Endometrial tissue concentrations of ceftiofur derivatives following intrauterine infusion in both non-infected mares and mares with experimentally induced endometritis

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Abstract

Intrauterine infusion of antimicrobials is common practice for the treatment of bacterial endometritis, however evidence based guidelines are lacking. The objective of this study was to determine endometrial concentrations of ceftiofur derivatives following intrauterine infusion in non-infected mares and those with experimentally induced endometritis. It was hypothesized that endometrial tissue concentrations of desfuroylceftiofur-acetamide (DCA; active metabolite of ceftiofur) would remain above the minimum inhibitory concentration (MIC) for common uterine pathogens for greater than 24 h in both groups of mares. Endometrial biopsy samples were collected from six mares during estrus immediately prior to intrauterine infusion of 1g ceftiofur and at 4, 8, 12, 24, 36 and 48 h after infusion. Desfuroylceftiofur-acetamide levels in endometrial tissue were measured using liquid chromatography-mass spectrometry (LC-MS) at each time point. Six further mares were then chosen that had been shown to be susceptible to breeding induced endometritis. Once in estrus they were inoculated with 10^7 colony-forming units (CFU) of *Streptococcus equi zooepidemicus (S. zooepidemicus)*. 24 h following inoculation, 1g of ceftiofur was infused into the uterus and endometrial biopsies taken immediately prior to and at 6, 12, 24, 48, 72 and 96 h after infusion to determine tissue concentrations of DCA. In non-infected mares, concentrations of DCA were above MIC_{90} for *S. zooepidemicus* and *Escherichia coli (E. coli)* for the 48 h-testing period. In mares with experimentally induced endometritis, concentrations of DCA were only above MIC_{90} for *E. coli* for 6 h and *S. zooepidemicus* for 24 h.

Keywords: Mare, bacterial endometritis, ceftiofur

Introduction

Bacterial endometritis is a leading cause of infertility in the mare and a major cause of economic loss to the equine breeding industry.[1] The use of intrauterine antimicrobials to treat endometritis is common practice;[2-4] evidence-based guidelines, however, for suitable antibiotic choice, effective dose rates, and treatment lengths are lacking. While *in vitro* susceptibility patterns can be determined following uterine culture of mares with endometritis[5,6] there is limited *in vivo* research of commonly used antimicrobials. This is especially true at sites of active infection[7] as pharmacokinetic studies are typically performed on non-infected mares rather than those with active endometritis.

Streptococcus equi zooepidemicus (S. zooepidemicus) and *Escherichia coli (E. coli)* are the two most common causes of bacterial endometritis in the mare.[5,6,8] Ceftiofur is widely used to treat bacterial endometritis caused by these agents, both via systemic administration and intrauterine infusion.[3,7] An online survey reported ceftiofur to be the most commonly used antimicrobial for intrauterine infusion.[9] Ceftiofur is a third generation cephalosporin that has good spectrum of activity against gram-positive and some gram-negative organisms due to increased resistance to gram-negative beta-lactamases,[10] it is hydrolysed in the liver to form its most prevalent metabolite; desfuroylceftiofuracetamide (DCA).[11] Ceftiofur is licensed in the United States for treatment of respiratory infections in horses caused by *S. zooepidemicus*,[10,12] but despite its widespread use for treating endometritis, there are limited data on its distribution in the uterus following intrauterine infusion.[13,14]

The objective of this study was to compare endometrial tissue concentrations of the ceftiofur derivative; DCA following intrauterine infusion in both non-infected mares and those with experimentally induced endometritis. It was hypothesized that endometrial tissue concentrations of DCA would remain

above target concentrations for greater than 24 h in both non-infected mares and those with experimentally induced endometritis.

Materials and methods

Selection of mares

Two groups of six, non-pregnant mares of mixed breeds, ages (range 5 to 21 yrs.) and weights (range 525 to 578 Kg) were selected. The mares were housed at the Center for Equine Health at the University of California, Davis on dry lots with *ad lib* access to hay and water and twice-daily feed of a balanced concentrate.

Non-infected mares. Twelve mares were examined daily by transrectal palpation and ultrasonography for evidence of follicular growth, uterine edema development, intrauterine fluid accumulation, and cervical tone. Once deemed to be in estrus, a baseline uterine biopsy was taken with a Kevorkian biopsy forcep in an aseptic manor as previously described[15] and histopathology evaluation was performed to rule out underlying uterine pathology such as the presence of inflammatory cells, periglandular fibrosis and lymphatic lacunae. The biopsies were graded using the Kenney-Doig scale.[16] Endometrial samples were also taken in an aseptic manor for bacterial culture using a double-guarded swab (MOFA Global, Verona, WI) and cytology using a cytobrush (MOFA Global, Verona, WI)[17] to confirm the absence of any bacterial infection. Any mares showing evidence of uterine pathology (grade IIB or III endometrium due to fibrosis or inflammatory changes or >3 to 4 polymorphonuclear neutrophils (PMN) per high power field) [14] were removed from the study. Six mares were selected to obtain the non-infected mare group.

Endometritis mares. A second group of mares were chosen to be experimentally induced with bacterial endometritis. These mares were older than ten years of age (range 11-22 years), with biopsy scores of IIA-III and were free from bacterial endometritis as determined by uterine culture and cytology. In addition these mares were tested to determine susceptibility to breeding induced endometritis, as described by Woodward et al.[18] In short, the mares were infused with killed spermatozoa during estrus and evaluated for their ability to clear induced uterine inflammation, with susceptible mares retaining intrauterine fluid 48 h and 96 h following insemination and resistant mares clearing the fluid within 48 h. The aim of selecting susceptible mares was to help ensure that they would not resolve the bacterial induced endometritis prior to the sampling process. Of sixteen mares tested, six mares fit the inclusion criteria.

Experimental design

Non-infected mares. Once the mares (n=6) were determined to be in estrus by transrectal ultrasound examination, i.e. a follicle of ≥30 mm in the presence of uterine edema, a sterile infusion pipette was manually guided through the cervix to facilitate intrauterine infusion of 1 g ceftiofur hydrochloride (Naxcel®, Zoetis, Florham Park, NJ) diluted in sterile saline to 60 ml total volume, as per standard infusion protocol. Endometrial biopsies were taken immediately prior to infusion (time point 0) and then at 4, 8, 12, 24, 36 and 48 h after infusion. The tissue samples were stored in sterile plastic tubes at -20° C until analysis. Desfuroylceftiofur-acetamide concentrations in the endometrial tissues were then measured by liquid chromatography–mass spectrometry (LC-MS; see below) at each time point.[19]

Endometritis mares. Once the susceptible mares were determined to be in estrus by criteria described above, the perineal area was aseptically prepared and they were inoculated with a total of 10^7 CFU of *S. zooepidemicus* in 10 ml of phosphate buffered saline (PBS). Twenty four hours following inoculation endometrial culture, cytology and ultrasound examination were performed to confirm inflammation i.e. positive endometrial culture, >3 to 4 PMNs per high power field on cytology and intrauterine fluid accumulation on ultrasound examination. Endometrial biopsies were then taken immediately prior to the intrauterine infusion of 1g ceftiofur hydrochloride (time point 0) and then again at 6, 12, 24, 48, 72 and 96 h after inoculation to determine tissue concentrations of DCA by LC-MS. Based on the results of the non-infected group of mares, where endometrial tissue concentrations were

above target concentrations for the duration of the 48 h testing period, it was decided to extend the duration of sampling in the endometritis mares to 96 h. In order to take the same number of total biopsy samples the 4 and 8 h biopsies were not taken and instead replaced by the 6 h sample. All of the biopsies were performed on the same estrous cycle to reduce the effects of potential variability in subsequent cycles. Multiple biopsies have not been shown to be harmful; no significant effect on cytokine expression was associated with repeat biopsies in a study by Christoffersen et al.[20] The Institutional Animal Care and Use Committee of the University of California, Davis, approved all experimental procedures.

Bacterial inoculation

The *S. zooepidemicus* isolate used as a model to induce endometritis in the susceptible mares was obtained from a clinical sample submitted to the Microbiology Laboratory at the University of California, Davis. The colonies were streaked on a blood agar plate and incubated for 24 h at 35° C in 5% CO_2. After incubation colonies were transferred to 10 mL sterile 0.85% saline (10^8 CFU per mL as determined by nephelometer) and then diluted 1:10 using sterile 0.85% saline to a final concentration of 10^7 CFU of *S. zooepidemicus* per 10 mL inoculum. The inocula were kept on ice until immediately prior to transcervical infusion via an infusion pipette aseptically passed through the cervix into the uterus.

Liquid chromatography tandem-mass spectrometry

Ceftiofur residues were quantified in endometrial tissue using a method similar to that described by Drillich et al.[19] The method converts all residues of ceftiofur into DCA prior to analysis, as DCA is a more stable analyte to measure. The concentration of DCA was measured in endometrial tissue by LC-MS. The technique was optimized to provide a lower limit of quantitation of 0.1 μg/g for DCA and a lower limit of detection of 0.03μg/g.

Statistical analysis

Descriptive statistical analysis was carried out using StatXact 11 (Cytel Software Corporation, Cambridge, MA). The distributions of concentrations from the non-infected horses were compared to those with endometritis at each of the separate sampling times using the exact Wilcoxon-Mann-Whitney test. Significance was set at $P < 0.05$. All values are represented as means ± standard error of the mean (SEM).

Results

Non-infected mares

Following intrauterine ceftiofur infusion, endometrial tissue concentrations of DCA declined from mean concentration of 873.3 μg/g (± 81.1) at 4 h, to 43.4 μg/g (± 23.6) at 48 h (Graph 1). At no stage did any mare have evidence of active endometritis either clinically or based on culture results. One mare was excluded from the study following the 24 h biopsy due to minor uterine bleeding.

Endometritis mares

Following inoculation with *S. zooepidemicus*, all six mares in the second experiment had bacteriological evidence of endometritis (positive culture results), as well as clinical evidence of uterine inflammation, including intrauterine fluid accumulation, positive cytology (>3 to 4 PMNs per high power field) and excessive uterine edema. Endometrial tissue concentrations of DCA declined from 173.7 μg/g (± 57.0) at 6 h, to 4.9 μg/g (± 4.1) at 96 h following intrauterine ceftiofur infusion (Graph 2).

Non-infected vs. endometritis mares

Initial DCA endometrial concentrations in the endometritis mares (173.7 μg/g [± 57.0] at 6 h) were lower than the non-infected mare group (873.3 μg/g [± 81.1] at 4 h) and remained so throughout the study. When comparing the same time points across the two groups, endometrial tissue concentrations of ceftiofur were significantly lower at 12 h in mares with experimentally induced endometritis compared to

non-infected mares and were lower at both 24 h (46.8 μg/g [± 37.3] vs. 171.0 μg/g [± 101.3]) and 48 h (20.4 μg/g (± 13.1) vs. 43.4 μg/g (± 23.6)) although not significant.

Discussion

MIC_{90} (0.25 μg/ml)[21] of *S. zooepidemicus* was exceeded in endometrial tissue for the duration of the 48h-testing period in the non-infected mares. This provides evidence that a single-dose ceftiofur infusion will be effective for at least 48 h in this population. In the mares with experimentally induced endometritis, tissue concentrations of DCA were above MIC_{90} for *S. zooepidemicus* in all six mares at 12 and 24 h, supporting the current practice of once daily infusions of 1g ceftiofur.[4] However at 48 h tissue concentrations were below MIC_{90} for *S. zooepidemicus* in one of the infected mares (0.02 μg/g), suggesting that an every-other-day infusion protocol could result in treatment failure in a proportion of clinical cases of *S. zooepidemicus* endometritis.

Based on an MIC_{90} of 2.0 μg/ml for *E.coli*,[21] endometrial tissue concentrations of DCA were above target concentration in all non-infected mares for the 48 h testing period. In the endometritis group, tissue concentrations were only above MIC_{90} for 6 h in all mares; they were below 2.0 μg/g in one mare at 12 h, three mares at 24 h and four mares at 48 h. Published MIC_{90} for *E. coli* do vary, with older reports quoting 0.5 μg/ml.[22] This may not, however, reflect the current situation and unpublished data from *E. coli* isolates obtained in the microbiology laboratory at University of California, Davis suggest an MIC_{90} of 4.0 μg/ml. When deciding if ceftiofur is an appropriate antimicrobial to use for *E. coli* endometritis, it is advisable to determine the specific MIC_{90} of the isolate in question.

To our knowledge this is the first study directly comparing the distribution of ceftiofur following intrauterine infusion in both non-infected and endometritis mares. When comparing the same time points across the two experiments, tissue concentrations of DCA were significantly different at 12 h in the endometritis mares than the non-infected mares and lower at both 24 h and 48 h. This suggests that care should be taken when extrapolating data from studies performed on non-infected mares, as they may not be reflective of those with endometritis. Ideally mares with experimentally induced endometritis would be more suitable models for future pharmacokinetic studies evaluating the efficacy of antimicrobials for the treatment of bacterial endometritis.

It is speculated that mares with experimentally induced endometritis may have had local prostaglandin release or increased blood flow due to inflammation, which may have affected the clearance of DCA, and hence explain the differences detected between the two groups. Clinical signs of uterine inflammation were seen in all mares experimentally induced with endometritis but histological examination of the endometrium was not performed as in other studies.[23]

Determining the DCA levels in uterine luminal secretions was not performed and is a limitation of the study. Mares in the non-infected group did not have any evidence of uterine fluid at any stage and hence measuring luminal concentrations was unlikely to be relevant. In the endometritis mares, however, it would have been interesting to determine DCA levels in the luminal fluid, since bacteria often reside within the uterine lumen and hence would be targeted in this location.

Desfuroylceftiofur-acetamide levels in systemic circulation were also not determined following intrauterine infusion. While ceftiofur is absorbed by the mucosa and would therefore be expected to be present in the circulation, there is limited evidence to support this in the case of other antimicrobials including clavulanate and amikacin, hence the decision not to measure systemic levels.[23,25]

An endometrial biopsy was taken prior to the infusion of the ceftiofur hydrochloride to ensure that endometrial levels of DCA were non-detectable at this stage. It is unknown, however, if the minor trauma inflicted at the biopsy site may have affected the endometrial absorption of the antimicrobial. It was felt that the importance of having a time point 0 (baseline result) outweighed this concern.

The individual variability seen was interesting and while the repeatability of this could be determined with a larger sample size, it is likely to reflect the spectrum of mares with endometritis that are treated and both their ability to absorb ceftiofur and the potential for uterine evacuation of antimicrobials. While the use of systemic antimicrobials has been discussed to overcome these issues,

DCA concentrations in endometrial tissue following intrauterine infusion were higher in this study in non-infected mares (873.3± 81.1 μg/g) and in endometritis mares (173.7 ± 57.0μg/g) compared to those reported following systemic treatment (1.7 ± 0.5μg/g).[26] In addition there have been conflicting results in studies testing the penetration of endometrial tissue concentrations of ceftiofur following systemic administration.[26,27]

Conclusion

This study supports the use of once daily intrauterine infusions of 1 g ceftiofur for cases of *S. zooepidemicus* endometritis. However, when treating bacterial endometritis caused by pathogens with a higher MIC_{90} such as *E. coli*, ceftiofur may not an appropriate choice for a once daily infusion protocol. Further research would be justified to determine steady state endometrial tissue concentrations following repeated intrauterine dosing of ceftiofur and the effect of increasing the initial dose of ceftiofur on endometrial tissue concentrations.

Acknowlegement

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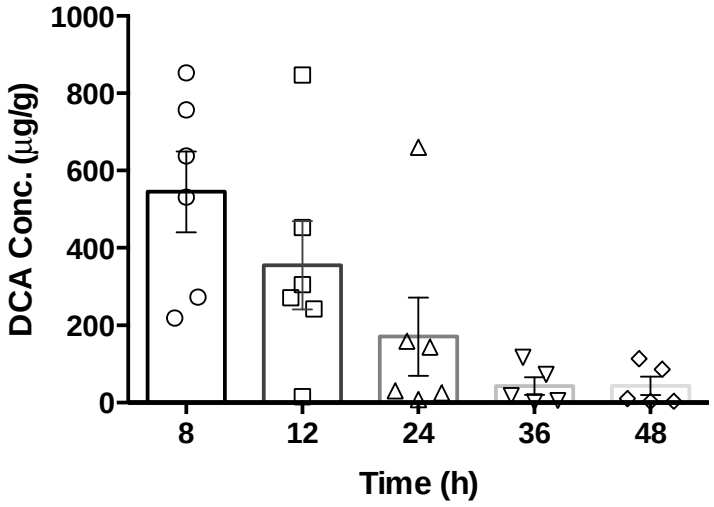
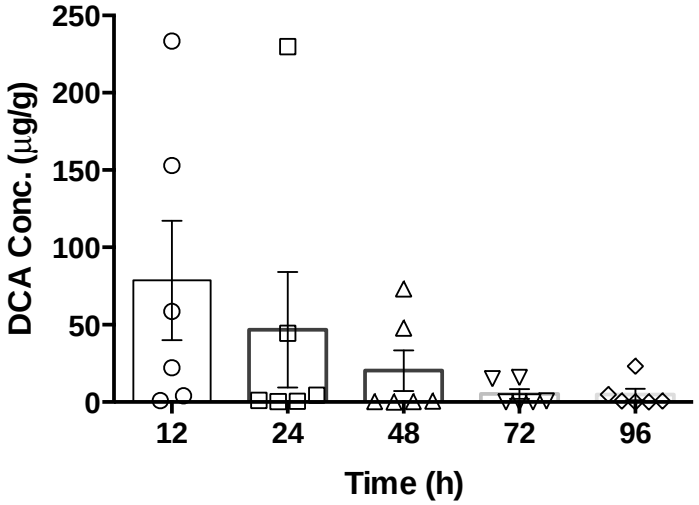
References

1. Riddle WT, LeBlanc MM, Stromberg AJ: Relationships between uterine culture, cytology and pregnancy rates in a Thoroughbred practice. Theriogenology 2007;68:395-402.
2. Causey RC: Uterine therapy for mares with bacterial infections. In: McKinnon, editor. Current therapy in equine reproduction. St. Louis: W.B. Saunders; 2007. p. 105-115.
3. Lu KG, Morresey PR: Reproductive tract infections in horses. Vet Clin North Am Equine Pract 2006;22:519-552.
4. Brinsko SP, Blanchard TL, Varner DD, et al: Endometritis. In: Hartman, editor. Manual of equine reproduction. 3rd ed. Saint Louis: Mosby; 2011. p. 73-84.
5. Davis HA, Stanton MB, Thungrat K, et al: Uterine bacterial isolates from mares and their resistance to antimicrobials: 8,296 cases (2003–2008). J Am Vet Med Assoc 2013;242:977-983.
6. Albihn A, Baverud V, Magnusson U: Uterine microbiology and antimicrobial susceptibility in isolated bacteria from mares with fertility problems. Acta Vet Scand 2003;44:121-129.
7. LeBlanc MM: The current status of antibiotic use in equine reproduction. Equine Vet Educ 2009;21:156-167.
8. Hurtgen JP: Pathogenesis and treatment of endometritis in the mare: A review. Theriogenology 2006;66:560-566.
9. Dascanio, JJ: How and when to treat endometritis with systemic or local antibiotics. Proc Annu Conv Am Assoc Equine Pract 2011. p. 24-31.
10. Haggett EF, Wilson WD: Overview of the use of antimicrobials for the treatment of bacterial infections in horses. Equine Vet Educ 2008;20:433-448.
11. Brown SA, Jaglan PS, Banting A: Ceftiofur sodium: disposition, protein-binding, metabolism, and residue depletion profile in various species. Acta Vet Scand Suppl 1991;87:97-99
12. Folz SD, Hanson BJ, Griffin AK, et al: Treatment of respiratory infections in horses with ceftiofur sodium. Equine Vet J 1992;24:300-304.
13. Bermudez V, Sifontes L, Navarro N, et al: Effects of intrauterine infusion of sodium ceftiofur on the endometrium of mares. Proc Am Assoc Equine Pract; 1995. p.261-263.
14. Ricketts SW: Treatment of equine endometritis with intrauterine irrigations of ceftiofur sodium: a comparison with mares treated in a similar manner with a mixture of sodium benzylpenicillin, neomycin sulphate, polymixin B sulphate and furaltadone hydrochloride. Pferdeheilkunde 1997;13:486-489.
15. Brinsko SP, Blanchard TL, Varner DD, et al: Breeding soundness examination of the mare. In: Hartman, editor. Manual of equine reproduction. 3rd ed. St. Louis: Mosby; 2011. p. 39-53.
16. Kenney RM, Doig PA: Equine endometrial biopsy. In: Morrow DA, editor. Current therapy in theriogenology. 2nd ed. Philadelphia: WB Saunders; 1989: p. 723-729.
17. Cocchia N, Paciello O, Autetta L, et al: Comparison of the cytobrush, cottonswab, and low-volume uterine flush techniques to evaluate endometrial cytology for diagnosisng endometritis in chronically infertile mares. Theriogenology 2012;77:89-98.
18. Woodward EM, Christoffersen M, Campos J, et al: Susceptibility to persistent breeding-induced endoemtritis in the mare: Relationship to endometrial biopsy score and age, and variations between seasons. Theriogenology 2012;78:495-501.
19. Drillich M, Arlt S, Kersting S, et al: Ceftiofur derivatives in serum, uterine tissues, cotyledons, and lochia after fetal membrane retention. J Dairy Sci 2006; 89:3431-3438.
20. Christoffersen M, Woodward EM, Bojesen AM, et al: Effect of immunomodulatory therapy on the endometrial inflammatory response to induced infectious endometritis in susceptible mares. Theriogenology 2012;78:991-1004.

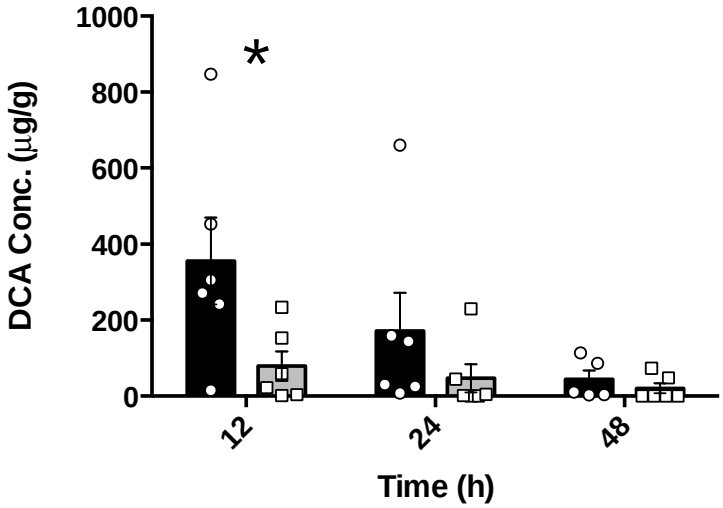
21. Wayne PA: Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals (secondinformational supplement). Clinical and Laboratory Standards Institute; 2013.
22. Salmon SA, Watts JL, Yancey RJ, Jr: In vitro activity of ceftiofur and its primary metabolite, desfuroylceftiofur, against organisms of veterinary importance. J Vet Diagn Invest 1996;8:332-336.
23. Parlevliet JM, Paccamonti DL, Barker SA: Cefquinome concentrations in endometrium after intrauterine treatment of cobactan 4.5% in mares and inflammatory response of the endometrium to this treatment. Reprod Domest Anim 2009;44:189-193.
24. Van Camp SD, Papich MG, Whitacre MD: Administration of ticarcillin in combination with clavulanic acid intravenously and intrauterinely to clinically normal oestrous mares. J Vet Pharmacol Therap 2000;23, 373-378.
25. Orsini JA, Park MI, Spencer PA: Tissue and serum concentrations of amikacin after intramuscular and intrauterine administration to mares in estrus. Can Vet J 1996; 37:157-160.
26. Witte TS, Bergwerff AA, Scherpenisse P, et al: Ceftiofur derivates in serum and endometrial tissue after intramuscular administration in healthy mares. Theriogenology 2010;74:466-472.
27. Cervantes CC, Brown MP, Gronwall R, et al: Pharmacokinetics and concentrations of ceftiofur sodium in body fluids and endometrium after repeated intramuscular injections in mares. Am J Vet Res 1993;54:573-575.



Graph 1: Non-infected mares: Endometrial tissue concentrations of DCA (desfuroylceftiofur-acetamide) at 8, 12, 24, 36 and 48 h.



Graph 2: Mares with experimentally induced endometritis: Endometrial tissue concentrations of DCA (desfuroylceftiofur-acetamide) at 12, 24, 48, 72 and 96 h.



Graph 3: Endometrial tissue concentrations of DCA (desfuroylceftiofur-acetamide) in non-infected mares (black bars and o) and mares with experimentally induced endometritis (grey bars and ❑) at 12, 24 and 48 h post intrauterine infusion of 1 g ceftiofur. An asterisk indicates significant differences in DCA tissue concentrations at 12 h.