Survival capacity of *Mycoplasma agalactiae* and *Mycoplasma mycoides* subsp *capri* in the diluted semen of goat bucks and their effects on sperm quality

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ABSTRACT

This study examines the viability of *Mycoplasma agalactiae* (Ma) and *Mycoplasma mycoides* subsp *capri* (Mmc) during 150 minutes of incubation at 37 °C in contaminated diluted semen (DS) doses. The effects of the presence of both microorganisms on sperm viability, motility, and morphology were also examined. In a second experiment, the viability of Ma and its effects on sperm viability were determined in ejaculate samples and skimmed milk semen extender samples. Ma and Mmc were able to survive in DS at concentrations considered infectious, and no significant differences in mean concentrations were detected (7.1 log colony-forming units [CFU]/mL). However, initial concentration of Ma declined (P < 0.05) from 7.5 to 6.9 log CFU/mL and Mmc declined (P < 0.05) from 7.7 to 7.1 log CFU/mL after incubation. Conversely, ejaculate concentrations of Ma increased significantly (from 7.1 to 7.4 log CFU/mL, P < 0.05). These observations suggest that the natural breeding medium is more suitable for Ma than the medium used for artificial insemination (AI). The presence of Mmc slightly reduced sperm viability in the DS (from 21.7% to 16.6%, P < 0.05). The absence of major effects on sperm quality could lead to the unnoticed use of semen contaminated with Ma and Mmc for AI. As both bacteria were able to survive the conditions of ejaculates and semen doses, these findings suggest a risk of venereal transmission of contagious agalactia and support the use of mycoplasma-free semen samples for (AI).

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1. Introduction

Mycoplasma agalactiae (Ma) and *Mycoplasma mycoides* subsp *capri* (Mmc) are the main etiologic agents of contagious agalactia (CA), a serious infectious syndrome that affects small ruminants. Production losses in dairy herds have prompted research targeted at the diagnosis and monitoring of both microorganisms in dairy goat herds or at examining their effects on the bulk tank milk [1,2]. This has determined that other factors related to CA, such as the reproductive implications of the disease in infected herds, have not been examined and are likely underestimated, as it has already been suggested [3]. In effect, it has been reported that Ma and Mmc can colonize and damage the urogenital tract in small ruminants [2,4,5].

Recent studies in goats addressing the role of the buck in the epidemiology of CA have detected the presence of Ma and Mmc in asymptomatic seronegative auricular

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carriers [6] even in artificial insemination (AI) centers [7]. Infected bucks at the AI center showed the systemic presence of Ma and Mmc including the urogenital tract and semen. This has incited protocols to prevent the future entry of asymptomatic carriers in AI centers [6,8,9].

In other mammal species, the genital tropism of some mycoplasma species allows for venereal transmission both by natural breeding and by AI with negative impacts on reproduction. In cattle, *Mycoplasma mycoides* subsp *mycoides* small colony is excreted in the semen of asymptomatic bulls [10] and *M canadense*, *M bovigenitalium* (Mbv), or *Ureaplasma diversum* show high infection rates of the semen of breeding bulls [11,12]. *Mycoplasma bovis* (Mb) has been associated with infertility and reproductive failure in cattle [13]. In horses, species such as *M subdolum*, *M equirhinis*, or *M equigenitalium* have been identified in semen with negative effects on fertility [14,15]. In dogs, *Mycoplasma* spp. and *Ureaplasma* spp. are also thought to be transmitted *via* the venereal route [16]. Even in humans, the presence of *U parvum*, *U urealyticum* (Uu) or *M hominis* has been linked to infertility, and their venereal transmission has been reported [17–19]. The venereal transmission of CA has yet to be confirmed.

If the agents responsible for CA in goat bucks are excreted in semen, this could affect sperm quality and the subsequent fertility of these small ruminants. Accordingly, Ak et al. [20] confirmed that Ma reduced the semen characteristics such as volume, motility, and concentration and increased the proportion of morphologically abnormal spermatozoa in experimentally infected rams. The capacity of Mmc to affect the sperm motility and morphology of bull semen has also been reported [21]. The presence of *Ureaplasma* spp. in experimentally infected ram semen has also been found to compromise sperm viability [22]. Although the literature lacks information on the effects on sperm quality of the presence of Ma and Mmc in goat semen, we cannot rule out possible detrimental effects, as described for other mycoplasma and host species.

To assess the real risk of venereal transmission of CA in goats, this study examines the survival of Ma and Mmc in semen and in the diluent used to prepare semen doses using a model of contaminated goat semen described previously [23]. This model was also used to determine the effects of both mycoplasmas on sperm quality in terms of sperm viability, motility, and morphology using techniques routinely used at AI centers.

2. Materials and methods

This study was made following the code of good practice in research and scientific publications, proposed by the ethics committee of the University of Murcia.

2.1. Goat bucks

Semen samples were collected from nine Murciano-Granadina breed bucks aged 2 to 6 years at two AI centers. These animals met the conditions set out in the Commission Regulation (EU) No 176/2010 on goat sperm donor [24]. Although there was no clinical history of CA in the centers, the animals were checked to confirm their mycoplasma-free status by conducting two blood tests to detect antibodies against Ma (ELISA test, Pourquier Institute, France) in the year before the onset of the study. In addition, in this year leading up to the study, the bucks were screened twice and four times by subjecting ear canal samples (2) and semen (1), respectively, to culture and polymerase chain reaction (PCR) using a protocol to detect CA carriers, as described elsewhere [6]. The animals were kept in stress-free conditions and fed with their normal diet. Animal handling and health measures were those recommended for this species under current legislation [24].

2.2. Experimental design

Two experiments were performed. In the first, diluted semen (DS) samples previously inoculated with Ma or Mmc were incubated for 150 minutes at 37 °C and tested for mycoplasma viability, sperm viability, motility and tail abnormalities, and pH. In the second experiment, the same protocol was used to examine mycoplasma viability and sperm viability in untreated ejaculate samples (stored for 1 or 3 hours at 4 °C) and semen extender samples inoculated with Ma.

2.2.1. Experimental contamination of DS with Ma and Mmc

Ejaculates were obtained from five bucks using an artificial vagina. Each ejaculate was diluted in a medium prepared using skimmed milk without antibiotics (10 g of powdered cow milk and 0.2 g of α-D-glucopyranose in 100 mL of distilled water) and the semen suspension adjusted to 200×10^6 cells per mL [25]. This sperm suspension (DS) was gradually cooled to 4 °C over 90 minutes in a cold room and kept for 10 minutes at room temperature before experimental contamination. A pool of semen samples from all animals was prepared, and after its homogenization, three aliquots of 1460 μL were placed into sterile Eppendorf tubes and each one inoculated with $1 \times 10^{7-8}$ viable colony-forming units/mL (CFU/mL, 40 μL) of Ma (PG2, NCTC 10123) and Mmc (Y-goat, NCTC 11706) or incubated with an equal volume of liquid medium specific for mycoplasmas as a negative control [23]. The remaining pool was tested by culture and PCR to confirm the absence of *Mycoplasma* spp. in the semen used. All three prepared aliquots were incubated at a temperature of 37 °C (ideal for the growth of *Mycoplasma* spp.) on a heating block (VWR, Radnor). During the experiment, we determined mycoplasma viability and sperm viability in each experimental group at the following incubation times: 0, 30, 45, 60, 75, 90, 105, 120, and 150 minutes. The spermatic motility parameters were evaluated at the following incubation times: 0, 60, and 90 minutes. In preliminary studies (data not shown), we observed that motility parameters of the negative control and contaminated conditions tended to 0 after 90 minutes after contamination, and this could interfere with the means and statistical analyses. Sperm tail morphology was examined at 30, 90, and 150 minutes of incubation. Finally, at these latter three time points, pH was determined in the original DS pool and in the three spiked DS aliquots. Three replicates of this experiment were conducted on different days.

2.2.2. Experimental contamination of semen extender with Ma

Two 1.5-mL aliquots of the same semen extender (skimmed milk) without spermatozoa were prepared in sterile Eppendorf tubes at room temperature. In one aliquot, 40 μL was replaced with an equal volume of an inoculum ($1 \times 10^{7-8}$ CFU/mL) of Ma (PG2, NCTC 10123), whereas the other aliquot was left untreated. Both tubes were incubated at 37 °C on a heating block (VWR, Radnor). Mycoplasma viability and pH were determined as described previously at the same time points. This experiment was also performed as three replicates on different days.

2.2.3. Experimental contamination of ejaculates with Ma

In this experiment, ejaculates obtained from the nine bucks by the artificial vagina method were placed in sterile tubes at 37 °C in a water bath for 15 minutes. The tubes were then gradually cooled to a final temperature of 4 °C over 90 minutes. Then, the ejaculates were placed at room temperature for about 10 minutes and pooled, and some of this pool split in two aliquots of 1460 μL in sterile Eppendorf tubes. One aliquot was inoculated with $1 \times 10^{7-8}$ CFU/mL (40 μL) of Ma (PG2, NCTC 10123), and the other was spiked with an equal volume of mycoplasma liquid medium as a negative control [23]. The remaining volume of pool ejaculate was used for the corresponding controls as previously described. Both aliquots were incubated at 37 °C and mycoplasma and sperm viability determined as described previously (see Section 2.2.1). pH was also monitored during the experiment (ejaculate pool and at 30, 90, and 150 minutes of incubation). Six replicate experiments were conducted using ejaculates refrigerated for about 1 hour before pooling in three of these replicates and ejaculates stored at 4 °C for 3 hours before pooling in the remaining trials.

2.3. Inoculum preparation

The strains PG-2 (Ma) and Y-goat (Mmc) were used to prepare the inocula. Both bacteria were cultured for 48 hours in liquid medium at 37 °C until the logarithmic phase of growth [26]. Subsequently, viable bacteria were quantified according to a method described previously [27], and a concentration of $1 \times 10^{7-8}$ CFU/mL was used to inoculate the DS, raw ejaculates, or extender. Because Ma and Mmc level of excretion in semen is not known, these concentrations were used because it is within the excretion range described for Ma in milk (10^{6-8} CFU/mL) [28] or Mmc in colostrum (10^{6-10} CFU/mL) [29].

2.4. Mycoplasma viability

Ma or Mmc viability was determined using the method of serial dilutions as reported previously [27]. In brief, 50 μL of inoculated or control DS, ejaculate, or extender were plated onto agar medium for mycoplasmas [9] in microtiter plates and the plates incubated for 48 hours at 37 °C in a humidified atmosphere enriched with 5% CO_2. Mycoplasma viability was always determined in duplicate.

2.5. Sperm quality (sperm viability, motility, motion parameters, and morphology)

Sperm viability was determined by eosin–nigrosin staining (2.5% eosin and 5% nigrosin in PBS pH 6.7) at 37 °C [30], examining 200 sperm cells per sample in duplicate.

Using a computer-assisted sperm analysis system (ISAS, Proiser, Valencia, Spain) [31], we determined percentages of total motility and progressive motility, curvilinear velocity (VCL, μm/s), straight-line velocity (VSL, μm/s), average path velocity (VAP, μm/s), linearity of the curvilinear trajectory (ratio of VSL/VCL, %), straightness (ratio of VSL/VAP,%), wobble of the curvilinear trajectory (ratio of VAP/VCL, %), amplitude of lateral head displacement (ALH, μm), and beat cross-frequency (Hz).

A 7-μL drop aliquot of the sperm sample was placed on a warmed (37 °C) slide (Superfrost Menzel-Glaser, Braunschweig, Germany) and covered with a 24 × 24-mm cover slip. The setting parameters were the following: 100 frames were captured with a Basler digital camera (50 frames/sec), in which spermatozoa had to be present in at least 15 to be counted, imagines obtained at ×100 magnification in a negative contrast phase microscope. Spermatozoa with a VAP less than 10 μm/s were considered immotile. A minimum of 5 fields per sample were evaluated, counting a minimum of 200 spermatozoa per sub-sample.

To examine sperm morphology, we used a 4% saline solution formulated to fix the spermatozoa. A 50-μL volume of this solution was added to 50 μL of semen, and the samples stored at 4 °C until examination using a phase contrast microscope (at ×100). In each sample, 200 sperm cells were examined and results expressed as percentages of normal and abnormal (coiled and bent) sperm tails [32]. Each determination was performed in duplicate.

2.6. pH measurement

pH was measured using an electronic pH meter (Hamilton Minitrode, Bonaduz, Switzerland) which was calibrated according to the manufacturer's instructions before each experiment.

2.7. Mycoplasma culture and PCR

Solid and liquid mycoplasma media [9] were used to test the presence of mycoplasmas. Liquid media were inoculated with 200 μL of each sample and incubated at 37 °C in a 5% CO_2 humid atmosphere for 48 hours. Isolates from previously cloned single colonies were used for preliminary identification on the basis of biochemical and serologic tests as previously described [6].

In addition, DNA was extracted from 200 μL of each sample using the High Pure PCR Template Preparation Kit (Roche Diagnostics) according to the manufacturer's instructions. The PCR procedures used were specific for Ma and Mp [33,34]. To detect the presence of Mmc or *M capricolum* subsp *capricolum* (Mcc), an initial PCR was conducted to detect all members of the *M mycoides* cluster [35]. Whenever the result of this test is positive, the

identity of the species is confirmed by amplifying the *fusA* gene [36] for sequencing and alignment [37].

2.8. Statistical analysis

For bacterial viability, determinations were transformed into log CFU/mL to normalize the distribution of data. Bacterial viability was the dependent variable; independent variables were the treatment, time, and their interaction, and statistical analysis was performed using a general linear procedure with Statistical Analysis System 6.11 (SAS, 1996). In addition, for sperm viability (%), sperm motility, and sperm morphology variables, a two-way ANOVA model was used to assess the effects of treatment, time, and their interaction. When ANOVA revealed a significant effect, this was confirmed by Tukey HSD (Honestly significant differences) test.

3. Results

All nine animals used in this study were seronegative for Ma. The absence of Ma, Mmc, Mcc, and Mp in the ear canal and in raw semen was also confirmed before the study. All semen samples tested negative when subjected to culture and PCR for mycoplasmas. No *Mycoplasma* spp. colony growth was observed in any of the negative controls for the DS, raw ejaculate, or semen extender samples.

The survival capacity of the mycoplasmas in the different conditions examined was determined through microbial counts. Figure 1 provides the concentrations in CFU/mL of mycoplasmas recorded at each time point in the inoculated in contaminated DS, ejaculated, acidified ejaculated semen, and semen diluent. In DS, no significant differences in mean Ma and Mmc concentrations were detected (7.1 log CFU/mL; standard error of the mean [SEM], ±0.09) during incubation. This mean concentration was similar to those recorded for the ejaculate samples (7.3 log CFU/mL; SEM, ±0.11; refrigerated for 1 or 3 hours before inoculation) and semen extender (7.4 log CFU/mL; SEM, ±0.12) inoculated with Ma.

Figure 2 provides the pH values recorded for the different inoculated samples and corresponding non-inoculated controls at each time point during incubation. In each case, pH differences between inoculated samples and their negative controls did not exceed ±0.1 (data not shown). Diluted semen samples spiked with Ma and Mmc showed similar pHs, with a mean value of 6.2. A lower pH was recorded in the pool of ejaculates stored for 3 hours compared with 1 hour before contamination. In the semen extender and ejaculate samples (stored for 1 and 3 hours) inoculated with Ma, means of 6.5, 6.7, and 5.9 were recorded during the 2.5 hours of incubation.

The percentages of viable sperm recorded at each incubation time in the inoculated DS (Ma and Mmc) and ejaculate (Ma) samples are provided in Figure 3. A significant effect of time was observed such that sperm viability decreased during incubation (P < 0.01). The presence of Mmc in the DS samples (mean, 16.68%; SEM, ±2.97)
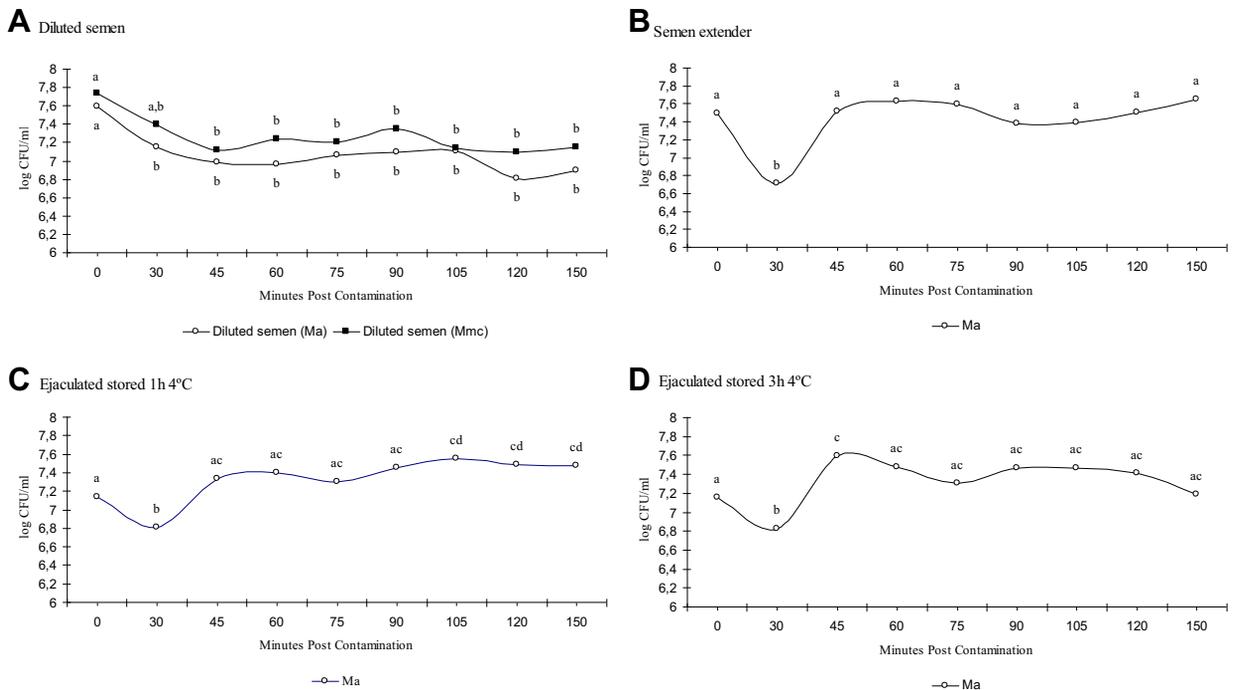

Fig. 1. Average values over time of viability (log colony-forming units [CFU]/mL) of *Mycoplasma agalactiae* (Ma) or *Mycoplasma mycoides* subsp *capri* (Mmc) used to contaminate samples of diluted semen (n = 15) (A), semen extender (n = 3) (B) or ejaculates (n = 54) (C and D) and incubated at 37 °C for 150 minutes. In all the experimental groups, a significant effect of time and of the interaction time × sample was observed (Tt*time: P < 0.05, time: P < 0.05). [a,b,c,d]Means with different superscripts differ significantly (P < 0.05) along the time. (For interpretation of the references to color in this figure, the reader is referred to the Web version of this article.)
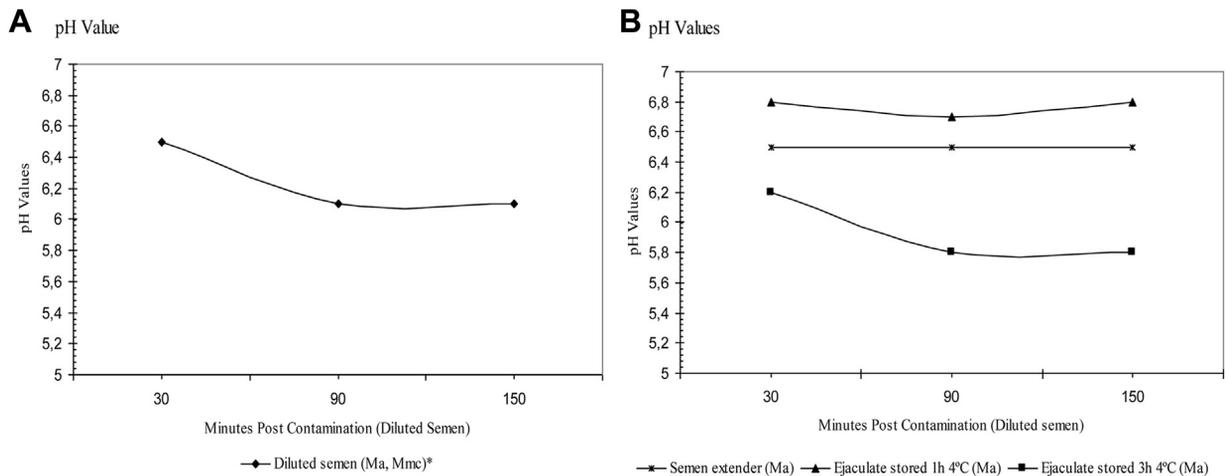
A

pH Value

B

pH Values

Fig. 2. In these graphs, pH values of different samples are shown along 150 minutes of contamination (37 °C). (A) Diluted semen (n = 15) contaminated with *Mycoplasma agalactiae* (Ma) and *Mycoplasma mycoides* subsp *capri* (Mmc) in the first experience. (B) Contaminated semen extender (n = 3) and two conditions of contaminated ejaculates (n = 54) with Ma. The average pH values obtained in the pools before the contamination were 6.0 (diluted semen), 6.5 (semen extender), 7.1 (ejaculate stored for 1 hour, 4 °C), and 6.4 (ejaculate stored for 3 hours, 4 °C). *In both contamination conditions (Ma and Mmc), identical pH values were observed along the contamination time.

induced a reduction in sperm viability (P = 0.02; mean in negative control, 21.7%; SEM, ±4.80), whereas the presence of Ma in the DS (mean, 18.33%; SEM, ±3.71) or raw ejaculate samples had no such effect (mean in contaminated ejaculate, 27.25%; SEM, ±3.67; mean in negative control, 26.48%; SEM, ±3.64).

Figure 4 shows sperm motility variables measured by computer-assisted sperm analysis recorded in the contaminated DS samples after 90 minutes of incubation. The presence of Ma and Mmc in DS had no significant effect on sperm motility parameters evaluated. An effect of time (P < 0.05) was observed in total motility and progressive motility. An effect of treatment × time (P < 0.05) was observed in total motility, progressive motility, and ALH. However, the values of total and progressive motility observed in the last time evaluated were not significantly lower than observed in controls.

Percentages of abnormal sperm tails were not significantly affected by the presence of Ma or Mmc, the incubation time, or the interaction between both factors. The mean abnormal sperm tail percentage recorded was 7.1%.

4. Discussion

This experimental study examines the survival of Ma and Mmc in goat semen and their effects on sperm quality. In the first experiment, DS samples were contaminated with Ma or Mmc to assess mycoplasma viability and effects on sperm viability, motility, and tail abnormalities. In the second phase, the determinations were made in ejaculates (mycoplasma viability and sperm viability) and semen extender samples (mycoplasma viability) contaminated with Ma.
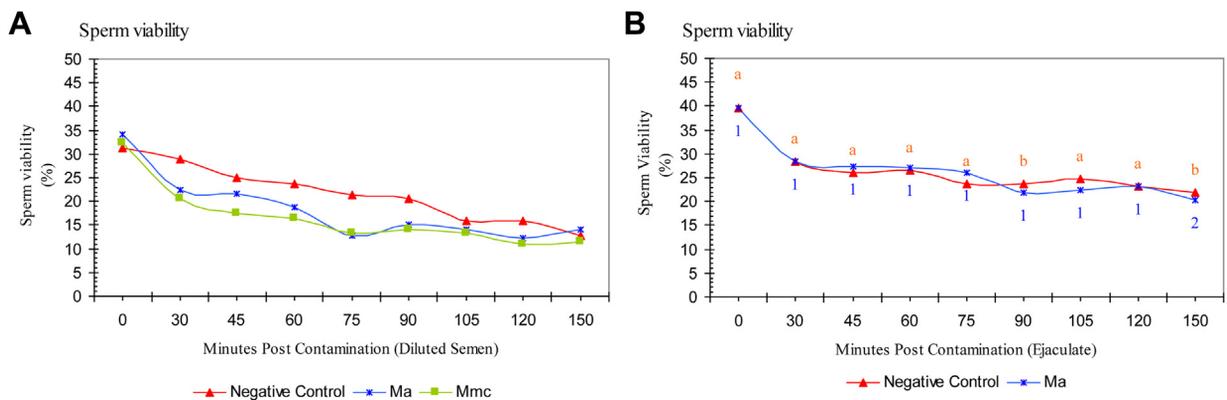
A

Sperm viability

B

Sperm viability

Fig. 3. (A) Average values over time of sperm viability recorded in contaminated diluted semen (n = 15) with *Mycoplasma agalactiae* (Ma) and *Mycoplasma mycoides* subsp *capri* (Mmc) and incubated for 150 minutes (treatment: P < 0.05, time: P < 0.05). (B) Ejaculate samples (n = 54) experimentally contaminated with Ma and also incubated for 150 minutes (time P < 0.05). Means with different superscripts differ significantly (P < 0.05) along the time in negative control ([a,b]) and contaminated ejaculates ([1,2]). (For interpretation of the references to color in this figure, the reader is referred to the Web version of this article.)
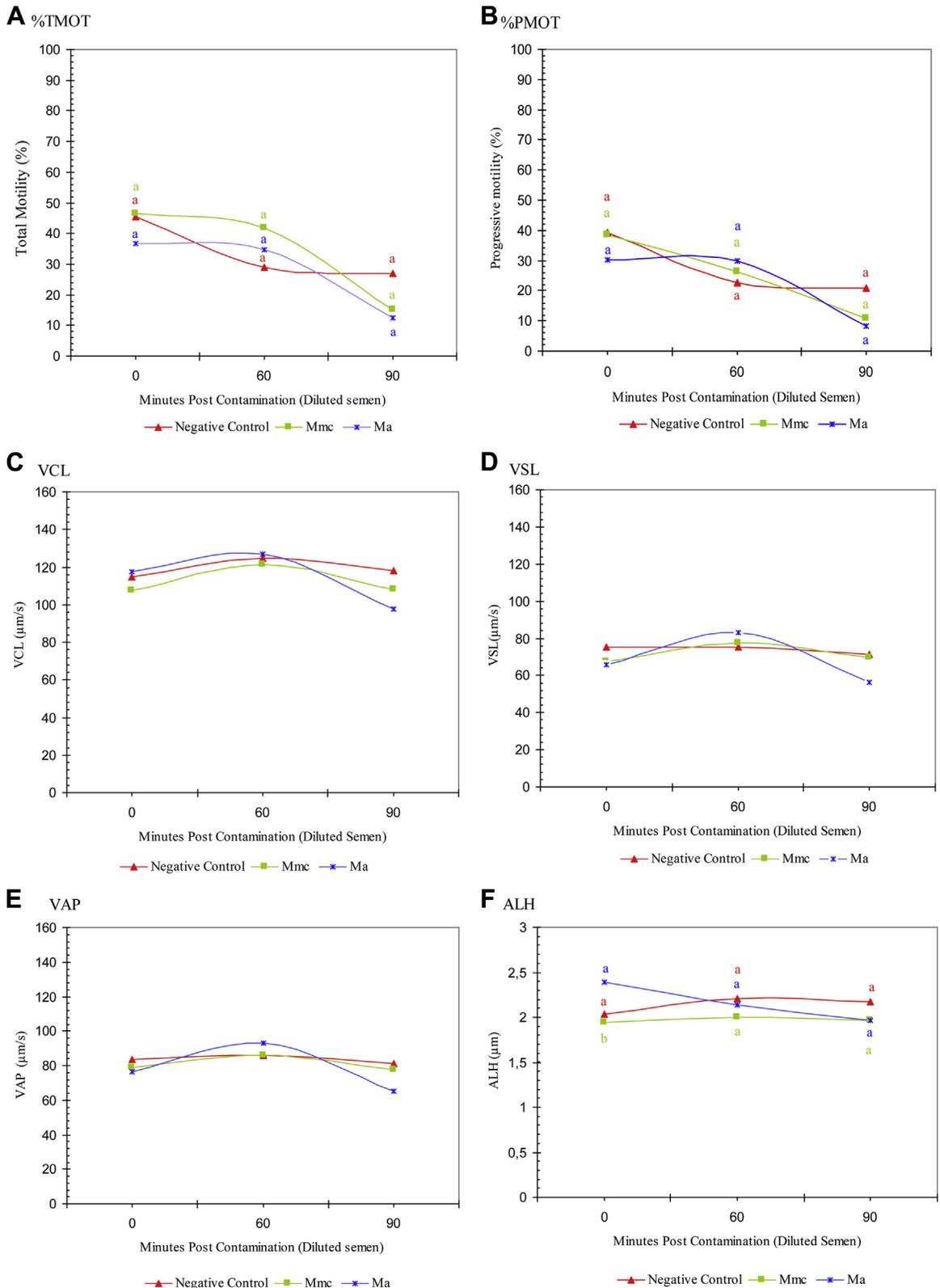
Fig. 4. Average values over time of sperm motility variables measured by computer-assisted sperm analysis in diluted semen (n = 15) inoculated with *Mycoplasma agalactiae* (Ma) and *Mycoplasma mycoides* subsp *capri* (Mmc). (A) Total motility (TMOT; Tt × time: P = 0.04; time: P = 0.00); (B) progressive motility (PMOT; Tt × time: P = 0.02; time: P = 0.04); (C) curvilinear velocity (VCL); (D) straight-line velocity (VSL); (E) average path velocity (VAP); (F) amplitude of lateral head displacement (ALH; Tt × time: P = 0.02); (G) beat cross-frequency (BCF); (H) linearity of the curviliner trajectory (LIN: VSL/VCL); (I) straightness (STR: VSL/VAP); (J) wobble (WOB: VAP/VCL). [a,b]Superscripts describe differences (P < 0.05) among treatment within time. (For interpretation of the references to color in this figure, the reader is referred to the Web version of this article.)
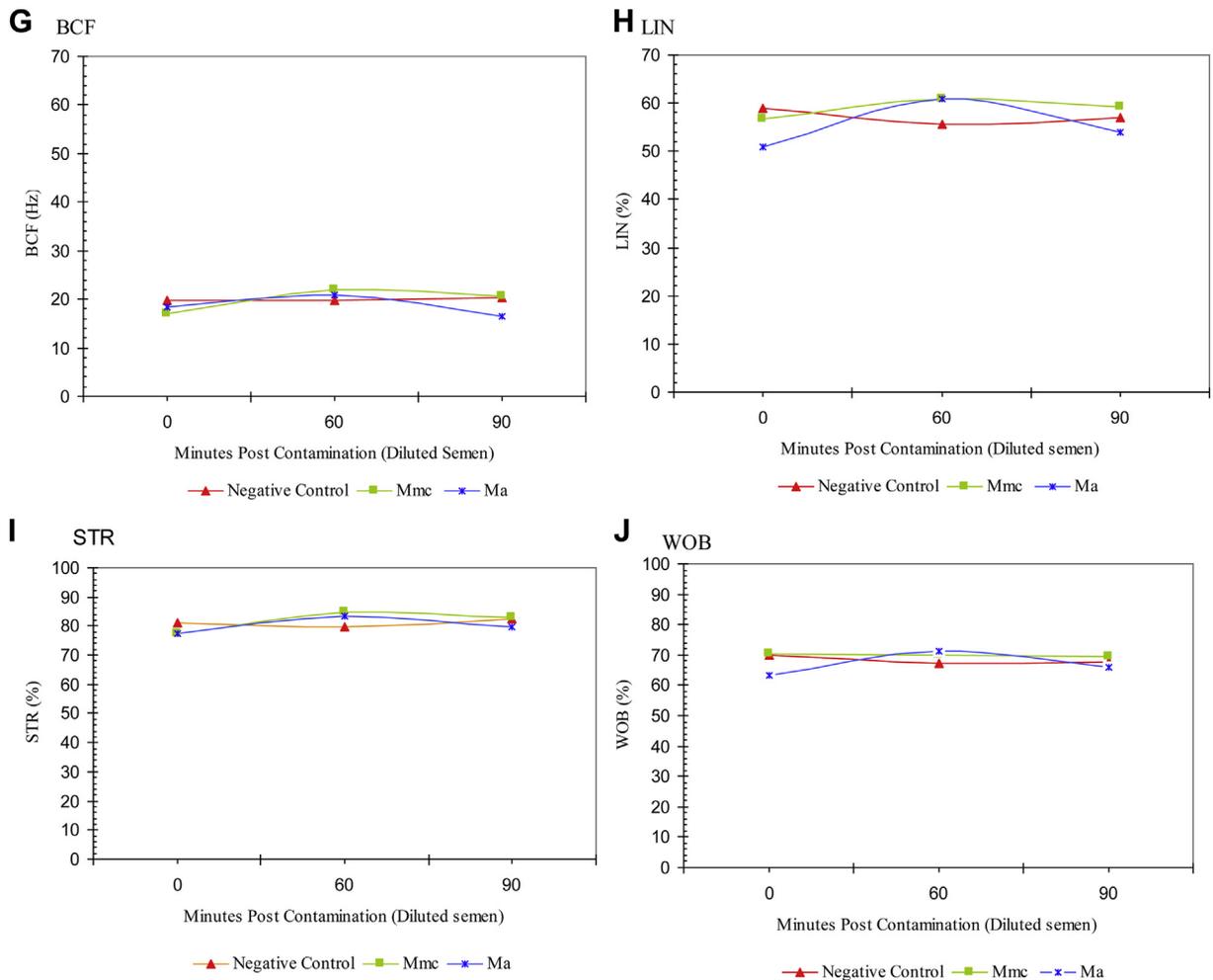
G BCF

H LIN

I STR

J WOB

Fig. 4. (*continued*).

Referring to the results of bacterial viability, after 2.5 hours of incubation in DS, similar viability was observed for Ma and Mmc (average concentration) despite a significant decrease in starting concentrations of less than one logarithmic unit (Fig. 1). This bacterial concentration is within the range of 1×10^6–10^7 CFU/mL reported as infective [38] and suggests that Ma and Mmc are able to remain viable in DS at potentially infective concentrations. Although Ma and Mmc may be excreted in the semen of naturally infected carrier bucks [6,8], the venereal transmission of CA has not been confirmed. The survival capacity of CA mycoplasmas, herein observed along with the observation that they may damage the reproductive tract of goats or sheep, support this hypothesis [2]. Hence, the risk of transmission of CA-mycoplasmas *via* AI should not be ruled out, and the treatment of semen doses with antibiotics against Ma and Mmc should be considered. The ability of mycoplasmas and ureaplasmas to survive in fresh semen (46% of isolates) and processed semen (31% of isolates) has been reported in cattle. In this sense, it has also been

shown in cattle that Mc and Mbv can transmit infection by natural breeding and AI [11,13], and this has prompted the use of different antibiotic treatments for DS to prevent the risk of venereal infection [39].

The viability of Ma in ejaculates was also examined, and it was observed that Ma can also survive in this medium at infective concentrations similar to those observed in DS (Fig. 1). Thus, as observed in cattle [11,13], there is also a risk of venereal transmission of Ma by natural breeding in goats, which requires confirmation. However, contrary to what was observed in DS, Ma concentration increased significantly during incubation in raw ejaculate samples that had been kept for 1 hour at 4 °C before their contamination, indicating that this is a suitable medium for Ma growth and that natural breeding could be an easier route of Ma transmission than AI.

Our results also indicate that during the first few minutes of contact with DS, both Ma and Mmc underwent a significant decrease in concentration (Fig. 1). As we observed no detrimental effects of sperm cells on the viability of Ma, the negative effects of DS on the

mycoplasma viability could be influenced by the physico-chemical properties of the diluent. This was assessed by contaminating the semen diluent with Ma, although significant effects on the Ma concentration were not observed after 30 minutes of incubation (Fig. 1). This tolerance of Ma to the milk-based extender is not surprising given that this species has tropism for the mammary gland and is excreted in milk [1]. These findings point to a negative effect on bacterial survival of the presence of sperm cells in the diluent.

A possible explanation could be a detrimental effect of pH due to sperm metabolism in the diluent. Other authors have observed that sperm glycolytic activity produces acidification of the medium [40] in agreement with our results (Fig. 2). In effect, the DS pool showed a lower initial pH (6) than the sperm diluent (6.5). In addition, it should be noted that the pH of the raw ejaculate-contaminated samples (stored for 1 hour at 4 °C) was close (7.1) to the optimal pH reported for the growth of mycoplasmas (7.4). This could explain the higher Ma concentration observed in these samples during incubation. Interestingly, in another assay of this work, ejaculates were stored for 3 hours and this caused the metabolic sperm acidification of the samples at the beginning of the experiment (6.6). This acidification of the medium inhibited the increase in the concentration of Ma as it was observed in ejaculates stored only for 1 hour (Fig. 1). These results highlight the effect of pH on the viability of this bacterium. Indeed, more acidic external media may cause the death of bacterial populations less adapted to such changes [41], given the sensitivity of these microorganisms lacking a cell wall to external environmental changes and soil acidification. The important role of pH in the infectivity of mycoplasma species with genital tropism has been recently reported in humans, whereby an abnormally high vaginal pH promotes the survival of M genitalium and therefore increases the risk of infection [42]. New strategies that consider the influence of this factor on CA mycoplasmas should be investigated in the future.

Besides bacterial viability, we also explored the effects of the presence of Ma or Mmc in DS on sperm viability and sperm motility. In addition, we also examined the effects of Ma on sperm viability in raw ejaculates. Our result represent the first evidence that the presence of Mmc in DS can produce adverse effects on the sperm viability (Fig. 3) of goat buck semen, which could give rise to an underestimated reproductive failure, as previously suggested [2,3]. Several simultaneous circumstances could explain these adverse effects. One could be the possible attachment of Mmc to the sperm cells of the buck, which could affect sperm membrane integrity as has already been described in other mycoplasmosis [40,43]. Furthermore, the sperm adhesion capacity of Mmc has been experimentally observed in bull semen [21]. This event was also related to a decline in total and progressive sperm motility that was not observed in our study on contaminated goat sperm with Ma or Mmc.

We observed detrimental effect on DS sperm viability produced by Mmc that was not observed by Ma (Fig. 3). We propose that the glucose content of the diluent [30]

could be another factor affecting sperm quality in contaminated DS. Mmc is able to ferment sugars to supply its metabolism and could compete with sperm for this energy source. In contrast, the Hominis cluster to which Ma belongs cannot ferment the glucose present in the DS, as also reported for Mh [40]. Furthermore, these authors did not observe any effect on sperm viability in human semen inoculated with this mycoplasma species, consistent with the effect of Ma observed here. In another study in which DS was spiked with Mb [44], another species of this cluster that does not ferment glucose [45], no apparent effect on sperm motility was detected. This suggests that the presence of sugar in contaminated sperm diluents could induce sperm damage by glucose-fermenting mycoplasma species.

Finally, we also assessed the effects of Ma and Mmc on the morphology of sperm tails in the spiked DS samples. The capacity of Mmc to produce ultrastructural lesions to bovine sperm tails was detected after 30 minutes of experimental contamination [21], which could lead to tail morphoabnormalities. We observed any significant effects of Ma or Mmc on this factor in our DS samples. In contrast, it was observed [40] several degrees of coiling and bending of the tail in a low proportion of spermatozoa exposed to Uu. However, according to these authors, this seminal contamination would have no major effects on viability, motility, and sperm morphology in the short term. Indeed, the mild effects detected here of Mmc on sperm viability (Fig. 3) and the absence of Ma and Mmc effects on sperm motility and tail sperm morphology could lead to the inadvertent use of semen contaminated with these two species for AI.

4.1. Conclusions

Under the conditions of our study, Ma and Mmc were able to survive for 2.5 hours after the incubation at 37 °C in DS at concentration considered infective in the model used. Given the survival capacity of these mycoplasma species in the female reproductive tract, the risk of venereal transmission should be considered. Natural breeding could be more favorable for Ma transmission compared to AI because this bacterium was better able to withstand the conditions of the ejaculate samples compared to the DS samples. Although sperm viability of DS samples was only slightly affected by Mmc, this determines that the presence of Ma and Mmc could be undetected in routine sperm quality checks performed in AI centers. Our data reinforce the need to design AI programs that consider the safety of goat semen doses.

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