—Technical Note—

The Efficacy of the Water Purification System with an Ultra Violet Lamp and Ultrafilter for the Preparation of Bovine Embryo Culture Media

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Abstract. The efficacy of a water purification system including a high-intensity 185 nm ultra violet lamp with ultrafilter (UV-UF) for eliminating endotoxins was examined. The activity of endotoxins was below the detectable level in UV-UF water. In contrast, ultra-purified water from a system without a UV lamp (UP) showed a high level of endotoxins, despite water resistivity of more than 18 M Ω -cm. Bovine IVM/IVF zygotes were cultured for 174 h in protein-free media prepared with UV-UF and UP water. The developmental rate to blastocysts at 150 h was significantly higher in the medium prepared with UV-UF water. The results demonstrate that the UV-UF system is beneficial for eliminating endotoxins from ultra-purified water, and for preparing protein-free media for culture of bovine embryos.

Key words: Bovine, Embryo, Endotoxin, Water quality.

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Water is a major component of embryo culture medium, and high-quality water is necessary for its preparation [1–4]. It is recommended to prepare culture media using reverse-osmosis (RO), deionized and ultra-filtrated water with a resistivity of at least 18 M Ω -cm [5]. However, it has been demonstrated that the water ultra-purified with a resistivity of more than 18 M Ω -cm contains some organic carbons, endotoxins and planktonic bacteria [6, 7]. Endotoxins in the media suppress the embryonic development in mice [8–11] and in the human [12, 13]. UV irradiation in the water purification system reduces the planktonic bacteria population in the system, and decreases the concentrations of total organic carbons and endotoxins in the purified water [6]. Further reduction in the concentrations of total organic carbons and endot-

oxins can be expected by the addition of an ultrafilter located downstream from the UV lamp, since endotoxins are relatively large molecules.

The present study evaluated the efficacy of a water purification system including a UV lamp with an ultrafilter for the elimination of endotoxins. Moreover, bovine embryonic development was determined by culturing embryos using protein-free media prepared with water from the above system, since there is no report which studies the effect of endotoxins on the development of bovine embryos using protein-free media.

Materials and Methods

Water for media preparation

We prepared ultra-purified water using the following two water purification systems (Fig. 1): UV-UF; The system had a four-cartridge purifica-

tion pack (Q-pack; Nihon Millipore Ltd., Tokyo, Japan), which included activated-carbon and ion-exchange resins housed within 4 cylinders, a high-intensity 185 nm UV lamp plus a polishing cartridge (Milli-Q SP TOC, Nihon Millipore) with an ultrafilter (Nihon Millipore) to remove organic carbons. This system was provided with water purified by a Milli-RX 12 Plus (RO-treated and deionized water, Nihon Millipore). UP; The system included an ultrafilter, ion-exchange fibers, and was provided with RO-treated and deionized water. The function of this system was equivalent to that of Q-pack. A final filter (pore size, 0.22 μm) was also a part of the system at the dispensing point of both units. Water provided from both systems showed resistivity of more than 18 M Ω -cm. Total organic carbons in UV-UF water were analyzed with an A-100P portable analyzer (Anatel Co. Ltd., Colorado, USA). Endotoxin activities of the water from the two systems were determined using a Toxicolor endotoxin kit (Toxicolor system LS-6 Set; Seikagaku Co. Ltd., Tokyo, Japan). Endotoxin from *Escherichia coli* 0111:B4 (Seikagaku Co. Ltd.) was used as the standard.

Source of zygotes

Bovine zygotes were obtained after *in vitro* maturation and fertilization as described elsewhere [14]. In brief, bovine cumulus-oocyte complexes (COCs) from small antral follicles of abattoir ovaries were cultured for about 22 h in a Hepes-buffered TCM 199 (Gibco Laboratories, Grand Island, NY, USA) supplemented with fetal calf serum, follicle-stimulating hormone, estradiol-17 β , sodium pyruvate and gentamicin sulfate. *In vitro* fertilization was conducted using frozen semen from a Holstein bull. The motile sperm were separated using a discontinuous gradient with 45 and 90% Percoll (Pharmacia BioProcess, Uppsala, Sweden). The COCs were co-incubated with sperm at the concentration of 5×10^6 cell/ml in 100- μl droplets of modified Brackett and Oliphant isotonic medium (mBO medium) containing fatty-acid free BSA and theophylline covered with paraffin oil at 39 C in a humidified atmosphere of 5% CO₂, 5% O₂ and 90% N₂. We used UV-UF water to prepare mBO medium. After 18 h of co-incubation, presumptive zygotes were stripped of cumulus cells by vortexing in Hepes-buffered Tyrode's medium.

Fig. 1. Diagrams of the water purification systems. UV-UF: The system had a four-cartridge purification pack (Q-pack), a high-intensity 185 nm UV lamp plus a polishing cartridge and an ultra filter. UP: The system included an ultrafilter and ion-exchange fibers. The function of this system was equivalent to that of the Q-pack.

In vitro culture of zygotes

A modified synthetic oviduct fluid supplemented with 20 amino acids, 10 $\mu\text{g}/\text{ml}$ insulin and 1 mg/ml polyvinyl alcohol (mSOFai) [14] was used as a protein-free medium. Cumulus-free zygotes were cultured for another 174 h in 40- μl droplets (about 40 zygotes per droplet) of the protein-free culture medium prepared with UP or UV-UF water covered with paraffin oil at 39 C in a humidified atmosphere of 5% CO₂, 5% O₂ and 90% N₂. Embryonic development was evaluated at 150 and 174 h of culture in the protein-free media.

Results and Discussion

Endotoxins were below the detectable level (<0.001 EU/ml) in UV-UF water, but were detected in UP water (0.225 EU/ml = 78 pg/ml). UV-UF water contained a low level (2 $\mu\text{g}/\text{l}$) of total organic carbons. Although the concentration of total organic carbons in UP water was not measured,

Table 1. Development of bovine zygotes in protein-free media prepared with ultrapure water from two different types of water purification system

Item	Water source	
	UP ^{a)}	UV-UF ^{a)}
No. of replicates (No. of oocytes)	6 (213)	6 (220)
% of cleavage	78.8 ± 5.6	80.0 ± 4.0
% of blastocysts at 150 h	19.5 ± 3.3 ^{c)}	26.4 ± 6.3 ^{d)}
174 h	31.8 ± 11.8	38.6 ± 2.7
% of hatching rate ^{b)} at 174 h	22.3 ± 19.0	23.6 ± 13.4

% Values are means ± SD.

a) See Fig. 1.

b) % based on the number of blastocysts.

c, d) Values with different superscripts differ significantly ($p < 0.05$).

UP water should contain a higher level of total organic carbons than UV-UF water, since there is a positive correlation between total organic carbon contents and endotoxins concentration [6]. It was not clear why UP water showed such a high activity of endotoxins. In the conventional water purification system without a UV-UF unit, the contamination with a high level of endotoxins in the source water and the bacterial growth in a water purification system result in the high activity of endotoxins in the ultra-purified water [6]. The endotoxins activity in the source water for the UP system was over the measurable range of the Toxicolor endotoxin kit even after 11-times dilution. Whereas the activity of endotoxins was not detected in UV-UF water, in spite of the source water for the UV-UF system was contaminated with endotoxins at the activity beyond the measurable range. These results suggested the efficiency of the UV-UF system in the elimination of endotoxins. Further it is highlighted that the importance of routine evaluation of total organic carbons and/or endotoxins in ultra-purified water derived from water purification systems without a UV-UF unit, even if they could provide an ultra-purified water with the resistivity of more than 18 MΩ·cm.

There were no differences in the proportions of cleaved zygotes and hatching blastocysts between the two media (Table 1). However, the developmental rate of embryos to blastocysts at 150 h of culture was significantly higher in the medium prepared with UV-UF water than in the medium prepared with UP water ($P < 0.05$; Student's *t*-test). Same tendency ($P = 0.10$; Student's *t*-test) was seen in the percentages of blastocyst at 174 h of culture. These results demonstrated that UV-UF water was suitable for preparing protein-free media for cul-

ture of bovine embryos. This may be due to the lower activity of endotoxins in UV-UF water. Endotoxin contamination was reported not to affect the *in vitro* development of bovine embryos when they were co-cultured with somatic cells in medium containing serum [15]. In the presence of somatic cells and proteins, the detrimental effects of endotoxin might be eliminated [16]. As we used protein-free media, we could make the effect clear.

A reduction in the pregnancy rates has been demonstrated after the transfer of human embryos cultured in media containing relatively low concentrations (1 pg/ml) of endotoxins [12, 13, 17], without a reduction in blastocyst formation rate [17]. Thus, it is likely that the pregnancy rate decreases after the transfer of bovine embryos cultured in the medium prepared with UP water due to a high level (78 pg/ml) of endotoxins. Further studies are needed to determine the dose-dependent effects of endotoxins on the *in vitro* development of bovine embryos and on their subsequent development *in vivo* after being transferred to the recipient animals.

It is concluded that the UV-UF system is beneficial to the elimination of endotoxins from ultra-purified water, and to the preparation of protein-free media for *in vitro* culture of bovine embryos.

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