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

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Abstract

Bovine Viral Diarrhea Virus (BNDV) 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 BVD virus on the process of in vitro fertilization (IVF). One hundred compact cumulus oocyte- complexes were selected after aspiration of antral follicles (2-6 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 $10^{5.5}$ and $10^{6.3}$ tissue culture infectious dose ($TCID_{50}$/ml) > respectively. Frozen thawed sperm of a BVDV free bull was put on top of a Percoll gradsant (45% and 90%).The final concentration of sperm was adjusted to 10^6 and 10^5 cells /ml. Sperm cells were incubated with $10^{5.2}$, $10^{4.2}$ and $10^{3.2}$ CP BVDV or $10^{5.2}$, $10^{5.2}$ and $10^{4.3}$ NCP BVDV $TCID_{50}$/ml during 2 hours at 38.5 C with 5% CO2 and maximum humidity. Twenty five l of 10^6 or 10^5 BVDV incubated sperm cells was added to a 25 l droplet under oil. Ten cumulus-free oocyte were added to each droplet and incubated overnight allowing to be fertilized Presumed zygotes 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 ($10^{5.2}$ $TCID_{50}$/ml) in combination with the lowest final sperm concentration (10^5 sp/ml) decreased fertilization significantly compared with the control (4% v. 19%) ($P<0.05$).

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 untill well after calving. It is classified into 2 biotype: Cytopathic (CP) and Noncythopathic (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 antral follicles from slaughterhouse ovaries. Maturation was accomplished in groups of 100 oocyte in 500 l 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
sodium pyruvat

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 subject to in vitro fertilization (IVF).

Virus

The origin of the virus was for the cythopathic strain (CP BVDV) the Oregon C24V isolate and for the noncythopathic strain (NCP BVDV) isolate No. 22146 (11). They were propagated in MEM + 5% Fetal Calf Serum (FCS).

Experimental design

Frozen sperm of a bull was thawed in a water bath at 37 C and put on top of a Percoll gradient (45%- 90%). To separated living from dead spermatozoa, the sperm was 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 75 rpm. The resulting sperm pellet was resuspended to obtain a final concentration of 10^7 sperm cells/ml in IVF- TALP with heparin 10
C. Then the sperm cells were centrifuged again, resuspended in IVF- TALP medium without heparin and diluted to obtain a concentration of 10^6 and 10^5 sperm cells/ml, respectively.

The groups where as follows:

Controls: For influence of MEM- medium on fertilization:

1 ml of MEM- medium was diluted with 1 ml of sperm cells at 10^6 Sp/ml
1 ml of MEM- medium was diluted with 1 ml of sperm cells at 10^5 Sp/ml
1 ml of IVF- no heparin medium was diluted with 1 ml of sperm cells at 10^6 Sp/ml
1 ml of IVF- no heparin medium was diluted with 1 ml of sperm cells at 10^5 Sp/ml

CP & NCP BVDV

1 ml of pure CP & NCP BVD virus suspension (titer=$10^{5.5}$ and $10^{6.3}$, respectively) was diluted with 1 ml of sperm cells at 10^6 Sp/ml.
1ml of virus suspension diluted 1:10 in MEM medium was diluted with 1 ml of sperm cells at 10^6 and 10^5 Sp/ml.
1ml of virus suspension diluted 1:100 in MEM medium was diluted with 1 ml of sperm cells at 10^6 and 10^5 Sp/ml.

All these groups were incubated for 2 hours in the CO2 incubated 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 suspention was added to a 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% formaldhyde, 2% glutaraldehyde in PBS overnight and then stained with Hoechst 1 or 10 mg/ml N ethanol. After monting in DABCO, they were evaluated for the presence of 2 pronouclei by means of fluoresence 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 (10^5 Sp/ml) decreased fertilization significantly compared with thye control (4% v. 19%) ($P<0.05$) (Table.1).Whereas NCP BVDV had no significant influence on the fertilization rate at the three infectious titers which were used ($P<0.05$) (Table. 2).

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

Sperm/ ml	control	CP ($TCID_{50}$/ml) $10^{3.5}$	$10^{4.5}$	$10^{5.5}$
10^5	19% (14/75)	9% (6/69)	11% (8/75)	4% (3/76)*
10^6	57% (42/74)	71% (53/75)	56% (40/72)	68% (50/73)

Significant difference ($P<0.05$) with the fertilizanon rate in the control within the same row

Table 2. fertilization rates using sperm cells incubated with NCP BVDV virus in IVF.

Sperm/ ml	control	CP ($TCID_{50}$/ml)		
		$10^{4.5}$	$10^{5.5}$	$10^{6.5}$
10^{5}	19% (14/75)	24% (18/70)	16% (13/82)	13% (9/68)*
10^{6}	57% (42/74)	60% (46/77)	52% (39/75)	72% (54/75)

Non significant difference (P<0.05) with the fertilization rate in the control within the same row

This study showed that infected sperm cell (10^5 sp/ml) with CP BVDV ($10^{5.5}$ $TCID_{50}$/ml) affected significantly on fertilization but NCP BVDV did not influence in compare with control group.

IVF are subjectedto more manipulation and longer cultivation in the presence of biological products derived from cattle, resulting in a higher risk of becoming infected as compared to in vivo- produced embryo. (1&12).

Use of infected semen frequently results in reduced fertility due to impaired embryonic development and embryonic death, as showen by IVF and in vitro culture (7).

It is reported that in vitro inoculation of in vivo embryos with CP BVDV had no adverse effect on survival embryonic development (3&9). Since there are some considerable cellular differences between in vitro and in vivo- produced embryos, extrapolation of in vivo results to in vitro- produced embruos is difficult (10&13).

Bielananskiand Dubuc (2) showed that after the use of semen from persistently infected with NCP BVDV , cleavage rates and development to the blastocyst stage were not significantly reduced in IVF. Whereas Guerin 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 biotype is not clear. First, it must be mentionedthat little is known about molecilar mechanisms responsible for virus entry into suceptible bovine cells. Glycoproteins, espicially gp^{48} and gp^{53}, present in the viral envelop are thought to be involved in attachment of the virus to and/or penetration of the virus into the host cells (5&6). They suggested that multiple receptors for BVDV attachment may be present and that different virus strains do not necessarly have the same receptor. In addition, it is thought that BVDV utilize more than one receptor for attachment and that binding of virous 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 , furthur research is being conducted to invesigate the interaction between BVDV and spermatoza in IVF.

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