Experimental infection of bovine cumulus oocyte complexes with *Mycoplasma bovis*

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Backgound

Mycoplasma bovis (*Mb*) is an important bovine pathogen causing mastitis, pneumonia, arthritis and genital infections. Animal contact is the main source of *Mb* infection. Other known risks include artificial insemination with contaminated semen. Experimental studies have shown that *Mb* inoculation into uterus or insemination with *Mb* infected semen causes bursitis, salpingitis, abortion and infertility in female cattle. Information on the potential risk of embryos transferring *Mb* to recipient cows/heifers is scarce.

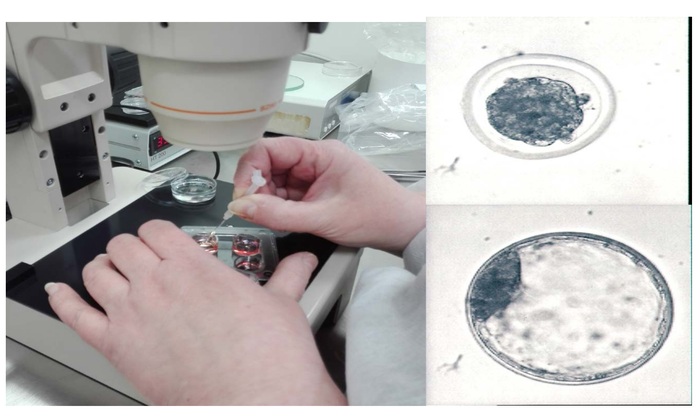
Zona Pellucida (ZP) is an acellular matrix that surrounds the oocyte and early embryo. Most studies agree that ZP protects the embryo from infection with different pathogens. However, some pathogens can become firmly attached to the ZP. The organism can still infect the recipient following transfer or the embryo after hatching. Previously Riddell et al. (1989) and Bielanski et al. (1989) have shown that *Mb* adheres tightly to ZP.

Our aim is to study the risk of *Mb* in embryo transfer using *in vitro* produced embryos. As a first step in these studies, we examined the interaction of *Mb* with bovine oocytes.

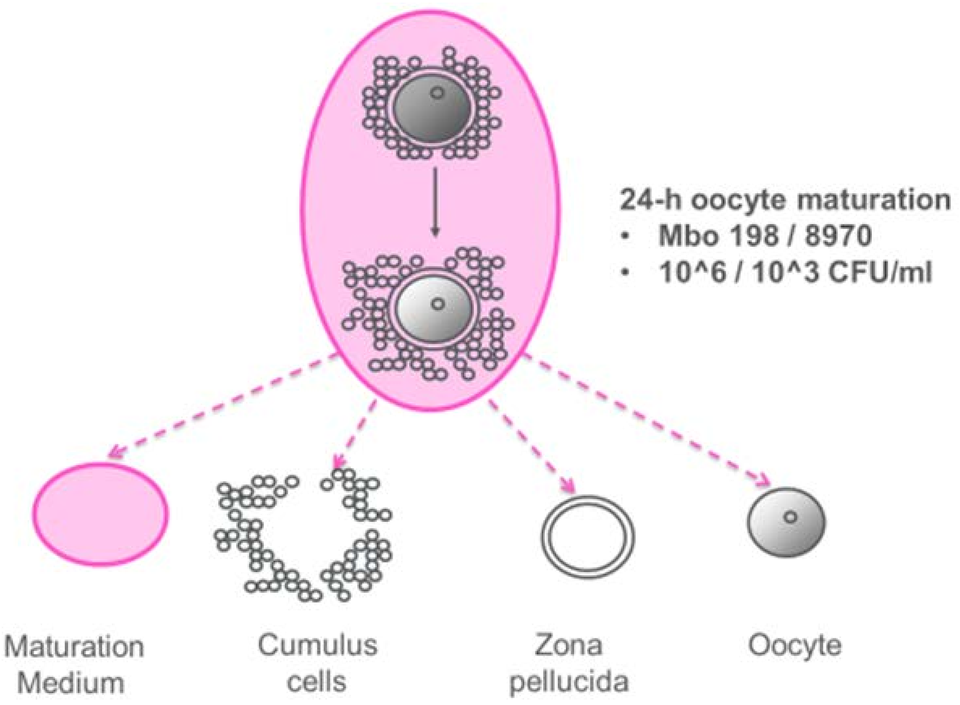
Materials and Methods

Cumulus oocyte complexes (COC) were aspirated from ovarian follicles of cows within 6 h after slaughter. All media used in COC treatments were previously tested and showed no growth inhibition of *Mb*. COCs were matured in maturation medium (Räty et al., 2011) in 5% CO_2 at 37 °C for 24 h. The maturation medium was contaminated with two different *Mb* strains, either with strain 198 (isolated from bull semen) or strain 8970 (isolated from mastitis). Two concentrations of *Mb* were used (10^6 and 10^3 cfu/ml). After maturation, COCs were washed 3 times, then cumulus cells (CCs) were removed from oocytes using combined mechanical and trypsin treatment. Three washes were repeated and after that ZP were removed using Tyrode's solution. Finally oocytes were washed three times. The maturation medium, CCs, ZP and oocytes were separately examined for *Mb* by culture method.

Ten-fold dilutions up to 10^{-3} were made into F broth in tightly closed tubes. Broth cultures were incubated at 37 °C for 14 days. The growth and colour change were monitored every other day. *Mb* was identified using *oppD* real-time PCR (Sachse et al., 2010).



In vitro oocyte maturation



Results

Similar results were obtained for both strains used. No *Mb* was detected from ZP or oocytes, whereas maturation medium and cumulus cells were positive at high *Mb* concentrations.

Table 1. Detection of *Mycoplasma bovis* from cumulus oocyte complexes.

Sample	Strain and concentration			
	198		8970	
	10^6	10^3	10^6	10^3
Maturation medium	+	-	+	-
Cumulus cells	+	-	+	-
Zonae pellucidae	-	-	-	-
Oocytes	-	-	-	-

Conclusions

Our results indicate that *Mb* does not pass Zona Pellucida and does not infect the oocyte. Also we could not confirm the earlier results showing that *Mb* adheres tightly to ZP as we could not detect *Mb* in ZP culture even in the high concentration.

Our next step will be studying if *Mb* contaminated sperm cells can infect *in vitro* produced embryos.

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