BHV-1) can infect genital organ and cause abortion or infertility in cow [7]. In the present study, we examined the growth activity and the growth region of BHV-1 in bovine follicular oocytes with cumulus cells.

Bovine follicular oocytes were collected from small follicles (25 mm or less) of bovine ovaries by means of aspiration of syringes. The ovaries were removed from slaughtered cows which were seronegative to BHV-1. The oocytes were divided into two groups; with and without cumulus cells. The medium used for washing and maturation of the oocytes was TCM-199 (25 mM HEPES, Earle salt) with 5% fetal bovine serum that was found seronegative for antibody to BHV-1, 100 μg/ml streptomycin and 100 μg/ml penicillin. BHV-1 Los Angeles strain (ATCC-VR188) was prepared from the Madin Darby bovine kidney (MDBK) cell culture. In experiment 1 (Exp. 1), 25 of the bovine follicular oocytes with cumulus cells were exposed to the BHV-1 at the level of 4×10^5 plaque forming unit (pfu)/ml for 1 hr at 37°C. After the incubation, the oocytes were washed by passing them through ten 100 μl drops of medium under mineral oil. A fresh micropipette was used to transfer the oocytes to each successive drop for washing. Five of the oocytes were collected at the 2, 4, 6, 8 and 10th washings. Each of the samples was added to 1 ml of Earle's balanced salt solution (0.5% lactalbumin hydrolysate, 0.2% bovine serum albumin, 100 μg/ml streptomycin, 100 μg/ml penicillin, and 5 μg/ml fungizone) and treated by ultrasonic. They were assayed by means of plaque production with monolayers of the MDBK cells in 6 well microplates (Falcon, U.S.A.). In Exp. 2, 10 or 12 of the bovine follicular oocytes with and without cumulus cells were exposed to BHV-1 at 4×10^5 pfu/ml and 4×10^6 pfu/ml for 1 hr at 37°C, respectively. After 8 times washing, half (5 or 6) of the oocytes were frozen immediately and assayed as virus titer of 0 hr. Five or six of the remaining oocytes were cultured to mature for 24 hr at 39°C under 5% CO2 in air. They were assayed by the plaque method. In Exp. 3, 20 of the bovine follicular oocytes with cumulus cells were exposed to BHV-1 at 4×10^5 pfu/ml and incubated for 72 hr after 8 times washing. Five of the oocytes were collected at each time (0, 24, 48 and 72 hr) and assayed by the same method as Exp 2. As control, unoinoculated oocytes with cumulus cells were prepared to observe the normal form of the oocytes and the cumulus cells in both Exp. 2 and Exp. 3. An indirect immunofluorescent technique was performed to determine the replication of BHV-1 in bovine follicular oocytes with cumulus cells at 24 hr after exposure using immune rabbit serum of neutralizing antibody titer 1:128 against BHV-1.

Fig. 1. Relationship between the number of washings and the virus titer of bovine follicular oocytes with cumulus cells exposed to BHV-1 at 4×10^5 pfu/ml. The results are shown as the mean values and standard deviations for four experiments.
The relationship between the number of washings and the titer of recovered virus in follicular oocytes is shown in Fig. 1. Exposed virus was almost washed out by 6 times. In growth activity of BHV-1, the virus could replicate in the follicular oocytes with cumulus cells, but not in the oocytes without the cells (Fig. 2). The virus titer was observed to reach the peak at 24 hr and it generally decreased after that (Fig. 3). BHV-1 antigen was detected in the cumulus cells by fluorescent antibody examination (Fig. 4) and not in the uninoculated cumulus cells. No remarkable difference in form was observed between the infected oocytes and non-infected control oocytes with cumulus cells at 24 hr by a stereoscopic microscope.

In handling of bovine embryo, washing 10 times is stipulated to remove pathogens according to the International Embryo Transfer Society manual [12]. However, Singh [10] observed that exposed BHV-1 could not be completely removed from bovine embryos, even if the embryos were washed 10 times. The present experiments demonstrated that the most of BHV-1 were washed out by 6 times but a few viruses were still absorbed in the bovine follicular oocytes with cumulus cells after 10 times washings.

Previous works indicated that many viruses did not
penetrate to the zona intact embryo [6] and some viruses were difficult to replicate in zona free embryo [1, 3, 8, 9]. All the experiments in this study were performed by embryos after fertilization and it is still unknown how viruses affect the follicular oocytes with cumulus cells. Meanwhile, in the relationship between BHV-1 and embryos, BHV-1 attached to the zona pellucida of embryos and could not penetrate its structure to gain access to the embryonic cells. However, BHV-1 could replicate in zona free embryos and induced the embryo death [1, 2, 5].

In the present study, BHV-1 could replicate in the bovine follicular oocytes with cumulus cells similar to zona free embryos, but not in the oocytes without the cells. Moreover, the detection of the specific fluorescence for BHV-1 was coincident with the virus recovery from the oocytes with cumulus cells. These findings strongly suggest that the growth activity of BHV-1 depends on the existence of cumulus cells. And the oocytes infected with viruses are difficult to be distinguished from the non-infected oocytes by means of gross examination, though the virus titer increased over $10^3$ pfu/ml in mature culture. It should therefore be exercised with great caution to handle bovine follicular oocytes with cumulus cells for IVF to prevent the infectious agents, especially viruses. Further study will be necessary to determine whether BHV-1 has any effect on the maturation and development process of bovine follicular oocytes and to test the susceptibility of other viruses for the cumulus cells.

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REFERENCES