Original Article

The Impact of Cytopathogenic and Non-cytopathogenic Biotypes of Bovine Viral Diarrhea Virus on Total Antioxidant Capacity of Bovine Oocytes In-vitro

Amirmahdi Roshanzamir[1], Massoud Talebkhan Garoussi[1*], Jalil Mehrzad[2]

1. Department of Theriogenology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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ABSTRACT

Background: Bovine viral diarrhea virus (BVDV) is the most common pathogen in dairy cattle herds.

Objectives: This study aims to examine the impact of cytopathic (CP) and non-cytopathic (NCP) BVDV biotypes on the total antioxidant capacity (TAC) of bovine oocytes in vitro.

Methods: Oocytes were obtained from slaughtered bovine ovaries, washed, and matured in a maturation medium. Oocytes were divided into five groups, each consisting of at least 60. The control group was not exposed to BVDV biotypes. Oocytes were challenged with CP and NCP, BVDV at two concentrations of 10^4 and 10^5 tissue culture infectious doses $(TCID)_{50}$/mL. To determine the antioxidant capacity of oocytes, a TAC assay was conducted after two hours of incubation. Graph Pad Prism software, version 8.4.3 was utilized to analyze the data. A one-way analysis of variance (ANOVA) was performed, and a post-hoc Tukey's honestly significant difference (HSD) test was accompanied.

Results: The results indicated that only the CP biotype of BVDV significantly decreased the TAC of infected oocytes compared to the control group, whereas the NCP biotype did not significantly alter the TAC of the infected groups.

Conclusion: Our study showed that only CP BVDV in 10^4 and 10^5 $TCID_{50}$/mL doses affected the infected oocytes and significantly decreased the oocyte's TAC. At the same time, the NCP biotype did not significantly alter the infected oocyte's TAC.

Keywords: Bovine viral diarrhea virus (BVDV), Cytopathic (CP), Non-cytopathic (NCP), Oocyte, Total antioxidant capacity (TAC) assay

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*** Corresponding Author:**
Massoud Talebkhan Garoussi, Professor.
Address: *Department of Theriogenology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.*
Phone: *+98 (21) 66929532*
E-mail: *garoussi@ut.ac.ir*

Introduction

Bovine viral diarrhea virus (BVDV) is a highly consequential pathogen in the dairy cattle industry. It is responsible for diverse clinical manifestations, ranging from subclinical moderate infections to severe acute disease (Abdelsalam et al., 2020). BVDV belongs to the pestivirus genus of the Flaviviridae family, which is characterized by its single-stranded ribonucleic acid (RNA) genome, enveloped structure and the presence of two biotypes: Non-cytopathogenic (NCP) and cytopathogenic (CP) depending on their effect on cultured cells. The NCP biotype is prevalent and often leads to transient infections, while the CP biotype is associated with fatal mucosal diseases (Dabiri et al., 2021; Talebkhan Garoussi et al., 2011). Transmission occurs through direct contact with infected animals or fomites, with persistently infected (PI) calves playing a critical role as viral reservoirs. The economic effect of BVDV is profound, stemming from reduced reproductive performance, increased mortality, and immunosuppression, which exacerbate secondary infections (Veysi & Hajibemani, 2020). Control measures include vaccination, biosecurity and the identification and culling of PI animals. Despite these efforts, BVDV remains a pervasive challenge in terms of its extensive host range and genetic diversity (Meyer et al., 2021).

The effect of BVDV on the reproductive tract and mammary glands of cattle is of critical concern in veterinary medicine and animal husbandry. BVDV is known to cause a range of reproductive disorders, including infertility, abortions and congenital abnormalities. The virus can cross the placental barrier, leading to fetal infection, which can result in the birth of PI calves (Oguejiofor et al., 2019). These PI calves often appear normal at birth but can shed the virus throughout their lives, serving as a source of infection for the rest of the herd (Garoussi et al., 2019). The effect of the virus on the reproductive tract can manifest as ovarian dysfunction, decreased sperm quality, and disruption of the estrous cycle. Moreover, BVDV was associated with the development of uterine diseases, such as endometritis, which further impairs reproductive performance (Oguejiofor et al., 2019). The economic implications of these reproductive challenges are substantial because they can lead to decreased calving rates, increased culling of affected animals, and overall reduced herd productivity.

BVDV significantly affects the quality of bovine oocytes, which is crucial for the reproductive success of cattle. Studies have shown that BVDV infection can lead to a decrease in the developmental competence of oocytes, affecting their ability to fertilize and develop into viable embryos (Pinto et al., 2017). The virus can have direct CP effects on oocytes or indirectly affect them by altering the ovarian follicular environment. This can result in fewer oocytes retrieved during ovum pick-up procedures and a lower proportion of oocytes reaching maturity (Kagawa et al., 2022). Moreover, the presence of BVDV in follicular fluid was associated with alterations in the levels of critical growth factors and cytokines that are essential for oocyte maturation (Wang & Pang, 2024).

Oxidative stress occurs when cellular antioxidant defenses fail to counterbalance the production of reactive oxygen species (ROS), which can injure cells. The overproduction of ROS, chemically reactive molecules involved in cell signaling and homeostasis, can harm DNA, lipids, proteins, and cell structures (Dayal et al., 2014). Total antioxidant capacity (TAC) indicates the cell's capability to counteract ROS, as antioxidants inhibit the progression of oxidative damage (Asadi et al., 2017). Viral infections, including those caused by BVDV, can disrupt this balance, increasing oxidative stress. This can result in the overproduction of ROS. If antioxidants are not adequately counteracted, cellular damage can be induced, and affecting various physiological processes (Rajput et al., 2014). It is imperative to comprehend the intricate relationship between oxidative stress, TAC, and viral infections to devise efficacious therapeutic approaches for managing viral diseases. BVDV is known to induce oxidative stress in various cell types, and, probably, bovine oocytes are similarly affected (Al-Kubati et al., 2021). An imbalance between the generation of ROS and antioxidant defenses in bovine oocytes can impair normal cellular activities, including those essential for successful fertilization and early embryonic development (Rajput et al., 2014). Understanding how BVDV affects oocyte levels of oxidative stress is crucial due to its critical function in reproduction.

This study aims to examine how BVDV affects the overall antioxidant capacity of infected bovine oocytes.

Material and Methods

Bovine ovaries were acquired from a commercial slaughterhouse in the suburban areas of Tehran and Alborz Provinces, Iran, immediately after slaughter and were submerged in phosphate-buffered saline (PBS). Thereafter, ovaries were transferred to the laboratory in one hour and subjected to two more washes in fresh PBS containing streptomycin at a concentration of 100 mg/

mL, penicillin at a concentration of 100 IU/mL, and fungizone at a concentration of 25 µg/mL. Cumulus-oocyte complexes (COCs) were aspirated from ovarian follicles measuring 3-6 mm in diameter using an 18-gauge needle connected to a 10 mL syringe. Before starting any experimental procedures, follicular fluid extracted from each batch of ovaries was regularly tested using the Garoussi and Mehrzads' methodology for the presence of BVDV antigen using polymerase chain reaction (PCR) (Garoussi & Mehrzad, 2011).

Washing oocytes

Before the IVM of oocytes, they were subjected to a washing procedure based on the Stringfellow method (Stringfellow et al., 1997). This method involved ten washing cycles. Initially, the oocytes were grouped into sets of ten and placed within one-milliliter droplets of washing medium. Subsequently, they underwent a single wash in antiseptic solution for 90 s. According to the protocol, the washing medium contained 100 mL of PBS, 4 g of bovine serum albumin (BSA), 10000 IU of Penicillin G Potassium, and 10 mg of streptomycin sulfate. The antiseptic solution contained 100 mL of hank's balanced salt solution (HBSS), 0.4 mL trypsin-EDTA (0.25%), phenol red, 10000 IU penicillin G potassium, and 10 mg streptomycin sulfate.

In vitro maturation (IVM)

Using the established procedures outlined by Garoussi and Mehrzad (2011), bovine oocytes were matured in vitro. Oocytes were immersed in a maturation medium, repeatedly washed and surrounded by layers of dense follicular cells. Tissue culture medium-199 (TCM-199) supplemented with Earle's salts, 10% BVDV-free fetal bovine serum (FBS), one µg/mL estradiol, 60 µg/mL Folltropin, 2 IU/mL HCG, 50 ng/mL epidermal growth factor (EGF), 0.4 mM glutamine, 0.2 mM sodium pyruvate and 50 µg/mL gentamicin made up the maturation medium. Four-well culture containers containing 700 µL of maturation medium were used to culture oocytes in a controlled incubator. The incubation conditions comprised 38.5 °C temperature, 5% carbon dioxide (CO_2) concentration, and 100% humidity for 24 hours. After maturation, cumulus cells were removed from the oocytes by vortexing for 90 s in 2 mL minimal essential medium (MEM) containing 2% BVDV-free fetal calf serum (FCS).

Virus culture and preparation

The madin-darby bovine kidney (MDBK) cell line was used for the laboratory culture of BVDV. CP and NCP biotypes of BVDV were obtained from the Department of Virology, Faculty of Veterinary Medicine, University of Tehran, and the Virology Department of the Razi Vaccine and Serum Research Institute, respectively. Initially, MDBK cells were cultured in minimum essential medium (MEM) supplemented with 5% FCS. Adherent cells were subcultured after reaching 70%-80% confluence. The virus was propagated in MDBK cells and frozen at -70 °C. After eight passages, the presence of the virus was confirmed by reverse transcription PCR (RT-PCR). The virus was then frozen and titrated using the Reed-Muench formula (Reed & Muench, 1938).

Experimental groups

The experimental groups in this study were established based on the BVD virus's CP and NCP biotypes. Two different viral doses (10^5 and 10^4) were expressed as a 50% tissue culture infectious dose ($TCID_{50}$/mL), as described by (Garoussi & Mehrzad, 2011).

The oocytes were divided into five distinct groups, each consisting of at least 60 oocytes and replicated three times. The control group was not exposed to BVDV biotypes. The second and third groups were infected with 10^4 and 10^5 $TCID_{50}$/mL of NCP BVDV, respectively. The fourth and fifth groups were infected with 10^4 and 10^5 $TCID_{50}$/mL CP BVDV, respectively. The duration of infection in each group was 2 hours.

TAC assay

The TAC test was used to assess the samples' overall antioxidant capacity with and without extract. The TAC test was performed using a KiazistTM TAC Assay kit. The experiment's concept and methodology included the reduction of Fe^{+2} ions. The process produced a dye when the chromogen 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) was used as an appropriate substrate for the peroxidase enzyme. The absorbance of light at 593 nm was measured, and the optical density (OD) was recorded. A higher antioxidant power in the sample indicates increased involvement of the chromogen in the reactions, resulting in higher OD values. An optimization process was performed to quantify the antioxidant levels in the samples, and a standardized method was employed to report the results. Light absorption was measured using a standard working solution. To conduct the assay, five microliters of the sample were added to a

96-well plate, while five microliters of PBS were added to the wells serving as the control group. Subsequently, 250 µL of the working solution was added to each well. The plates were incubated for approximately 5 minutes at ambient temperature. The concentration of Fe^{+2}, representing the antioxidant capacity and the presence or absence of antioxidants in the samples, was determined and reported as µM Fe^{+2} /L.

Statistical analysis

The results were analyzed using GraphPad Prism software, version 8.4.3. One-way analysis of variance (ANOVA) with post-hoc Tukey's honestly significant difference (HSD) test was used to determine statistical significance between groups. A $P \leq 0.05$ was considered significant. The results were presented as the Mean±SD.

Results

TAC assay

Table 1 presents the effects of several BVDV biotypes on the oocytes. The TAC of the control group was 659.3±50.44. TAC levels were similar in the control, 10^4 $TCID_{50}$/mL and 10^5 $TCID_{50}$/mL NCP groups, with no significant differences ($P>0.05$). Nevertheless, a significant decrease in TAC was observed between the 10^4 $TCID_{50}$/mL and 10^5 $TCID_{50}$/mL CP groups compared to the control group ($P \leq 0.05$) (Table 1).

Discussion

The current study investigated how CP and NCP BVDV biotypes affected the overall antioxidant capacity of bovine oocytes in vitro. We measured the TAC of bovine oocytes following BVDV infection to assess their overall antioxidant response, focusing on endogenous mechanisms rather than on the effects of exogenous antioxidants. TAC was chosen because it is an excellent representative of the capacity to combat oxidative stress, and it provides a comprehensive measure of both enzymatic and non-enzymatic antioxidants within cells and oocytes, reflecting their natural defense mechanisms against oxidative stress (Ghorbanian et al., 2017; Lira Ferrari & Bucalen Ferrari., 2011). While specific markers, such as malondialdehyde, could offer additional insights into oxidative damage, we focused on TAC, as it effectively gauges the oocytes' capacity to counteract oxidative stress. This study showed that although the NCP biotype did not have a comparable impact, varying amounts of CP BVDV might influence the overall antioxidant capacity of bovine oocytes. In brief, both doses of the CP biotype significantly decreased the TAC of the infected oocytes (Table 1).

The complex interaction between the body's antioxidant defense systems and the production of ROS results in a complex phenomenon known as oxidative stress (Bohm et al., 2023). ROS are naturally produced during cellular metabolicprocesses, such as mitochondrial respiration, enzymatic reactions, and inflammation. Although ROS are crucial signaling molecules and contribute to normal cellular functions, their excessive production or impaired elimination can lead to oxidative stress (Szarka et al., 2022).

ROS, such as superoxide anions, hydroxyl radicals, and hydrogen peroxide, can damage cellular components in terms of their reactivity (Katakwar et al., 2016). Nucleic acids, proteins, and lipids are susceptible to oxidative damage. Amino acid residues within proteins are susceptible to oxidation, which can result in structural and functional changes. Lipid oxidation can produce reactive products, including lipid peroxides, which can disrupt the integrity and function of cell membranes (Gaschler & Stockwell, 2017). Oxidative damage to DNA can result in mutations, alterations in nucleotide bases, and DNA strand breaks, which are detrimental to genetic integrity (Rahimian et al., 2020). As an elaborate antioxidant defense mechanism, cells have been developed to counteract the harmful effects of ROS. This system has enzymatic and non-enzymatic components (Khazaei & Aghaz., 2017). Enzymatic antioxidants, including catalase, superoxide dismutase, and glutathione peroxidase, collaborate to catalyze the conversion of ROS into less deleterious forms. Vitamins C and E, glutathione, and various phytochemicals are non-enzymatic antioxidants that scavenge ROS by donating electrons to stabilize and neutralize free radicals (Gupta et al., 2021). However, under conditions of increased ROS production, such as exposure to environmental toxins, chronic inflammation, or viral infections, the antioxidant defense system can become overwhelmed. This results in oxidative stress due to an imbalance between ROS production and antioxidant capacity (Alfadda & Sallam, 2012). A cascade of detrimental events, including dysregulation of cellular processes and activation of stress signaling pathways, can be initiated by oxidative stress.

The current study demonstrated that CP BVDV substantially impacted the overall antioxidant capacity of bovine oocytes. Recent studies have provided valuable insights into the effects of BVDV infection on oxidative stress and antioxidant capacity in animal cells. A study conducted by Schweizer found that CP BVDV induces

Table 1. The effect of CP and NCP BVDV on the TAC of bovine oocytes

Control	104 NCP	105 NCP	104 CP	105 CP
659.3±50.44[a]	582.3±48.25[a]	640.7±47.54[a]	488.1±40.25[b]	447.6±20.79[b]

[a, b]Symbols on line with different alphabetic letters are significantly different.

apoptosis in cultured cells through oxidative stress, caspase activation, and DNA fragmentation. This process is characterized by increased ROS levels early in apoptosis, preceding caspase activation and DNA fragmentation. Certain antioxidants, such as butylated hydroxyanisole and ebselen, effectively protect cells from apoptosis induced by the CP biotype of BVDV. In contrast, antioxidants, such as N-acetylcysteine, pyrrolidine dithiocarbamate, lipoic acid, dihydrolipoic acid and tiron, were ineffective in preventing apoptosis. These results suggest that oxidative stress plays a crucial role in apoptotic cell death induced by the CP biotype of BVDV (Schweizer & Peterhans, 1999). A study conducted by Villalba et al. (2016) reported responses to CP BVDV infection in MDBK cells. Notably, the study observed downregulation of genes involved inROS metabolism, suggesting increased oxidative stress (Villalba et al., 2016). It was demonstrated that BVDV infection interferes with cellular metabolic processes, increasing the generation of ROS and causing oxidative stress, which is consistent with the current study (Liu et al., 2018). Furthermore, excessive production of ROS triggered by BVDV infection can initiate intracellular signaling pathways that mediate various cellular responses, including inflammation and programmed cell death (Zhou et al., 2017).

Moreover, BVDV infection in cattle has been linked to several clinical symptoms, such as reproductive problems, respiratory diseases, and immunosuppression. The observed consequences may be associated with oxidative stress caused by BVDV since oxidative stress involves these symptoms (Pinior & Köfer, 2016). Recent research has provided new insights into the involvement of BVDV in initiating mitophagy, a specific type of autophagy that targets the breakdown of impaired or harmed mitochondria. Since mitochondria are essential for controlling ROS generation and antioxidant defense mechanisms, activating mitophagy by BVDV infection may affect the cellular response to oxidative stress (Li et al., 2024). Oxidative stress is a critical factor in the reproductive biology. In the context of oocyte quality and embryo development, oxidative stress can have profound consequences (Jiang et al., 2021). Oocytes, which are highly metabolically active, are particularly vulnerable to oxidative damage due to their limited antioxidant capacity. Consequently, any disruption in the delicate balance between ROS production and antioxidant defense mechanisms can significantly impact oocyte function and subsequent embryo development (Barrozo et al., 2021).

Antioxidants, such as superoxide dismutase, catalase, glutathione, and vitamin E, play crucial roles in neutralizing ROS and protecting against oxidative damage (Dontha, 2016). However, BVDV infection disrupts the antioxidant defense system, leading to oocytes' diminished ability to effectively scavenge ROS. This compromised antioxidant capacity further exacerbates oxidative stress within oocytes, amplifying detrimental effects on oocyte quality and reproductive success (González Altamiranda et al., 2013).

Conclusion

In conclusion, only the BVDV CP biotype can significantly alter the TAC of the infected oocytes, but the NCP biotype did not have such an effect. The current study demonstrated that the BVDV CP biotype reduced the overall antioxidant capacity of infected oocytes compared to the control group. However, further research is needed to determine the processes underlying the loss of TAC in BVDV-infected oocytes.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization, methodology, resources, investigation, validation, visualisation, formal analysis, and writing: All authors; Data curation: Massoud Talebkhan Garoussi, and Amirmahdi Roshanzamir; Supervision,

funding acquisition, and project administration: Massoud Talebkhan Garoussi and Jalil Mehrzad; Software: Amirmahdi Roshanzamir, and Jalil Mehrzad.

Conflict of interest

The authors declared no conflict of interest.

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