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

2
3 Juan E. Romano^{1a}, Andrea Barbarossa^{2,3}, Giampiero Pagliuca^{2,3}, Graciela B. Villadóniga⁴,
4 Teresa Gazzotti^{2,3}, Beatrice Mislei⁵, Elisa Zironi^{2,3}, Gaetano Mari^{2,5}

5
6 ¹Large Animal Clinical Sciences, College of Veterinary Medicine & Biomedical Sciences,
7 Texas A&M University, College Station, TX , USA

8 ²Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia,
9 Bologna, Italy

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

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

13 ⁵AUB-INFA, National Institute of Artificial Insemination, University of Bologna, Cadriano,
14 Bologna, Italy

15
16 Abstract

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

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20 healthy and satisfactory potential breeders, were used. The route of administration either
21 subcutaneous or intramuscular did not affect the mean values for 10 mg/kg dose in plasma (1,470
22 ng/mL vs. 1,330 ng/mL; $P = 0.82$) or seminal plasma (5,710 ng/mL vs. 5,390 ng/mL; $P = 0.88$),
23 or for 20 mg/kg dose in plasma (2,540 ng/mL vs. 2,590 ng/mL; $P = 0.96$) or seminal plasma
24 (25,600 ng/mL vs. 19,400 ng/mL; $P = 0.58$), respectively. Comparison between the 10 mg/kg
25 and 20 mg/kg doses showed a difference in terms of mean plasma levels (1400 ng/mL vs. 2570
26 ng/mL; $P = 0.07$) and mean seminal plasma levels (6,480 ng/mL vs. 26,200 ng/mL; $P = 0.004$),
27 respectively. After the dose of 10 mg/kg, plasma C_{max} was 2,841 ng/mL at 12 hours (T_{max}) with a
28 half-life of 20.1 hours; seminal plasma C_{max} was 11,515 ng/mL at 24 hours (T_{max}) with a half-life
29 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
30 a half-life of 18.1 hours; seminal plasma C_{max} was 55,040 ng/mL at 24 hours (T_{max}) with a half-
31 life of 15.7 hours. Oxytetracycline long-acting may be an appropriate antibiotic, owing to its
32 pharmacokinetic properties, that could be used for treating bulls' genital infections when its
33 usage is indicated.

34

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

36

37 ^aCorresponding author: juanromano@live.com

38

39 Introduction

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

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

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

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

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

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

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

76 2. Material and Methods

77 2.1. Animals

78 Six Simmental bulls with excellent temperament and healthy appearance were selected.
79 Each one had a comprehensive physical examination, including a breeding soundness
80 examination, in accordance with the guidelines of the Society for Theriogenology [14]. In
81 addition, blood was collected from the tail vessels and analyzed for CBC and chemical panel to
82 rule out any subclinical liver or kidney disease. None of these showed any abnormalities. Thus,
83 all the bulls were diagnosed as healthy and satisfactory potential breeders. Four of these bulls
84 were randomly selected for this investigation. The age of the bulls was 15.3 ± 0.3 months (range:
85 15–16 months). The weight was 648.9 ± 25.9 kg (range: 645–680 kg). The body condition score
86 was 6.0 ± 0.4 (range: 5.5–6.5) [15]. The bulls were maintained in individual pens and received a
87 ration of corn silage, mixed hay, and alfalfa with water ad libitum. Each bull received 2.5 kg of
88 pellets concentrate containing 14% of crude protein once a day.

89 2.2. Experimental design

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

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

108 2.3. Oxytetracycline analysis

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

119 The LC system consisted of a Waters Acquity UHPLC binary pump, equipped with a Waters
120 Acquity BEH C18 (50 × 2.1 mm, 1.7 µm) column (Waters, Milford, MA, USA), maintained at 40
121 °C. The mobile phase consisted of a mixture of 5 mM ammonium formate in water (A) and water

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122 containing methanol 95:5 (v/v) with 5 mM ammonium formate (B) at a flow rate of 0.3 mL/min
123 under programmed conditions. The chromatographic run started with 90% A for the first 0.5 min,
124 then gradually switched to 10% A over 1.5 min, and, after 1 min in these conditions, went back to
125 90% A in 0.5 min, allowing the column to equilibrate for one more minute. The LC was interfaced
126 to a Waters Quattro Premier XE triple quadrupole mass spectrometer (Waters, Milford, MA,
127 USA), operating in positive electrospray ionization (ESI+) mode. The capillary voltage was set at
128 3.00 kV, while the source and desolvation temperatures were 120 °C and 400 °C, respectively.
129 Cone gas was set at 100 L/h and desolvation gas at 650 L/h. Argon was used as the collision gas.
130 Oxytetracycline and demeclocycline eluted at 2.04 and 2.11 min, respectively; the quantifying ion
131 was 461.1 > 425.8 *m/z* for oxytetracycline and 465.0 > 447.6 *m/z* for demeclocycline. Data were
132 acquired and processed using MassLynx 4.1 software (Waters, Milford, MA, USA).

133 During each day of analysis, calibration curves were freshly prepared by spiking 200 µL aliquots
134 of each matrix with oxytetracycline (Toronto Research Chemicals, North York, ON, Canada) at
135 optimal concentration ranges (20–10,000 ng/mL for plasma, 20–100,000 ng/mL for seminal
136 plasma). Quality control (QC) samples were also prepared in triplicates at three different
137 concentrations for each curve. Peak area ratios between oxytetracycline and the internal standard
138 were plotted against their concentration. Then a linear least square regression model was applied.
139 The resulting correlation coefficient (R²) was always ≥ 0.99, and all the calibration standards were
140 within ± 15% of the nominal value, confirming the good linearity of the method. The lower limit
141 of quantification (LLOQ), intended as the lowest concentration of oxytetracycline tested, giving a
142 signal/noise ratio ≥ 10, was calculated to be 20 ng/mL for the three matrices. Accuracy expressed
143 as the relative difference between the measured value and expected concentration resulted within
144 ± 15% at all QC concentrations and all the three matrices. Similarly, precision, defined as the
145 coefficient of variation (CV%) among repeated individual measures, was always < 15%.

146

147 2.4. Pharmacokinetic parameters

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148 Noncompartmental analysis was performed to estimate the pharmacokinetic parameters
149 in plasma and seminal plasma for each animal. Standard software, PK-Solver add-in for Excel
150 [17], was used to estimate the pharmacokinetic parameters. The following variables were
151 calculated for plasma and seminal plasma of each animal: time of peak drug concentration
152 (T_{max}), peak drug concentration (C_{max}), apparent elimination half-life ($t_{1/2}$, calculated as \ln
153 $(2)/\lambda_z$, λ_z being the first-order rate constant associated with the terminal portion of the time-
154 concentration curve as estimated by linear regression of time vs. log concentration). The area
155 under the time-concentration curve from time zero to the last observed concentration (AUC_{0-last})
156 was calculated by the linear trapezoidal rule. The area under the time-concentration curve from
157 time zero extrapolated to infinity (AUC_{0-inf} , calculated by adding the last observed concentration
158 divided by λ_z to the AUC_{0-last}), area under the moment curve from time zero to last observed
159 concentration ($AUMC_{0-last}$), area under the moment curve from time zero extrapolated to infinity
160 ($AUMC_{0-inf}$), mean resident time estimated using time zero to last observed concentrations
161 (MRT_{0-last} , calculated as $AUMC_{0-last}/AUC_{0-last}$), and mean residence time estimated using time
162 zero to infinity (MRT_{0-inf} , calculated as $AUMC_{0-inf}/AUC_{0-inf}$).

163

164 2.5. Statistical Analysis.

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

169 3. Results

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

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174 Oxytetracycline administered either SC or IM at 10 mg/kg dose resulted in no difference
175 in mean plasma levels ($1,470 \pm 1,090$ ng/mL vs. $1,330 \pm 990$ ng/mL; $P = 0.82$) and mean seminal
176 plasma levels ($5,710 \pm 4,640$ ng/mL vs. $5,390 \pm 3,160$ ng/mL; $P = 0.88$), respectively. Because
177 no significant differences were noticed at the two sites of administration, the means for plasma
178 and seminal plasma were combined. Oxytetracycline administered either SC or IM at 20 mg/kg
179 dose resulted in no differences in mean plasma levels ($2,540 \pm 1,970$ ng/mL vs. $2,590 \pm 2,030$
180 ng/mL; $P = 0.96$) and mean seminal plasma levels ($25,600 \pm 22,900$ ng/mL vs. $19,400 \pm 17,200$
181 ng/mL; $P = 0.58$), respectively. As no significant differences were detected at the two sites of
182 administration, the means for plasma and seminal plasma were combined. Means of plasma and
183 seminal plasma levels for both the 10 mg/kg dose and the 20 mg/kg dose were different ($1,400 \pm$
184 990 vs. $6,480 \pm 3520$ ng/mL; $P = 0.001$ for the former and $2,570 \pm 1,910$ vs. $26,200 \pm 18,700$
185 ng/mL; $P = 0.001$ for the latter), respectively. The mean ratio plasma/seminal plasma
186 oxytetracycline levels for 10 mg/kg dose was 5.92 ± 2.16 (3.44–8.45; $P = 0.001$) and for the 20
187 mg/kg dose was 11.48 ± 3.78 (5.92–15.76; $P = 0.001$). Oxytetracycline doses of 10 mg/kg and 20
188 mg/kg resulted in different mean plasma levels (1400 ± 990 vs. $2,570 \pm 1,910$ ng/mL; $P = 0.07$)
189 and different mean seminal plasma levels ($6,480 \pm 3,520$ ng/mL vs. $26,200 \pm 1,870$ ng/mL; $P =$
190 0.004), respectively. Oxytetracycline plasma levels above 1,000 ng/mL persisted for 48 hours for
191 the 10 mg/kg dose as compared to 66 hours ($P = 0.001$) for the 20 mg/kg dose. Oxytetracycline
192 seminal plasma levels above 1,000 ng/mL remained elevated for over 96 hours for both the 10
193 mg/kg or 20 mg/kg dose and remained above that threshold longer than plasma levels (96 vs. 57
194 hours; $P = 0.0001$). At 96 hours, the oxytetracycline mean seminal plasma level for 10 mg/kg
195 dose was $1,700 \pm 260$ ng/mL and $3,340 \pm 260$ ng/mL for the 20 mg/kg dose.

196 The oxytetracycline long-acting plasma and seminal plasma pharmacokinetic parameters
197 are presented in Table 1. The oxytetracycline concentration in plasma and seminal plasma for the
198 10 mg/kg is presented in Figure 1, and oxytetracycline concentration in plasma and seminal
199 plasma for the 20 mg/kg is presented in Figure 2.

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200 Following either SC or IM administration at the dose of 10 mg/kg, the plasma C_{\max} was
201 2,841 \pm 401 ng/mL at 12 hours (T_{\max}) with a half-life of 20.1 \pm 5.9 hours. The plasma $AUC_{0-\text{last}}$
202 was 112,560 \pm 8,067 ng/mL/hour. The seminal plasma C_{\max} was 11,515 \pm 2,445 ng/mL at 24
203 hours (T_{\max}) with a half-life of 23.7 \pm 4.1 hours. The seminal plasma $AUC_{0-\text{last}}$ was 550,387 \pm
204 13,081 ng/mL/h. Following either SC or IM administration at the dose of 20 mg/mL, the plasma
205 C_{\max} was 5,269 \pm 111 ng/mL at 12 hours (T_{\max}) with a half-life of 18.1 \pm 0.4 hours. The plasma
206 $AUC_{0-\text{last}}$ was 204,281 \pm 3,104 ng/mL/hour. The seminal plasma C_{\max} was 55,040 \pm 10,605
207 ng/mL at 24 hours (T_{\max}) with a half-life of 15.7 \pm 1.2 hours. The seminal plasma $AUC_{0-\text{last}}$ was
208 2,153,942 \pm 384,669 ng/mL/h.

209

210 4. Discussion

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

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

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

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

247 In the present study, the 10 mg/kg dose showed a plasma C_{max} of 2,841 ng/mL at 12
248 hours (T_{max}), which was lower than what other studies reported (4,500–6800 ng/mL) with a T_{max}
249 from 5–10 hours depending on the brand of oxytetracycline long-acting used [22]. This
250 difference could be due to the fact that in the present study, the blood collection was performed
251 12 hours after administration, when the plasma oxytetracycline concentration was already in the

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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 $\geq 1,000$ 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.

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

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

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

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

331 Conclusion

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

335

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337

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345

346 Competing interests

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

349

350 References

- 351 1. Welles EG, Tyler JW, Wolfe DF, et al.: Eperythrozoon infection in young bulls with
352 scrotal and hindlimb edema, a herd outbreak. *Theriogenology* 1995;43:557–567.
- 353 2. Montes AJ, Wolfe DF, Welles EG, et al: [Infertility associated with Eperythrozoon](#)
354 [wenyonii infection in a bull](#). *J Am Vet Med Assoc* 1994;204:261–263.
- 355 3. Romano JE, Brinsko SP, Blanchard TL, et al : Male reproductive disorders. In: Smith BP,
356 Van Metre D, Pusterla N, editors. *Large Animal Internal Medicine*. 6th ed. Elsevier Inc:
357 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.

- 358 4. Ball L, Griner LA, Carroll EJ: The bovine seminal vesiculitis syndrome. Amer J Vet Res
359 1964;25:291–302.
- 360 5. Martínez MF, Arteaga AA, Barth AD: Intraglandular injection of antibiotics for the
361 treatment of vesicular adenitis in bulls. Anim Reprod Sci 2008; 104:201–211.
- 362 6. Martínez MF, Barth AD: Early detection and treatment of vesicular adenitis in bulls.
363 Anim Reprod Sci 2007;101:252–256.
- 364 7. Srinivasan A: Antibiotic stewardship: Why we must, how we can. Cleve Clin J Med.
365 2017;84:673–679.
- 366 8. Romano JE, Barbarosa A, Pagliuca G Villadóniga B, Gazzotti T, Mislei B, Zironi E, Mari
367 G. Pharmacokinetics of tulathromycin on plasma and semen of beef bulls.
368 Theriogenology 2022;177:50-55.
- 369 9. Bulitta JB, Kinzig M, Naber CK, et al: Population pharmacokinetics and penetration into
370 prostatic, seminal, and vaginal fluid for ciprofloxacin, levofloxacin, and their
371 combination. Chemotherapy 2011;57:402–416.
- 372 10. Frimodt-Møller PC, Dørflinger T, Madsen PO: Distribution of ciprofloxacin in the dog
373 prostate and various tissues. Urol Res 1984;12:283–286.
- 374 11. Naber CK, Steghafner M, Kinzig-Schippers M, et al: Concentrations of gatifloxacin in
375 plasma and urine and penetration into prostatic and seminal fluid, ejaculate, and sperm
376 cells after single oral administrations of 400 milligrams to volunteers. Antimicrob Agents
377 Chemother 2001;45:293–297.
- 378 12. Romano, JE, Brinsko S: Reproductive physiology of the male. Chapter 40. Textbook of
379 Veterinary Physiology. 6th Edition. Cunningham’s Textbook of Veterinary Physiology.
380 By Bradley G. Klein, editor. Saunders & Elsevier. 2020, p 471–479.
- 381 13. Baxter P, McKellar Q: Plasma and lung concentrations of oxytetracycline after its
382 intramuscular administration in rats. Lab Anim Sci 1995; 45:107–109.
- 383 14. Koziol JH, Armstrong CL: Manual for breeding soundness examination of bulls.
384 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.

- 385 15. Richards MW, Spitzer JC, Warner MB: Effect of varying levels of postpartum nutrition
386 and body condition at calving on subsequent reproductive performance in beef cattle. J
387 Anim Sci 1986;62:300–306.
- 388 16. Bayliss MAJ, Rigdova K, Kyriakides M, et al: Development, validation and application
389 of a novel HPLC-MS/MS method for the measurement of minocycline in human plasma
390 and urine. J Pharm Biomed Anal 2019; 30;169:90–98.
- 391 17. Zhang Y, Huo M, Zhou J, et al: PK-Solver: An add-in program for pharmacokinetic and
392 pharmacodynamic data analysis in Microsoft Excel. Comput Methods Programs Biomed
393 2010; 99:306–314.
- 394 18. Minitab 17: Minitab Inc. State College, PA, USA.
- 395 19. Rasmussen F, Ladefoged O: Tissue damage at the injection site after intramuscular
396 injection of drug preparations formulated by addition of polyvinyl-pyrrolidone. Acta vet
397 scand 1974;15:636–638.
- 398 20. Clarke CR, Wang Z, Cudd L, et al: Pharmacokinetics of two long-acting oxytetracycline
399 products administered subcutaneously and intramuscularly. J Vet Pharmacol Therap
400 1999; 22:65–67.
- 401 21. Nouws JFM, Vree TB: Effect of injection site on the bioavailability of an oxytetracycline
402 formulation in ruminant calves. Vet Quart 1983;5:165–170.
- 403 22. Mevius DJ, Nouws JFM, Breukink HJ, et al: Comparative pharmacokinetics,
404 bioavailability and renal clearance of five parenteral oxytetracycline-20% formulations in
405 dairy cows, Vet Quart 1986;8:285–294.
- 406 23. De Laistre Banting A, Fanneau de la Horie GC: Subcutaneous and intramuscular
407 injection of oxytetracycline in calves: Comparison of serum concentration and local
408 tolerance. J Vet Pharmacol Therap 1987;10:184–186.
- 409 24. Ames TR, Larson VL, Stowe CM: Oxytetracycline concentrations in healthy and
410 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.

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

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443

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

Unit	10 mg/kg				20 mg/kg			
	Plasma		Seminal plasma		Plasma		Seminal plasma	
	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

445

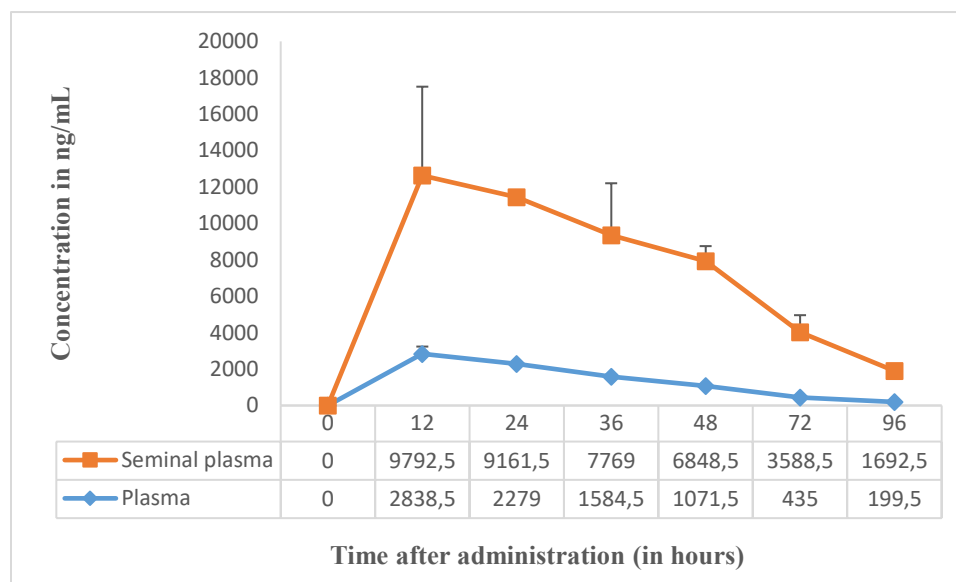
446 λ_z being the first-order rate constant associated with the terminal portion of the time-concentration curve; $t_{1/2}$: apparent elimination half-time
447 calculated as $\ln(2)/\lambda_z$; T_{max} : time of peak drug concentration; C_{max} : the peak of drug concentration; AUC_{0-last} : area under the time-concentration curve
448 from time zero to the last observed concentration; AUC_{0-inf} : area under the time-concentration curve from time zero extrapolated to infinity; $AUMC_{0-}$
449 $last$: area under the moment curve from time zero extrapolated to last observed concentration; $AUMC_{0-inf}$: area under the moment curve from time
450 zero extrapolated to infinity; MRT_{0-last} : mean resident time calculated as $AUMC_{0-last}/AUC_{0-last}$; MRT_{0-inf} : mean resident time calculated as $AUMC_{0-}$
451 inf/AUC_{0-inf} . mean (\pm SD) pharmacokinetic parameters in plasma and seminal plasma calculated via non-compartmental analysis.

452

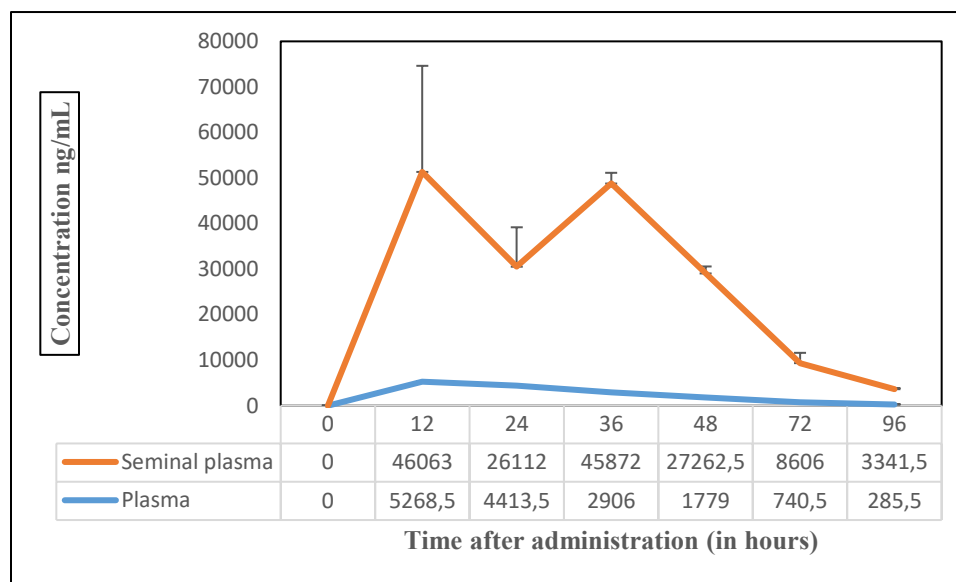
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454
455 Figure 1 – Plasma and seminal plasma concentration of oxytetracycline LA
456 (ng/ml; mean \pm SD) administered at 10 mg/kg.
457



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Figure 2- Plasma and seminal plasma concentration of oxytetracycline LA (ng/ml; mean \pm SD) administered at 20 mg/kg.

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