Interaction between Cytopathic and Non-cytopathic Bovine Viral Diarrhea Virus and Sperm Cells on In Vitro Fertilization of Bovine Oocyte

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Abstract

Bovine Viral Diarrhea Virus (BVDV) is a major cause of bovine reproductive failure. The objective of the present study was to detect a possible effect of incubation of sperm cells with CP and NCP BVDV virus on the process of in vitro fertilization (IVF). One hundred compact cumulus-oocyte complexes were selected after aspiration of all follicles (1.6 mm). They were matured during 24 hours in maturation medium (TCM 199 bicarbonate buffered) without oil at 38.5°C in 5% CO₂ and 100% humidity. The Oregon C24V strain of CP BVDV and isolate No 22146 strain of NCP BVDV were used at a virus stock titer 10⁻⁶ and 10⁻⁵ tissue culture infectious dose (TCID₅₀/ml) respectively. Frozen thawed sperm of a BVDV free bull was put on top of a Fertoll gradient (45% and 90%). The final concentration of sperm was adjusted to 30° and 15° cells/ml. Sperm cells were incubated with 10⁻⁶, 10⁻⁵ and 10⁻⁴ CP BVDV or 10⁻⁶, 10⁻⁵ and 10⁻⁴ NCP BVDV TCD₅₀/ml during 2 hours at 38.5°C with 5% CO₂ and maximum humidity. Twenty-five 1 of 15° or 10⁻⁵ BVDV incubated sperm cells was added to 24 1 droplets under oil. Ten cumulus-free oocytes were added to each droplet and incubated overnight allowing to be fertilized. Preovulated oocytes were vortexed to removed the sperm cells and fixed in 2% formaldehyde and 2% glutaraldehyde in PBS. Three replicates were performed. The logistic regression test was used for analysis of data (statistic 4.1). This study showed that only the highest infectious titer of CP BVDV (10⁻⁴ TCD₅₀/ml) in combination with the lowest final sperm concentration (10⁶ sperm/ml) decreased fertilization significantly compared with the control (5% vs 15%) (p<0.01).

Key words: BVD virus, IVF, Sperm.

Introduction

Bovine Viral Diarrhea Virus (BVDV), a small enveloped RNA virus that belongs to the genus Pestivirus in the family Flaviviridae (4). BVDV is a major cause of bovine reproductive failure from the time of conception until well after calving. It is classified into 2 biotypes: Cytopathic (CP) and Noncytopathic (NCP). The presence of CP and NCP BVD Virus during bovine In Vitro Fertilization (IVF) results in lower cleavage rates and embryo development (11). The objective of the present study was to detect a possible effect of incubation of sperm cells with CP and NCP BVD virus on the process of IVF.

Material and Methods

in vitro oocyte maturation

Bovine oocytes were matured using routine techniques. Briefly, compact cumulus oocyte complexes were selected after aspirating all follicles from slaughterhouse ovaries. Maturation was accomplished in groups of 100 oocyte in 500 μl of maturation medium (no oil overlay) during 24 hours of incubation at 38.5°C in 5% CO₂ in air. Maturation medium consisted of TCM199 bicarbonate buffered medium supplemented with 7.5% Hyclone serum (v/v), 0.5 mM pyruvate.

To obtain cumulus free oocytes, mature cumulus oocyte complexes were vortexed in Hepes TALP for 2-4 min at maximum speed to remove adherent cumulus cells before being subjected to in vitro fertilization (IVF).

Virus

The origin of the virus was the cytopathic strain (CP BVDV) the Oregon C24V isolate and for the non-cytopathic strain (NCP BVDV) isolate No. 22146 (11). They were propagated in MEM plus 5% Fetal Calve Serum (FCS).

Experimental design

Frozen sperm of a bull was thawed in a water bath at 37°C and put on top of a Fertoll gradient (45% 90%). To separated living from dead spermatozoa, the sperm were centrifuged for 30 minutes at 2000 rpm. After centrifuged, the supernatant was removed and live sperm were resuspended in TALP + BSA and centrifuged once more for 10 minutes at 35 rpm. The resulting sperm pellet was resuspended to obtain a final concentration of 10⁶ sperm cells/ml in IVF. TALP medium with heparin 10 U/ml was incubated for 30 min without heparin and diluted to obtain a concentration of 10⁴ and 10⁵ sperm cells/ml, respectively.
Controls for influence of MEM medium on fertilization:
1 ml of MEM medium was diluted with 1 ml of sperm cells at 10⁵ Sp/ml
1 ml of MEM medium was diluted with 1 ml of sperm cells at 10⁶ Sp/ml
1 ml of IVF medium was diluted with 1 ml of sperm cells at 10⁵ Sp/ml
1 ml of IVF medium was diluted with 1 ml of sperm cells at 10⁶ Sp/ml

CP & NCP BVDV
1 ml of pure CP & NCP BVD virus suspension (titer=10⁴.⁵ and 10⁴.⁶, respectively) was diluted with
1 ml of sperm cells at 10⁵ Sp/ml.
1 ml of virus suspension diluted 1:10 in MEM medium was diluted with 1 ml of sperm cells at 10⁶ and
10⁷ Sp/ml.
1 ml of virus suspension diluted 1:100 in MEM medium was diluted with 1 ml of sperm cells at 10⁶ and
10⁷ Sp/ml.

All these groups were incubated for 2 hours in the CO2 incubator at 39 C to allow virus
contamination of the sperm cells. They were centrifuged for 10 minutes at 1500 rpm and the
concentration was adapted with IVF-TALP - heparin to the double concentration of the initial
concentration. In the meantime vortexing of cumulus-oocyte complexes was performed. Twenty five
of the sperm suspension was added into 20 cumulus-free oocyte were added to each droplet and incubated overnight. Three replicates were performed.

Evaluation of fertilization:
After 18-20 hours of sperm-oocyte co-incubation, any remaining sperm cells were removed from
the oocytes by 1 minute of vortexing. Then the zygotes were fixed in 2% formaldehyde, 2%
guanidinethiohydrazide in PBS overnight and then stained with Hoechst 1 or 10 mg/ml N-ethanol. After mounting in DABCO, they were evaluated for the presence of 2 pronuclei by means of fluorescence microscopy.

The logistic regression test was used for analysis of data (statistic 4.1).

Result and Discussion:
The study showed that the highest infectious titer of CP BVDV (10 5.5 TCID 50/ml) in combination
with lowest final sperm concentration (10 ⁴ Sp/ml) decreased fertilization significantly compared with
type control (4% v. 19%) (P<0.05) (Table 1). Whereas NCP BVDV had no significant influence on the
fertilization rate at the three infectious titters which were used (P<0.05) (Table 2).

Table 1. Fertilization rate using sperm cells incubated with CP BVDV virus.

<table>
<thead>
<tr>
<th>Sperm/ml</th>
<th>control</th>
<th>CP (TCID50/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>10⁵</td>
<td>10¹⁵</td>
</tr>
<tr>
<td></td>
<td>19% (0-75)</td>
<td>7% (0-09)</td>
</tr>
<tr>
<td></td>
<td>10⁶</td>
<td>57% (42-74)</td>
</tr>
<tr>
<td></td>
<td>54% (40-72)</td>
<td>58% (50-75)</td>
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</tbody>
</table>

Significant difference (P<0.05) with the fertilization rate in the control within the same row
This study showed that infected sperm cell (10⁵ sp/ml) with CP BVDV (10⁻⁵ TCID₅₀/ml) affected significantly on fertilization but NCP BVDV did not influence in compare with control group.

It is reported that in vitroculture of in vivo embryos with CP BVDV had a severe adverse effect on survival embryonic development (5,6,9). Since there are some considerable cellular differences between in vivo and in vitro-produced embryos, extrapolation of in vivo results to in vitro-produced embryos is difficult (10,11).

Dehnavini (2) showed that after the use of semen from persistently infected with NCP BVDV, cleavage rate and development to the blastocyst stage were not significantly reduced in IVF. Whereas Gurin and coworkers demonstrated that embryonic development was significantly reduced in the presence of NCP BVDV (7). The reason for the differences in susceptibility to the two bovine is not clear. First, it must be mentioned that little is known about molecular mechanisms responsible for virus entry into susceptible bovine cells. Glycoproteins, especially gp120 and gp91, present in the viral envelope are thought to be involved in attachment of the virus to the virus. Hence, penetration of the virus into the host cell (5,6). They suggested that multiple receptors for BVDV attachment may be present and that different virus strains do not necessarily have the same receptor. In addition, it is thought that BVDV utilizes more than one receptor for attachment, and that binding of virions to the cell surface may be multiple event (8). Regarding these theories, it is possible that CP BVDV and NCP BVDV use different receptors.

At present, further research is being conducted to investigate the interaction between BVDV and spermatozoa in IVF.

References