Bacterial contamination of ram semen, antibiotic sensitivities, and effects on sperm quality during storage at 15 °C

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Abstract
This study was designed to determine the degree and type of bacterial contamination of ejaculated semen samples in fertile rams and its consequences on sperm quality during storage. In experiment 1, 68 ejaculates from 36 rams were divided into two aliquots, one of which was used for bacterial culture, while the other one was diluted, stored at 15 °C and assessed for plasma membrane integrity and motility at 0, 24 and 48 h after dilution. From the 68 ejaculates, 66 were positive for aerobic bacteria, including 20 species of bacteria from 14 genera. The most frequently isolated bacteria were *Escherichia coli*, *Proteus mirabilis*, *Enterobacter cloacae*, *Staphylococcus epidermis*, and *Staphylococcus aureus* species. These 5 bacteria were present in 97% of all contaminated samples. All contaminant bacteria were found to be sensitive to gentamicin and to ceftiofur, with variable percentages of resistance to the other antibiotics evaluated. In samples with total enterobacteria count lower than 100 colony-forming units (CFU)/ml, higher proportions of motile and progressive sperm and higher velocities of spermatozoa were observed at different times during storage. In experiment 2, pure cultures of the most frequently isolated bacteria were individually added to fresh semen samples of low contamination and tested for their effects on sperm quality during storage at 15 °C. Semen with *E. coli* showed a drastic reduction in motility, velocity and viability during storage. This reduction was also significant, but less drastic, in semen with *E. cloacae* and *P. mirabilis*, whereas it was partial and less pronounced in the other groups (*S. epidermidis* and *S. aureus*). In conclusion, the contamination of ram semen with enterobacterial species reduced sperm quality during storage at 15 °C, and the antibiotics gentamicin and ceftiofur showed the higher antimicrobial activities.

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1. Introduction
Semen collection in farm animal species is not a sterile procedure, and some degree of contamination with bacteria cannot be avoided (Clément et al., 1995; Varner et al., 1998; Althouse et al., 2000; Thibier and Guerin, 2000; Althouse and Lu, 2005; Aurich and Spergser, 2007; Bielanski, 2007). In rams, semen is usually collected with an open-ended artificial vagina that may be contaminated with bacteria from the surface of the penis and prepuce, collection area, equipment and people. As a consequence, bacteria might compromise semen quality during storage and contaminate the female’s reproductive tract. To minimize these adverse effects, antibiotics are included in the composition of the ram semen extenders to prevent bacterial growth (Maxwell and Salamon, 1993; Salamon and Maxwell, 2000).

Some bacteria are deleterious to the spermatozoa in a concentration-dependent manner. The most extensively studied is the effect of *Escherichia coli* in human spermatozoa survival. This Gram-negative bacterium reduces sperm...
motility through sperm adhesion and agglutination (Wolff et al., 1993; Monga and Roberts, 1994), causes morphological changes at the level of the midpiece, plasma membrane, and acrosome (Diemer et al., 2000), alters sperm function, and increases phosphatidylserine translocation (Villegas et al., 2005). In animal species, a variety of bacteria from different genera have been identified after aerobic culture of extended semen samples, and certain bacteria had detrimental effects on semen quality during low temperature storage (Althouse et al., 2000; Aurich and Spergser, 2007; Akhter et al., 2008).

Limited information is currently available describing the bacterial contamination of ejaculated semen samples in fertile rams. The aim of this study was to determine the degree and type of bacterial contamination of ejaculated semen samples in fertile rams and its consequences on sperm quality during storage at 15 °C.

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals, semen collection and preparation of samples

All animal procedures were performed in accordance with the Spanish Animal Protection Regulation RD223/1988, which conforms to European Union Regulation 86/609. A total of 68 ejaculates were collected from 36 genetically healthy adult Rasa Aragonesa rams (2–6 years old) using sterilized artificial vaginas and glass tubes. Rams were from the only insemination centre for Rasa Aragonesa (25 rams), and from a public regional research centre (11 rams), and were selected on the basis of a successful reproductive history and healthy appearance during general exploration and local examination of the genital organs. The undiluted semen was evaluated for motility under a phase contrast microscope at 400× (Olympus BX40 fluorescence microscope) following established guidelines (Watts, 1999).

2.1.2. Bacterial culture

Cultures were taken from single-sire whole ejaculate aliquots (n = 68; 36 rams) within 2 h after recovery. Each sample was plated on blood agar, chocolate agar and Mac-Conkey agar, and the plates were incubated in aerobic conditions at 37 °C. Aerobic cultures were inspected and bacterial growth recorded after 24 and 48 h of incubation. Bacterial contamination (expressed as colony-forming units—CFU/ml) was categorized into four groups for statistical analysis: <100, 100–999, ≥1000. Nonenteric bacteria were identified using the API 20NE system (BioMérieux S.A., Madrid, Spain), and enteric bacteria were identified using the API 20E (BioMérieux S.A., Madrid, Spain) or BBL Enterotube II (Becton Dickinson S.A., Madrid, Spain) systems. Antibiotic sensitivities were performed using the Kirby-Bauer Disk Diffusion Susceptibility Method (Becton Dickinson S.A., Madrid, Spain) following established guidelines (Watts, 1999).

2.1.3. Assessment of stored semen samples

2.1.3.1. Assessment of plasma membrane integrity

Sperm viability (membrane integrity) was assessed as described by Yániz et al. (2008). Briefly, semen samples diluted in a citrate-based extender (80.6 mM sodium citrate titrated to pH 7.0 using a 1 M citric acid solution, 55.6 mM glucose) to 50 × 10^6 sperm/ml. Aliquots (0.5 ml) of diluted samples were pipetted into 1 ml Eppendorf centrifuge tubes, and 5 μl of PI solution (0.5 mg/ml in PBS) were added to the samples. Each aliquot was incubated for 8 min in the dark at 30 °C, and spermatozoa were immobilized with formaldehyde at a final concentration of 0.3%. Spermatozoa were examined and photographed under an Olympus BX40 fluorescence microscope at 100×. Two pictures where taken of each field under negative-phase contrast and fluorescence microscopy (Olympus BX40, Olympus Optical Co., Ltd., Japan; UMWIG3 filter block) using a digital camera adapted to the microscope (Canon Eos 400D controlled using the computer through a remote control program). The images were processed using UTH-SCSA Image-Tool open software (Version 3.0, available on-line at http://ddsdx.uthscsa.edu/dig/download.html). The percentage of membrane-damaged spermatozoa was calculated as the number of propidium–iodide positive cells (in the fluorescence microscopy image) divided by the total number of cells in the same field (negative-phase contrast microscopy image). At least 500 cells were examined per sample.

2.1.3.2. Sperm motility determination by CASA (computer-assisted sperm analysis)

Computer-assisted sperm analysis (ISAS®, Version 1.0, PROISER, Valencia, Spain) was used to assess sperm motility, as described by Yániz et al. (2008). Briefly, sample aliquots (5 μl) were placed in a pre-warmed Makler chamber, and at least 500 sperm cells were analysed by CASA for each sample. The semen variables recorded were motility percentage (MS, %), progressive motility percentage (PS, %), straight line velocity (VSL, μm/s), curvilinear velocity (VCL, μm/s), average path velocity (VAP, μm/s); linearity (LIN, as a measure of a curvilinear path, VSL/VCL), straightness (STR, as the linearity of the average path, VSL/VAP), wobble (WOB, oscillation measure of the actual path about the average path, VAP/VCL), and amplitude of lateral head displacement (ALH, μm).

2.2. Experiment 2

A total of 16 ejaculates were collected from 6 genetically healthy rams from an experimental centre as described previously, using meticulous hygiene measures to decrease bacterial contamination. The minimum visual motility criterion was 80%. Native semen was checked for bacterial contamination immediately after collection as in experi-
ment 1. Seminal samples with bacterial growth in native semen higher than 100 CFU/ml (n = 6) were not processed. Pure cultures of the most frequently isolated bacteria (E. coli, Enterobacter cloacae, Proteus mirabilis, Staphylococcus aureus and Staphylococcus epidermidis) were individually tested for confirmation of effects on sperm quality during storage at 15°C. Testing was performed by transferring each of the above bacteria diluted in sterilized saline solution or saline solution alone (control) into a sample aliquot of freshly milk-extended semen (8 x 10^6 sperm/ml) to a final concentration of 10^6 bacteria/ml. Bacteria concentration was determined by turbidity (Sutton, 2006). Immediately after addition of the bacteria or saline solution, and after cooled storage (15°C) for 24 and 48 h, membrane integrity (viability), and motility of diluted semen were determined as previously described.

2.3. Statistical analysis

The values obtained were expressed as mean ± standard error of the mean (SEM). Statistic analyses were performed using the SPSS package, version 15.0 (SPSS Inc., Chicago, IL, USA). Normality distributions and variance homogeneity of the median value score for each set were checked by the Kolmogorov–Smirnov and Levene tests, respectively. As all data were normally distributed parametric tests were used throughout. Differences in membrane integrity and motility between groups were examined through one-way analysis of variance (ANOVA) using generalized linear models. If the F value was significant, a Tukey test was used for a posteriori multiple comparisons. Statistical analysis were performed considering the parameters of sperm quality at a given time during storage as the dependent variables and bacterial contamination with aerobic bacteria (<100, 100–999, ≥1000), enterobacteria (<100, 100–999, ≥1000) (experiment 1), or the effect of different bacteria added to the semen aliquots (experiment 2) as independent factors.

3. Results

3.1. Experiment 1

Microbial results indicated that, from the 68 ejaculates, 66 were positive for aerobic bacteria. A single bacterial contaminant was obtained from 43% (29/68) of the ejaculates; 54% (37/68) contained 2 or more bacterial species, with 10 specimens containing 3–6 contaminants. The degree of bacterial contamination is shown in Fig. 1. Table 1 lists the bacteria isolated from contaminated semen samples with their respective antibiotic sensitivities. After aerobic culture, the growth of 20 species of bacteria from 14 genera was registered (Table 1). The most frequently isolated bacteria were from both enteric (E. coli, P. mirabilis, E. cloacae) and nonenteric (S. epidermidis, S. aureus) groups. Some of these 5 bacteria were present in 97% of all contaminated samples (64/66). Different enterobacteria were isolated in 69.1% (47/68) of samples, and E. coli in 60.3% (41/68) of samples. All contaminant bacteria were found to be sensitive to the aminoglycoside gentamicin and to cefotiofur. The following percentages of bacteria were found to be resistant to the other antibiotics evaluated: 20% to ampicillin, 53% to penicillin, 15% to streptomycin, 4% to spectinomycin, 47% to erythromycin, 33% to oxytetracycline, 13% to polymixin B, and 15% to co-trimoxazole (SXT).

No significant relationships were observed between the total aerobic bacterial counts, determined using standard plate count agar (PCA) method, and the parameters of sperm motility and viability during storage. However, when the total enterobacteria counts in MacConkey plates were lower than 100 CFU/ml, higher proportions of motile (Fig. 2a) and progressive (Fig. 2b) sperm and higher velocities (Fig. 2c–e) of spermatozoa were observed at different times during storage (P < 0.001), although no significant differences were observed in the other kinematic variables analysed.

3.2. Experiment 2

The effects of specific bacteria on the most significant parameters of sperm motility determined by CASA and viability are shown in Fig. 3. Immediately after addition of bacteria, only the percentage of spermatozoa with progressive motility was significantly higher in the control group compared to all other groups (Fig. 3b). After 24 h of storage, semen with E. coli showed a drastic reduction in motility (Fig. 3a), velocity (Fig. 3c–i) and viability (Fig. 3j) compared to the other groups. This reduction was also important, but less apparent, in semen with E. cloacae. The other groups (P. mirabilis, S. epidermidis and S. aureus) differed significantly with the control in some kinematic variables at this time period (progressive motility, VSL, VAP, WOB, STR, ALH), but differences were less pronounced (Fig. 3). At 48 h, all spermatozoa in the E. coli group were immotile (Fig. 3a) and had damaged membranes (Fig. 3j). Significantly (P < 0.001) lower motility (Fig. 3a) and viability (Fig. 3j) parameters were found in semen with E. cloacae and P. mirabilis in comparison with the other groups. Differences in viability, motility and velocity parameters between semen with Gram-positive bacteria and the control were not significant at this time (Fig. 3).

4. Discussion

Semen is normally colonized by a variety of microorganisms that may reduce semen preservation and fertility,
Table 1
Antibiotic sensitivity test for the isolated and identified bacteria.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Antibiotic (disc concentration)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ampicillin (10 µg)</td>
</tr>
<tr>
<td></td>
<td>Penicillin G (6 µg)</td>
</tr>
<tr>
<td></td>
<td>Streptomycin (10 µg)</td>
</tr>
<tr>
<td></td>
<td>Gentamicin (10 µg)</td>
</tr>
<tr>
<td></td>
<td>Spectinomycin (100 µg)</td>
</tr>
<tr>
<td></td>
<td>Erythromycin (15 µg)</td>
</tr>
<tr>
<td></td>
<td>Oxytetracycline (30 µg)</td>
</tr>
<tr>
<td></td>
<td>Polymixin b (300 UI)</td>
</tr>
<tr>
<td></td>
<td>SXT (1.25/23.75 µg)</td>
</tr>
<tr>
<td></td>
<td>Cefotaxim (30 µg)</td>
</tr>
<tr>
<td>Acinetobacter radioresistens (n=2)</td>
<td>S</td>
</tr>
<tr>
<td>Burkholderia cepacia (n=1)</td>
<td>S</td>
</tr>
<tr>
<td>Enterobacter cloacae (n=5)</td>
<td>R</td>
</tr>
<tr>
<td>Escherichia coli (n=1)</td>
<td>R/S</td>
</tr>
<tr>
<td>Escherichia hermannii (n=1)</td>
<td>S</td>
</tr>
<tr>
<td>Klebsiella oxytoca (n=1)</td>
<td>S</td>
</tr>
<tr>
<td>Klebsiella pneumoniae (n=1)</td>
<td>R</td>
</tr>
<tr>
<td>Morganella morganii (n=1)</td>
<td>R</td>
</tr>
<tr>
<td>Pantoea spp1 (n=1)</td>
<td>S</td>
</tr>
<tr>
<td>Pantoea spp3 (n=1)</td>
<td>S</td>
</tr>
<tr>
<td>Proteus mirabilis (n=9)</td>
<td>R</td>
</tr>
<tr>
<td>Proteus vulgaris (n=1)</td>
<td>S</td>
</tr>
<tr>
<td>Providencia rettgeri (n=1)</td>
<td>S</td>
</tr>
<tr>
<td>Pseudomonas alcaligenes (n=1)</td>
<td>S</td>
</tr>
<tr>
<td>Salmonella cholerasuis (n=1)</td>
<td>S</td>
</tr>
<tr>
<td>Serratia liquefaciens (n=1)</td>
<td>S</td>
</tr>
<tr>
<td>Staphylococcus aureus (n=7)</td>
<td>R/S</td>
</tr>
<tr>
<td>Staphylococcus epidermidis (n=36)</td>
<td>R/S</td>
</tr>
<tr>
<td>Streptococcus α-hemolytic (n=1)</td>
<td>S</td>
</tr>
<tr>
<td>Vibrio fluvialis (n=1)</td>
<td>R</td>
</tr>
</tbody>
</table>

n = samples from which the bacteria were isolated.
SXT: sulphametoxazol–trimethoprim, 1.25/23.75 µg.
S = sensitive; R = resistant; R/S = strain resistant and sensitive.
and transmit diseases to the female. The new features of the present study are: (1) bacteria were isolated from the same ejaculates that were tested for sperm quality, and the concentration of enterobacteria was related to sperm quality in normal ejaculates of fertile males (experiment 1); (2) the direct effect of some bacteria (P. mirabilis, S. epidermidis) on spermatozoa was evaluated for the first time; (3) to our knowledge, this work constitutes the first time that levels and type of bacterial contamination, antibiotic sensitivities of specific bacteria and their effect on spermatozoa have been evaluated in apparently healthy and fertile rams. We must emphasize the necessity to perform studies on bacteriospermia in each animal species, as differences in anatomy, physiology and management may determine the presence of different levels and type of contamination, sensitivities of bacteria to antibiotics, and effects on spermatozoa. This study may be useful in helping to design specific working routines to minimize bacterial contamination and effects on semen quality in the ram; for example, most semen diluents currently used in Spanish ovine AI programs include penicillin and streptomycin (Yániz et al., 2005), probably because little is known of their effectiveness against bacterial contaminants.

Our observations indicate that ejaculated semen from commercial rams can frequently contain bacterial flora, in concentrations of up to $10^8$ CFU/ml. The origin may be the systemic and local infections of the reproductive tract, as well as the introduction of microorganisms during collection, processing or storage. The use of semen from apparently healthy rams, and the culture of bacteria directly from the ejaculates without processing, determine that the main incorporation of bacteria in semen probably took place during the collection process, although other sources of contamination should not be discounted. During semen collection, it is difficult to avoid contamination with the saprophytic bacteria of the prepuce or with bacteria from the environment, as evidenced in other animal species (Althouse et al., 2000; Aurich and Spergser, 2007; Akhter et al., 2008).

The most common microorganisms isolated were E. coli, P. mirabilis, E. cloacae, S. epidermis and S. aureus. These commensal bacteria are also common in the semen of other species. For example, E. coli is one of the most frequent microorganisms isolated in human (Teague et al., 1971; Auroux et al., 1991; Wolff et al., 1993; Diemer et al., 1996), equine (Varner et al., 1998), canine (Ling and...
Fig. 3. Effect of specific bacteria on kinetic parameters and viability of ram spermatozoa during storage (**P < 0.001, for each storage period).

Ruby, 1978; Allen and Dagnall, 1982; Bjurstrom and Linde-Forsberg, 1992) and porcine (Althouse et al., 2000) semen. The presence of Staphylococcus spp. has been described in semen from dogs (Ling and Ruby, 1978; Allen and Dagnall, 1982; Bjurstrom and Linde-Forsberg, 1992), stallions (Varner et al., 1998), and bulls (Wierzbowski, 1985). Proteus spp. were present in some ejaculates of dogs (Ling and Ruby, 1978; Allen and Dagnall, 1982; Bjurstrom and Linde-Forsberg, 1992), stallions (Varner et al., 1998), and bulls (Wierzbowski, 1985) and boars (Althouse et al., 2000). Finally, E. cloaca were also described in extended porcine seminal samples (Althouse et al., 2000). More interestingly, in a recent study in rams with suspected infertility (Otter, 2008), the most common isolates included E. coli, staphylococci, and Proteus species. These bacteria were recovered in similar proportions from seminal samples with normal and reduced quality, which suggested that they were probably either commensals of the reproductive tract, or contaminants (Otter, 2008). These aerobic organisms may colonize the penile and preputial mucosa, and perhaps distal urethra, as has been described in canine (Ling and Ruby, 1978; Allen and Dagnall, 1982; Bjurstrom and Linde-Forsberg, 1992).

In both experiments 1 and 2, it was found that the presence of enterobacterial species in semen was related to a reduced sperm quality during storage. Seminal samples with low levels (<100 CFU/ml) of enterobacterial contamination in study 1 had higher viable and motile sperm from 0 to 48 h of preservation. Of the 5 bacterial species tested in study 2, the three species of enterobacteria had clearly detrimental effects on sperm quality parameters. The adverse effects were more pronounced in the case of E. coli, followed by E. cloaca, and less important and manifested later with P. mirabilis. In agreement with our results, inoculation of pure cultures of E. coli and E. cloaca into freshly extended semen caused an important depression in several sperm quality parameters during storage in boars (Althouse et al., 2000).
Bacterial–spermatozoal interactions have been studied in some detail in the case of *E. coli*, and it has been established that the spermicidal effects of this bacteria are concentration-dependent (Aurox *et al.*, 1991; Diemer *et al.*, 1996). Detrimental effects were observed in semen samples only after a sperm:bacterial ratio of 1:1 or greater was achieved. In experiment 2, we used an initial sperm:bacterial ratio of 8:1, and a decrease in the percentage of progressive sperm was observed immediately after addition of bacteria, although the other quality parameters analysed were not affected until the second analysis at 24 h. During storage at 15 °C, multiplication of bacteria determines that sperm:bacterial decreases with time, increasing the adverse effects on spermatozoa. In other species (porcine and equine), the effects of bacterial contamination were not immediate and usually required 24–48 h of storage before depressions in sperm quality were evident (Althouse *et al.*, 2000; Aurich and Spergser, 2007). However, in these studies the evaluation of the percentage of spermatozoa with progressive motility was not undertaken and, consequently, it is difficult to know if the immediate effect of the bacteria in the present study could be attributed to a higher sensitivity of the ram spermatozoa to bacteria or to differences in the analysis technique. In ovine, cervical insemination is usually performed around 8 h after collection (Maxwell and Salamon, 1993; Salamon and Maxwell, 2000). This is an important limitation for a more extensive use of AI, but in terms of bacterial contamination represents the advantage that the amount of time available for bacterial multiplication is limited.

The effects of *E. coli* on human spermatozoa include reduction in sperm motility, sperm agglutination (Wolff *et al.*, 1993; Monga and Roberts, 1994), alteration of sperm function, increased phosphatidylserine translocation (Villegas *et al.*, 2005; Schulz *et al.*, 2010) and induction of morphological changes at the level of the midpiece, plasma membrane, and acrosome (Diemer *et al.*, 2000; Schulz *et al.*, 2010). The inhibitory effect of *E. coli* on sperm motility has been attributed to adhesion by mannose binding structures to receptors in the sperm membrane, a phenomenon also responsible for sperm agglutination. However, there is increasing evidence that toxic soluble factors released by the bacteria, such as α-hemolysin, Shiga-like toxin, lipopolysaccharides and peptidoglycan fragments may also have an important deleterious effect on spermatozoa (Schulz *et al.*, 2010). It is our current assumption that the mechanism(s) of action by which other Gram-negative bacteria identified in this study, such as *E. cloacae* and *P. mirabilis*, affect sperm may also be attributed to phenomena triggered by bacterial adhesion, or due in part to soluble factors released by the bacteria.

Addition of Gram-positive bacteria *S. epidermidis* and *S. aureus* caused a less pronounced decrease only in some motility parameters during storage, whereas other sperm quality variables were not affected. In a previous study with stallion semen (Aurich and Spergser, 2007), *S. aureus* failed to cause any changes in semen quality during storage over 72 h. In Gram-positive bacteria, the mechanisms that may alter sperm function have not so far been identified (Aurich and Spergser, 2007). Different strategies may be taken to minimize the effects of bacterial contamination on extended semen, as the bacterial concentration remains below a threshold level, so fertility is not affected (Althouse *et al.*, 2000). The first and most viable option is to enhance the hygienic measures during semen collection and processing. Dilution of the ejaculates with sterile diluents will further decrease the concentration of contaminants (Biełanski, 2007), although this aspect has low influence in ovine because of the high sperm concentration employed for AI. Finally, control of bacterial growth is usually performed by the use of semen extenders containing antibiotics with broad-spectrum bactericidal or bacteriostatic activity (Maxwell and Salamon, 1993; Salamon and Maxwell, 2000). Perhaps too much reliance is often placed on this method of bacterial control in ovine semen. In this species, necessary short storage periods for semen determine that the control of bacterial multiplication may be less important than in other animal species in which successful long-life semen extenders have been developed. Interestingly, 13% of identified bacteria were simultaneously resistant to penicillin and streptomycin, the most common preservative antibiotic combination used in ovine semen extenders, whereas *E. coli* was the bacteria most frequently resistant to both antibiotics (31.7%, 13/41). Antibiotics with higher antimicrobial activities in the present study were gentamicin and ceftiofur. However, their inclusion in the composition of ram semen extenders should be preceded by a study of their effects on sperm function. The effect of gentamicin (0.25 and 0.5 g/l) on the motility and viability of ram spermatozoa during storage at 15 °C up to 48 h has been determined (data not shown) and no apparent adverse effects in comparison to gentamicin-free extender were observed. In stallions, gentamicin reduce the sperm motility parameters during storage (Aurich and Spergser, 2007), although in this study a high gentamicin concentration (1 g/l) was used. The same authors stated that when gentamicin concentration was lowered to 0.25 g/l the adverse effects were not longer apparent. When this antibiotic was included in the composition of semen diluents in other species, its concentration oscillates between 200 and 500 mg/ml, that is not longer apparent. When this antibiotic was included in the composition of semen extenders containing antibiotics with broad-spectrum bactericidal or bacteriostatic activity (Gadea, 2003; Akhter *et al.*, 2008). Ceftiofur has been recently included in the composition of boar semen diluents (Gadea, 2003), though no conclusive results are available yet. In the light of the present study, this antibiotic may also be a good alternative to be included in ram semen diluents, although its impact on ram spermatozoa needs to be tested.

5. Conclusions

In conclusion, the contamination of ram semen with enterobacterial species reduced sperm quality during storage at 15 °C. The degree of resistance to the different antibiotics was variable, but all detected bacteria were found to be sensitive to the aminoglycoside gentamicin and to ceftiofur. These antibiotics could be a good alternative to the traditional penicillin/streptomycin combination used in ovine.
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