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Juan E. Romano[a], Anisa Bardhi[b], Giampiero Pagliuca[bc], Graciela B. Villadóniga[d], Andrea Barbarossa[bc]

[a] Cooperative of Agriculture and Research Center, College of Agriculture, Food and Natural Resources, Prairie View A&M University, Prairie View, TX, 77446, USA
[b] Department of Veterinary Medical Sciences, University of Bologna, 40064, Ozzano dell'Emilia, Bologna, Italy
[c] Health Sciences and Technologies-Interdepartmental Center for Industrial Research (CIRI-SDV), University of Bologna, 40064, Ozzano dell'Emilia, Bologna, Italy
[d] St Joseph Regional Health Center, Pediatric Services, Bryan, TX, 77802, USA

Abstract

The objectives of this study were to compare the serum and seminal plasma pharmacokinetic profiles of florfenicol (FLO) and florfenicol amine (FLA) after the administration of FLO either by IM or SC routes in beef bulls. Four clinically healthy Hereford bulls underwent a comprehensive physical exam, including breeding soundness examination, CBC, and chemistry profile panel. Bulls were healthy and classified satisfactory potential breeders. In one group (n = 2), a single dose of FLO was administered SC in the middle of the neck at a dose of 40 mg/kg of body weight. In the second group (n = 2), a single dose was administered IM in the muscles of the neck at a dose of 20 mg/kg. Concentrations of FLO and FLA in serum and seminal plasma were determined by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). Blood and semen samples were collected before the administration of FLO and at 12, 24, 36, 48, 72, 96, 120, 144, and 168 h after injection. The blood was collected from the coccygeal vessels, and semen was collected by electroejaculation. All samples were immediately refrigerated, processed within the first hour after collection, and finally stored at -80 °C. The mean level of total FLO in serum was higher when administered by the SC route (1,415.5 ng/mL) than by the IM route (752.4 ng/mL; P = 0.001). Differences were observed between the percentage of FLA in serum (1.8%; ranging from 1.3 to 2.9) and in seminal plasma (27.5%; ranging from 15.9 to 34.2; P = 0.0001). The mean level (±SD) of FLA was higher in seminal plasma compared to serum (467 ± 466 ng/mL and 18 ± 16 ng/mL, respectively; P = 0.001). The mean level of total FLO in seminal plasma was 1,454.8 ng/mL for the SC route and 1,872.9 ng/mL for the IM route without differences between the two routes (P = 0.51). Differences in the mean level of total FLO between serum and seminal plasma were detected (1,187 ± 2,069 ng/mL and 1,748 ± 1,906 ng/mL, respectively; P = 0.04). From the present investigation, it was concluded that FLO is a suitable antibiotic based on its pharmacokinetic attributes and may be employed for the treatment of bull genital infections when its use is indicated. To study the pharmacokinetics of FLO in seminal plasma, the analysis of FLA should be incorporated.

Keywords

Bulls, Florfenicol, Florfenicol amine, Pharmacokinetics, Serum, Semen

1. Introduction

In bulls, the use of antibiotics is essential in various reproductive clinical conditions [[1], [2], [3]]. One of the most common reproductive diseases in young and old bulls is the vesicular adenitis syndrome [3,4], and its treatment often involves the use of local or systemic antibiotics [3,5]. In bulls, a variety of microorganisms have been isolated from cases of vesicular adenitis syndrome including Actinobacillus actinoides, Aeromonas hydrophila, Brucella abortus, Chlamydophila psittaci, Corynebacterium renale, Corynebacterium pseudotuberculosis, Escherichia coli, Histophilus somni, Mycobacterium tuberculosis, Mycobacterium paratuberculosis, Mycoplasmas, Proteus mirabilis, Streptococcus, Staphylococcus, Ureaplasmas, Trueperella pyogenes, and Tritrichomonas foetus [3]. In bulls the most frequent microorganism isolated is Trueperella pyogenes [3,5]. However, most of the studies the selection of antibiotics for this last condition and other genital

infections (e.g. epididymitis) was based on personal experience, anecdotal evidence, extrapolation from other species, and few were based on microbiological culture with identification and sensitivity test to the antibiotics [3,5]. To ensure the appropriate choice of antibiotics, it is crucial to rely on microbiological culture, isolation, identification, and sensitivity tests [3,5,6]. Administering the chosen antibiotic at the precise dose, route, and frequency for an appropriate duration (antibiotic stewardship) is essential [7]. Careful and responsible use of antibiotics is critical in minimizing the risk of microbial resistance. Recent research has reported new information about two families of antibiotics found in bull's seminal plasma: macrolides and tetracycline [8,9]. This new knowledge is essential not only for designing effective treatment regimens but also for avoiding the unnecessary use of antibiotics and preventing the development of antibiotic-resistant microorganisms. To minimize medication errors and potential harm, following the "five rights" is proposed—ensuring the right patient, the right drug, the right dose, the right route, and administering the treatment for the appropriate duration [10]. Adhering to these principles can significantly improve treatment outcomes and patient well-being.

Florfenicol exhibits a broad spectrum of antibacterial activity that includes all microorganisms sensitive to chloramphenicol that comprised gram-negative bacilli, gram-positive cocci, several anaerobes, such as Bacteroides fragilis, Rickettsia and Chlamydia spp., among other atypical microorganisms such as Ureaplasma and Mycoplasma [[11], [12], [13], [14]]. It belongs to the family of antibiotics known as amphenicols, in which mode of action is by inhibiting microbial protein synthesis through binding to the 50S subunit of the 70S ribosome, thereby impairing peptidyl transferase activity and preventing peptide elongation. The typical effect is bacteriostatic, but high concentrations can exhibit bactericidal properties against certain microorganisms. Thiamphenicol and FLO, while structurally related to chloramphenicol, have modifications that enhance their efficacy, reduce toxicity, and, in the case of FLO, decrease bacterial resistance by containing fluorine molecules [11,12]. Florfenicol is considered a time-dependent antibiotic, although some information suggests that it may also exhibit concentration-dependent or codependent behavior [[11], [12], [13], [14]]. It possesses characteristics such as high bioavailability, lipophilicity, and adequate tissue penetration, enabling it to achieve high levels within cells and cross certain anatomical barriers, making it effective against intracellular pathogens [11,[13], [14], [15]]. Importantly, FLO is not susceptible to the actions of acetyltransferase, an enzyme used by bacteria to develop resistance to chloramphenicol and thiamphenicol [16,17]. Florfenicol amine is the major metabolite of degradation of FLO by acid-catalyzed hydrolysis. Florfenicol amine has not antibiotic activity but is an important standard for monitoring animal and environmental residues of FLO [18].

The uses of FLO in veterinary medicine include the prevention and treatment of bovine respiratory disease (BRD) associated with Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni. It is also employed for the treatment of bovine interdigital phlegmon associated with Fusobacterium necrophorum and Bacteroides melaninogenicus [11,12]. In addition, FLO has demonstrated efficacy in treating calves with either naturally occurring or experimentally induced keratoconjunctivitis [18,19], and its presence has been detected in synovial fluid after regional intravenous perfusion [20] and parenteral administration [21].

To the best of our knowledge, the pharmacokinetics of FLO have not been investigated in either bulls or semen. Given its known features mentioned above, this antibiotic presents an intriguing opportunity for examination of its presence in semen. In addition, FLO was the most effective antibiotic against Trueperella pyogenes, the most common pathogen of vesicular adenitis, with more than 95% of in vitro susceptibility of 144 isolates [22]. Moreover, having a long-acting antibiotic available would reduce the frequency of administration and animal handling, thereby minimizing animal stress and improving compliance. Investigating the pharmacokinetic parameters of FLO at two doses, 20 mg/kg, or 40 mg/kg body weight, not only adds new knowledge but also has practical significance. Bulls are large animals, requiring high-volume doses. It is recommended to administer no more than 10 mL per injection site with at least a 10 cm space between administration sites [23]. Therefore, dispensing FLO in mature bulls would necessitate multiple application sites.

The objectives of this study were to compare the serum and seminal plasma pharmacokinetic profile of FLO and its major active metabolite FLA after administration of FLO either by IM or SC routes in beef bulls.

2. Material and methods

2.1. Animals

Eight Hereford bulls were selected for the study based on their excellent temperament and healthy appearance. Each bull underwent a comprehensive physical examination, including a breeding soundness examination following the guidelines provided by the Society for Theriogenology [24]. Out of these eight bulls, four were randomly chosen for the investigation. Additionally, blood samples were collected from the coccygeal vessels and analyzed for CBC and chemistry profile to rule out any subclinical liver or kidney disease. The bulls were healthy, and the CBC, as well as the chemical profile, showed that liver and kidney functions were normal. Therefore, no potential interference of these organs that could have affected the pharmacokinetic parameters of these bulls was detected. The age of the bulls was 22.3 ± 5.6 mo (range: 17.0–28.0). The weight was 366 ± 64 kg (307–455). The body condition score was 6.0 ± 0.4 (5.5–6.5) [25]. The bulls were kept together in a common pasture and had access to free choice coastal hay and water ad libitum. Additionally, each bull received 2.0 kg of concentrate cubes once a day, containing 14% crude protein, and had access to mineral salts.

2.2. Experimental design

The bulls used had not received any FLO administration. In one group (n = 2), a single dose of FLO was administered SC route in the neck at a dose of 40 mg/kg of body weight (Nuflor, Intervet/Merck Animal Health NJ, 07065). In the second group (n = 2), it was administered IM route in the muscles of the neck at a dose of 20 mg/kg of body weight. If the dose was greater than 10 mL, additional administration sites located within 10 cm of the original site were used. After injection, the sites were massaged vigorously to enhance the distribution of the drug solution into the tissues.

The order of sample collection was blood and semen, collected at 0, 12, 24, 36, 48, 72, 96, 120, 144, and 168 h after FLO administration. Blood was collected from the coccygeal vessels using vacuum tubes without additives and permitted to clot. Semen was collected from each bull by electroejaculation by using an electro-ejaculator in automatic mode; the same set-up was used for all the bulls (Pulsator V, Lane Manufacturing, Denver, CO, USA) using a two-electrode rectal probe of 60 mm diameter, as previously reported [26]. All the bull's behavior responses during and after electroejaculation were monitored following previous criteria already stated [26]. All the samples were immediately refrigerated, then centrifuged at 1,300 g for 30 min, processed within the first hour, and stored at -80 °C. The procedures used in this investigation were performed according to the standards for the "Use of Animals in Research and Education" by the World Organization for Animal Health [27].

2.3. Florfenicol and florfenicol amine analysis

Florfenicol and florfenicol amine were measured in serum and seminal plasma samples using ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), as previously described [28]. Before the experiment, the analytical method was validated following the EMA/CHMP/ICH/172948/2019 guidelines [29]. The concentration ranges were appropriate for the levels observed in actual samples: for FLO, it was 0.05–10 μg/mL in both serum and seminal plasma, and for florfenicol amine, it was 0.002–0.2 μg/mL in serum and 0.005–1 μg/mL in seminal plasma. The acquisition of two specific MS/MS transitions for each analyte and the use of the deuterated internal standard FLO-d3 ensured reliable quantification of the compounds of interest.

2.4. Pharmacokinetic parameters

Noncompartmental analysis was utilized to estimate the pharmacokinetic parameters in serum and seminal plasma for each individual animal. The standard software PK-Solver add-in for Excel [30] was employed to estimate these pharmacokinetic parameters. The following variables were calculated for both serum 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, where λz represents the first-order rate constant associated with the terminal portion of the time-concentration curve, estimated by linear regression of time versus log concentration; area under the time-concentration curve from time zero to the last observed concentration ($AUC_{0\text{-last}}$), calculated using the linear trapezoidal rule; 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

2.5. Statistical analysis

Statistical software [31] was utilized to calculate parameters such as the mean, standard deviation, and range. The Student's T-test was employed for independent and paired samples. Furthermore, analysis of variance for repeated samples using the General Linear Model was applied. Additionally, the software program PK-Solver, as mentioned earlier [30], was used for pharmacokinetic parameter calculations. An alpha error of 5% was adopted to accept the alternative hypothesis.

3. Results

The administration of FLO in both routes was well tolerated by all bulls throughout the study period, with mild swelling and tenderness during the first two days, which disappeared by the end of the study. All the behavioral responses during electroejaculation were moderate, mild, or light [26]. No changes in appetite, behavior, urination, or feces consistency were noticed. Interestingly, the two routes of administration presented differences in the pharmacokinetic parameters of FLO in serum but not in seminal plasma.

The mean level of total FLO in serum was higher with the SC route (1,415.5 ng/mL) compared to the IM route (752.4 ng/mL; P = 0.001; Fig. 1). Differences were also observed in the mean levels of FLO and FLA between SC (666 versus 10.98 ng/mL; P = 0.02) and IM (252.86 versus 21.13 ng/mL; P = 0.03) routes of administration, respectively.

In serum, the percentage of FLA was 1.98% (range 1.3–2.9) for the IM route and 1.69% (1.5–1.8) for the SC route (P = 0.38). The mean level of total FLO in seminal plasma was 1,454.8 ng/mL for the SC route and 1,872.9 ng/mL for the IM route, with no significant differences between the two routes (P = 0.51; see Fig. 2).

The curves of total FLO (florfenicol + florfenicol amine) concentration (ng/mL) in seminal plasma through the experimental period after single administration of FLO by IM (20 mg/kg) or SC (40 mg/kg) routes are presented in Fig. 2. In seminal plasma, the percentage of FLA was 29.0 % (range 24.1–34.2%) for the IM route and 25.9% (range 15.9 and 32.3%) for the SC route (P = 0.52). The mean level (±SD) of FLA was higher in seminal plasma compared with serum (467 ± 466 and 18 ± 16, respectively; P = 0.001). Differences in the mean levels of total FLO between serum and seminal plasma were detected (1,187 ± 2,069 ng/mL and 1,748 ± 1,906 ng/mL, respectively; P = 0.04). Fig. 3 presents the concentration of total FLO (ng/mL) in serum and seminal plasma throughout the investigation period.

Pharmacokinetics parameters calculated for non-compartmental analysis of serum and seminal plasma are described in Table 1. The total FLO in seminal plasma concentrations presented a C_{max} of 7,084.20 ng/mL at 24 h by the SC route and a C_{max} of 5,330.5 ng/mL at 42 h for the IM route, without differences between both routes (P = 0.39). No differences in the other seminal plasma pharmacokinetics parameters such as half-life, AUC, AUMC, and MRT between SC and IM routes were detected.

4. Discussion

In previous reports, the administration of FLO by IM route (20 mg/kg) in six lactating Holstein cows resulted in C_{max} in serum of 2.3 μg/mL at 3 h [32]; a C_{max} of 2.7 μg/mL at 4.4 h when injected by the IM route (20 mg/kg) in five 12-month-old. Black Pied heifers [33]; a C_{max} in plasma of 2.98 μg/mL at 12 h in six lactating Holstein cows injected by the SC route (40 mg/kg) [34]; a C_{max} in serum of 3.07 μg/mL (harmonic mean) at 18.3 h detected in ten calves between 3 and 6 months of age injected by the IM route (20 mg/kg) [35]; a C_{max} in plasma of 3.18 μg/mL at 0.38 h in six male calves Holstein-Friesian injected by the IV route (20 mg/kg) [13]; a C_{max} of 3.2 μg/mL at 6.8 h in five 12-month-old Black Pied heifers injected by the SC route (40 mg/kg) [33]; a C_{max} of 3.21 μg/mL at 3.3 h in ten calves receiving by the IM route (20 mg/kg) [15]; a C_{max} in plasma of 3.42 μg/mL at 1.19 h in six 6-month-old castrated calves by the SC route (40 mg/k) [14]; a C_{max}

of 4.9 µg/mL 7.6 h in five mature cows by the SC route (40 mg/kg) [36]; a C_{max} of 5.4 µg/mL at 4 h after oral administration to six male veal Holstein calves (11 mg/kg) [37]; a C_{max} in serum of 5.90 µg/mL at 0.63 h when it was applied via IV route (2.2 mg/kg) in the dorsal common digital vein of six cows [20]; a C_{max} in serum of 9.41 µg/mL at 3.3 h in six Holstein calves between 1 and 8 weeks old when orally administered (22 mg/kg) [38]. From all these studies, a wide variation in C_{max} and T_{max} was noticed. In the present study, the levels of total FLO in serum were higher in the SC route than the IM route throughout the experimental period, with a C_{max} of 2,830.2 ng/mL at 12 h for the IM route (20 mg/kg) and 3,668 ng/mL at 12 h when SC administered (40 mg/kg). These findings agreed with some of the previous reports and with a recent study that compared both routes in 12-month-old heifers [33]. In the present investigation, the values in serum of total FLO for both routes were in consonance with those previous reports, allowing us to be confident that the outcomes in seminal plasma were reliable.

The half-life of FLO in plasma was affected by the route of administration, with a half-life of 2–3 h after IV, 18 h after IM, and 27 h after SC administration [11]. In the present study, the outcomes agree with those findings, showing that the IM route had a shorter half-life than the SC route in serum [33]. Interestingly, the half-life of FLO in the seminal plasma presented no differences between routes.

The effectiveness of any antimicrobial is established by both its pharmacokinetics (PK) and pharmacodynamics properties (PD) [39,40]. In veterinary medicine, three PK/PD indices are routinely used: the ratio of the area under the curve of the free drug plasma concentration to the minimum inhibitory concentration (MIC) (AUC/MIC), the peak antibiotic concentration to MIC ratio (C_{max}/MIC), and the time that free plasma concentration exceeds the MIC over the dosing interval (T > MIC). Among these, the AUC higher than MIC for a specific microorganism (AUC/MIC) is considered the primary pharmacokinetic/pharmacodynamics forecaster for clinical effectiveness.

Therefore, prior to using FLO in bulls, it is important to understand its pharmacokinetics, particularly the duration of time that the concentrations of this medicine are maintained above the MIC for targeted pathogens throughout the treatment period. As shown in this study, FLO was present in both serum and seminal plasma throughout the experimental period of seven days. Moreover, the levels of FLO in seminal plasma were higher than in serum. This information will be critical in designing appropriate treatment protocols based on the levels of FLO in seminal plasma at specific times after administration.

The use of FLO for the treatment of bovine respiratory disease was based on two schemes of administration: a single SC injection at a dose of 40 mg/kg or two IM injections at a dose of 20 mg/kg with a two-day interval [11,12]. In the present study, the pharmacokinetic values in serum for both routes of administration showed that the level of FLO above 500 ng/mL was between 48 and 72 h for the IM route and between 120 and 144 h for the SC route, in agreement with a recent investigation [33].

In a previous investigation, when oxytetracycline long-lasting was administered to Simmental bulls at 10 mg/kg or 20 mg/kg, differences between doses in the mean level of oxytetracycline in seminal plasma, as well as C_{max}, AUC and AUMC, were detected [9]. However, in the present study, no differences in seminal plasma were observed in the mean total FLO concentrations, as well as in all pharmacokinetics parameters compared (C_{max}, T_{max}, $T_{1/2}$, AUC, AUMC, and MRT) between doses. This outcome was unexpected, especially considering that the bulls injected by SC received a double dose of FLO compared to the IM route. The reasons for this observation are unknown, but one probable reason could be that there was a threshold of transport of FLO through the accessory sexual glands. Consequently, even though the levels were higher in serum for the SC route, a level of saturation could have been reached for the male genital tract tissues. This finding has multiple remarkable implications. On one side, the administration of FLO by IM was as effective as the SC route in all pharmacokinetics parameters compared, resulting in a reduced volume of administration, decreased treatment costs, and a significant reduction in the withdrawal time as well [11,12]. It is necessary to remark that the use of FLO in bulls for reproductive diseases such as vesicular adenitis syndrome will be considered in the USA as extra label use because no indication was specified in the label; therefore, it is necessary to determine that the meat withdraw time will be longer that for the those recommended in the label (28 days for IM versus 38 days for SC routes) [11,12].

The administration of FLO by the IM route (20 mg/kg) in lactating Holstein cattle resulted in a serum C_{max} of 2.3 µg/mL at 3 h and a C_{max} in milk of 1.6 µg/mL at 10 h [32]. In a second study, the administration of FLO by the SC route (40 mg/kg) in lactating cows also resulted in a C_{max} in plasma of 2.98 µg/mL at 12 h and a C_{max} in milk of 1.74 µg/mL at 12 h [34]. From these studies, it was observed that C_{max} in milk was between 58 and 70% of the plasma or serum level. In pigs, the C_{max} of FLO in plasma after IM administration (15 mg/kg) was 3.58 µg/mL at 1.64 h, and the C_{max} in synovial fluid was 2.73 µg/mL at 3.4 h [41]. Therefore, the maximum level in synovial fluid was 76% of the plasma level. When FLO was administered to beef cows by the SC route (40 mg/kg), and synovial samples were collected from the metatarsophalangeal joint for 10 days, the C_{max} was 2.7 µg/mL, which was 50% of the estimated plasma C_{max} used from another study [21]. In interstitial fluid, it was found that the FLO levels were below the plasma level [14]. FLO concentrations in the brain, cerebrospinal fluid, and aqueous humor were one fourth to one-half of the serum level [14,37]. However, a further independent study found that FLO presented a C_{max} higher (47%) in the cerebrospinal fluid compared to serum levels [13]. Finally, in the use of a tissue cage model for calf pneumonia microorganisms, calves that received 40 mg/kg by the SC route resulted in a serum C_{max} of 5.91 µg/mL, which was higher than those of exudate (3.39 µg/mL) and transudate (2.84 µg/mL) [42]. From all these previous studies, it was observed that FLO levels in tissues were lower than those in plasma or serum, except for the lungs, where levels were more than 200% of serum levels [14,36]. In the present study, the persistence of levels ≥500 ng/mL of total FLO was longer in seminal plasma (5 days) than in serum (3 days).

In the present study, the level of total FLO in seminal plasma was above serum levels during all the experimental periods. Moreover, using serum levels of FLO as a source of information to establish a correct treatment regimen for genital infections in bulls could be considered a misleading approach based on the pharmacokinetic parameters of FLO from the present investigation. In two recent studies, using different families of antibiotics such as macrolide (Tulathromycin) or tetracycline (Oxytetracycline), the concentrations were higher and persisted longer compared with plasma levels [8,9]. Consequently, further investigation is necessary to determine if the male genital tract has a special affinity for these different families of antibiotics due to the high levels in seminal plasma compared with serum or plasma levels.

One remarkable aspect of the present investigation was the assessment of both FLO and FLA in serum and seminal plasma. This methodology allowed us to observe that the FLA levels between both fluids were not only different, being almost 2% in serum and around 27.5% for seminal plasma, but it also revealed differences in the total level of FLO between both fluids. Without this new approach of analysis for both products, these differences in FLO levels between serum and seminal plasma would not have been possible to detect. More investigation to determine the high levels of FLA in seminal plasma compared with serum is required. It seems that the male genital tract maybe produced this metabolite or has the capacity to accumulate it.

In the present research, several weaknesses were identified, including a limited number of bulls, bulls of the same age, a single breed, and the inclusion of only clinically healthy bulls. Additionally, wide variations in the levels of FLO, as described in former experiments, were also noticed. Nevertheless, the evidence generated could be considered a starting point not only for establishing a primary treatment regime for bulls that requires the use of FLO but also for future studies on this topic. The effects of FLO on reproductive performance have not been determined yet, therefore, precautions about its use-on animals of breeding age are recommended. Studies about FLO for the treatment of vesicular adenitis cases are necessary. Moreover, toxicity studies in dogs, rats, and mice have been associated with testicular degeneration and atrophy. Further investigations are necessary to enhance the current information on this matter.

Based on the present study, it has been concluded that FLO exhibits pharmacokinetic attributes that make it a suitable antibiotic for the treatment of bull genital infections, provided its use is appropriately indicated by the sensitivity of the microorganism isolated to FLO. To further study the pharmacokinetics of FLO in seminal plasma, the analysis of FLA should be incorporated.

Conflicts of interest

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

Credit authorship contribution statement

Juan E. Romano: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Anisa Bardhi:** Analysis of samples by UHPLC-MS/MS. Manuscript review. **Giampiero Pagliuca:** Analysis of samples by HP-LC. Manuscript review. **Graciela B. Villadóniga:** Pharmacokinetic analysis. Writing and manuscript review. **Andrea Barbarossa:** Analysis of samples by UHPLC-MS/MS. Manuscript review.

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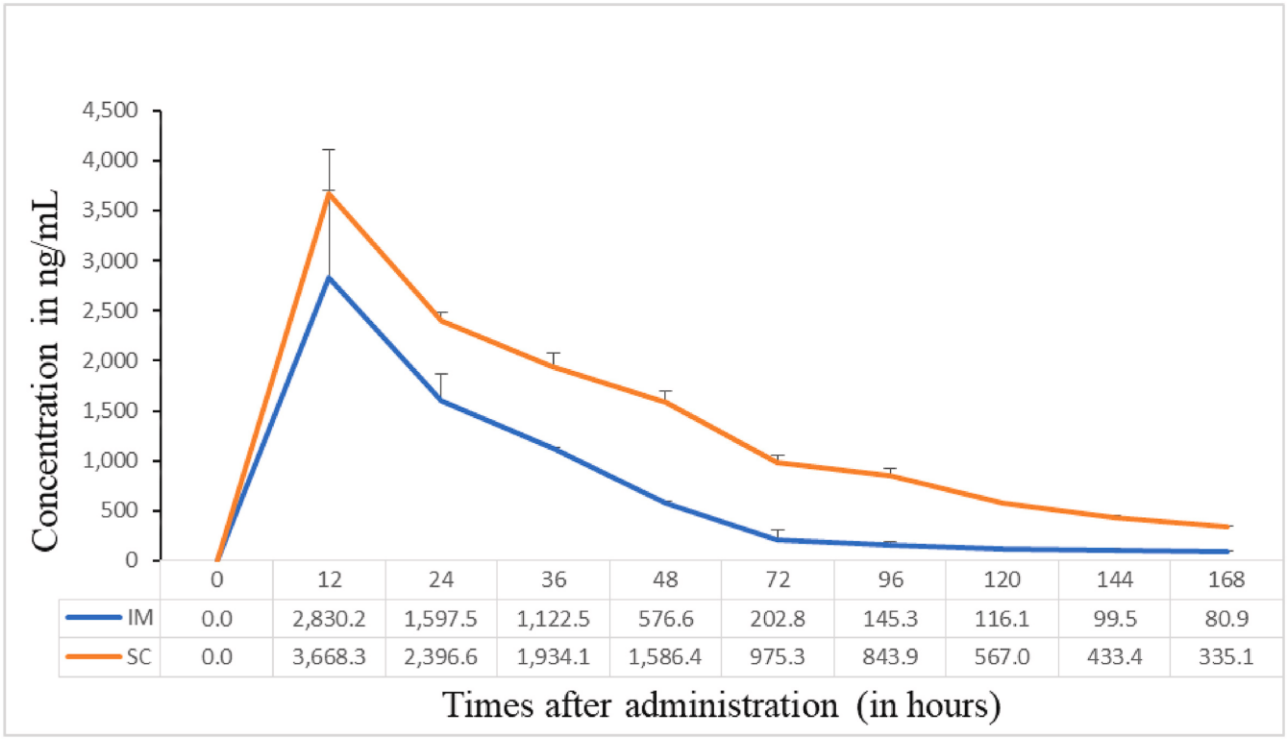
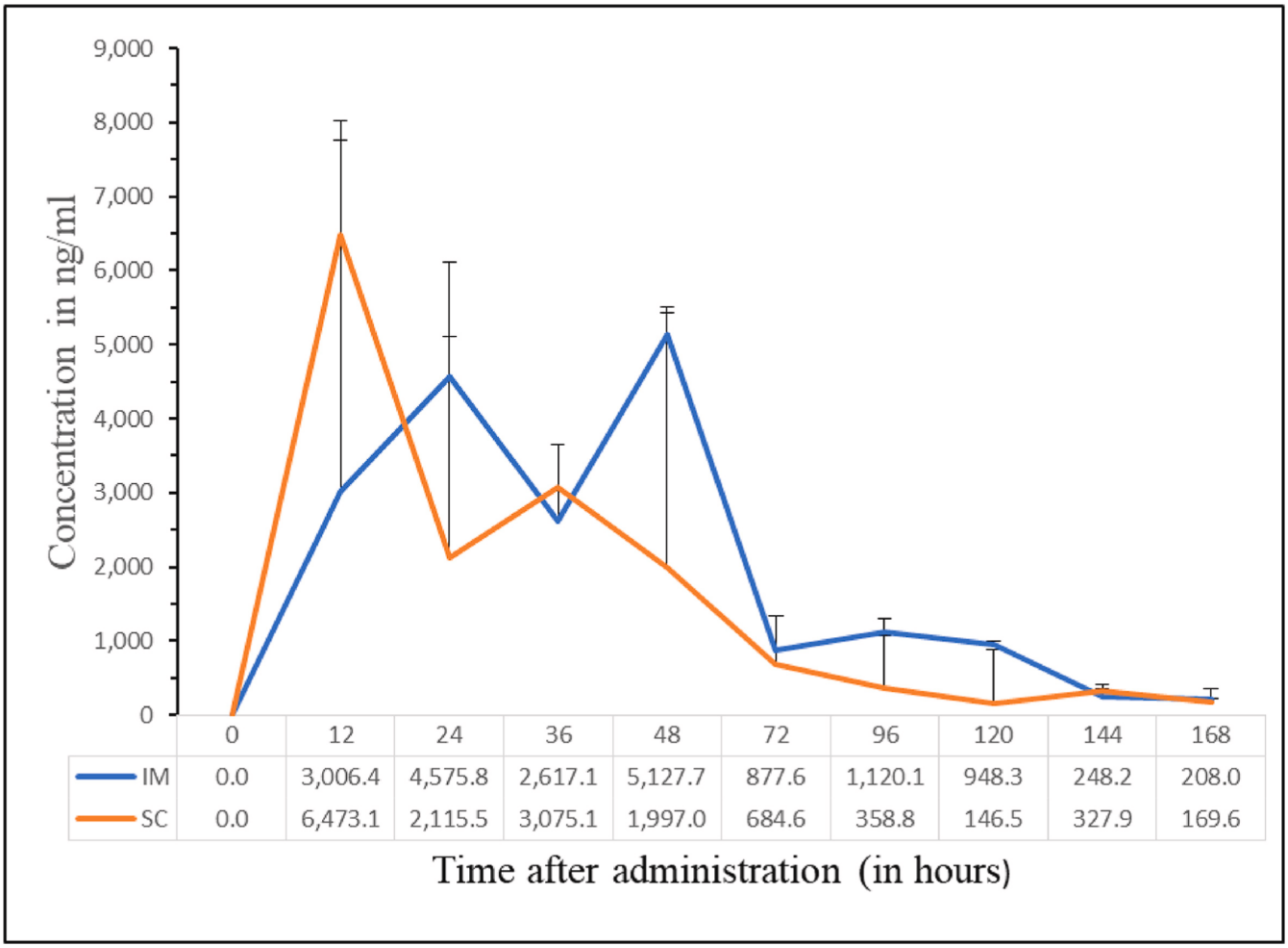


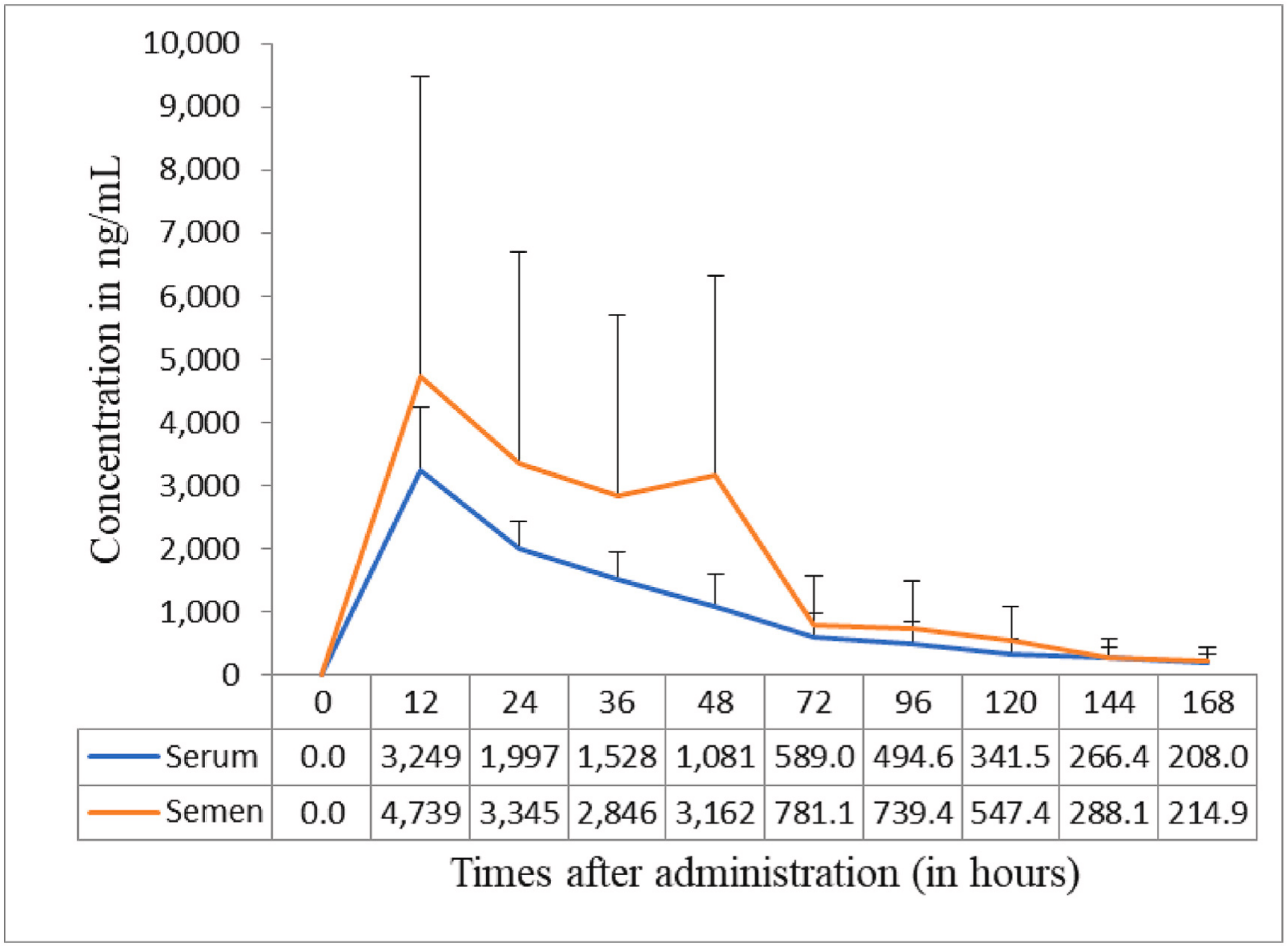
	0	12	24	36	48	72	96	120	144	168
IM	0.0	2,830.2	1,597.5	1,122.5	576.6	202.8	145.3	116.1	99.5	80.9
SC	0.0	3,668.3	2,396.6	1,934.1	1,586.4	975.3	843.9	567.0	433.4	335.1

Fig. 1. Mean (+1 SD) total florfenicol (florfenicol + florfenicol amine) concentration (ng/mL) in serum after single administration either by IM or SC routes in four Hereford bulls.



	0	12	24	36	48	72	96	120	144	168
IM	0.0	3,006.4	4,575.8	2,617.1	5,127.7	877.6	1,120.1	948.3	248.2	208.0
SC	0.0	6,473.1	2,115.5	3,075.1	1,997.0	684.6	358.8	146.5	327.9	169.6

Fig. 2. Mean (+ SD) seminal plasma of total florfenicol (florfenicol + florfenicol amine) concentration (ng/mL) after single administration of florfenicol either by IM (20 mg/kg) or SC (40 mg/kg) routes in four Hereford bulls.



	0	12	24	36	48	72	96	120	144	168
Serum	0.0	3,249	1,997	1,528	1,081	589.0	494.6	341.5	266.4	208.0
Semen	0.0	4,739	3,345	2,846	3,162	781.1	739.4	547.4	288.1	214.9

Fig. 3. Mean (+ SD) serum and seminal plasma of total florfenicol (florfenicol + florfenicol amine) concentration (ng/mL) after single administration (SC and IM combined) in four Hereford bulls.

Table 1

Serum and seminal plasma pharmacokinetics parameters of total florfenicol (florfenicol + florfenicol amine) administered by IM route (20 mg/kg) and SC route (40 mg/kg) in beef bulls.

Parameter	Unit	Serum		Probability	Seminal Plasma		Probability
		IM	SC		IM	SC	
Lambda z (ʎz)	1/h	0.01733	0.01112	P = 0.06	0.02137	0.02009	P = 0.46
T 1/2	h	40.7	62.7	P = 0.04	38.3	38.1	P = 0.49
T max	h	12.0	18.0	P = 0.43	24.0	42.0	P = 0.16
C max	ng/mL	2,830.2	3,668.3	P = 0.04	7,084.2	5,330.5	P = 0.39
$AUC_{0\text{-last}}$	ng/mL*h	1,421.1	1,840.1	P = 0.06	200,595.3	293,844.9	P = 0.34
$AUC_{0\text{-inf}}$	ng/mL*h	2,125.7	2,754.2	P = 0.04	215,804.2	304,312.8	P = 0.34
$AUMC_{0\text{-last}}$	ng/ml*h^2	1,773.4	2,297.2	P = 0.001	7,915,593.6	15,417,021.6	P = 0.26
$AUMC_{0\text{-inf}}$	ng/mL*h^2	1,949.5	2,525.7	P = 0.004	11,579,231.9	17,757,147.6	P = 0.30
$MRT_{0\text{-last}}$	h	37.8	55.4	P = 0.03	45.8	51.1	P = 0.40
$MRT_{0\text{-inf}}$	h	47.4	82.6	P = 0.02	62.8	60.6	P = 0.48

ʎz 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)/ʎz; Tmax: time of peak drug concentration; Cmax: peak of drug concentration; AUC0-last: area under the time-concentration curve from time zero to the last observed concentration; AUC0-inf:area under the time-concentration curve from time zero extrapolated to infinity; AUMC0-last: area under the moment curve from time zero extrapolated to last observed concentration; AUMC 0-inf: area under the moment curve from time zero extrapolated to infinity; MRT 0-last: Mean resident time calculated as AUMC 0-last/AUC 0-last; MRT0-inf: Mean resident time calculated as AUMC 0-inf/AUC 0-inf. Mean pharmacokinetics parameters in plasma and seminal plasma calculated via noncompartmental analysis.