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Characterization of the *Campylobacter fetus* subsp. *venerealis* Adhesion to Bovine Sperm Cells

Caracterización de la Adhesión de *Campylobacter fetus* subsp. *venerealis* a Espermatozoides Bovinos

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SUMMARY: *Campylobacter fetus* is extracellular bacteria of the genital tract of cattle. They cause infertility and abortion, but there is no documented information on the susceptibility of bovine sperm cells to this bacteria. The aim of this present work was to study the effects provoked by *Campylobacter fetus* subsp. *venerealis* when in interaction with bovine sperm cells. The bovine spermatozoa were obtained frozen bovine semen pooled from uninfected bulls, and were exposed to living campylobacter over different periods of time. Light microscopy and scanning electron microscopy first revealed a tropism, then a close proximity followed by tight adhesion between these two different cells. A decrease in the spermatozoa motility was observed. Motile bacteria were observed during the next 3 h, this process began with a tight membrane–membrane adhesion. The adhesion between *Campylobacter fetus* to the sperm cell occurred either by the flagella or by sperm head. Results from this study demonstrated with light microscopy scanning electron microscopy allowed us to characterize some aspects of the interaction of *Campylobacter fetus* subsp. *venerealis* and bovine sperm while preserving the cellular and bacterial structure. This *ex vivo* model might be useful for studies on adhesion and cytopathogenicity of different field strains of *Campylobacter fetus*.

KEY WORDS: Adhesion; *Campylobacter fetus* subsp. *venerealis*; Sperm cells.

INTRODUCTION

Successful mammalian reproduction requires that sperm migrate through a long and convoluted female reproductive tract before reaching oocytes. For many years, fertility studies have focused on biochemical and physiological requirements of sperm (Barth, 1995). There are a large number of microorganisms that propagate for the seminal route. The pathogen can adhere to the sperm in the tail and/ or the head, or can infect the various components of the seminal fluid as sperms, seminal plasma and leukocytes, among others) (Bielanski & Dubuc, 1993; Bielanski *et al*., 1994; Bielanski & Dubuc, 1994; Bielanski & Loewen, 1994; Bielanski *et al*., 2004). Many of the existing studies on the association pathogen-sperm are not conclusive because, the detection of this union is complex and in some in some cases uncertain (Casadevall & Pirofski, 2000) as in the case of *Campylobacter fetus* (Bielanski *et al*., 1994).

In the project of *ex vivo* model for the study of the pathogenesis of the bovine genital campylobacteriosis (Chiapparrone *et al*., 2008; Chiapparrone *et al*., 2009; Chiapparrone *et al*., 2011; Chiapparrone *et al*., 2014) intends to describe the behavior of a strain of *Campylobacter fetus* subsp. *venerealis* (*C. fetus* subsp. *venerealis*) bacteria of preputial smegma of bull in its interaction with sperm cattle by light microscopy and scanning electron microscopy (Fletcher & Floodgate, 1973).

MATERIAL AND METHOD

Bacterial strain. The *C. fetus* subsp. *venerealis* strain was isolated in beef bovine of Tandil - Argentina, from preputial tract of Aberdeen Angus bull and identified using phenotypic test and PCR. The strain was grown for 48 h at 37 °C incubated under microaerophilic conditions which correspond to the logarithmic growth phase. All experiments

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were performed with *C. fetus* subsp. *venerealis*-1 strain at a concentration of $1x10^9$ cells/mL.

Sperm cells. We used frozen bovine semen pooled from fertile bulls for artificial insemination. For interaction purposes, straws of frozen semen were thawed by immersion in a water bath at 37 °C for 30 s, centrifuged and the entire medium removed. The cells were washed once in PBS and transferred to Minimum Essential Medium Eagle (MEM) culture medium (M0643 Sigma-Aldrich) supplemented with 10 % fetal bovine serum.

Viability tests were based on visual morphology and motility under dark field microscopy, each sperm cell was categorized as belonging to one of four motility categories (rapid progressive, slow progressive, non-progressive and immotile) and viability staining with Nigrosin-Eosin stain (Rodríguez-Martinez, 2013).

Adherence assay. For interaction analysis, suspensions of sperm cells were counted in a Neubauer chamber and exposed to *C. fetus* subsp. *venerealis*-1 in a cell ratio of 1:1 bacteria–sperm cells, for 3 h at 37 °C incubated under 5 % CO_2. For control experiments, the same bacteria and sperm cells free were used. The bacterial motility can be determined under dark field microscopy.

Giemsa stain. Sperm cells were fixed with acetone at 4° C and stained with Giemsa 10 %. Bacterial adhesion was observed and quantified by optical microscopy.

Scanning electron microscopy. After checking the adhesion by Giemsa staining, innoculated cell cultures were processed according to Electron Microscopy Laboratory protocol of the College of Sciences, Universidad Nacional de Mar del Plata (UNMdP). Briefly, the sperm cells were fixed with 3 % glutaraldehyde in sodium cacodylate buffer and washed with 0.1 M sodium cacodylate buffer. After dehydration with ethanol they were exposed to hexamethyldisilazane overnight, and then gold and palladium metallizing (Denton Vacuum Desk II). The samples were observed with scanning electron microscope (Jeol JSM-6460 LV). The system used was an EDAX Genesis XM4 - Sys 60, equipped with Multichannel Analyzer EDAX mod EDAM IV, Sapphire Si (Li) detector and super ultra-thin Window of Be, and EDAX Genesis version 5.11 software.

RESULTS

Of the sperm cells 78 % were alive, before bacteria - sperm interaction. This number reduced to 56 % in the third

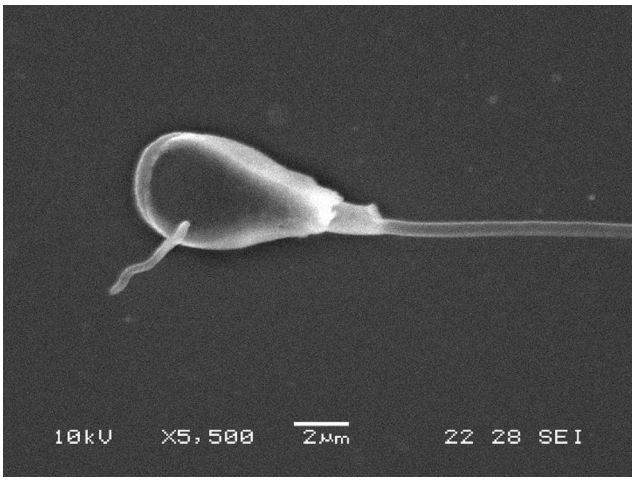

Fig. 1. *Campylobacter fetus* subsp. *venerealis* adhered to head of sperm cells for the apical end. Scanning electron microscopy. 5500X.

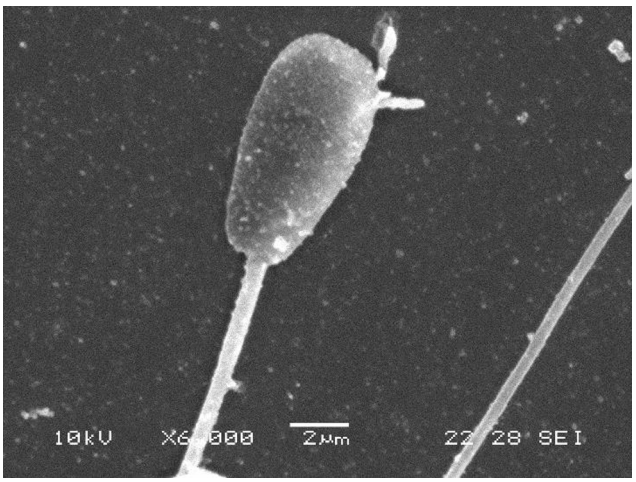

Fig. 2. *Campylobacter fetus* subsp. *venerealis* adhered to head of sperm cells for the apical end. Scanning electron microscopy. 6000X.

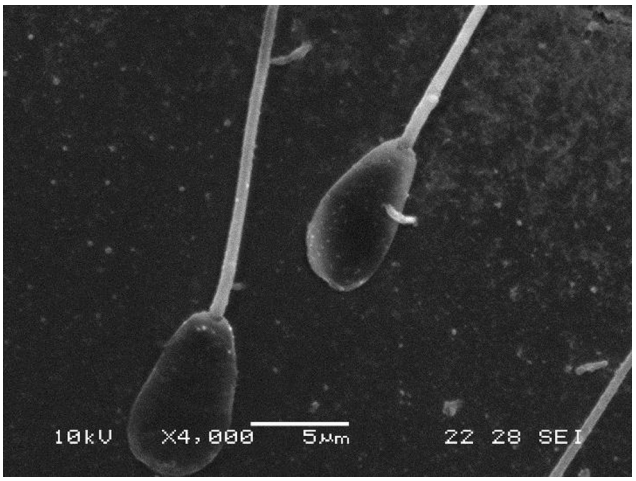

Fig. 3. *Campylobacter fetus* subsp. *venerealis* adhered to head and tail of sperm cells for the apical end. Scanning electron microscopy. 4000X.

hour, when bacteria and sperm cells were under interaction. Sperm that were white (unstained) were classified as alive, and those that showed any pink or red coloration were classified as dead, with the sole exception for sperm with a slight pink or red appearance restricted to the neck region ('leaky necks'), which were assessed as live. Motile bacteria were observed during the next 3 h.

By scanning electron microscopy sperm cell treated with *C. fetus* subsp. *venerealis* showed adhesion and alterations in the bovine spermatozoa. All the superficial structures of spermatozoa such as head (66 %), mid piece, neck, and tail (34 %) were involved in bacterial adhesion.

The capacity of adherence to different parts of the sperm, the number of adherent *C. fetus* subsp. *venerealis* and the characteristics of interaction were analyzed. In the images,

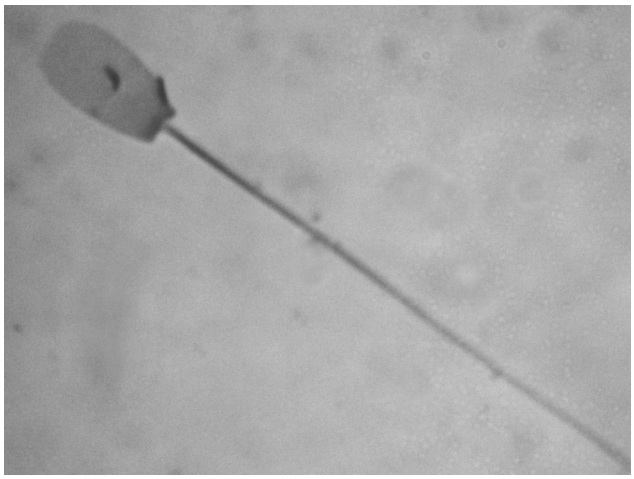

Fig. 4. *Campylobacter fetus* subsp. *venerealis* adhered to head of sperm cells. Giemsa stain. 100X.

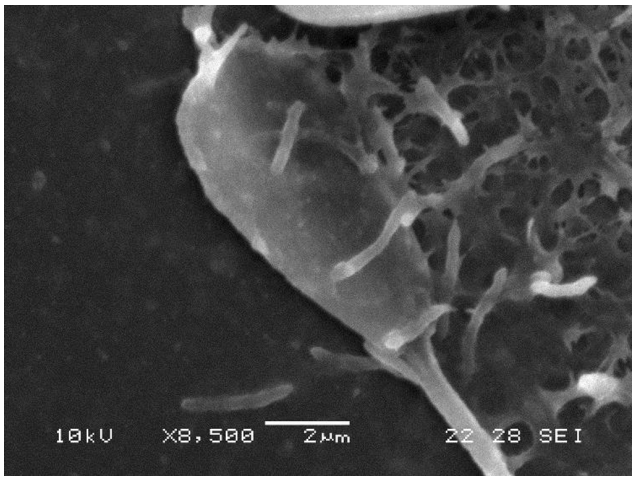

Fig. 5. Observation of more than one *Campylobacter fetus* subsp. *venerealis* adhered to sperm cells. Scanning electron microscopy. 8500X.

the presence of one or more *C. fetus* subsp. *venerealis* adhered by the apical body, mainly to the sperm head (Figs 1, 2 and 3), was observed and confirmed by routine colorations (Fig. 4). In others cases, it was possible to determine the presence of bacterial shapes in an extracellular matrix, adhered to the external membrane of the head and tail of sperm, grouping with biofilm characteristics (Figs. 5 and 6).

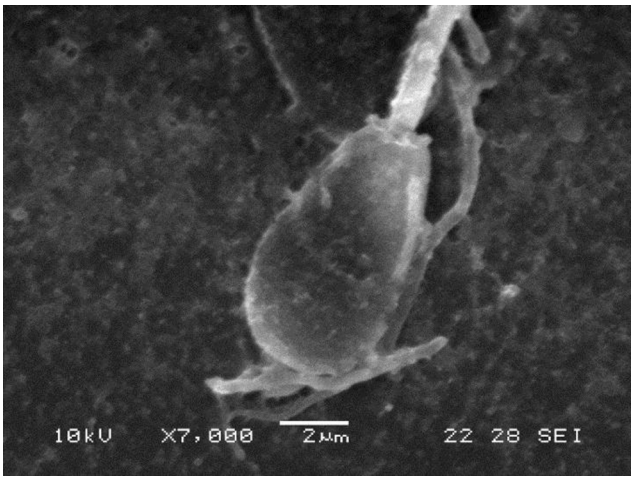

Fig. 6. Observation of grouping of *Campylobacter fetus* subsp. *venerealis* adhered to head of sperm cells. Scanning electron microscopy. 7000X.

DISCUSSION

C. fetus subsp. *venerealis* is one bacteria that cause infertility in cows (Campero, 2002; Catena, 2002; Catena, 2003). However, there is no documented information about Campylobacter effect on sperm cells viability (Bhavsar & Kapadnis, 2006; Brenner *et al*., 2005; de Melo & Pechére, 1990; Graham & MacDonald, 1988; Joens *et al*., 2010) and there is no complete agreement on the detrimental role of bacteria presence in the semen.

The ability of bacteria to bind to host cells is important as it represents an early event in the creation establishment in vivo microorganism - cell relationship (Casadevall & Pirofski, 2000; Benchimol *et al*., 2008; Chiapparrone *et al*., 2014). Sometimes, such binding process is also a requirement for pathogenicity. Understanding the capability of adherence of *C. fetus* subsp. *venerealis* to the host cell (Chiapparrone, 2011) is significant for a thorough knowledge of the initial steps involved in the pathogenesis of bovine genital campylobacteriosis.

The light microscopy and scanning electron microscopy allowed us to characterize some aspects of the interaction of *C. fetus* subsp. *venerealis* and bovine sperm while preserving the cellular and bacterial structure.

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CHIAPPARRONE, M. L.; SOTO, P. & CATENA, M. Caracterización de la adhesión de *Campylobacter fetus* subsp. *venerealis* a espermatozoides bovinos. *Int. J. Morphol., 34(4)*:1419-1423, 2016.

RESUMEN: *Campylobacter fetus* subsp. *venerealis* es un patógeno extracelular del tracto genital de bovinos. En las hembras causa subfertilidad y aborto, mientras que los toros son portadores en el esmegma prepucial y se desconoce si provoca daño en los espermatozoides. El objetivo del presente trabajo fue estudiar los efectos de *Campylobacter fetus* subsp. *venerealis* sobre espermatozoides bovinos. Los espermatozoides obtenidos a partir de pajuelas de semen pertenecientes a toros no infectados, se coincubaron con una cepa de *Campylobacter fetus* subsp. *venerealis* por diferentes períodos de tiempo. Por microscopía óptica y electrónica de barrido se observó el tropismo inicial de la bacteria hacia los espermatozoides y la adhesión bacteriana, de forma colateral se observó su efecto en el espermograma. Post incubación los espermatozoides presentaron menor motilidad progresiva y mayor porcentaje de muertos con respecto al control. Se comprobó la viabilidad de la bacteria a las 3 h. Se registró la adhesión de *Campylobacter fetus* subsp. *venerealis* a la membrana celular de distintas porciones del espermatozoide: cabeza, pieza media, cuello y cola. Los resultados de este estudio permitieron caracterizar la interacción entre *Campylobacter fetus* subsp. *venerealis* y espermatozoides bovinos por microscopía óptica y electrónica de barrido. La aplicación de este modelo *ex vivo* permitirá profundizar los conocimientos referentes a los procesos de adhesión y citopatogenicidad de *Campylobacter fetus*.

PALABRAS CLAVE: Adhesión; *Campylobacter fetus* subsp. *Venerealis*; Espermatozoides.

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