ARCHIVIO ISTITUZIONALE
DELLA RICERCA

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Pharmacokinetics of oxytetracycline long-acting on plasma and semen of beef bulls

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:
Romano, J.E., Barbarossa, A., Pagliuca, G., Villadóniga, G.B., Gazzotti, T., Mislei, B., et al. (2022). Pharmacokinetics of oxytetracycline long-acting on plasma and semen of beef bulls. THERIOGENOLOGY, 186, 21-26 [10.1016/j.theriogenology.2022.03.032].

Availability:
This version is available at: https://hdl.handle.net/11585/897289 since: 2023-06-14

Published:
DOI: http://doi.org/10.1016/j.theriogenology.2022.03.032

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/).
When citing, please refer to the published version.

(Article begins on next page)

17 September 2026

This is the final peer-reviewed accepted manuscript of:

Romano, Juan E., Andrea Barbarossa, Giampiero Pagliuca, Graciela B. Villadóniga, Teresa Gazzotti, Beatrice Mislei, Elisa Zironi, e Gaetano Mari. «Pharmacokinetics of Oxytetracycline Long-Acting on Plasma and Semen of Beef Bulls». Theriogenology 186 (1 luglio 2022): 21–26.

The final published version is available online at:
https://doi.org/10.1016/j.theriogenology.2022.03.032

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

Pharmacokinetics of oxytetracycline long-acting on plasma and semen of beef bulls

Juan E. Romano[1a], Andrea Barbarossa[2,3], Giampiero Pagliuca[2,3], Graciela B. Villadóniga[4], Teresa Gazzotti[2,3], Beatrice Mislei[5], Elisa Zironi[2,3], Gaetano Mari[2,5]

[1]Large Animal Clinical Sciences, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX , USA

[2]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy

[3]Health Sciences and Technologies-Interdepartmental Centre for Industrial Research (CIRI-SDV) - University of Bologna, Ozzano dell'Emilia, Bologna, Italy

[4]St. Joseph Regional Health Center, Pediatric Services, Bryan, TX, USA

[5]AUB-INFA, National Institute of Artificial Insemination, University of Bologna, Cadriano, Bologna, Italy

Abstract

The objectives of this investigation were to evaluate the pharmacokinetic parameters of oxytetracycline long-acting in plasma and seminal plasma after a single administration through either subcutaneous or intramuscular route at 10 mg/kg or 20 mg/kg dose. Four Simmental bulls,

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

healthy and satisfactory potential breeders, were used. The route of administration either subcutaneous or intramuscular did not affect the mean values for 10 mg/kg dose in plasma (1,470 ng/mL vs. 1,330 ng/mL; P = 0.82) or seminal plasma (5,710 ng/mL vs. 5,390 ng/mL; P = 0.88), or for 20 mg/kg dose in plasma (2,540 ng/mL vs. 2,590 ng/mL; P = 0.96) or seminal plasma (25,600 ng/mL vs. 19,400 ng/mL; P = 0.58), respectively. Comparison between the 10 mg/kg and 20 mg/kg doses showed a difference in terms of mean plasma levels (1400 ng/mL vs. 2570 ng/mL; P = 0.07) and mean seminal plasma levels (6,480 ng/mL vs. 26,200 ng/mL; P = 0.004), respectively. After the dose of 10 mg/kg, plasma C_{max} was 2,841 ng/mL at 12 hours (T_{max}) with a half-life of 20.1 hours; seminal plasma C_{max} was 11,515 ng/mL at 24 hours (T_{max}) with a half-life of 23.7 hours. After the dose of 20 mg/kg, plasma C_{max} was 5,269 ng/mL at 12 hours (T_{max}) with a half-life of 18.1 hours; seminal plasma C_{max} was 55,040 ng/mL at 24 hours (T_{max}) with a half-life of 15.7 hours. Oxytetracycline long-acting may be an appropriate antibiotic, owing to its pharmacokinetic properties, that could be used for treating bulls' genital infections when its usage is indicated.

Keywords: Bull, oxytetracycline long-acting, pharmacokinetics, plasma, semen

[a]Corresponding author: juanromano@live.com

Introduction

In bulls, numerous reproductive clinical disorders demand the use of antibiotics [1-3]. Seminal adenitis syndrome represents one of the most frequent reproductive diseases in young and old bulls [3,4]. One of the ways of treating this syndrome is the administration of either local or systemic antibiotics [3,5,6]. Antibiotic selection for this clinical condition and other genital

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

infections is based on personal experience, anecdotes, extrapolation from other species, or the results of microbiological culture and sensitivity tests. The chosen antibiotic needs to be administered after determining the correct dosage, route, and frequency for an acceptable period (antibiotic stewardship) [7]. Furthermore, judicious use of antibiotics remains critical for minimizing the risk of creating microbial resistance. Scarce information is available on antibiotic levels in the bulls' genital tract or semen. Recently, new research showed that levels of tulathromycin, a macrolid antibiotic, was several times higher and persisted longer in seminal plasma compared with the plasma levels in beef bulls [8]. No studies have been published on the oxytetracycline in the bulls' genital tract or semen. Therefore, new information about this subject is of utmost importance not only to determine the suitable antibiotic but also to avoid its unnecessary usage.

The main evidence about pharmacokinetics of antibiotics in the male genital tract is derived from human and dog models [9-11]. However, the anatomy and physiology of these two species are different from ruminants [12]. Extrapolation of the information from such different species should only be carried out when no other data is available.

All tetracyclines are equally active and typically have the same broad-spectrum, which comprises both aerobic and anaerobic gram-positive and gram-negative bacteria, Mycoplasmas, Rickettsia, and Chlamydia, and even some protozoa such as Amoeba [13]. Oxytetracycline long-acting is a broad-spectrum antibiotic with a long half-life used in the treatment of a wide range of diseases. In cattle, the indications for using long-acting oxytetracyclines include bovine respiratory diseases, infectious bovine keratoconjunctivitis, foot rot, bacterial enteritis, wooden tongue, leptospirosis, wound infections, and acute metritis. Tetracyclines work by inhibiting the binding of the bacterial 30S ribosomal subunit, specifically at the aminoacyl-tRNA acceptor ("A") site on the mRNA ribosomal complex, thereby preventing ribosomal translation [13].

The pharmacokinetics of oxytetracycline in the bull's genital tract or semen has not been investigated. Due to oxytetracycline' above-mentioned characteristics, this antibiotic is a premier

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

candidate for further investigation its presence in semen. The availability of an antibiotic with long-acting effects would limit administration and animal handling frequency with the consequent reduction in animal stress. It will also improve compliance.

The objective of this investigation was to determine the pharmacokinetic parameters of oxytetracycline long-acting in plasma and seminal plasma after single-dose administration, either through subcutaneous or intramuscular route at 10 mg/kg or 20 mg/kg dose in beef bulls.

2. Material and Methods

2.1. Animals

Six Simmental bulls with excellent temperament and healthy appearance were selected. Each one had a comprehensive physical examination, including a breeding soundness examination, in accordance with the guidelines of the Society for Theriogenology [14]. In addition, blood was collected from the tail vessels and analyzed for CBC and chemical panel to rule out any subclinical liver or kidney disease. None of these showed any abnormalities. Thus, all the bulls were diagnosed as healthy and satisfactory potential breeders. Four of these bulls were randomly selected for this investigation. The age of the bulls was 15.3 ± 0.3 months (range: 15–16 months). The weight was 648.9 ± 25.9 kg (range: 645–680 kg). The body condition score was 6.0 ± 0.4 (range: 5.5–6.5) [15]. The bulls were maintained in individual pens and received a ration of corn silage, mixed hay, and alfalfa with water ad libitum. Each bull received 2.5 kg of pellets concentrate containing 14% of crude protein once a day.

2.2. Experimental design

The bulls had no history of oxytetracycline administration. A single dose, either subcutaneous (SC) or intramuscular (IM) of oxytetracycline long-acting (Terramicina long-acting, Zoetis Italy, Rome) at 10 mg/kg or 20 mg/kg (day 0 time 0) was administered. This antibiotic is approved to be used IM. Two of the bulls received the lower dose (10 mg/kg body weight), either IM or SC, on the right side of the neck. The remaining two bulls received the

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

higher dose (20 mg/kg body weight), either IM or SC, on the right side of the neck. Therefore, each bull represented a unique dose and route of administration. The total dose each bull was administered with was not more than 10 mL per injection site. The order of sample collections was plasma and semen, collected at 0, 12, 24, 36, 48, 72, and 96 hours after oxytetracycline administration. Blood was collected from the tail vessels using vacuum tubes containing lithium heparin (10 mL). Semen was collected by electroejaculation using an electro-ejaculator under automatic control (Pulsator V, Lane Manufacturing, Denver, CO, USA), having a two-electrode rectal probe of 60 mm. All the samples were immediately refrigerated. They were then centrifuged at 600 g for 30 minutes, processed within the first hour, and stored at -80 °C. The bulls were monitored twice daily for demeanor, appetite, and swelling at the injection site throughout the sample collection period and one week after that. Procedures used in this investigation were approved by the Committee for Animal Welfare, University of Bologna (Prot. n.0005783).

2.3. Oxytetracycline analysis

Oxytetracycline concentrations in plasma and seminal plasma were measured using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), adapting the approach described by Bayliss et al [16]. Briefly, after being thawed at room temperature, 200 µL of the sample was transferred to a 1.5 mL Eppendorf microtube, and an equal amount of the internal standard solution consisting of demeclocycline (Toronto Research Chemicals, North York, ON, Canada) in 10% trichloroacetic acid was added. The sample was then agitated on a vortex mixer for 30 sec and centrifuged at 21,000 × *g* for 10 min at 4 °C. A 50 µL aliquot of the supernatant was diluted in 450 µL water containing methanol 90:10 (*v/v*) with 5 mM ammonium formate and 0.1% formic acid. Extracted samples were kept in the autosampler at 20 °C, and 10 µL from each vial was injected in LC-MS/MS.

The LC system consisted of a Waters Acquity UHPLC binary pump, equipped with a Waters Acquity BEH C18 (50 × 2.1 mm, 1.7 µm) column (Waters, Milford, MA, USA), maintained at 40 °C. The mobile phase consisted of a mixture of 5 mM ammonium formate in water (A) and water

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

containing methanol 95:5 (*v/v*) with 5 mM ammonium formate (B) at a flow rate of 0.3 mL/min under programmed conditions. The chromatographic run started with 90% A for the first 0.5 min, then gradually switched to 10% A over 1.5 min, and, after 1 min in these conditions, went back to 90% A in 0.5 min, allowing the column to equilibrate for one more minute. The LC was interfaced to a Waters Quattro Premier XE triple quadrupole mass spectrometer (Waters, Milford, MA, USA), operating in positive electrospray ionization (ESI+) mode. The capillary voltage was set at 3.00 kV, while the source and desolvation temperatures were 120 °C and 400 °C, respectively. Cone gas was set at 100 L/h and desolvation gas at 650 L/h. Argon was used as the collision gas. Oxytetracycline and demeclocycline eluted at 2.04 and 2.11 min, respectively; the quantifying ion was 461.1 > 425.8 *m/*z for oxytetracycline and 465.0 > 447.6 *m/*z for demeclocycline. Data were acquired and processed using MassLynx 4.1 software (Waters, Milford, MA, USA).

During each day of analysis, calibration curves were freshly prepared by spiking 200 µL aliquots of each matrix with oxytetracycline (Toronto Research Chemicals, North York, ON, Canada) at optimal concentration ranges (20–10,000 ng/mL for plasma, 20–100,000 ng/mL for seminal plasma). Quality control (QC) samples were also prepared in triplicates at three different concentrations for each curve. Peak area ratios between oxytetracycline and the internal standard were plotted against their concentration. Then a linear least square regression model was applied. The resulting correlation coefficient (R2) was always ≥ 0.99, and all the calibration standards were within ± 15% of the nominal value, confirming the good linearity of the method. The lower limit of quantification (LLOQ), intended as the lowest concentration of oxytetracycline tested, giving a signal/noise ratio ≥ 10, was calculated to be 20 ng/mL for the three matrices. Accuracy expressed as the relative difference between the measured value and expected concentration resulted within ± 15% at all QC concentrations and all the three matrices. Similarly, precision, defined as the coefficient of variation (CV%) among repeated individual measures, was always < 15%.

2.4. Pharmacokinetic parameters

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

Noncompartmental analysis was performed to estimate the pharmacokinetic parameters in plasma and seminal plasma for each animal. Standard software, PK-Solver add-in for Excel [17], was used to estimate the pharmacokinetic parameters. The following variables were calculated for plasma and seminal plasma of each animal: time of peak drug concentration (T_{max}), peak drug concentration (C_{max}), apparent elimination half-life (t1/2, calculated as ln (2)/λz, λz being the first-order rate constant associated with the terminal portion of the time-concentration curve as estimated by linear regression of time vs. log concentration). The area under the time-concentration curve from time zero to the last observed concentration ($AUC_{0\text{-last}}$) was calculated by the linear trapezoidal rule. The area under the time-concentration curve from time zero extrapolated to infinity ($AUC_{0\text{-inf}}$, calculated by adding the last observed concentration divided by λz to the $AUC_{0\text{-last}}$), area under the moment curve from time zero to last observed concentration ($AUMC_{0\text{-last}}$), area under the moment curve from time zero extrapolated to infinity ($AUMC_{0\text{-inf}}$), mean resident time estimated using time zero to last observed concentrations ($MRT_{0\text{-last}}$, calculated as $AUMC_{0\text{-last}}/AUC_{0\text{-last}}$), and mean residence time estimated using time zero to infinity ($MRT_{0\text{-inf}}$, calculated as $AUMC_{0\text{-inf}}/AUC_{0\text{-inf}}$).

2.5. Statistical Analysis.

A statistical software [18] was used to determine parameters such as mean, standard deviation, and range. Student's t-test for paired samples was conducted. Besides, a software program (PK-Solver) for pharmacokinetic parameters, as previously mentioned, was used [17]. An alpha error of 5% was considered to accept the alternative hypothesis.

3. Results

All the bulls remained clinically healthy throughout the study period. However, every bull presented with mild swelling at the area of injection. The area around the SC injection site was larger than around the IM route. The swelling began to decrease by Day 2 after injection and was resolved in all bulls by Day 7.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

Oxytetracycline administered either SC or IM at 10 mg/kg dose resulted in no difference in mean plasma levels (1,470 ± 1,090 ng/mL vs. 1,330 ± 990 ng/mL; P = 0.82) and mean seminal plasma levels (5,710 ± 4,640 ng/mL vs. 5,390 ± 3,160 ng/mL; P = 0.88), respectively. Because no significant differences were noticed at the two sites of administration, the means for plasma and seminal plasma were combined. Oxytetracycline administered either SC or IM at 20 mg/kg dose resulted in no differences in mean plasma levels (2,540 ± 1,970 ng/mL vs. 2,590 ± 2,030 ng/mL; P = 0.96) and mean seminal plasma levels (25,600 ± 22,900 ng/mL vs. 19,400 ± 17,200 ng/mL; P = 0.58), respectively. As no significant differences were detected at the two sites of administration, the means for plasma and seminal plasma were combined. Means of plasma and seminal plasma levels for both the 10 mg/kg dose and the 20 mg/kg dose were different (1,400 ± 990 vs. 6,480 ± 3520 ng/mL; P = 0.001 for the former and 2,570 ± 1,910 vs. 26,200 ± 18,700 ng/mL; P = 0.001 for the latter), respectively. The mean ratio plasma/seminal plasma oxytetracycline levels for 10 mg/kg dose was 5.92 ± 2.16 (3.44–8.45; P = 0.001) and for the 20 mg/kg dose was 11.48 ± 3.78 (5.92–15.76; P = 0.001). Oxytetracycline doses of 10 mg/kg and 20 mg/kg resulted in different mean plasma levels (1400 ± 990 vs. 2,570 ± 1,910 ng/mL; P = 0.07) and different mean seminal plasma levels (6,480 ± 3,520 ng/mL vs. 26,200 ± 1,870 ng/mL; P = 0.004), respectively. Oxytetracycline plasma levels above 1,000 ng/mL persisted for 48 hours for the 10 mg/kg dose as compared to 66 hours (P = 0.001) for the 20 mg/kg dose. Oxytetracycline seminal plasma levels above 1,000 ng/mL remained elevated for over 96 hours for both the 10 mg/kg or 20 mg/kg dose and remained above that threshold longer than plasma levels (96 vs. 57 hours; P = 0.0001). At 96 hours, the oxytetracycline mean seminal plasma level for 10 mg/kg dose was 1,700 ± 260 ng/mL and 3,340 ± 260 ng/mL for the 20 mg/kg dose.

The oxytetracycline long-acting plasma and seminal plasma pharmacokinetic parameters are presented in Table 1. The oxytetracycline concentration in plasma and seminal plasma for the 10 mg/kg is presented in Figure 1, and oxytetracycline concentration in plasma and seminal plasma for the 20 mg/kg is presented in Figure 2.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

Following either SC or IM administration at the dose of 10 mg/kg, the plasma C_{max} was 2,841 ± 401 ng/mL at 12 hours (T_{max}) with a half-life of 20.1 ± 5.9 hours. The plasma $AUC_{0\text{-last}}$ was 112,560 ± 8,067 ng/mL/hour. The seminal plasma C_{max} was 11,515 ± 2,445 ng/mL at 24 hours (T_{max}) with a half-life of 23.7 ± 4.1 hours. The seminal plasma $AUC_{0\text{-last}}$ was 550,387 ± 13,081 ng/mL/h. Following either SC or IM administration at the dose of 20 mg/mL, the plasma C_{max} was 5,269 ± 111 ng/mL at 12 hours (T_{max}) with a half-life of 18.1 ± 0.4 hours. The plasma $AUC_{0\text{-last}}$ was 204,281 ± 3,104 ng/mL/hour. The seminal plasma C_{max} was 55,040 ± 10,605 ng/mL at 24 hours (T_{max}) with a half-life of 15.7 ± 1.2 hours. The seminal plasma $AUC_{0\text{-last}}$ was 2,153,942 ± 384,669 ng/mL/h.

4. Discussion

In cattle, oxytetracycline has been reported to produce local irritation and extensive tissue damage following IM administration [19]. Sterile and non-sterile abscess at the places of administration resulted in trimming at the injection site, with subsequent loss of good quality beef. The degree of tissue irritation at the site of administration depends on multiple factors such as the type and concentration of the drug, volume injected, vehicle used, number of administrations, and temperature of the drug at the time of administration [19-21]. Therefore, SC administration of a drug could be a potentially better option. Nevertheless, the pharmacokinetics of the drug should be considered before an alternative route of administration is recommended.

In the present study, both routes of administration produced inflammation, evidenced by moderate swelling and pain at the injection sites. The current formulation was approved for IM administration. The signs were evident for 4 days following administration. However, the swelling lasted for a shorter period than previously reported [20]. On the other hand, inflammation that lasted for 5 days was detected when 10% oxytetracycline was administered through the IM route [21]. The possible difference among reports could have been due to the different oxytetracycline formulations and dosage used [20,22].

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

In general, it is thought that IM administration of drug results in a higher peak maintained for a shorter time, and the SC causes a lower peak but is maintained longer [20]. However, in the present study, the route of administration did not affect the mean plasma or seminal plasma concentrations for both doses. This is in accordance with previous studies which showed no differences in plasma pharmacokinetics parameters when both routes of administration were compared using different drug concentrations as 5%, 10%, and 20% [20,21,23]. Interestingly, various degrees and duration of inflammation were noticed at the site of administration after different formulations of oxytetracycline long-acting at 20% were used through IM route [22]. The formulation that exhibited the lowest clinically noticeable inflammation presented the highest peak plasma oxytetracycline concentration [22]. The similarities in terms of pharmacokinetics between routes of administration observed in the present study could have been due to the inflammation produced by the SC route eliciting a rapid absorption of the oxytetracycline long-acting.

Initial studies that compared 20 mg/kg doses of standard formulation of 10% oxytetracycline with 20% long-acting oxytetracycline resulted in different plasma pharmacokinetic profiles. The long-acting formulation leads to a lower peak concentration, a later time peak concentration, a longer half-time, and a bigger AUC [24,25]. An additional study showed slight divergences in pharmacokinetics between two distinct formulations of oxytetracycline long-acting but no differences due to different routes of administration [20]. Formulations of 20% concentration of oxytetracycline have an important additional advantage because of the smaller injection volume required.

In the present study, the 10 mg/kg dose showed a plasma C_{max} of 2,841 ng/mL at 12 hours (T_{max}), which was lower than what other studies reported (4,500–6800 ng/mL) with a T_{max} from 5–10 hours depending on the brand of oxytetracycline long-acting used [22]. This difference could be due to the fact that in the present study, the blood collection was performed 12 hours after administration, when the plasma oxytetracycline concentration was already in the

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

252 descending phase. Furthermore, the comparison between oxytetracycline levels after 24 hours
253 agreed with the profile of that study [22].

254 The plasma C_{max} was 5,269 ng/mL for the 20 mg/kg dose. Previous C_{max} values reported
255 include 3,300 ng/mL [25], 3,890 ng/mL [26], 4,000 ng/mL [27], 5,200 ng/mL [28], 5,700 ng/mL
256 [29], 6,500 ng/mL [30], and from 6,210–7,500 ng/mL [20]. These previous investigations
257 reported a T_{max} of 1.5 hours [27], 3.9 hours [26], 6 hours [30], 4.7–6.2 hours [20], and 8 hours
258 [27]. Differences in peak concentration among some of those studies could have arisen because
259 the blood collection, as previously mentioned for 10 mg/kg dose, was done 12 hours after
260 administration, during which oxytetracycline concentration already enters the falling phase. In
261 the present study, a plasma concentration of ≥ 1,000 ng/mL was maintained for up to 60 hours,
262 and a level of ≥ 500 ng/mL was maintained for 84 hours.

263 Following either SC or IM at the dose of 10 mg/kg, the seminal plasma C_{max} was 11,515
264 ng/mL at 24 hours (T_{max}) with a half-life of 23.7 hours. The seminal plasma $AUC_{0\text{-last}}$ was
265 550,387 ng/mL/hour. Next, with either SC or IM administration at the dose of 20 mg/mL, the
266 seminal plasma C_{max} was 55,040 ng/mL at 24 hours (T_{max}) with a half-life of 15.7 hours. The
267 seminal plasma $AUC_{0\text{-last}}$ was 2,153,942 ng/mL/hour.

268 The plasma $AUC_{0\text{-last}}$ was 112,560 ng/mL/hour for the 10 mg/kg dose. The plasma $AUC_{0\text{-last}}$
269 for the 20 mg/kg dose was 204,281 ng/mL/hour and almost in agreement with previously
270 reported studies (231,000–260,000 ng/mL/hour, Clarke et al., 1999) [20,27]. However, it was
271 higher than other previous reports of 161,410 ng/mL/hour [26] or 149,000 ng/mL//hour [24].

272 The mean residency time between plasma and seminal plasma for the 10 mg/kg or 20
273 mg/kg did not differ significantly despite the mathematical contrast between the values. This
274 outcome could probably be due to the low number of bulls used for each dose. Furthermore,
275 when the comparison included all plasma versus all seminal plasma for both doses, a significant
276 difference was observed. This result indicates that the residency time of oxytetracycline was
277 longer in seminal plasma than in plasma.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

In general, the present investigation showed that plasma pharmacokinetic parameters for both routes of administration were similar to those previously reported [20,21,24], therefore, this finding validated the current outcomes not only for plasma but also for seminal plasma.

The ejaculate consists of spermatozoa suspended in a fluid called seminal plasma (SP). The SP constituents are created from the rete testis, epididymis, and accessory sex glands (AG) of the male reproductive tract [12,31,32]. The spermatozoa present in the ejaculate originate from the tail of the epididymis and ampulla [12,31-33]. Therefore, the presence of oxytetracycline in the seminal plasma could be considered a strong indication that the antibiotic was released from the tail of the epididymis and/or accessory sexual glands. The initial step for a successful antibiotic treatment requires a drug selection based on the microbiological results and the drug's pharmacokinetic properties to establish an adequate antimicrobial dose regime. For the first time, the present study showed that the concentration and its permanency of oxytetracycline in seminal plasma for both doses were higher and longer compared to the plasma levels. The reason why oxytetracycline achieved a higher concentration in seminal plasma is that it is a lipophilic drug with a high volume of distribution. Consequently, high tissue concentrations were achieved [26]. It was shown that long-acting oxytetracycline was well distributed within the tissues, with a serum/ tissue ratio of 6.45 for kidney and 2.39 for liver, being equal to or being one for lungs, and lower for muscle, spleen, and tears in clinically normal animals [21,34]. In the present study, the mean seminal plasma oxytetracycline concentrations were found to be nearly 6 times higher than in plasma for the 10 mg/kg dose and almost 12 times higher greater for the 20 mg/kg dose. These findings have important clinical implications such as decreased animal handling and stress without affecting the therapeutic concentration of oxytetracycline in semen. The level of active drug in the target tissues is a factor of great significance in antibiotic therapy, because it is a well-accepted fact that only free drug acts against microorganisms. It was showed that oxytetracycline long-acting has a bioavailability of more than 80% [25,27]. When administering bacteriostatic antibiotics such as long-acting oxytetracycline, the serum or seminal plasma levels should not decrease below the effective

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

minimum inhibitory concentrations during treatment. Therefore, the selection of dosing intervals and the desired minimum seminal plasma concentrations requires basic pharmacokinetic information as a guideline. Two other essential factors that should be considered are against which specific microorganism the oxytetracycline has to be applied and the clinical–pathological condition. Most of the information about pharmacokinetics parameters originated from healthy animals; notably, the disease process may alter the kinetic pattern. It was showed that the concentration of oxytetracycline administered parenterally was higher in pneumonic lung tissues than in normal ones and in quarters affected with mastitis compared to normal ones [21,24]. In general, a serum concentration between 500 and 1,000 ng/mL has been suggested as the effective therapeutic level. The minimum inhibitory concentration values for tetracyclines against most susceptible pathogenic microorganisms in cattle (Bacillus anthracis, Mycoplasma spp, Pasteurella spp, Staphylococcus aureus, Streptococcus pyogenes, and Streptococcus pneumoniae) ranged between 120 and 1,000 ng/mL. In a recent study, the same specific microorganism presented variations in vitro minimum inhibitory concentrations due to origin of the herd, age of the animal, and phylogenic background [35]. The present investigation showed that the levels in seminal plasma were not correlated to plasma levels. Therefore, for the treatment of bull genital infections, the seminal plasma pharmacokinetic parameters could be considered a more accurate approach than studying plasma dynamics. The final proof of any calculated antibiotic regimen resided in its clinical effectiveness for the specific reproductive pathology.

This investigation has multiple limitations due to the following facts: only a small number of healthy bulls were included, animals were of same age, same breed, and the sampling was done for only 96 hours. However, the information generated should be considered as an essential baseline for further investigations. Supplementary pharmacokinetics studies in bulls with specific reproductive diseases, such as seminal adenitis, are mandatory before any treatment recommendation can be made with certainty.

Conclusion

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

It was concluded that oxytetracycline long-acting should be considered as an appropriate antibiotic owing to its pharmacokinetic properties and that it could be used for the treatment of genital infections in bulls when indicated.

Acknowledgment

Part of the current information was presented at the Annual Meeting of the Society for Theriogenology and American College of Theriogenologists to be held on July 21–25, 2021, at Omaha, NE. This research was supported by funds from AUB, University of Bologna, Italy (Prot. N. 0005783). The present experiment was performed in AUB-INFA, National Institute of Artificial Insemination, University of Bologna – Cadriano, Bologna, Italy. The authors also would like to thank Ms. Giulia Cristoni, Mr. Angelo Ferrari, and Mr. Fabrizio Lollini for their helpful assistance during this project.

Competing interests

All authors declare that no conflict of interest could be perceived as prejudicing the impartiality of the research reported.

References

1. Welles EG, Tyler JW, Wolfe DF, et al.: Eperythrozoon infection in young bulls with scrotal and hindlimb edema, a herd outbreak. Theriogenology 1995;43:557–567.
2. Montes AJ, Wolfe DF, Welles EG, et al: Infertility associated with Eperythrozoon wenyonii infection in a bull. J Am Vet Med Assoc 1994;204:261–263.
3. Romano JE, Brinsko SP, Blanchard TL, et al : Male reproductive disorders. In: Smith BP, Van Metre D, Pusterla N, editors. Large Animal Internal Medicine. 6th ed. Elsevier Inc: 2020. p 1505–1519.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

4. Ball L, Griner LA, Carroll EJ: The bovine seminal vesiculitis syndrome. Amer J Vet Res 1964;25:291–302.
5. Martínez MF, Arteaga AA, Barth AD: Intraglandular injection of antibiotics for the treatment of vesicular adenitis in bulls. Anim Reprod Sci 2008; 104:201–211.
6. Martínez MF, Barth AD: Early detection and treatment of vesicular adenitis in bulls. Anim Reprod Sci 2007;101:252–256.
7. Srinivasan A: Antibiotic stewardship: Why we must, how we can. Cleve Clin J Med. 2017;84:673–679.
8. Romano JE, Barbarosa A, Pagliuca G Villadóniga B, Gazzotti T, Mislei B, Zironi E, Mari G. Pharmacokinetics of tulathromycin on plasma and semen of beef bulls. Theriogenology 2022;177:50-55.
9. Bulitta JB, Kinzig M, Naber CK, et al: Population pharmacokinetics and penetration into prostatic, seminal, and vaginal fluid for ciprofloxacin, levofloxacin, and their combination. Chemotherapy 2011;57:402–416.
10. Frimodt-Møller PC, Dørflinger T, Madsen PO: Distribution of ciprofloxacin in the dog prostate and various tissues. Urol Res 1984;12:283–286.
11. Naber CK, Steghafner M, Kinzig-Schippers M, et al: Concentrations of gatifloxacin in plasma and urine and penetration into prostatic and seminal fluid, ejaculate, and sperm cells after single oral administrations of 400 milligrams to volunteers. Antimicrob Agents Chemother 2001;45:293–297.
12. Romano, JE, Brinsko S: Reproductive physiology of the male. Chapter 40. Textbook of Veterinary Physiology. 6th Edition. Cunningham's Textbook of Veterinary Physiology. By Bradley G. Klein, editor. Saunders & Elsevier. 2020, p 471–479.
13. Baxter P, McKellar Q: Plasma and lung concentrations of oxytetracycline after its intramuscular administration in rats. Lab Anim Sci 1995; 45:107–109.
14. Koziol JH, Armstrong CL: Manual for breeding soundness examination of bulls. Society for Theriogenology. 2nd edition. 2018, p 147.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

15. Richards MW, Spitzer JC, Warner MB: Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. J Anim Sci 1986;62:300−306.
16. Bayliss MAJ, Rigdova K, Kyriakides M, et al: Development, validation and application of a novel HPLC-MS/MS method for the measurement of minocycline in human plasma and urine. J Pharm Biomed Anal 2019; 30;169:90–98.
17. Zhang Y, Huo M, Zhou J, et al: PK-Solver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. Comput Methods Programs Biomed 2010; 99:306–314.
18. Minitab 17: Minitab Inc. State College, PA, USA.
19. Rasmussen F, Ladefoged O: Tissue damage at the injection site after intramuscular injection of drug preparations formulated by addition of polyvinyl-pyrrolidone. Acta vet scand 1974;15:636–638.
20. Clarke CR, Wang Z, Cudd L, et al: Pharmacokinetics of two long-acting oxytetracycline products administered subcutaneously and intramuscularly. J Vet Pharmacol Therap 1999; 22:65–67.
21. Nouws JFM, Vree TB: Effect of injection site on the bioavailability of an oxytetracycline formulation in ruminant calves. Vet Quart 1983;5:165–170.
22. Mevius DJ, Nouws JFM, Breukink HJ, et al: Comparative pharmacokinetics, bioavailability and renal clearance of five parenteral oxytetracycline-20% formulations in dairy cows, Vet Quart 1986;8:285–294.
23. De Laistre Banting A, Fanneau de la Horie GC: Subcutaneous and intramuscular injection of oxytetracycline in calves: Comparison of serum concentration and local tolerance. J Vet Pharmacol Therap 1987;10:184–186.
24. Ames TR, Larson VL, Stowe CM: Oxytetracycline concentrations in healthy and diseased calves. Am J Vet Res 1985;44:135–31357.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

411 25. Davey LA, Ferber MT, Kaye B: Comparison of the serum pharmacokinetics of a long
412 acting and a conventional oxytetracycline injection. Vet Rec 1985;117. 426–429.
413 26. Mestorino N, E. Mariño Hernández E, Marchetti L, et al: Pharmacokinetics and tissue
414 residues of an oxytetracycline/diclofenac combination in cattle. Rev Sci Tech Off Int
415 Epiz 2007;26:679–690.
416 27. Toutain PL, Raynaud JP: Pharmacokinetics of oxytetracycline in young cattle:
417 comparison of conventional vs long-acting formulations. Amer J Vet Res 1983;44:1203–
418 1209.
419 28. Craigmill AL, Holland RE, Robinson D, et al: Serum pharmacokinetics of
420 oxytetracycline in sheep and calves and tissue residues in sheep following a single
421 intramuscular injection of a long-acting preparation. J Vet Pharmacol Therap 2000;
422 23:345–352.
423 29. Kumar R, Malik JK: Some pharmacokinetic parameters and dosage regimes for a long-
424 acting formulation of oxytetracycline in 6- to 8- month -old male calves. Vet Res
425 Commun 1998;22:533–544.
426 30. Breeze R, Gay C: Plasma levels of a long-acting oxytetracycline in cattle. Bov Pract
427 1981;16:22–23.
428 31. Mann T, Lutwak-Mann C: Biochemistry of seminal plasma and male accessory fluids;
429 application to andrological problems. In: Male Reproductive Function and Semen.
430 Springer, London; 1981, p 269–336.
431 32. Aalbers JG: The contributions of the epididymis and the main accessory glands to
432 ejaculates of bull semen. Inter J Fert 1966;77:7–13.
433 33. Seidel GE, Foote RH: Compartmental analysis of sources of the bovine ejaculate. Biol
434 Reprod 1970;2:189–196.
435 34. Punch PI, Costa ND, Chambers ED, et al: Plasma and tear concentrations of antibiotics
436 administered parenterally to cattle. Res Vet Sci 1985;39:179–187.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

35. de Boer M, Cord Heuer C, Hussein H, McDougall S: Minimum inhibitory concentrations of selected antimicrobials against Escherichia coli and Trueperella pyogenes of bovine uterine origin. J Dairy Sci 2015;98:4427–4438.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

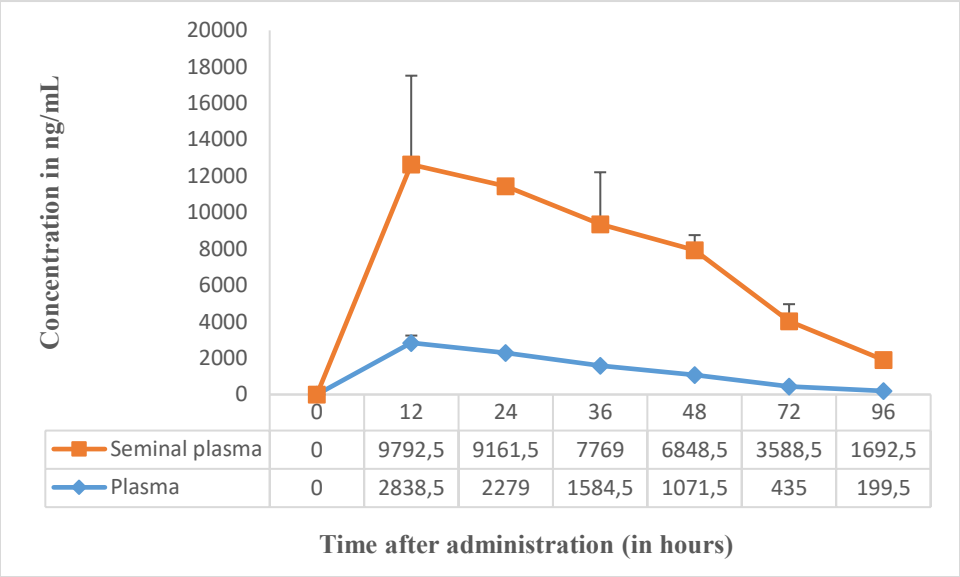
Table 1. Plasma and seminal plasma pharmacokinetics parameters (mean ± SD) of oxytetracycline long-acting administered at 10 mg/g or 20 mg/kg

			10 mg/kg						20 mg/kg		
	Plasma			Seminal plasma			Plasma			Seminal plasma	
Unit	Mean	SD		Mean	SD		Mean	SD		Mean	SD
1/h	0.0362	0.0108		0.0296	0.0051		0.0382	0.0009		0.04419	0.0033
h	20.1	6		23.7	4.1		18.2	0.4		15.7	1.2
h	12	0		24	16.9		12	0		24	16.9
ng/ml	2.841	401		11,515	2,445		5,269	111		55,040	10,605
ng*h/mL	112,560	8,066		550,387	13,081		204,281	3,104		2,153,942	384,669
ng*h/mL	118,706	12,379		609,088	5,737		211,746	2,689		2,229,992	396,140
ng*h^2/mL	3,656,347	316,386		21,472,266.00	1,742,682		6,430,752	63,843		76,246,176	7,693,129
ng*h^2/mL	4,442,702	908,243		29,172,884	4,538,396		7,342,886	8,444		85,282,668	9,185,550.00
h	32.5	0.5		39.1	4.1		31.5	0.2		35.6	2.8
h	37.2	3.8		47.9	7		34.7	0.4		38.5	2.7

λ_z being the first-order rate constant associated with the terminal portion of the time-concentration curve; t1/2: apparent elimination half-time calculated as ln(2)/ λ_z; T_{max}: time of peak drug concentration; C_{max}: the peak of drug concentration; $AUC_{0\text{-last}}$: area under the time-concentration curve from time zero to the last observed concentration; $AUC_{0\text{-inf}}$: area under the time-concentration curve from time zero extrapolated to infinity; $AUMC_{0\text{-last}}$: area under the moment curve from time zero extrapolated to last observed concentration; $AUMC_{0\text{-inf}}$: area under the moment curve from time zero extrapolated to infinity; $MRT_{0\text{-last}}$: mean resident time calculated as $AUMC_{0\text{-last}}$ /$AUC_{0\text{-last}}$; $MRT_{0\text{-inf}}$: mean resident time calculated as $AUMC_{0\text{-inf}}$ / $AUC_{0\text{-inf.}}$ mean (± SD) pharmacokinetic parameters in plasma and seminal plasma calculated via non-compartmental analysis.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.

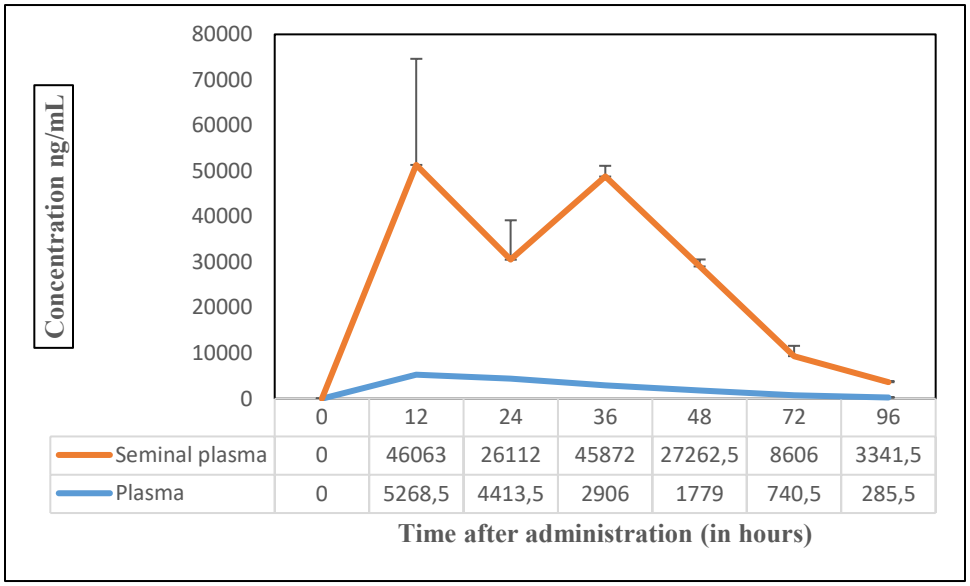


	0	12	24	36	48	72	96
Seminal plasma	0	9792,5	9161,5	7769	6848,5	3588,5	1692,5
Plasma	0	2838,5	2279	1584,5	1071,5	435	199,5

Figure 1 – Plasma and seminal plasma concentration of oxytetracycline LA (ng/ml; mean ± SD) administered at 10 mg/kg.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.



458
459
460 Figure 2- Plasma and seminal plasma concentration of oxytetracycline LA
461 (ng/ml; mean ± SD) administered at 20 mg/kg.

462

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/)

When citing, please refer to the published version.