Effects of Storage Conditions of Bovine Ovaries and Oocytes on the Success Rate of *in Vitro* Fertilization and Culture

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Abstract. The present study was carried out to examine effects of storage conditions of bovine ovaries and/or oocytes on the success rate of *in vitro* fertilization (IVF) and subsequent *in vitro* culture (IVC) of the zygotes. Ovaries obtained from a slaughter house were stored in Ringer solution at 39C (group 1), 20C (group 2), or 4C (group 3). In another group, immature oocytes were isolated from ovaries and stored in TCM-199 medium at 39C (group 4). The entire collection procedures of the ovaries took approximately 3 h at the slaughter house from the time of slaughter. The ovaries and/or the oocytes were stored under the conditions described above for 5 h before the experiment. In the control group, oocytes were isolated from the ovaries and cultured immediately to induce maturation. Following IVF and IVC, none of the zygotes developed to morula stage in group 1 and 3. In group 2, some zygotes developed to morula or blastocyst stage (11%, 7% respectively), but the rates were significantly lower than in the control group (32%, 15%). Group 4 gave high success rates (38%, 13%) similar to those attained in the control group.

Key words: Ovary storage, *In vitro* fertilization, Bovine oocytes.

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To carry out *in vitro* fertilization (IVF) of bovine oocytes, the ideal situation would be to proceed IVF immediately after the collection of ovaries at a slaughter house. Practically, however, it usually takes a long time to collect ovaries and bring them from a remote slaughter house to a laboratory. This is particularly true in Japan where only a small number of slaughter houses cover large areas. The method for storing the ovaries for several hours is particularly useful.

By culturing 8-cell stage IVF embryos in rabbit oviducts, Shioya *et al.* [1] reported that storage of bovine ovaries at 38C for several hours reduced the rate of blastocysts formation. If oocytes were isolated from ovaries at a slaughter house within 40 min after slaughter, 28.6% of 8-cell stage embryos developed to blastocysts, whereas only 14.3% of 8-cell stage embryos did so, if ovaries had

been stored for 3 h. The object of this paper is to find an optimal storage condition of bovine ovaries and oocytes for IVF and the subsequent culture of the zygotes *in vitro* (IVC).

Materials and Methods

Storage of ovaries and immature oocytes

Ovaries of adult cows and heifers were obtained from a slaughter house. They were divided into 5 experimental groups. In group 1–3, the ovaries were stored in Ringer solution (RS) at different temperatures of 39C (group 1), 20C (group 2), or 4C (group 3). In group 4, the ovaries were stored in RS at 39C during their collection, and then immature oocytes were sucked from the follicles having a diameter of 1–5 mm into a syringe with an 18G needle [2], stored in 10 ml of TCM-199 medium supplemented with 25 mM HEPES in a

glass test tube kept at 39C. Group 5 was a control, where immature oocytes were isolated from the follicles immediately after the collection of the ovaries, and cultured to induce oocyte maturation.

Since it took about 3 h for the collection of the enough number of ovaries in the slaughter house, the average duration of the storage of the ovary is considered as 1.5 h. The ovaries in group 1–3 and the oocytes in group 4 were kept for additional 5 h at the designated temperature in a thermos bottle.

IVF and IVC

The procedures of IVF and IVC are same as those described by Nakao and Nakatsuji [3]. Collected oocytes of all groups were cultured for 20 h in TCM-199 to complete the maturation process. The matured oocytes thus prepared were fertilized by the capacitated sperm following ionophore treatment [4]. Ova which retained their cumulus cells were cultured in plastic culture dishes (Falcon 3001) containing TCM-199 for 48 h. When the cumulus cells dissociated themselves, the embryos were transferred and cultured further on the monolayer culture of cumulus cells [5] in TCM-199 medium supplemented with 2% FCS at 39C under an atmosphere of 5% CO₂, 5% O₂, and 90% N₂.

Results and Discussion

Table 1 shows results of the experiment. We obtained no embryonic development beyond 8-cell stage in group 1 and 3.

The oocytes from the ovaries stored at 20C

(group 2) developed to morula and blastocyst stages at significantly lower rate than in the control group. On the other hand, group 4 gave a similar rate of development to morula and/or blastocyst stage as the control group.

Shioya *et al.* [1] reported that storage of the isolated oocytes in TCM-199 at 39C immediately after the collection of the ovary improved the rate of the successful development to the blastocyst stage, in comparison with the ovaries stored *in toto* at a slaughter house. Thus the isolated oocytes can be stored for a longer period than those *in toto*.

In excised ovaries, lack of the blood circulation will produce deteriorating conditions for the oocytes, such as low oxygen tension and accumulation of toxic metabolites. Lowering the storage temperatures of the excised ovary or isolation of oocytes shortly after the slaughter will avoid these effects.

Yang *et al.* [6] investigated the cleavage rate of the bovine embryos derived from IVF of the oocytes obtained from the ovaries stored in PBS at 37C, 25C, or 4C, found a drastic decrease of the cleavage rate (from 69.4% to 8.0%) when the ovaries had been stored for 8 h at 37C, and concluded that the storage of ovaries at 25C was optimal in this regard. In our study for the development of embryos, the storage of ovaries at 20C gave results better than the storage at 39C or 4C. The reason is unknown for why follicular oocytes stored in lower temperature (4C) gave worse result than those stored in higher temperature (20C).

Table 1. Development of bovine IVF embryos after storage of ovaries and/or oocytes under various conditions.

	Condition of storage				No. of oocytes	No. of embryos (%)		
	stored as	temperature	time	medium		8-cell	morula ^{a)}	blastocyst ^{b)}
Group 1	ovary	39C	6.5h	RS ^{c)}	114	1 ^c	0	0
Group 2	ovary	20C	6.5h	RS	172	34(20%) ^b	19(11%) ^b	12(7%) ^b
Group 3	ovary	4C	6.5h	RS	153	5(3%) ^c	0	0
Group 4	ovary	39C	1.5h	RS	138			
	↓			↓	↓			
	oocyte	39C	5.0h	TCM-199	138	51(37%) ^a	38(28%) ^a	18(13%)
Group 5	ovary	39C	1.5h	RS	94	46(49%) ^a	30(32%) ^a	14(15%) ^a

a): Examined at 120h after insemination.

b): Examined at 168h after insemination.

c): Ringer solution.

Means with different superscripts are significantly different ($p < 0.05$).

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