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Influence of vedaprofen (Quadrisol®) on quality and freezability of stallion semen

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Abstract

The objective of this study was to evaluate the effect of the non-steroidal anti-inflammatory drug (NSAID) vedaprofen (Quadrisol®) on quality and freezability of stallion semen. Experiments were performed using 22 Franches Montagnes stallions from the National Stud in Avenches (Switzerland) randomly divided into a control and test group. Vedaprofen was given orally to all stallions of the test group at the recommended therapeutic dose (initial dose of 2 mg/kg followed by 1 mg/kg body weight every 12 h) for 14 days. Control animals received the same amount of carrier substance. During treatment, blood samples of five stallions in both test and control group were collected for $PGF_{2\alpha}$-metabolite (PG-metabolite) determination. Ejaculates from all stallions were collected and cryopreserved weekly for 14 weeks from September to December. Concentrations of PG-metabolite, PGF and PGE were measured in the seminal plasma of ejaculates collected 2 weeks before, during and 2 weeks after treatment. In fresh semen the volume, concentration, motility and number of normal sperm and sperm with major defects (acrosome defects, abnormal heads, nuclear vacuoles, proximal droplets, midpiece defects) were evaluated. In frozen-thawed semen samples motility as well as viability (SYBR-14/PI) were tested and the hypoosmotic swelling test (HOS) was performed. Results demonstrate that vedaprofen had no effect on blood plasma concentration of PG-metabolite

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but significantly inhibited both, PGF and PGE concentrations in seminal plasma. Furthermore, all quality parameters in fresh and frozen-thawed semen were not affected by vedaprofen treatment but the time of semen collection had a significant ($P < 0.05$) effect on motility, normal sperm and sperm with nuclear vacuoles in fresh semen.

Keywords: Stallion; Semen; NSAID; Vedaprofen; Prostaglandin

1. Introduction

Vedaprofen is a non-steroidal anti-inflammatory drug (NSAID) of the new generation licensed in the European Union and many other countries for use in the horse [1]. Its mode of action is by inhibition of cyclooxygenase (COX) enzymes which catalyze the biosynthesis of various prostanoids from arachidonic acid [2]. There are two distinct isoforms of COX; COX-1 which is constitutively expressed in most tissues and COX-2 which is markedly induced by inflammation [3]. According to product information (Intervet International BV, The Netherlands) vedaprofen is known to selectively inhibit COX-2 and thereby reduce inflammation without side effects on stomach and kidney, occurring when non-selective COX inhibitors such as acetylsalicylate or phenylbutazon are used.

Many breeding stallions combine stud duties with competition—this increases the risk of injuries and often leads to treatment with NSAIDs. In literature, there is little information regarding the possible side effects of NSAIDs on semen quality and fertility in the stallion. Mc Donnell et al. [4] demonstrated that 1 g phenylbutazon given orally twice daily for 4 weeks to three stallions had no effect on quality of fresh and cooled semen. However, another study with stallions [5] has shown that daily oral administration of 3 g phenylbutazon for 24 days can significantly decrease seminal plasma concentrations of $PGF_{2\alpha}$ metabolites.

In men, a decrease in prostaglandin in seminal plasma has been observed after using lysine salicylate, flurbiprofen and sulphasalazine [6] as well as naproxen [7] with only sulphasalazine resulting in a specific effect on semen quality. From another study in men [8] we know that indomethacin or oxaprozin also lowered the $PGF_{2\alpha}$-concentration but had no influence on sperm motility. This contrasts with results from infertile oligospermic men who experienced an increase in sperm number and motility after diclofenac treatment for 30 days [9]. In the rat, the application of acetylsalicylate, indomethacin but not phenylbutazon significantly decreased PGE_2 in seminal plasma although only indomethacin affected pregnancy rate [10]. In rabbits, phenylbutazon given during 9 days lowered the concentrations of $PGF_{2\alpha}$ and PGE_2 in seminal plasma but increased the ejaculate volume and sperm motility [11].

Because of the contradictory data on the effects of NSAIDs on semen quality in various species and since experiments about the effect of vedaprofen are lacking in the horse, the aim of the present study was to investigate the influence of vedaprofen on prostaglandin concentrations in blood and seminal plasma as well as on semen quality and freezability in the stallion.

2. Materials and methods

2.1. Experimental design

Experiments were performed using 22 healthy Franches Montagnes stallions, aged between 3 and 8 years, from the National Stud Farm in Avenches (Switzerland). The animals were kept in box stalls bedded with straw and were fed hay, oats and pellets supplemented with minerals. All animals were exercised daily for at least 1 h. Before starting the experiment the stallions were trained to mount the phantom and extragonadal sperm reserves were minimized by five daily semen collections. The stallions were divided randomly into a control and a test group of 11 animals each. Vedaprofen gel (Quadrisol®, Intervet International BV, The Netherlands) was given orally to all stallions of the test group ($n = 11$) at the recommended therapeutic dose (initial dose of 2 mg/kg followed by 1 mg/kg body weight every 12 h) for 14 days. Control animals ($n = 11$) received the same amount of carrier substance. Blood samples (EDTA) from five randomly selected stallions from each group, were obtained by jugular venipuncture immediately before and 4 h after vedaprofen application every other day during the 2-week treatment period. The 4 h bleeding interval was based on a report [1] indicating highest vedaprofen concentrations 2–3 h after its application. The samples were immediately centrifuged ($3000 \times g$, 10 min) and the decanted plasma frozen (-18 °C) until analysis. Semen was collected and evaluated and cryopreserved weekly for 14 weeks from September to December, starting 2 weeks before treatment commenced, ending 10 weeks after the last vedaprofen treatment. From all ejaculates collected 2 weeks before, during and 2 weeks after treatment an aliquot of 5 mL was centrifuged ($2400 \times g$, 3 min) and seminal plasma frozen (-18 °C).

2.2. Semen processing and examination

After semen collection and removal of the gel fraction (in-line gel filter), the volume (graduated cylinder) and the concentration (spectrophotometer) were estimated. Progressive motility was assessed with a Mika Motion Analyzer (Cell Motion Analyzer SM-CMA-1074, MTM, Switzerland) in fresh diluted semen using standardized threshold values for stallion semen [12]. For morphological examination five drops of fresh semen were fixed in 2 mL Hancock solution and smears prepared [13]. At least 200 spermatozoa were evaluated by phase contrast microscopy (Olympus BX50, UplanFl 100×/1.30) and abnormal spermatozoa classified in major and minor defects [14,15]. The gel-free semen was diluted with centrifugation medium (INRA 82-Hepes + 2% (v/v) egg yolk) to a concentration of 100×10^6 spermatozoa/mL and centrifuged at $1000 \times g$ for 2 min. After centrifugation, the supernatant was removed and the sperm pellet resuspended in freezing medium (75 mL lactose solution 11% (w/v), 20 mL egg yolk, 5 mL glycerol) to a final concentration of 300×10^6 spermatozoa/mL. Thereafter, semen was cooled to 4 °C during at least 30 min, packaged into 0.5 mL straws and frozen in an automatic freezer (Minidigitcool 700 ZB 290, IMV, France) at a cooling rate of 60 °C/min from +4 to -100 °C and 30 °C/min from -100 to -140 °C.

The straws were then stored in liquid nitrogen until examination. From each ejaculate three straws were thawed (37 °C, 30 s), pooled and diluted with 3 mL centrifugation medium to determine the motility, viability and hypoosmotic swelling (HOS). Viability was performed by dual DNA staining (LIVE/DEAD® Sperm Viability Kit, Molecular Probes Europe, Leiden, NL) using SYBR-14 in combination with propidium iodide (PI). SYBR-14 (component A) was diluted with anhydrous dimethyl sulfoxide (DMSO) 1:10 (SYBR-14 working solution), while PI (component B) was used in original concentration. Two microliters of the SYBR-14 working solution was added to 1 mL of diluted thawed semen. After incubation of 10 min at 37 °C, 5 μL of component B were added. Five minutes later, 5 μL of stained semen were placed on a slide, covered with a coverslip (24 mm × 24 mm) and examined using fluorescence microscopy (Olympus BX50, UPlanApo 40×/0.85, FITC filter U-MWIB, high pressure Hg-lamp). Different sequences of fluorescence stained spermatozoa were monitored by connecting a video camera (SANYO VCC-2972) to the microscope. At least 500 sperm cells were counted on the screen and the percentage of green fluorescing sperm was defined as viability [16]. The functional membrane integrity of frozen-thawed semen was assessed with the HOS test according to Neild et al. [17]. A sample of 100 μL thawed semen was incubated for 30 min at 37 °C in 1 mL of lactose 50 mOsm in distilled water. At least 200 spermatozoa were counted at a magnification of 400× and classified according to the presence of a swollen tail (curled/coiled principal or end piece). The percentage of HOS-positive spermatozoa (number of spermatozoa with swollen tails per total number of spermatozoa × 100) was recorded for each sample [17,18].

2.3. Prostaglandin analysis

Blood samples were assayed for the stable metabolite of $PGF_{2\alpha}$, 15-ketodihydro-$PGF_{2\alpha}$ (PG-metabolite). In seminal plasma the concentrations of PG-metabolite, PGF (sum of 1 and 2 series) and PGE (sum of 1 and 2 series) were determined. Analysis was performed by radioimmunoassay according to Granström and Kindahl [19]. The sensitivity of the assay was 30 pmol/L for PG-metabolite and 0.5 nmol/L for PGF and PGE [20].

2.4. Statistical analysis

Data were analyzed using StatView 5.0 software program (SAS Institute, Switzerland). A multivariate analysis of variance (ANOVA) with repeated measures was carried out to asses the effects of treatment, time of collection and interaction between time and treatment on semen characteristics and on prostaglandin concentrations in blood and seminal plasma. Non-normally distributed values were logarithmized for this purpose. Group (vedaprofen, control) differences at individual time points were compared by unpaired *t*-test in normally (semen characteristics) and by Mann–Whitney test in non-normally (PG-metabolite, PGF and PGE) distributed variables. Differences were considered significant at a probability level of $P < 0.05$.

3. Results

3.1. Blood concentrations of PG-metabolite

Results from ANOVA demonstrate that during the 14 days of medication neither the time of blood collection nor the treatment and the interaction between time and treatment significantly ($P > 0.05$) influenced blood plasma PG-metabolite concentrations. Mean PG-metabolite values on treatment days 1, 3, 5, 8, 10, 12 and 14, 1 h before and 4 h after the application were 223.8 ± 19.4 and 222.1 ± 19.1 in the vedaprofen and 179.0 ± 22.1 and 154.2 ± 19.0 in the control group.

3.2. Seminal plasma concentrations PG-metabolite, PGF and PGE

In seminal plasma collected during and 2 weeks after application, a significant ($P < 0.05$) effect of treatment and time and the interaction between treatment and time of collection on PGE concentration was seen. A significant ($P < 0.05$) influence of time and the interaction between treatment and time was also observed on PGF but not on PG-metabolite values (Table 1). During treatment, significant differences ($P < 0.05$) were present between PGF and PGE concentrations in the vedaprofen and control group (Figs. 1 and 2). Median values for PGF ranged between 1.8 and 19.7 nmol/L in the vedaprofen group and between 4.4 and 21.8 nmol/L in control stallions. Median values for PGE ranged between 3.3 and 169.5 nmol/L in the vedaprofen group and between 36.0 und 262.3 nmol/L in control stallions. During the 2-week treatment period PGF and

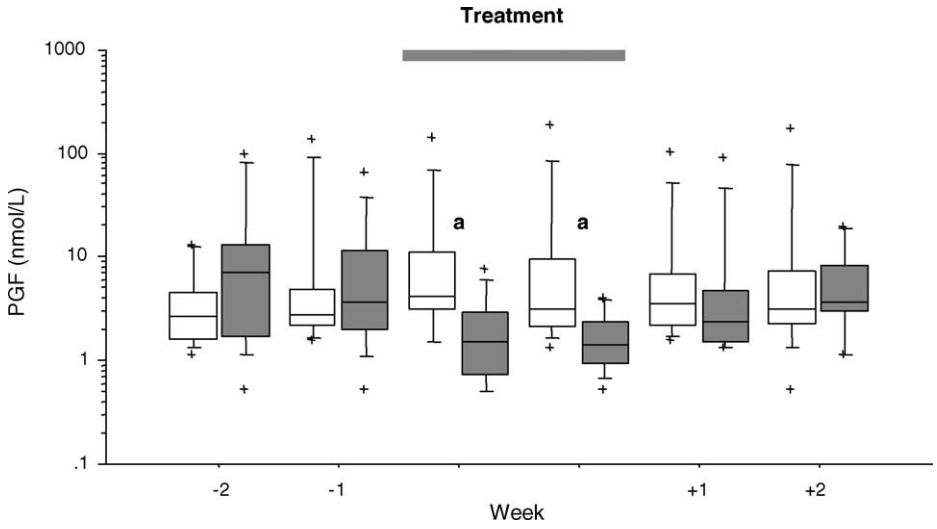

Fig. 1. Box plot of seminal plasma PGF concentrations in stallions ($n = 11$ each) with (▩) and without (□) vedaprofen before, during and after end of treatment. Letter 'a' indicates significant difference ($P < 0.05$, Mann–Whitney test).

Table 1
Mean ± S.E.M. of seminal plasma concentrations of PG-metabolite, PGF and PGE from weekly collected ejaculates during 4 weeks in vedaprofen treated and control stallions ($n = 11$ each) and the effect of treatment, time of semen collection and interaction between treatment and time

Parameter	Vedaprofen (mean ± S.E.M.)	Control (mean ± S.E.M.)	Treatment (P)	Time (P)	Interaction (P)
PG-metabolite (nmol/L)	2.6 ± 0.1	2.7 ± 0.1	0.7992	0.6101	0.1021
PGF (nmol/L)	5.7 ± 2.0	18.4 ± 6.4	0.1415	0.0011*	<0.0001*
PGE (nmol/L)	49.3 ± 21.3	162.1 ± 61.7	0.0127*	0.0006*	<0.0001*

* Significant ($P < 0.05$).

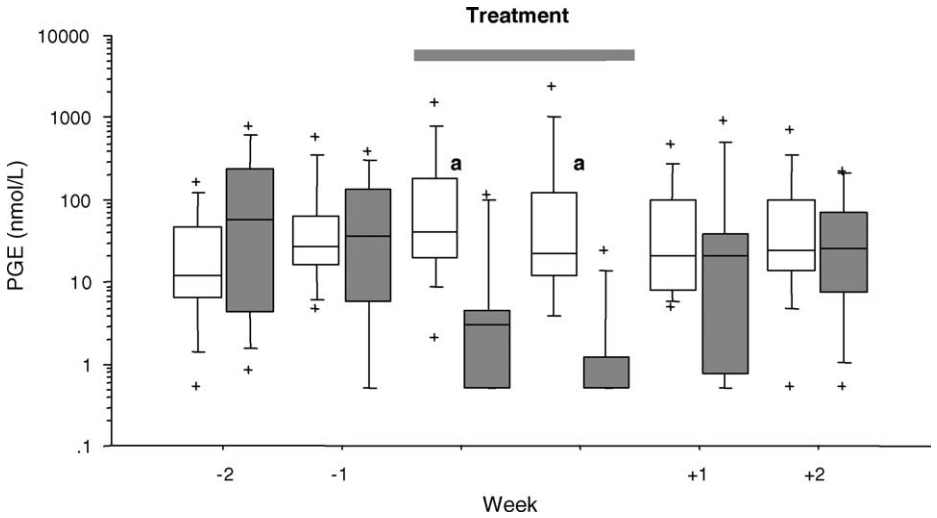

Fig. 2. Box plot of seminal plasma PGE concentrations in stallions ($n = 11$ each) with (■) and without (□) vedaprofen before, during and after end of treatment. Letter 'a' indicates significant difference ($P < 0.05$, Mann–Whitney test).

Table 2
Mean ± S.E.M. of semen characteristics from weekly collected ejaculates during 12 weeks in vedaprofen treated and control stallions ($n = 11$ each) and the effect of treatment, time of semen collection and interaction between treatment and time

Parameter	Vedaprofen (mean ± S.E.M.)	Control (mean ± S.E.M.)	Treatment (P)	Time (P)	Interaction (P)
Volume (mL)	24.3 ± 1.5	22.7 ± 1.2	0.7261	0.6610	0.9742
Concentration ($\times 10^6$ ML)	248.7 ± 9.7	266.5 ± 7.3	0.6188	0.1759	0.0860
Total sperm ($\times 10^9$)	5.6 ± 0.3	5.8 ± 0.3	0.8157	0.1736	0.9540
Motility fresh semen (%)	82.9 ± 0.9	84.6 ± 0.6	0.4956	0.0384*	0.1145
Normal spermatozoa (%)	24.4 ± 1.0	23.7 ± 1.2	0.8809	0.0383*	0.7185
Major defects (%)	63.1 ± 1.3	63.9 ± 1.5	0.9004	0.0677	0.4973
Acrosome defects (%)	9.9 ± 0.8	8.9 ± 0.8	0.7992	0.5225	0.9527
Nuclear vacuoles (%)	21.2 ± 1.4	29.8 ± 1.9	0.2920	0.0001*	0.3429
Motility frozen-thawed semen (%)	52.5 ± 1.4	54.7 ± 1.5	0.7261	0.6610	0.9742
Viability frozen-thawed semen (%)	57.0 ± 0.9	56.1 ± 1.0	0.6188	0.1759	0.0860
HOS frozen-thawed semen (%)	43.3 ± 0.7	39.5 ± 0.7	0.8157	0.1736	0.9540

* Significant ($P < 0.05$).

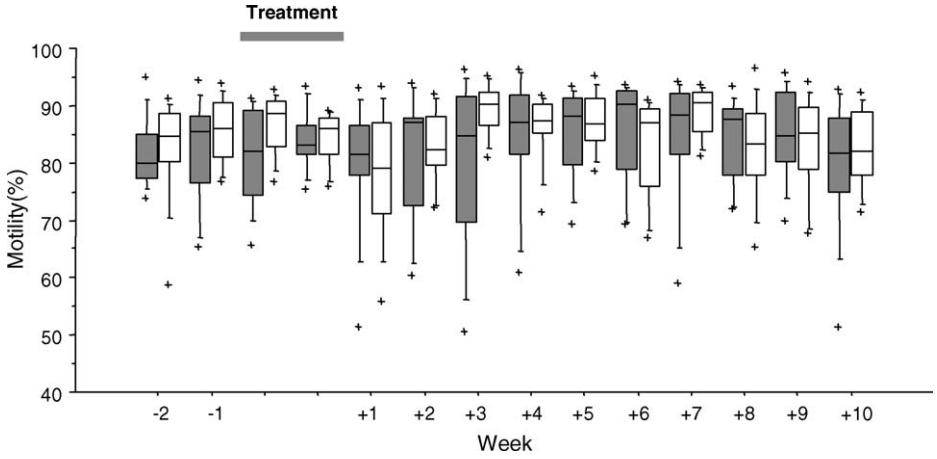

Fig. 3. Box plot of motility in fresh semen collected weekly from September to December in stallions ($n = 11$ each) with (■) and without (□) vedaprofen.

PGE values in seminal plasma were significantly ($P < 0.05$) lower in the vedaprofen than in the control group.

3.3. Semen characteristics

Data of semen characteristics in fresh and frozen-thawed semen collected during and until 10 weeks after treatment analyzed by ANOVA are shown in Table 2. None of the parameters measured were significantly ($P > 0.05$) influenced either by treatment or by the interaction between treatment and time of collection. However, the time of collection had a significant ($P < 0.05$) effect on motility, normal sperm and nuclear vacuoles in fresh semen (Figs. 3–5).

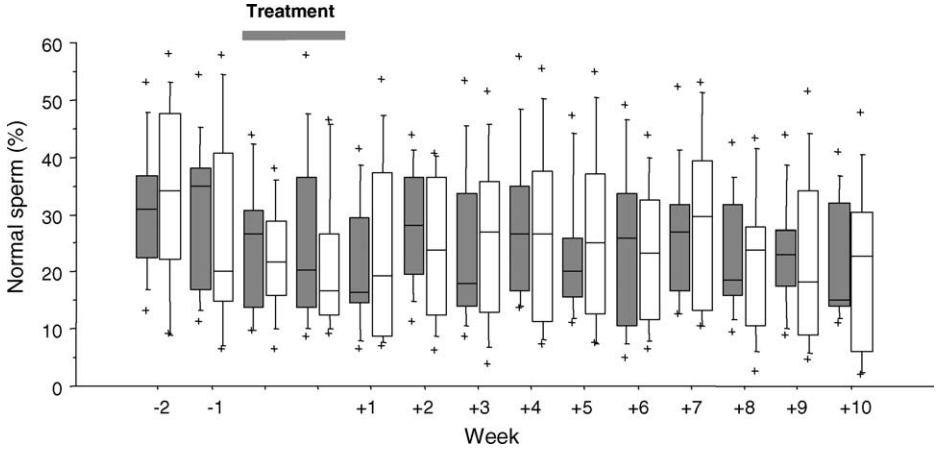

Fig. 4. Box plot of normal sperm in fresh semen collected weekly from September to December in stallions ($n = 11$ each) with (■) and without (□) vedaprofen.

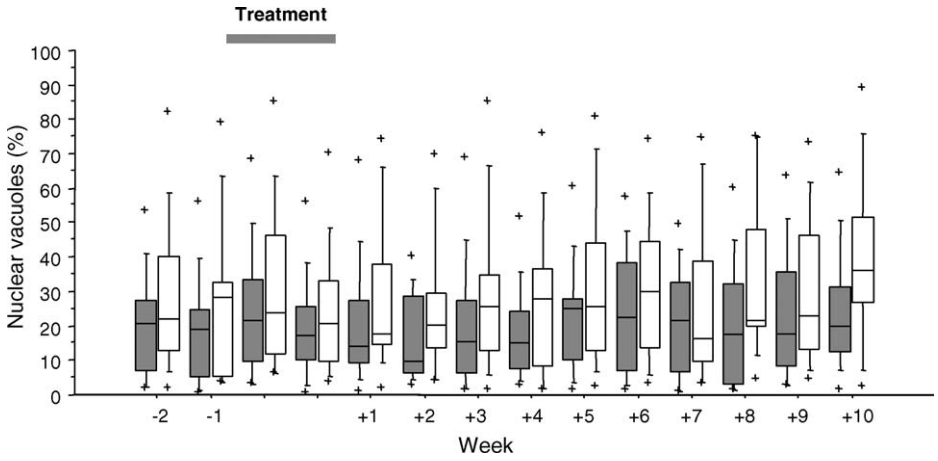

Fig. 5. Box plot of nuclear vacuoles in fresh semen collected weekly from September to December in stallions ($n = 11$ each) with (■) and without (□) vedaprofen.

4. Discussion

The results of the present investigation clearly demonstrate that vedaprofen given twice daily during 14 days did not significantly affect circulating PG-metabolite concentrations in the blood of the stallions. These findings agree with those of Larson et al. [5] in which no changes in blood PG-metabolite concentrations were found in three stallions after oral administration of 3 g phenylbutazon during 24 days. Differing results were obtained in cows when carprofen was given twice daily at a dose of 0.7 mg/kg body weight intravenously during 5 days [21] or flunixine meglumine at a dose of 2.2 mg/kg body weight intramuscularly during 10 days [22] as both drugs elicited a dramatic decrease of blood PG-metabolite. However, in this context it must be mentioned that these experiments were performed in the early puerperium when prostaglandin production is usually high and that the medication was administrated parenterally. The absence of significant differences in PG-metabolite concentrations between treated and untreated stallions in our study may be explained by the fact that vedaprofen is declared a specific COX-2 inhibitor, but in healthy animals, prostaglandin secretion is mainly regulated by constitutional COX-1.

Prostaglandin analysis in seminal plasma during vedaprofen treatment showed that concentrations of PGF and PGE were significantly lower in treated compared to control stallions. This finding is most likely the result of the inhibitory effect of vedaprofen on COX-2 activity, which is known to be constitutively produced in the testes [2]. On the other hand, PG-metabolite values in seminal plasma were not influenced by vedaprofen, the reason most likely being cross-reaction with other prostaglandin metabolites either produced in the genital tract or deriving from circulation. This contrasts with results from Larson et al. [5] who found a significant reduction of PG-metabolite after treatment of three stallions with 3 g phenylbutazon during 24 days. However, this study was conducted using only a small number of animals without controls and furthermore, the possibility of a

seasonal effect cannot be excluded during an investigation lasting from May until December.

Little is known about the function of prostaglandins in seminal plasma of the stallion, especially regarding possible effects on semen quality and never was fertility measured after treatment with NSAIDs. Treatment of three stallions with 1 g phenylbutazon twice daily during 4 weeks did not influence the quality of fresh and cooled semen [4], although PG-metabolite concentrations in seminal plasma were reduced by daily treatment with 3 g phenylbutazon during 24 days [5]. From these results it may be concluded that low prostaglandin values are not detrimental to semen quality in the stallion. This assumption is also supported by our study, as all semen characteristics in fresh and frozen-thawed semen remained unchanged during treatment and in the 10 weeks following vedaprofen application. In contrast, direct intramuscular application of $PGF_{2\alpha}$ 1 h before semen collection resulted in an increase of the ejaculate volume and a decrease of sperm concentration, while total sperm, as well as the motility and the percentage of sperm defects remained unchanged [23].

Concerning the relationship between subfertility and seminal prostaglandins in the horse, no information is present in the literature. In man it has been shown that infertility is often associated with low PGE [24,25] but high $PGF_{2\alpha}$ [26] concentrations in seminal plasma. In addition, a negative correlation between $PGF_{2\alpha}$ and sperm motility in normal fertile men has been found [26]. In vitro studies showed that the addition of $PGF_{2\alpha}$ and PGE to the incubation medium significantly increased sperm velocity but only PGE also clearly increased penetrating ability in the zona-free hamster oocyte penetration assay [27]. After inactivation of most prostaglandins in the seminal plasma through prostaglandin-15-hydroxydehydrogenase, a strong fall in sperm motility has been found in men [26] and the rabbit [28]. In the latter, a selective inactivation of PGE and $PGF_{2\alpha}$ through specific antisera resulted in lower and higher motility, respectively, but fertility rate was consistently reduced [28]. These results indicate that prostaglandins may also influence the fertilizing process in the female. It was demonstrated that intrauterine injection of prostaglandin antagonists in mice significantly reduced the number of implantation sites [29]. In the sow, however, a positive effect on farrowing rate and litter size has been reported [30] after $PGF_{2\alpha}$ was injected into the mucosa of the vulva at insemination. This effect could be attributed to an increase in uterine and tubal contractions [31] as well as to an advancement of ovulation, thereby shortening the interval between onset of estrus and ovulation [32]. Whether the low PGF and PGE concentration in seminal plasma during the vedaprofen treatment seen in our study may compromise fertility in the mare is not known and needs further research.

From our results it can be concluded, that in the stallion, the application of vedaprofen during 14 days has no influence on blood and seminal plasma PG-metabolite as well as on semen quality and freezability but clearly lowered PGF and PGE concentrations in seminal plasma.

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