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Theriogenology 78 (2012) 1199–1208

Theriogenology

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Effects of oral treatment with N-acetylcysteine on the viscosity of intrauterine mucus and endometrial function in estrous mares

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Received 23 February 2012; received in revised form 16 May 2012; accepted 22 May 2012

Abstract

Persistent breeding-induced endometritis is ranked as the third most common medical problem in the adult mare and leads to enormous economic loss in horse breeding. In mares suffering from persistent breeding-induced endometritis, increased amounts of intrauterine (i.u.) fluid or viscous mucus in estrus or after breeding may act as a barrier for sperm and can contribute to low fertility. Current therapies of these mares aim to eliminate i.u. fluid and mucus by uterine lavage and/or administration of ecbolic drugs. Recently, i.u. administration of N-acetylcysteine (NAC) has been shown to support therapy in mares with endometritis. It was the objective of the present study to investigate effects of an oral administration of NAC on the viscosity of i.u. fluid in estrous mares. It was hypothesized that oral treatment with NAC reduces the viscosity of i.u. fluid and has a positive effect on the inflammatory response of the endometrium. Mares ($n = 12$) were included in the study as soon as estrus was detected (ovarian follicle >3.0 cm and endometrial edema), which was defined as Day 1. They were randomly assigned to a treatment (10 mg/kg NAC on Days 1–4) or a control group (no treatment). On days 1 and 5 i.u. mucus was collected and its rheologic properties were accessed. On Day 5, endometrial biopsies were obtained and evaluated for integrity of the luminal epithelium, number of polymorphonuclear neutrophils (PMN), staining for cyclooxygenase 2 (COX2), staining with Kiel 67 antigen (Ki-67), lectins and periodic acid Schiff (PAS). In the treatment group, viscosity of i.u. mucus increased significantly between Days 1 and 5 ($P < 0.05$), while no differences were found in control mares (n.s.). At no time were significant differences between treated and control mares seen. Integrity of epithelium was not affected. After NAC treatment the mean number of PMN in endometrial biopsies was significantly lower compared to mares of the control group (1.9 ± 0.3 vs. 4.8 ± 0.4; $P < 0.05$). Nuclear immunostaining for COX2 was significantly lower after NAC treatment compared to control mares ($P < 0.05$). Score for PAS and Alcain staining of mucus in deep uterine glands differed significantly between groups (both $P < 0.05$). We conclude that oral NAC treatment does not reduce viscosity of uterine mucus but has an antiinflammatory effect on the equine endometrium.

Keywords: N-acetylcysteine; Mucus; Endometritis; Mare; Reproduction; Flow behavior

1. Introduction

Endometritis is the most important reason for low pregnancy rates in mares and therefore contributes to significant economic loss in horse breeding [1–4]. Endo-

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http://dx.doi.org/10.1016/j.theriogenology.2012.05.013

metritis is often accompanied by accumulation of intrauterine (i.u.) fluid and mucus. In mares suffering from persistent mating-induced endometritis (PMIE) accumulation of i.u. fluid occurs in response to breeding but independent from bacteria [1]. Mares with bacteria-induced endometritis will usually be excluded from breeding. However, estrous mares with i.u. fluid not related to infectious endometritis but caused by insufficient uterine clearance will be bred. In these mares, spermatozoa may be hampered on their route to the site of fertilization by pronounced amounts of i.u. secretions. A close relationship between sperm progression and the rheological behavior of uterine fluid is assumed. In humans, a strong correlation between viscoelasticity of cervical mucus and sperm penetration scores (SPR) exists. Therefore, SPR is a predictive value for fertility [5,6]. In estrous cows cervical mucus becomes watery, whereas the influence of progesterone during the luteal phase leads to more viscous mucus quality [7–9]. Increased amounts of fluid or viscous mucus of mares suffering from endometritis act as a barrier for sperm and can lead to low fertility rates [10–12].

Current therapies of mares suffering from endometritis aim to eliminate i.u. fluid and mucus by uterine lavage and/or administration of ecbolic drugs [2,3,13]. Recently, i.u. administration of N-acetylcysteine (NAC) has been shown to support antibiotic therapy in mares with endometritis [14,15]. N-acetylcysteine disrupts disulfide bonds between mucin polymers and thus exhibits mucolytic properties [16,17]. It is effectively used for mucolytic therapy of chronic obstructive pulmonary disease in horses [18]. Furthermore, it possesses antioxidant properties [19,20], can protect the colon mucosa after reperfusion injury [21] and has protease-inhibiting capacities [22,23].

The i.u. treatment with NAC has positive effects on fertility of mares with endometritis [14,15]. We showed recently that i.u. administered NAC does not adversely affect endometrial function in estrous mares but has antiinflammatory properties [24]. Oral application of NAC, however, would be advantageous and a simple alternative. Intrauterine manipulation in mares susceptible to endometritis could be avoided and administration of the drug without veterinary intervention would be allowed. In a current field study, orally administered NAC had an anti-inflammatory effect but did not affect clearance of i.u. fluid accumulation in mares suffering from PMIE [25]. However, no information on effects of oral treatment with NAC on the quality of i.u. fluid or mucus is available. Therefore, the objective of the present study was to investigate the viscosity of i.u. fluid in healthy estrous mares orally treated with NAC. It was hypothesized that oral treatment with NAC reduces the viscosity of i.u. fluid. For further evaluation of the inflammatory response of the endometrium, the presence of polymorphonuclear neutrophils (PMN) and staining for cyclooxygenase 2 (COX2) was evaluated. Kiel-67 (Ki-67) immunostaining in the endometrial tissue was analyzed for characterization of proliferative activity and secretion by the endometrium. Furthermore, periodic acid–Schiff reaction (PAS), Alcian blue staining and lectin binding patterns were evaluated to analyze carbohydrates as a parameter for changes in mucus quality in response to NAC treatment. We hypothesized that an oral treatment with NAC has a positive effect on the inflammatory response of the endometrium and reduces Ki-67 immunostaining, PAS, Alcain staining and lectin binding patterns.

2. Materials and methods

2.1. Animals

The study was conducted at the Clinic for Animal Reproduction, Freie Universität Berlin, Germany. Twelve mares of mixed breeds aged 2 to 8 years (mean 4.7 ± 1.7 yrs) weighing 500 to 600 kg (mean 558.3 ± 63.4 kg) were included. The mares were kept outdoors in an open shelter with access to pasture. Water was freely available and hay was given *ad libitum*. All experimental procedures used in this study were reviewed and approved by the Institutional Animal Care Committee of the Freie Universität Berlin, Germany.

2.2. Clinical examination

At the beginning of the experiment all mares underwent a breeding soundness examination (transrectal palpation and ultrasonography, Picker CS 9100, Physia, GmbH, Neu-Isenburg, Germany) for determination of genital health and cycle stage. Only mares without any signs of genital pathologies and with negative uterine swabs were included in the study. The experiment commenced for each mare as soon as estrus was detected, according to the presence of an ovarian follicle ≥3.0 cm and detection of endometrial edema. Day 1 was defined as first day of estrus detection.

2.3. Experimental design

Mares were equally assigned to either a treatment (T) or a control (C) group. In both groups, uterine mucus was collected by a cotton Salivette (Cortisol Salivette, Sarstedt, Nümbrecht, Germany) on Day 1 of

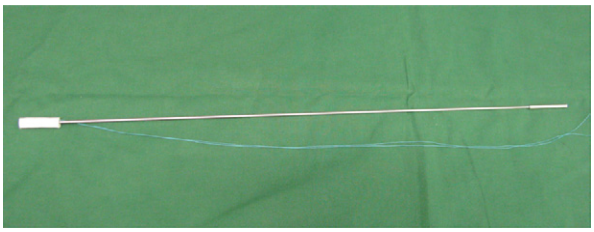

Fig. 1. The Salivette modified for i.u. mucus collection.

the experiment. On Day 1 and on the following three days all mares of the treatment group (n = 6) received NAC orally (10 mg Acetylcysteine/kg body weight; Equimucin; Cp Pharma, Burgdorf, Germany) twice daily. Mares of the control group (n = 6) remained untreated. On Day 5, uterine mucus was collected again by a cotton Salivette and an endometrial biopsy was taken.

2.4. Treatment solution and sample collection

The N-acetylcysteine was diluted in 10 mL of water and filled in a 20 mL syringe. The solution was administered orally to the mares through the syringe.

Before collection of i.u. mucus, the tail of each mare was wrapped; the perineum and vulva were washed three times with warm water and a disinfectant soap and dried with paper towels. For collection of the i.u. mucus, a suture (2/0 USP; WDT, Hannover, Germany) was lead through the cotton Salivette with the two ends being approximately 1 m long. Afterward, the Salivette was put on a sterile metal rod and the suture ends were fixed to the end of the metal rod (Fig. 1). The rod-mounted Salivette was covered with a hygienic sheath (Minitüb, Tiefenbach, Germany). With a sleeved arm, the Salivette-rod combination was guided through the vagina and cervix. Then, the Salivette was inserted into the uterus and the hygienic sheath was removed. The metal rod and the sleeved arm were withdrawn while the Salivette remained in the uterus of the mare for 10 min. Immediately after removal, the mucus was collected from the Salivette by centrifugation at 1000*g* for 5 min and subsequently kept at 4°C. Within the next hour, the mucus sample was transported to the Technical University, Berlin for further rheological examination.

Endometrial biopsies were collected from the base of either uterine horn with a Kevorkian biopsy forceps (Hauptner, Solingen, Germany) using standard procedures [26]. In brief, the biopsy forceps was manually introduced through the spread vulva into the vagina and through the cervical canal. After the biopsy forceps were placed at the base of one uterine horn the sleeved arm was withdrawn from the vagina and inserted into the rectum to guide the forceps to the desired place and to put endometrial mucosa into the forceps. The endometrial biopsies were immediately transferred into 4% formaldehyde and sent to the Institute for Histology, University for Veterinary Science, Vienna, Austria for further processing and evaluation.

2.5. Rheological examination

Viscosity behavior was measured as a function of shear rate using an MCR301 Rheometer (Physica, Anton Paar, GmbH, Graz, Austria) equipped with a cone–plate-system CP 25–2 and Peltier P–PTD200. A sample volume of only 160 μL was used. Shear rate examination profile was fitted as a downward curve from 100 $s^{-1} >> 0.1\ s^{-1}$. The data were collected every 2 s at each state measurement. Measurements were performed at a controlled temperature of 10°C (±0.1°C).

The effective viscosity of the mucus samples was calculated with the software RHEOPLUS/32 Multi6 V3.40 (Anton Paar, GmbH, Graz, Austria). The flow curves showed unequivocal non-Newtonian behavior. Therefore, the power law model was used for the calculation of the effective viscosity. The equation of state of the power law model is as follows:

$$\tau = \mathrm{K} \cdot \dot{\gamma}^{n} \quad (1)$$

τ is shear stress (Pa), K ($Pa{\cdot}s^{n}$) is consistency index, is shear rate (s^{-1}) and n is flow behavior index.

The effective viscosity is calculated through the followed equation

$$\eta_{\mathrm{eff}}(\dot{\gamma}) = \frac{\tau}{\dot{\gamma}} = K \cdot \dot{\gamma}^{n-1} \quad (2)$$

2.6. Histology and immunohistochemistry

The formalin fixed biopsy specimens were embedded in Histocomp (Vogel, Giessen, Germany) and cut at a thickness of 5 μm and stained with hematoxylin and eosin (H&E) as described [27]. Luminal epithelium was evaluated and classified as very well preserved (1), well preserved (2) and not well preserved (3). The PMN were morphologically differentiated, photographed and counted in six randomly selected fields (340 × 260 μm) with a light microscope at 40× magnification (Reichert, Vienna, Austria) as described previously [28]. Three fields were located in the stratum compactum and epithelium and the other three fields were chosen in the stratum spongiosum. The mean number of PMN per field was calculated. Photomicroscopy was performed with a microscope (Polyvar, Reichert, Austria) equipped with a color camera head

Table 1a
Rheological parameters of uterine mucus collected from estrous mares with an increase in effective viscosity from the first to the second measurement.

Sample	K Pas^n	**n** —	**r** —	**s** Pa	η_{eff} at $\gamma = 1\ s^{-1}$ mPas
Mare T-1A	0.017	0.728	0.988	0.02	17.00
Mare T-1B	0.280	0.273	0.971	0.05	280.00
Quotient	16.47	0.38			16.47
Mare T-2A	0.260	0.262	0.992	0.02	260.00
Mare T-2B	0.523	0.174	0.773	0.12	523.00
Quotient fsecond sample/first sample second sample/first sample	2.01	0.66			2.01
Mare T-5A	0.329	0.344	0.990	0.06	329.00
Mare T-5B	0.554	0.313	0.985	0.09	554.00
Quotient	1.68	0.91			1.68
Mare T-6A	0.352	0.229	0.958	0.05	352.00
Mare T-6B	0.703	0.196	0.925	0.11	703.00
Quotient	1.99	0.86			1.99
Mare C-3A	0.013	0.866	0.991	0.03	13.00
Mare C-3B	0.351	0.190	0.901	0.05	351.00
Quotient	27.00	0.22			27.00
Mare C-4A	0.357	0.308	0.983	0.06	357.00
Mare C-4B	0.648	0.176	0.889	0.11	648.00
Quotient	1.81	0.57			1.81
Mare C-6A	0.117	0.476	0.968	0.08	117.00
Mare C-6B	0.571	0.148	0.930	0.06	571.00
Quotient	4.88	0.31			4.88

K, consistency index; γ, shear rate (s^{-1}); n, flow behavior index; r, correlation ratio; s, standard deviation; η_{eff}, effective viscosity; T, treatment group; C, control group; A, first sample (before treatment); B, second sample (after treatment).

(DsFi1, Nikon, Japan) and NIS-Elements software (Nikon Instruments, Japan).

For analysis of carbohydrates, sections were stained with PAS and Alcian blue (pH 2.5) according to Romeis [27]. The presence of mucus on the luminal epithelium, in the superficial and in the deep glands was classified and scored as absent (0), mild (1), moderate (2), or strong (3).

Histochemical analysis with lectins *Helix pomatia* (HPA), *Ulex europaeus* I (UEA) and *Triticum vulgaris* (WGA) was performed as described elsewhere [24,29–31]. Binding of lectins was classified by the intensity of staining of the epithelium (cytoplasm and glycocalyx), the stroma, the superficial and the deep uterine glands (cytoplasm and glycocalyx) as absent (0), mild (1), moderate (2), or strong (3) as described [24,31,32].

Table 1b
Rheological parameters of uterine mucus collected from estrous mares with a decrease in effective viscosity from the first to the second measurement.

Sample	K Pas^n	n —	r —	s Pa	η_{eff} at $\gamma = 1\ s^{-1}$ mPas
Mare T-3A	0.142	0.290	0.966	0.03	142.00
Mare T-3B	0.095	0.497	0.943	0.11	95.00
Quotient	0.66	1.71			0.66
Mare C-1A	0.168	0.321	0.965	0.05	168.00
Mare C-1B	0.119	0.319	0.979	0.03	119.00
Quotient	0.71	0.99			0.71
Mare C-2A	0.435	0.332	0.975	0.09	435.00
Mare C-2B	0.061	0.836	0.995	0.03	61.00
Quotient	0.14	2.52			0.14
Mare C-5A	0.664	0.129	0.941	0.05	664.00
Mare C-5B	0.116	0.717	0.995	0.09	116.00
Quotient	0.17	5.56			0.17

K, consistency index; γ, shear rate (s^{-1}); n, flow behavior index; r, correlation ratio; s, standard deviation; η_{eff}, effective viscosity; T, treatment group; C, control group; A, first sample (before treatment); B, second sample (after treatment).

Table 1c
Rheological parameters of uterine mucus collected from estrous mares with negligible differences in effective viscosity from the first to the second measurement.

Sample	K Pas^n	n —	r —	s Pa	η_{eff} at $\gamma = 1\ s^{-1}$ mPas
Mare T-4A	0.378	0.281	0.991	0.04	378.00
Mare T-4B	0.495	0.235	0.991	0.04	495.00
Quotient	1.31	0.84			1.31

K, consistency index; γ, shear rate (s^{-1}); n, flow behavior index; r, correlation ratio; s, standard deviation; η_{eff}, effective viscosity; T, treatment group; C, control group; A, first sample (before treatment); B, second sample (after treatment).

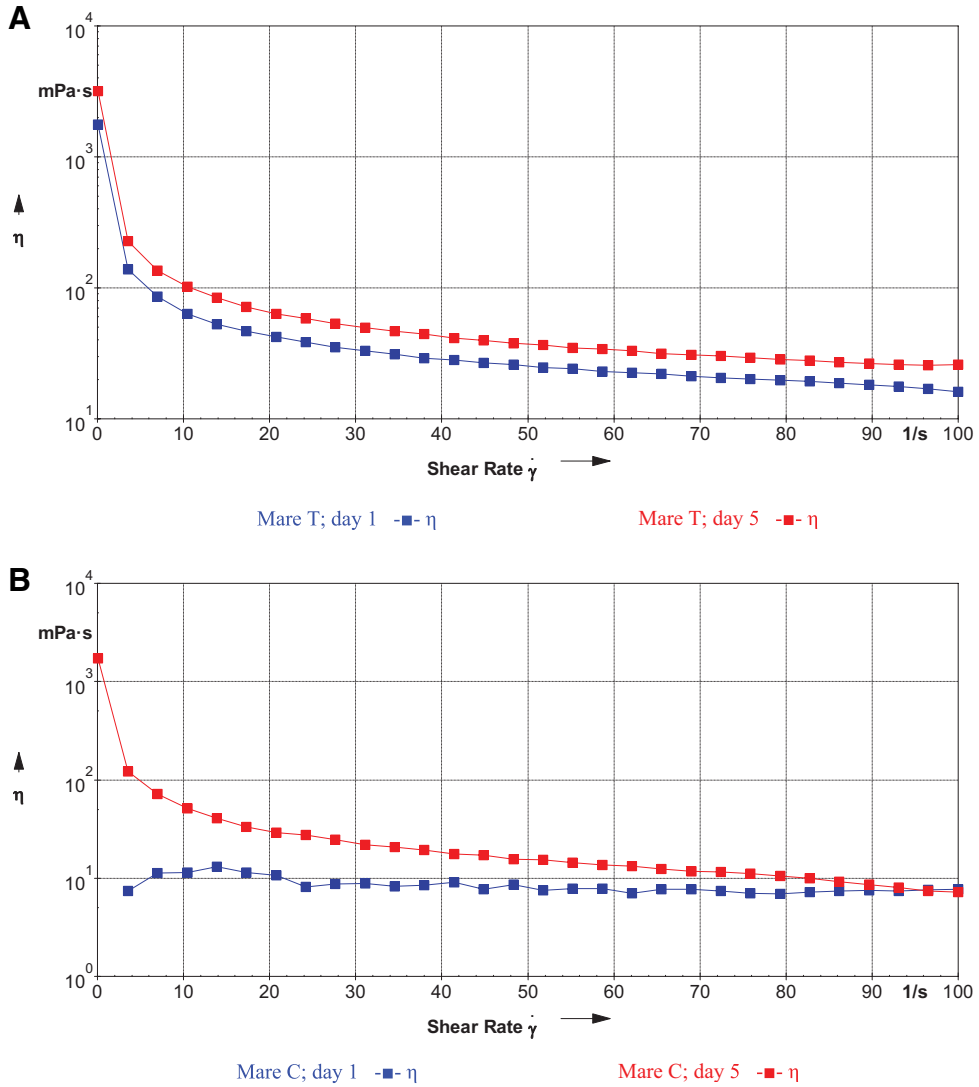

Fig. 2. Flow curves of the i.u. mucus from a representative mare of the treatment group (Fig. 2 a) and the control group (Fig. 2 b) with an increase in effective viscosity from the first (Day 1) to the second (Day 5) sample.

Blue: first sample (Day 1); red: second sample (Day 5); η: viscosity.

The expression of Ki-67 and COX2 was determined by immunohistochemical analysis according to Jischa, et al. [31] and Koblischke, et al. [28]. Primary antibodies (Ki-67: mouse monoclonal Ki-67/MM1: dilution 1:200, Novocastra, Newcastle, England; COX2: goat polyclonal, 1:500, Santa Cruz, CA, USA) and secondary antibody (anti-mouse ImmunoVision, Brisbane, USA; Vector Laboratories, CA, USA; or biotinylated antigoat, Santa Cruz, CA, USA) were used. Ki-67-positive cells were counted and photographed with a light microscope (40× magnification). At least 100 cells were counted in each slide in three randomly selected fields of the superficial epithelium, including the endometrial glands. The COX2 positive cells were also counted at 40× magnification. At least 200 cells were counted in the superficial epithelium and in the superficial glandular epithelium and analyzed for positive staining according to Willmann, et al. [33]. In addition, the intensity of staining of the epithelial cells was classified as absent (0), mild (1), moderate (2), or strong (3).

2.7. Statistical analysis

Statistical analysis was performed with the PASW statistic package (SPSS, Inc., Chicago, IL). Viscosity

Table 2
Histologic evaluation of endometrial biopsies collected on Day 5 after oral treatment with NAC (NAC) or no treatment (control). Values are means ± SEM for integrity of endometrial epithelial cells (score from 0 to 3), number of PMN/field, percentage of cells staining positive for Ki-67 (%) or COX2 (%) and intensity of Alcian and PAS staining (score from 0 to 3) at different locations of the endometrium.

Treatment	NAC	Control
Condition of endometrial epithelial cells (score)	1.6 ± 0.2	1.6 ± 0.2
Number of PMN/field (n)	1.9 ± 0.3[a]	4.8 ± 0.4[b]
Epithelial cells staining positive for Ki-67 (%)	38.8 ± 6.3	30.5 ± 5.2
Cells in superficial uterine glands staining positive for Ki-67 (%)	25.4 ± 8.6	12.2 ± 4.7
Cells in deep uterine glands staining positive for Ki-67 (%)	26.2 ± 8.6[a]	1.7 ± 1.3[b]
COX2 staining of endometrial epithelial cells (score)	1.5 ± 0.2	2.1 ± 0.1
Epithelial cells staining positive for COX2 (%)	6.6 ± 1.8	6.5 ± 1.2
COX2 staining of nucleus of the epithelium (score)	0.5 ± 0.2[a]	1.5 ± 0.2[b]
Cells in superficial uterine glands staining positive for COX2 (%)	12.5 ± 2.4	6.6 ± 1.3
COX2 staining of the nucleus of the superficial uterine glands (score)	0.3 ±. 0.2	0.8 ± 0.1
Alcian staining: mucus on epithelial cells (score)	2.0 ± 0.2	1.3 ± 0.2
Alcian staining: mucus in superficial uterine glands (score)	1.3 ± 0.4	0.6 ± 0.3
Alcian staining: mucus in deep uterine glands (score)	0.8 ± 0.1[a]	0.0 ± 0.0[b]
PAS staining: mucus on epithelial cells (score)	1.8 ± 0.3	1.1 ± 0.1
PAS staining: mucus in superficial uterine glands (score)	1.1 ± 0.1	0.6 ± 0.3
PAS staining: mucus in deep uterine glands (score)	1.1 ± 0.3[a]	0.3 ± 0.2[b]

[a,b] Different superscripts mark differences between treatments ($P < 0.05$).

between groups was compared by *t* test for independent samples; differences between Days 1 and 5 within groups were compared by *t* test for paired samples. The following parameters were evaluated as scores. H&E: integrity of endometrial surface epithelial cells; COX2: intensity of staining of endometrial epithelial cells; PAS: mucus on endometrial surface epithelial cells and in superficial and deep uterine glands; the lectins HPA, UEA and WGA in endometrial epithelial cells, stroma as well as in superficial and deep uterine glands. Therefore, non-parametric tests were used for evaluation. Comparisons between groups (paired samples) were performed by Wilcoxon test, comparisons between times by Mann-Whitney-*U* test. A P value <0.05 was considered significant. Values are means ± standard error of mean (SEM).

3. Results

3.1. Animals and clinical examination

All mares displayed estrus during the experiment and ovulated 1 to 2 days (mean 1.3 ± 0.4 days) after the end of the respective experiment. Per collection, a volume of 0.5 to 1.5 mL mucus was collected. A transrectal ultrasound examination of all mares was conducted 1 and 2 days after the end of the study and none of the mares showed i.u. fluid. In two mares small amounts of i.u. air were detectable on Day 1 after the end of the experiment, but were no longer detectable the following day.

3.2. Rheological examination

All mucus samples showed non-Newtonian shear thinning behavior. A strong decrease in viscosity by increase of the shear rate was apparent. The degree of decrease, however, varied among horses and day before ovulation.

For the calculation of the effective viscosity the power law model was used. Based on the changes of the effective viscosity calculated by $\gamma = 1\ s^{-1}$ the horses were divided in three classes: 1) Increase of the effective viscosity from the first to the second sample (Table 1a); 2) Decrease of the effective viscosity from the first to the second sample (Table 1b); and 3) Negligible difference in effective viscosity between first and second sample (Table 1c). In the majority of mares the effective viscosity of i.u. mucus increased from the first to the second sample (i.e., 4/6 and 3/6 horses of the treatment and the control group, respectively). Figure 2 shows the changes in the effective viscosity in mares as an example of the first group. During the whole study the flow curve of the measurement on Day 1 was higher than the flow curve on Day 5. In one mare of the treatment group and three mares of the control group a decrease of the effective viscosity of the mucus between Day 1 and Day 5 was observed (Table 1b). Only 1 of 12 mares showed a negligible difference in the consistency factor, in the flow behavior index and therefore in the calculated effective viscosity (Table 1c). In the control group there was no significant difference in mean viscosity between Day 1 and 5. In the

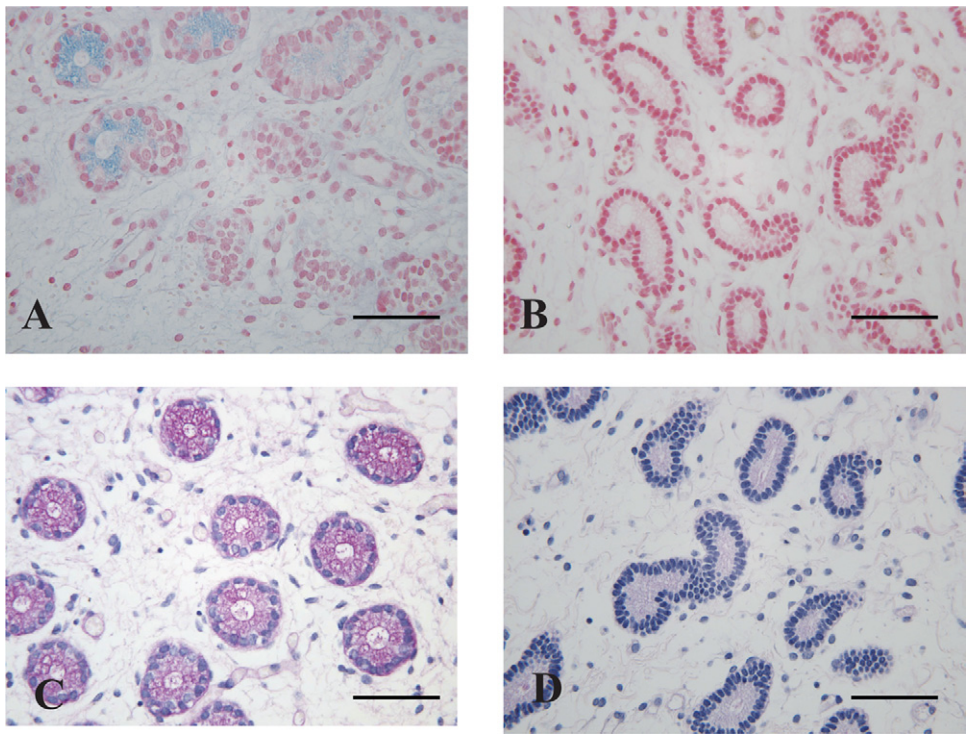

Fig. 3. Photomicrographs of endometrial biopsies collected from mares at Day 5 after oral NAC treatment. (A) Alcian staining with high amount of mucus. (B) Alcian staining with no mucus. (C) periodic acid Schiff (PAS) staining with high amount of mucus. (D) PAS staining with low amount of mucus. 165 × 250 mm (300 × 300 DPI).

treatment group, mean viscosity between Days 1 and 5 significantly increased ($P < 0.05$). In samples of single mares, however, effective viscosity did not differ between groups (Table 1).

3.3. Histology and immunohistochemistry

Histology of H&E-stained specimens of the endometrium did not show differences in integrity of the epithelium between groups. After NAC treatment the mean number of PMN in endometrial biopsies was significantly lower compared to mares of the control group ($P < 0.05$; Table 2). The number of cells staining for COX2 of the nuclei of the epithelium was significantly lower in NAC treated mares compared to control mares ($P < 0.05$). In deep uterine glands, scores for PAS and Alcain staining were significantly greater in the treatment compared to the control group ($P < 0.05$, Fig. 3). In the endometrial surface epithelium and the superficial uterine glands, however, differences in staining for PAS and Alcain could not be detected (Table 2). Staining for the proliferation marker Ki-67 in the deep uterine glands was significantly lower in the control group compared to the treatment group ($P < 0.05$). In the endometrial epithelium and the superficial uterine glands, staining for Ki-67 showed no differences (Table 2). Lectins HPA, UEA and WGA stained luminal epithelium, deep and superficial glands and stroma. The binding pattern of HPA in the glycocalyx of deep uterine glands was significantly greater in the treatment compared to the control group ($P < 0.05$). Differences in binding patterns of UEA and WGA did not exist between treatment and control group (Table 3).

4. Discussion

In the present study, mean viscosity of i.u. fluid significantly increased in estrous mares after treatment with NAC. This change did not occur in untreated estrous control mares. The results of effective viscosity on Day 1 in single samples showed a high variability. Therefore, in some samples of single mares the effective viscosity on Day 1 was higher than on Day 5 of another mare (Table 1a). A mucolytic effect of oral NAC treatment in samples of single mares, however, could not be confirmed. Recently, it has been shown that i.u. NAC-treatment supports the therapy of endo-

Table 3
Histologic evaluation of lectin binding patterns of endometrial biopsies collected on Day 5 after oral treatment with NAC (NAC) or no treatment (control). Values are means ± SEM (score from 0 to 3) for staining for the lectins *Helix pomatia* agglutin in (HPA), *Ulex europaeus* I agglutinin (UEA) and *Triticum vulgaris* agglutinin (WGA) at different locations of the endometrium in mares after oral administration of NAC (N-acetylcysteine) or control (no treatment).

Treatment	NAC	Control
Lectin HPA		
Epithelium cytoplasm	2.5 ± 0.2	1.8 ± 0.1
Epithelium glycocalyx	2.0 ± 0.2	2.1 ± 0.1
Stroma	0.3 ± 0.2	0.0 ± 0.0
Superficial glands	2.3 ± 0.2	1.5 ± 0.2
Cytoplasm		
Superficial glands glycocalyx	2.1 ± 0.1	1.6 ± 0.2
Deep glands cytoplasm	1.5 ± 0.2	0.6 ± 0.2
Deep glands glycocalyx	2.0 ± 0.3[a]	0.6 ± 0.2[b]
Lectin UEA		
Epithelium cytoplasm	2.5 ± 0.2	1.6 ± 0.2
Epithelium glycocalyx	1.6 ± 0.3	1.6 ± 0.4
Stroma	0.0 ± 0.0	0.0 ± 0.0
Superficial glands cytoplasm	1.8 ± 0.3	1.5 ± 0.2
Superficial glands glycocalyx	1.8 ± 0.3	1.3 ± 0.2
Deep glands cytoplasm	1.1 ± 0.3	1.0 ± 0.0
Deep glands glycocalyx	1.5 ± 0.3	1.0 ± 0.2
Lectin WGA		
Epithelium cytoplasm	2.6 ± 0.2	1.8 ± 0.1
Epithelium glycocalyx	2.0 ± 0.0	2.0 ± 0.2
Stroma	1.5 ± 0.2	1.8 ± 0.1
Superficial glands cytoplasm	2.1 ± 0.3	1.3 ± 0.2
Superficial glands glycocalyx	2.1 ± 0.3	1.5 ± 0.2
Deep glands cytoplasm	1.5 ± 0.2	1.3 ± 0.2
Deep glands glycocalyx	1.8 ± 0.3	1.0 ± 0.0

[a,b] Different superscripts mark differences between treatments ($P < 0.05$).

metritis in mares, enhances pregnancy rates and does not negatively affect endometrial function in healthy estrous mares [14,23,24]. However, i.u. administration and manipulation bears the risk of irritation and contamination of the endometrium. Besides, an oral application would allow an administration without veterinary intervention. Therefore, effects of oral application were investigated. The present study clearly demonstrates that oral administration of NAC influences endometrial secretions. This is demonstrated by changes in effective viscosity.

Periodic acid Schiff, Alcain staining and lectin binding characterize carbohydrates in secretions of the endometrium [29–31]. In the present study, deep uterine glands had a stronger staining after PAS and Alcain staining in mares treated with NAC indicating a higher amount of mucus production (Fig. 3). However, staining of epithelial cells and superficial uterine glands was not affected. No secreted mucus was detectable on the endometrial surface; this could be a result of destruction of mucus during collection and processing of biopsy samples. Thus, differences in mucus production due to treatment could not be assessed. NAC treatment did not affect endometrial lectin binding patterns. This is again in agreement with findings after i.u. NAC treatment [24]. As lectin staining presents information on the carbohydrate secretions and glycocalyx properties of the endometrial epithelium, altered binding patterns coincide with degenerative changes in the endometrium [30,31]. Thus, the present data confirm that NAC does not negatively influence endometrial function with regard to lectin staining.

In conclusion, NAC treatment seems to induce mucus production and in some samples an increase in viscosity. These findings are contradictory to the assumption that NAC should have mucolytic properties and thus reduce the viscosity of mucus by disrupting disulfide bonds between mucin polymers [15–17]. In the present study, oral NAC treatment induced an increase in viscosity in most of the mares (Table 1a). In one mare of the treatment group, however, a decrease in viscosity was detected (Table 1b). Information on quality of mucus produced by the equine endometrium is rare. The total amount of protein of uterine flushings is affected by the day of the estrous cycle with maximal amounts of proteins occurring during the luteal phase [34,35]. In dairy cows, progesterone dominance during the luteal phase increases the viscosity of uterine mucus [7–9]. Significant changes in viscosity of mucus did not occur in mares of the control group. All mares were clearly in estrus and none of the mares ovulated during the experimental phase. Therefore, changes in mucus quality in treated mares are most probably caused by NAC treatment, but cannot be explained by estrous cycle-dependent influences. It is unclear why NAC has disrupting effects on disulfide bonds and decreases viscosity of mucus in airways but has an increasing effect on the viscosity of uterine secretions in most of the mares. As higher mucus viscosity is believed to be associated with lower fertility [14] it could be hypothesized that oral NAC treatment of estrous mares is detrimental to conception. However, in a recent study fertility of mares with PMIE was not negatively affected by oral NAC administration [25].

The N-acetylcysteine also influenced endometrial function. The mean number of PMN in endometrial biopsies and the intensity of staining for COX2 of the epithelial nuclei were significantly lower in NAC treated mares. Both parameters have been used for the characterization of endometrial inflammation [24,28]. Results of the present study confirm the findings of an antiinflammatory treatment of NAC on the endometrium [23,24]. This effect seems to be independent from the route of administration. A nuclear localization of COX2 has been observed in other studies [36] including a reactive accumulation of COX2 due to drug treatment [37]. In the present study, however, the epithelial staining for COX2 of the nucleus in the superficial endometrium was significantly stronger in the control group compared to the treatment group ($P < 0.05$; Table 2). Cyclooxygenase 2 is involved in the synthesis of $PGF_{2\alpha}$ and has been shown a reliable marker for the presence of endometrial inflammation [28]. A reduction in epithelial staining for COX2 following treatment with NAC might be due to its antioxidative properties [20,21]. Furthermore, NAC treatment also increased epithelial proliferation which may support regeneration of the endometrium. The present data also confirm that NAC does not negatively influence endometrial function with regard to lectin binding.

5. Conclusions

Results of the present study demonstrate that oral treatment with NAC does not reduce the viscosity of uterine mucus in estrous mares. Furthermore, the oral treatment of mares with NAC shows a similar antiinflammatory effect on the equine endometrium as does i.u. treatment.

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