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Comparison of ticarcillin and piperacillin in Kenney's semen extender

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Abstract

Ticarcillin and piperacillin were compared to determine their effect on sperm motility and bacterial growth of equine semen samples diluted in Kenney's glucose skim milk semen extender. Each ejaculate ($n=11$) was divided into three portions and glucose skim milk semen extender solution was added. The control semen extender solution contained extended semen and no antibiotic, whereas ticarcillin and piperacillin solutions contained extended semen plus 1.0 mg/mL of ticarcillin or piperacillin, respectively. An aliquot was removed (1 h after collection) to evaluate sperm motility. All three solutions were stored at 4 °C and aliquots were obtained at 24 and 48 h to determine sperm motility and microbial concentration. Mean percentages of motile and progressively motile sperm did not differ significantly among control and antibiotic-containing solutions after storage. Control extended semen samples from ejaculates of stallions ($n = 11$) were contaminated with aerobic gram positive and gram negative bacteria. In solutions that contained either antibiotic, growth of these microbes was inhibited after 1, 24, and 48 h at 4 °C. Semen samples from stallions ($n = 5$) were extended with Kenney's glucose skim milk extender containing no antibiotic, ticarcillin or piperacillin and then inoculated with approximately 5×10^2 CFU/mL *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*; there was no significant difference between antibiotics in the inhibition of microbial growth. In conclusion, piperacillin was an appropriate alternative to ticarcillin in extenders for equine semen.

Keywords

Equine; Semen Extender; Antibiotic; Bacteriology; Spermatozoa

1. Introduction

Semen extenders are an important component of equine AI programs because they enhance the viability of sperm and allow for storage and transport of semen for a few days. Semen extenders provide substances to maintain the metabolic activity of spermatozoa, buffer against changes in pH that would otherwise disturb cell motility, and protect against cold shock during

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storage. Glucose and non-fat dry skim milk are the primary nutritive and cold-shock protective components of the Kenney semen extender used in this study. The use of effective semen extenders increased longevity of motility of spermatozoa when compared to the longevity of sperm motility of a raw sample [1].

Additionally, antibiotics in semen extenders inhibit growth of bacteria in the semen during storage [1]. The surfaces of the stallion's prepuce and penis are inhabited by a variety of bacteria. The normal bacterial microflora of stallion genitalia generally do not cause an infection in healthy mares. However, when the normal bacterial microflora of the penis or prepuce is disrupted, an overgrowth of common pathogenic bacteria such as *Klebsiella pneumonia* and *Pseudomonas aeruginosa* can cause infectious endometritis in mares and reduce fertility [2].

Urine contamination of sperm during ejaculation has also been reported as a cause of infertility in numerous species. The changes in pH and osmolarity of the ejaculate induced by urine have detrimental effects on stallion spermatozoa motility. An increased concentration of urine in an ejaculate was associated with a greater reduction in sperm motility. However, the addition of semen extender containing an antibiotic restored the motility of urine contaminated spermatozoa to that of the uncontaminated control [3].

Some studies [4] have indicated slight differences in sperm motility after storage in semen extender containing various antibiotics, as well as variation between individual stallions. However, some commonly used antibiotics such as ticarcillin disodium and amikacin sulfate do not have a detrimental effect on motility when exposed to spermatozoa during prolonged storage. Consequently, these antibiotics give satisfactory results increasing sperm longevity and motility.

Many AI programs, including the one at the New Bolton Center, predominantly used ticarcillin for several years. Ticarcillin, a derivative of penicillin, is a semi-synthetic antibiotic with a broad spectrum of bactericidal activity against many gram positive and negative aerobic and anaerobic bacteria, including *Ps. aeruginosa* [5]. Sperm from most stallions maintained good motility and fertility when extended in semen extenders containing ticarcillin. Additionally, this antibiotic was effective in controlling the overgrowth of bacteria routinely present in ejaculated semen samples from the stallion. For unexplained reasons, however, suppliers of ticarcillin recently limited the sale of this product. Breeding facilities using AI accumulated ticarcillin for the last breeding season and those supplies are now depleted or the drug has reached expiration.

In preliminary analysis conducted by members of the Section of Reproduction at the New Bolton Center, the broad-spectrum antibiotic piperacillin seemed to be as effective as ticarcillin in preventing contamination during AI. Piperacillin, another derivative of penicillin, is active *in vitro* against a variety of gram positive and negative aerobic and anaerobic bacteria such as *Ps. aeruginosa* and *K. pneumonia* [6].

The goal of this investigation was to provide quantitative evidence that piperacillin was a suitable replacement for ticarcillin in Kenney's glucose skim milk semen extenders. The parameters of this investigation included: (1) longevity of motility of spermatozoa; (2) inhibition of bacteria present in semen; and (3) control of the growth of *Ps. aeruginosa* and *K. pneumonia* in semen.

2. Materials and methods

Procedures for this experiment were adapted from the 1993 study published by Vaillancourt *et al* [7] and the 1998 study published by Varner *et al* [2]. All stallion ejaculates were collected

at the Georgia and Philip Hofmann Research Center for Animal Reproduction, New Bolton Center during June to August 2006 using a Missouri model artificial vagina (Nasco, Fort Atkinson, WI, USA).

2.1. Semen collection and preparation

Semen ejaculates were collected from horse stallions ($n = 11$). Each ejaculate was divided into three equal portions and semen was extended to obtain a final sperm concentration of 25×10^6 sperm/mL using one of the following three solutions. The control solution contained Kenney's glucose skim milk semen extender but no antibiotic. The ticarcillin solution contained Kenney's glucose skim milk semen extender plus 1.0 mg/mL ticarcillin, whereas the piperacillin solution contained Kenney's glucose skim milk semen extender plus 1.0 mg/mL piperacillin.

Sperm motility evaluation

The sperm motility of an aliquot of each solution was evaluated using a computer assisted sperm analyzer (IVOS Sperm Analyzer, Hamilton Thorne Biosciences, Beverly, MA, USA) 1 h after the ejaculate had been collected. Semen samples extended in all three solutions were stored until 24 h after collection in a commercially available equine semen transport system (Equitainer, Hamilton Research Inc., South Hamilton, MA, USA) which cooled the samples at a rate of -0.3 °C/min and then maintained the samples between 4 and 8 °C. Extended semen samples were transferred to a refrigerator that was maintained at 4 °C for 24 h after the semen had been collected. Sperm motility was analyzed in the cooled extended semen samples 24 and 48 h after the ejaculates had been collected. Two motility variables were evaluated: (1) percentage of motile spermatozoa and (2) percentage of progressively-motile spermatozoa.

2.3. Microbial evaluation of extended semen samples

A sample of each extended semen solution was analyzed for microbial concentration at 1, 24, and 48 h after semen collection by transferring a 10 μ L aliquot to a Trypsin Soy Blood Agar plate and a MacConkey plate which were then aerobically incubated for 24 h at 37 °C. A mean growth score was based on the number of colonies produced as follows: (1) 0 bacterial colonies; (2) 1-5 colonies; (3) 5-9 colonies; and (4) ≥ 10 bacterial colonies.

2.4. Microbial evaluation in experimentally inoculated specimens

Isolates of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* that had been harvested from the uterus of mares with endometritis were acquired from the culture collection maintained in the Research Microbiology Laboratory at New Bolton Center. These isolates were streaked onto BHI (Brain Heart Infusion agar) and a single colony was selected and transferred to BHI broth for overnight incubation at 37 °C. A fresh culture was grown for each experiment and cell concentration was estimated by spectrophotometric method correlated with a plate count.

Semen samples collected from some of the stallions ($n = 5$) were extended with Kenney's glucose skim milk extender containing no antibiotics, ticarcillin or piperacillin. An aliquot of each extended semen sample was inoculated with 5×10^2 CFU/mL *K. pneumoniae* or *Ps. Aeruginosa*, 4 h after the ejaculate had been obtained. At 1 h after inoculation, aliquots of the experimentally inoculated specimens were evaluated for microbial concentration by culturing for 24 h at 37 °C on Cystine-Lactose-Electrolyte-Deficient (CLED) agar or *Pseudomonas* Isolation agar, respectively. The experimentally inoculated extended semen samples were then cooled in an Equitainer® and aliquots were removed at 24 and 48 h and microbial concentrations were determined using the above described culture systems.

2.5. Statistical analysis

For the sperm motility and bacterial colonization data, the ladder test [9] was used to isolate normalizing transformations of the data. The percent motility observations were normalized using a square transformation and the colonization count in the inoculation data were normalized using a square-root transformation. The association between microbial growth and drug category was examined by Fisher's exact test [10].

Relationships between sperm motility and drug category and level of colonization and drug category were investigated using clustered regression analysis where clustering was on stallion. The statistical software STATA, Release 9.2 (Stata-Corp, College Station, TX, Stata Press) was used for the analysis, and a *P*-value for the clarity of definition of the odds ratio of less than 0.05 was considered indicative of an actual difference between the "interest" treatment and the reference state.

3. Results

3.1. Sperm motility evaluation

Extended stallion semen samples from stallions (*n* = 9) were analyzed for longevity of motility (Fig. 1). Mean percentages of motile and progressively motile sperm were not significantly different between control and antibiotic-containing semen extender solutions at 1 h (ambient temperature), and 24, or 48 h after collection when stored in an Equitainer® for 23 and 47 h.

3.2. Microbial evaluation of extended semen samples

Semen samples from stallions (*n* = 11) that were extended in Kenney's glucose skim milk semen extender containing no antibiotics, ticarcillin or piperacillin were analyzed for microbial concentration at 1 h after collection (ambient temperature) and after storage in an Equitainer® for 1, 23 and 47 h. (Fig. 2). Aerobic gram positive and gram negative bacteria grew in all control samples (no antibiotics). Both ticarcillin and piperacillin inhibited growth of these microbes in most samples taken at 1 h and 24 h after storage in an Equitainer®. After 48 h, a few colonies grew in three extended semen samples containing ticarcillin, but no bacterial colonies grew in extended semen samples containing piperacillin. There was a difference between the growth of bacterial colonies in the control samples versus the antibiotic-treated samples (*P* < 0.001). There was no difference between the growth of bacterial colonies in the extended semen samples containing ticarcillin or piperacillin (Fisher's exact = 0.000).

3.3. Microbial concentration evaluation in experimentally inoculated specimens

Extended semen samples from some of the stallions (*n* = 5) were inoculated with 5×10^2 CFU/mL *K. pneumonia* (Fig. 3) or *Ps. aeruginosa* (Fig. 4). There was a significant difference between the growth of bacterial colonies in the control samples and the antibiotic-treated samples, but no significant difference in inhibition of growth between extended semen samples containing ticarcillin or piperacillin.

4. Discussion

Piperacillin and ticarcillin did not decrease longevity of equine sperm motility in Kenney's glucose skim milk semen extender. Furthermore, piperacillin was as efficacious as ticarcillin for controlling the overgrowth of normal stallion genitalia microflora, *K. pneumonia*, and *Ps. aeruginosa*.

It is important to note that the ticarcillin used in this study, which was conducted from June to August 2006, expired in January 2005. If not expired, perhaps the ticarcillin would have been more effective than piperacillin in controlling the growth of normal stallion genitalia

microflora, *K. pneumonia*, or *Ps. aeruginosa* in an extended semen sample. However, piperacillin completely inhibited growth of the normal microflora for at least 48 h after collection. Therefore, when ticarcillin is not available for use in semen extenders, piperacillin would be an appropriate substitute for use in Kenney's glucose skim milk semen extender.

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Fig. 1. Percentage of progressively motile sperm in equine semen that was extended in Kenney's glucose skim milk semen extender containing no antibiotic, ticarcillin or piperacillin at 1 h (ambient temperature), and at 24 and 48 h (4 °C) in the Equitainer® (mean±SEM).

Fig. 2. Mean growth score of normal stallion genitalia microflora at 1, 24, and 48 h after collection and storage in an Equitainer® for 23 and 47 h. The bacterial growth was scored as: (1) 0 bacterial colonies; (2) 1 to 5 colonies; (3) 5 to 9 colonies; and (4) ≥ 10 bacterial colonies.

Fig. 3. Growth of *K. pneumoniae* after inoculation of extended equine semen samples containing no antibiotics, ticarcillin or piperacillin and storage in an Equitainer® for 24 and 48 h (mean $\pm$ SEM).

Fig. 4. Growth of *Ps. aeruginosa* after inoculation of extended equine semen samples containing no antibiotics, ticarcillin or piperacillin and storage in an Equitainer® for 24 and 48 h (mean± SEM).