

# Evaluation of equine electroretinographic responses by using two different electrodes and four different Alpha-2 agonist sedatives\*

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**ABSTRACT.** Rosa M., Botteon P.T.L., Pereira J.S., Brooks D.E. & Rosa M.V.D. **Evaluation of equine electroretinographic responses by using two different electrodes and four different Alpha-2 agonist sedatives.** [Avaliação da resposta eletroretinográfica de equinos utilizando dois tipos de eletrodos quatro sedativos Alfa-2 agonistas diferentes.] *Revista Brasileira de Medicina Veterinária*, 36(4):367-374, 2014. Centro de Pesquisa em Oftalmologia Veterinária (CEPOV), Avenida das Américas, 700, Bloco 8, Loja 103J, Barra da Tijuca, Rio de Janeiro, RJ 22640-100, Brasil. E-mail: marcmax@globo.com

The aim of this study was the evaluation and comparison of field electroretinographic responses in standing horses using two different active electrodes (DTL- plus<sup>TM</sup> and ERG-jet<sup>®</sup>), and four different alpha-2 agonist drugs Xylazine, Romifidine, Detomidine, Medetomidine. Forty healthy horses were evaluated by full field ERG. Horses were randomly allocated into eight groups according four sedative drugs and two electrodes types. In all groups a- and b- wave amplitudes and implicit times were investigated and compared for both eyes. Quality and costs of sedation drugs were estimated. This research led to the conclusion that there were no significant differences of a- and b-wave complex results between ERG-jet<sup>®</sup> and DTL-plus<sup>TM</sup> electrodes with any sedative type. The ERG-jet<sup>®</sup> lens proved to be more practical during the examination than the DTL-plus<sup>TM</sup> electrode. The use of a single dose of xylazine, romifidine, detomidine or medetomidine was sufficient to provide a good level of sedation and muscle relaxation during the ERG examination, although detomidine and medetomidine gave slightly superior results when compared with the other drugs in this study. The sedation with xylazine was the least when compared with the other drugs and it was also the cheapest to use. Any of the sedatives and either active electrode tested in this study should permit a good full field ERG evaluation in standing horses.

**KEY WORDS.** Electroretinography, DTL-plus<sup>TM</sup>, ERG-jet<sup>®</sup>, xylazine, romifidine, detomidine, medetomidine.

**RESUMO.** O objetivo deste trabalho foi estudar a eletrofisiologia da visão do equino, através do exame de eletroretinografia de campo total, com o uso de dois tipos de eletrodos (DTL- plus<sup>TM</sup> and ERG-

-jet<sup>®</sup>) e sedação com quatro diferentes drogas alfa-2 agonistas Xilazina, Romifidina, Detomidina, Medetomidina, com o animal na posição quadrúpede. Foram avaliados 40 equinos em posição quadrúpede.

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de pelo de exame de Eletroretinografia de Campo Total. Os animais foram aleatoriamente divididos em oito grupos em função de quatro sedativos e dois eletrodos. Em todos os grupos mensuraram-se os valores das amplitudes de pico das ondas "a" e "b", seus "implicit time", para os olhos direito e esquerdo. Também foram avaliados o tempo, a qualidade e o custo da sedação. Concluímos que houve diferença entre os eletrodos avaliados, e que o eletrodo ERG-jet mostrou-se mais adequado em razão da sua segurança e praticidade de utilização. As drogas estudadas não diferiram entre si quanto aos resultados do eletroretinograma, porém a Detomidina e Medetomidina apresentaram um desempenho superior em relação à Romifidina e à Xilazina na eficiência de contenção e facilitação do exame. A sedação com Xilazina foi a que representou menor custo de execução. Todos os sedativos e eletrodos testados permitiram uma boa avaliação do ERG de campo total nos cavalos em posição quadrúpede.

**PALAVRAS-CHAVE.** Electroretinografia, DTL-plus™, ERG-jet®, xylazine, romifidine, detomidine, medetomidine.

## INTRODUCTION

As one of numerous diagnostic procedures available in veterinary ophthalmology, the electroretinogram (ERG) is a useful tool for evaluation of the electrical activity of the outer retina. The ERG has been used to evaluate retinal function in Equine Recurrent Uveitis (ERU) a common cause of blindness in horses (Brooks 2002, Komáromy et al. 2003) and in Equine Congenital Stationary Night Blindness (CSNB), (Sandmeyer et al. 2007, Witzel et al. 1978). Animals with ocular trauma, cataract, glaucoma, retinal detachment or drug toxicity could also be assessed by ERG (Brooks 2005, Komáromy et al. 2003).

There are standard protocols for ERG in humans approved by the International Society for Electrophysiology of Vision (ISCEV), (Cooper 1933, Marmor 2004). For animal use there is guidelines for dogs (Granitz 1994, Komáromy et al. 1998, Narfström et al. 2002), cats (Narfström et al. 1985, Robson et al. 1996, Komáromy et al. 1998), rats (Hancock et al. 2004, Sandalon et al. 2008), mice, guinea pigs, monkeys (Rosolen et al. 2005) and rabbits (Gjörloff et al. 2004). There are also various ERG studies in other species such as horses (Komáromy et al. 2003, Nunnery et al. 2005, Sandmeyer et al. 2007). All of these ERG protocols evaluate the responses of retinal rods and cones photoreceptors using standardized light intensities based on human tests parameters (Marmor 2004). There are no guidelines,

standards or recommended ERG protocols specific for the horse at this time.

There are a large number of ERG electrodes on the commercial market. The most common are ERG-jet® monopolar and bipolar lens, the DTL fiber,™ Gold Foil,® Burién-Allen® and Goldlens® (Esakowitz et al. 1993, Narfström et al. 2002, Mentzer et al. 2005) most of them are for human purposes and can be adapted for animal testing to provide adequate comfort and reproducible ERG exams (Bayer et al. 1999). In this paper we studied the ERG response for DTL-plus™ and ERG-jet® electrodes (Mierdel 1995, Minami et al. 2000, Narfström et al. 2002, Yin 2004) in normal horses. The DTL-plus™ is a medical grade silver/nylon electrode with two sticky patches to facilitate its fixation to the patient. The ERG-jet® lens is a well-known and proven ERG electrode type in human and small animal ERG testing (Esakowitz et al. 1993, Komáromy 1998, Lam 2005, Mentzer et al. 2005)

Proper standing sedation for ophthalmic diagnostic procedures in horses is imperative to provide adequate security to the animals, people involved and equipment during the test (Feitosa et al. 1990, Geiser 1990, Muir et al. 1991, England et al. 1992, Daunt et al. 2002, Robertson 2004). The horse's natural behavior, body weight, and available sedative drugs are important variables to consider in equine ERG testing. Good muscle relaxation and analgesia with absence of sudden movements is desirable but not always easy to achieve in horses with a single dose of sedative drug (Clark et al. 1988, Bialski et al. 2003, Robertson 2004). There are several sedatives and anesthetics agents like alpha2 agonists, opiates, dissociative anesthetics, barbiturates and inhalants that can be used either single or combination dose for ERG testing in animals (Clark et al. 1988, Feitosa et al. 1990, Yonemura et al. 1996, Daunt et al. 2002, Norman et al. 2008). However some of these drugs protocols are known to cause significant alterations on the ERG a-b wave and implicit times parameters (Yonemura et al. 1996, Bayer et al. 1999, Norman et al. 2008). In this paper we analyzed sedation protocols for ERG testing in the horse with both commercially available electrodes and drugs.

## MATERIAL AND METHODS

In our study both eyes of forty healthy horses were evaluated. Evaluated among them 15 males and 25 mares, aging between 2 months to 18 years (mean age 4.6 years). The horses were randomly divided in 8 groups of 5 animals according 4 sedation drugs and 2 electrodes types used. The OD was the first eye examined in

all animals. The horses in this paper are used in sports, reproduction, and/or pleasure riding. All of them were stabled with free access to food and water. There were owner's prior approval for eye exams and ERG tests for this study. The minimum period of fasting was 8 hours for food and 6 hours for water. The horses were evaluated 2 days prior to ERG testing by tonometry, slit lamp biomicroscopy and direct ophthalmoscopy according to the owner's convenience.

Parameters like practical use, sedation protocol, cost effectiveness drug, quality of the ERG tracing (regarding the quality of generated signal), practicality of carrying out the ERG examination and the need of electrode repositioning and the difficulty for electrode placement in the horse eye were evaluated. The quality of sedation and costs were evaluated based on data collection regarding applied drug type. The sedation time regarding electrode type, the need of additional restraint, the need of head support, the ability of the horse to keep on standing position during the exam and the ease of exam implementation were also evaluated. The sedation cost is related to the amount of drug applied for 100 Kilogram (Kg) of live body weight.

Testing protocol and light stimulus sequence were proceeded according International Society of Clinical Electrophysiology of Vision (ISCEV) standards. The light source was a mini Ganzfield stimulator, a Handheld Multi-species ElectroRetinoGraph Instrument<sup>6</sup> (HMsERG<sup>TM</sup>). The ERG protocol used in this study was the QuickRetCheck<sup>®</sup>, a very short ERG protocol used in no anesthetized animal patients according to the HMsERG<sup>TM</sup> specifications in the user guide manual. This test has three steps: - S<sub>1</sub>- Rod test with flash intensity of 10mcd/s/m<sup>2</sup>, S<sub>2</sub>-Standard rod and cone test with flash intensity of 3 cd/ s/m<sup>2</sup> and S<sub>3</sub>- High intensity rod and cone test with flash intensity of 10cd.

The active electrodes were an ERG-Jet<sup>®7</sup> and a DTL-plus<sup>TM28</sup>. The reference electrode was a platinum subdermal right-angle needle model F-E7, and the ground electrode a platinum subdermal straight needle model F-E2. The DTL-plus<sup>TM2</sup> was placed on the cornea using its adhesive patches; the smaller end placed at the lateral canthus and the other extremity at the medial canthus. Methylcellulose<sup>9</sup> 2% was applied to both the DTL-plus<sup>TM2</sup> and ERG-jet<sup>®2</sup> to stick the electrodes fibers together, facilitate corneal attachment and increase electrical contact to the cornea (Figure 1). The reference and ground electrodes were placed subcutaneously 5cm from the lateral canthus and over the occipital bone respectively. All electrodes were connected to a pod input connected by a cable to HMsERG<sup>®</sup> Unit and the ERG exam performed.

The horses were tested inside closed barn stalls or in stocks at night with all lights turned off (Figure 2). Values found in these environments did not exceed 1 Lux measured with a lux meter Sinometer<sup>®</sup> Digital 4<sup>10</sup>. The pupil was dilated with tropicamide<sup>11</sup> 1% and the horses were dark adapted for 20 minutes. During dark adaptation the horses were sedated with a single dose of our randomly selected alpha2 agonist drug. After 5

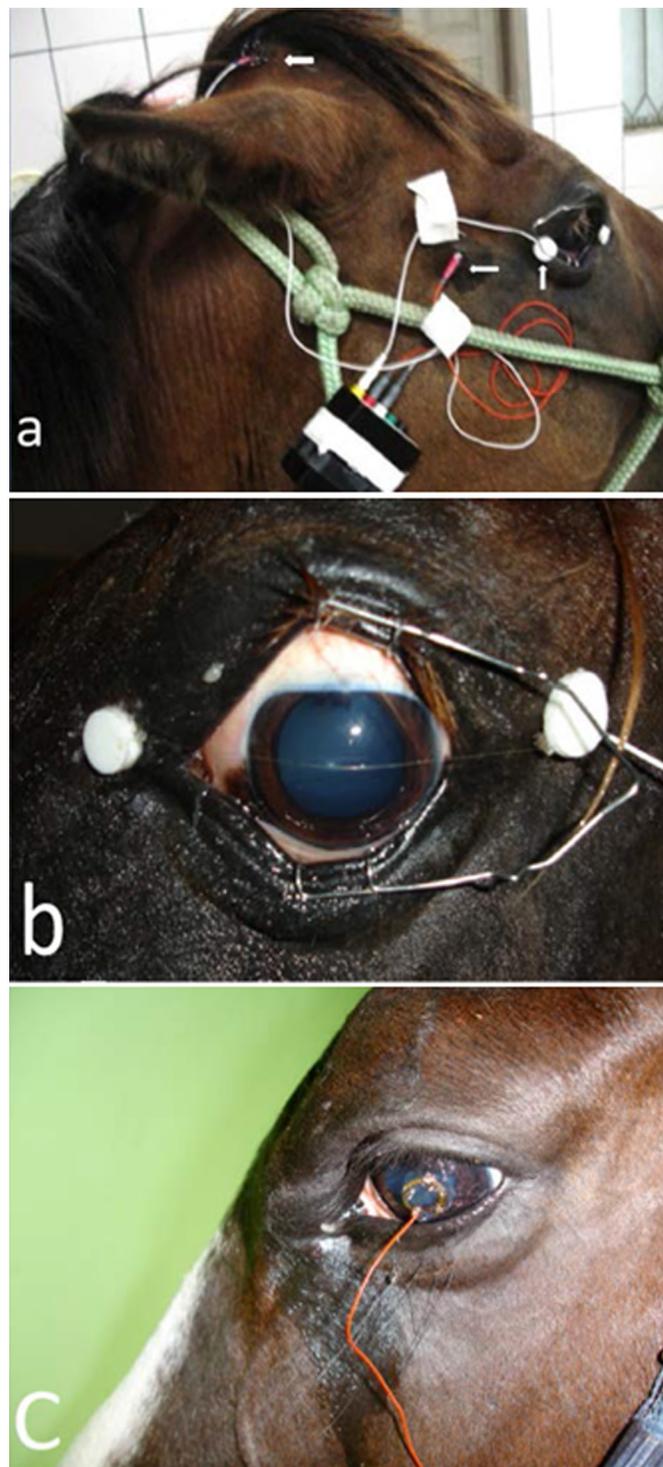


Figure 1. a - The active electrode DTL-plus is on the cornea, the reference electrode placed 5cm from lateral canthus and the ground electrode placed at the occipital bone of the horse. b - Dtl-plus close up. c - The electrode ERG-jet is attached to the corneal surface with methylcellulose.

<sup>6</sup> RetVet Corp Inc.

<sup>7</sup> ERG-jet<sup>®</sup>; Fabrinal SA Tuilerie 42, 2300 La Chaux-de-Fonds, Switzerland.

<sup>8</sup> DTL Plus Electrode<sup>®</sup> - Retina Technologies, Scranton PA, USA.

<sup>9</sup> Ophthalmos Ind. Com. Ltda, Brazil.

<sup>10</sup> Sinometer<sup>®</sup> Luxmeter LX1330B.

<sup>11</sup> Mydracyl<sup>®</sup> - ALCON, Brasil.

<sup>12</sup> Anestalcon<sup>®</sup> - ALCON, Brasil.



Figure 2. In this picture hole necessary equipment is assembled: the mini- Ganzfeld stimulator electrodes and the headstand.

Table 1. Mean and deviation of a- and b- wave amplitudes and implicit times for Photopic and scotopic stimuli in eyes of the horses, for the DTL-plus<sup>TM</sup>I and ERG-jet<sup>®</sup> electrodes.

	Photopic		Scotopic	
	DTL-plus <sup>TM</sup>	ERG-jet <sup>®</sup>	DTL-plus <sup>TM</sup>	ERG-jet <sup>®</sup>
"a" wave (μV)	127,7 ± 60,2 <sup>a</sup>	115,2 ± 56,3 <sup>a</sup>	9,4 ± 11,0 <sup>a</sup>	31,0 ± 40,8 <sup>b</sup>
Implicit time (ms)	15,5 ± 22,6 <sup>a</sup>	13,7 ± 15,4 <sup>a</sup>	13,5 ± 12,8 <sup>a</sup>	9,7 ± 9,7 <sup>a</sup>
"b" wave (μV)	275,5 ± 120,1 <sup>a</sup>	307,5 ± 135,3 <sup>a</sup>	122,2 ± 89,9 <sup>a</sup>	225,4 ± 140,0 <sup>b</sup>
Implicit time (ms)	68,1 ± 46,7 <sup>a</sup>	58,7 ± 27,4 <sup>a</sup>	85,3 ± 35,7 <sup>a</sup>	74,1 ± 31,6 <sup>a</sup>

SD= standard deviation. Different lowercases in lines represent statistical difference (P < 0.05).

minutes an auriculopalpebral nerve block with lidocaine was performed so a corneal topical anesthesia with proximetacaine hidrochloride<sup>12</sup>. After that the electrodes were placed.

All data of a- and b-wave complex amplitudes and implicit times obtained were analyzed by ERG viewer<sup>TM</sup> software from RetVet corp. Inc.

Data were statistical analyzed for normal distribution by Kolmogorov-Smirnov test. Normal data were analyzed by ANOVA. Data not normally distributed were analyzed by non-parametric tests. Comparison between groups were made by Friedman-test plus a Dunn test with 5% significance level and a Mann-Whitney U-test. Sedation data was analyzed by Kruskal-Wallis and plus a post hoc Dunn test with 5% significance.

## RESULTS AND DISCUSSION

Results in this study regarding the electroretinographic exam and standing sedation protocols with a single sedative drug dose were compared with the available literature for healthy animals. The use of a portable unit HMs ERG<sup>TM</sup> showed to

be practical to concentrate the light into the dilated pupil with horses once there were a small number of parts to handle in a dark room. Besides the fact the QuickRetCheck<sup>®</sup> have a short protocol characteristics and showed to be able to evaluate the hole retinal response of the horse in this paper.

Table 1 present the results of mean and standard deviation for a- and b-wave complex in right and left eyes for photopic and scotopic stimuli of DTL-plus<sup>TM</sup>I and ERG-jet<sup>®</sup> electrode. Responses of the right and left eyes of a- and b- wave and respective implicit time were compared in photopic and scotopic stimuli, and did not show statistical difference (P<0,5) between values Figure 3. Values in this experiment for DTL<sup>TM</sup> fiber electrode showed similarity between results achieved by Komáromy et al. 2003.

Comparison between the DTL-plus<sup>TM</sup>I and ERG-jet<sup>®</sup> showed the corneal placement of both types of electrodes was quite simple with no difference between them (Figure 2a, b, c). During the examination repositioning of ERG-jet<sup>®</sup> lens was not necessary in any of the 20 animals evaluated and was classified as appropriate. However the electrode type DTL-plus<sup>TM</sup>I had to be repositioned in 5 of 20 (25%) horses tested (P value <0.05) which was described as inadequate. This may be an important factor in choosing the electrode type since the frequent repositioning may result in injury or damage

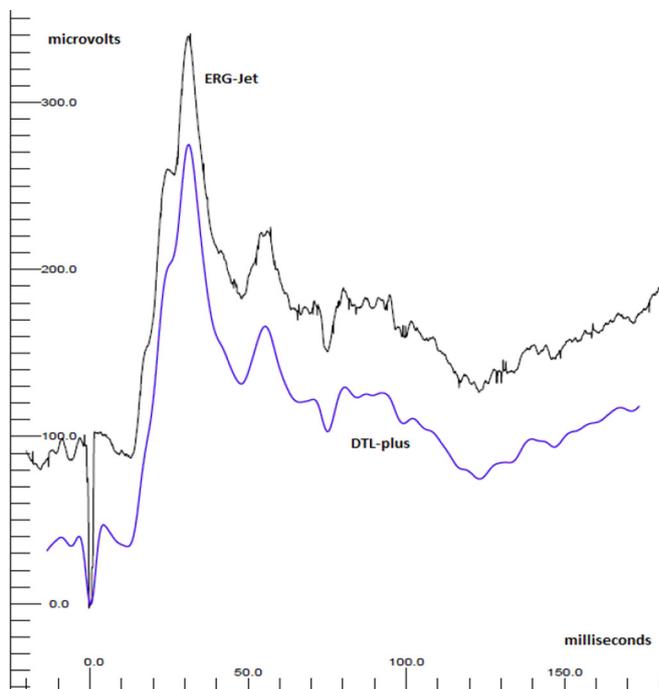


Figure 3. Graphic representation of a- and b- wave amplitudes and implicit times for Photopic stimuli. Representing a statistical difference between the ERG-jet<sup>®</sup> and DTL-plus<sup>TM</sup> electrodes (P < 0,05).

to the cornea and also delays the procedure. After evaluation of the graphs quality and generated signal of a- and b-waves complex recorded, no differences were observed between the electrodes.

The Comparison between amplitudes of a- and b- complex in scotopic phase (figure 4) in this paper were significantly higher (P value <0.05) for ERG-jet<sup>®</sup>, than those obtained with the DTL-plus<sup>™</sup> electrode (Table 1). This results had the same trend according other studies showing that smaller amplitudes can be obtained using the DTL-plus<sup>™</sup> when compared to the lens electrodes (Esakowitz et al. 1993, Hennessy et al. 1995, McCulloch et al. 1998). There are controversies about the best electrode type related to reproducibility and sensitivity in electrophysiological tests (Manami et al. 2000). This fact can be related to a difference in electrodes constituted materials; electrodes contact area with the corneal surface or positioning of the active and reference electrodes. The corneal placement of the electrode DTL-plus<sup>™</sup> can influence the ERG due its location in the conjunctival sac may result in atenuation of signal response due to its distance from the center cornea and or conjunctival contact. There is evidence that the amplitude of ERG becomes larger when the electrode is placed in the center of the cornea in a study with mice and rats (Bayer et al.1999) and changes in recordings due to electrodes types and reference electrode position in a study in dogs (Mentzer et al. 2005). Other studies do not show such difference between the electrodes types as in Yin et al.2004 who compared the results with DTL-plus<sup>™</sup> and ERG-Jet<sup>®</sup> contact lens in full-field electroretinogram in twenty normal human subjects.

The use of methylcellulose 2% not only kept the electrodes well attached to the horse cornea but protected it against erosion and dryness, and facilitated the electrical contact between the electrode and the cornea.

### Sedative evaluation regarding the drug type applied

The sedation protocol evaluated in this study was an intravenous single dose of the four different alpha 2-adrenoceptor agonists; xylazine<sup>3</sup>, romifidine<sup>4</sup>, detomidine<sup>5</sup> and medetomidine<sup>6</sup> in conjunction with regional nerve block and corneal topical anesthesia Geiser et al. 1990, Muir 1991, (Hobo et al. 1995). These drugs were able to provide a good level of sedation, restraint and comfort (Robertson et al. 2004, Sandalon et al. 2008) for both the placement and maintenance of the electrodes long enough for the ERG examinations.

The effects of the drugs on ERG was variable with the electrode analyzed. When we used DTL-plus, Xylazine decreased the b- wave amplitude in photopic phase and Romifidine prolonged the

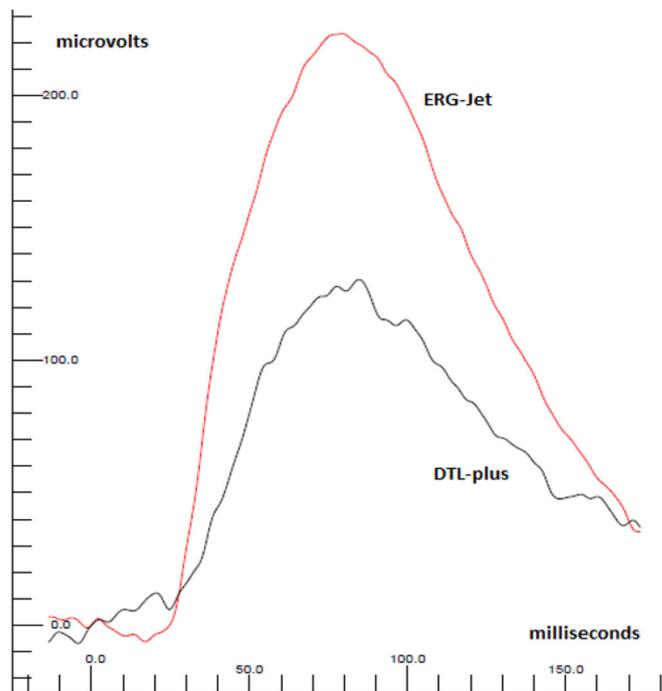


Figura 4. Graphic representation of a- and b- wave amplitudes and implicit times for Scotopic stimuli. Representing a statistical difference between the ERG-jet<sup>®</sup> and DTL-plus<sup>™</sup> electrodes (P < 0,05).

Table 2. Mean and deviation of a- and b- wave amplitudes and implicit times for Photopic and Scotopic stimuli in eyes of the horses, by the DTL-plus<sup>™</sup> and ERG-jet<sup>®</sup> electrode.

	Alpha-2 agonist drugs			
	Xylazine	Romifidine	Detomidine	Medetomidine
DTL-plus <sup>™</sup> in photopic phase				
"a" wave	150,2 <sup>a</sup>	130,6 <sup>a</sup>	109,4 <sup>a</sup>	120,7 <sup>a</sup>
Implicit time	15,1 <sup>a</sup>	8,3 <sup>a</sup>	17,8 <sup>a</sup>	21,0 <sup>a</sup>
"b" wave	215,6 <sup>a</sup>	301,6 <sup>b</sup>	253,9 <sup>ab</sup>	331,1 <sup>b</sup>
Implicit time	68,8 <sup>a</sup>	54,4 <sup>a</sup>	65,2 <sup>a</sup>	84,0 <sup>a</sup>
ERG-jet <sup>®</sup> in photopic phase				
"a" wave	95,7 <sup>ab</sup>	90,3 <sup>a</sup>	128,3 <sup>b</sup>	147,7 <sup>b</sup>
Implicit time	4,0 <sup>a</sup>	21,7 <sup>a</sup>	14,7 <sup>a</sup>	14,4 <sup>a</sup>
"b" wave	233,8 <sup>a</sup>	408,7 <sup>b</sup>	320,0 <sup>ab</sup>	227,9 <sup>a</sup>
Implicit time	38,2 <sup>a</sup>	71,9 <sup>a</sup>	69,3 <sup>a</sup>	55,5 <sup>a</sup>
DTL-plus <sup>™</sup> in scotopic phase				
"a" wave	6,2 <sup>a</sup>	11,2 <sup>a</sup>	50,7 <sup>b</sup>	21,5 <sup>ab</sup>
Implicit time	5,4 <sup>a</sup>	14 <sup>a</sup>	10,3 <sup>a</sup>	9,1 <sup>a</sup>
"b" wave	227,0 <sup>a</sup>	358,5 <sup>b</sup>	171,2 <sup>a</sup>	144,6 <sup>a</sup>
Implicit time	62,7 <sup>a</sup>	84,0 <sup>a</sup>	78,4 <sup>a</sup>	71,4 <sup>a</sup>
ERG-jet <sup>®</sup> in scotopic phase				
"a" wave	10,2 <sup>a</sup>	8,4 <sup>a</sup>	32,7 <sup>b</sup>	19,2 <sup>a</sup>
Implicit time	19,9 <sup>a</sup>	13,8 <sup>a</sup>	8,9 <sup>a</sup>	11,5 <sup>a</sup>
"b" wave	93,9 <sup>ab</sup>	76,0 <sup>a</sup>	167,3 <sup>b</sup>	161,7 <sup>b</sup>
Implicit time	101,3 <sup>a</sup>	76,6 <sup>a</sup>	79,3 <sup>a</sup>	83,9 <sup>a</sup>

Different lowercase letters represent statistical difference among drugs; different lowercase letters represent statistical difference among electrodes by Tukey test P < 0,05.

of b- wave amplitude in scotopic phase, compared with the other drugs. The latency of the implicit time were similar to all drugs.

Animals sedated with romifidine and using ERG-jet, lens showed a decrease of a-wave amplitude and a increasing in b-wave amplitude during photopic stimulation, however for scotopic phase there was a decrease of b-wave amplitude. Detomidine increased values of a-wave amplitude in scotopic phase (Table 2).

There were no significative difference between the implicit time results due to the electrode type or sedative drug used.

The grade of ataxia and need for a head support can be parameters for drug selection. In this study the observation of the first signs of ataxia and head lowering were considered as landmarks of onset sedation. Marked ataxia lowering the head and loss of balance was observed in animals treated with medetomidine<sup>VI</sup> and detomidine<sup>V</sup> in this experiment. This signs are cited in studies by Bryant et al. 1991, Bialski et al. 2003, Clark et al. 1988, respectively.

All sedative drugs tested in this experiment had a quickly and smoothly action and did not alter significantly cardiopulmonary parameters. Animals had a quick recover after our minimally invasive procedures (Komáromy et al. 1998, Bialski et al. 2003). All sedatives allowed the examination to be carried out with the animal in the standing position. The frequency of results according of the maintenance of standing position during the ERG examination regarding electrode and drug type applied is seen in Table 3. However when postural stability of the animals during the examination was assessed there was a significant difference ( $P < 0.05$ ) between sedation drugs used. The group sedated

with romifidine<sup>IV</sup> had the best stability and the detomidine group presented the greater instability during the examination. Groups sedated with xylazine and medetomidine had the intermediate positions and did not differ significantly from the others. The type of electrode used did not influence the results in this parameter.

Another parameter evaluated was the head positioning (Komaromy et al. 1998, Gelatt book 1999, Lam book 2005) during the examination. There was significant difference for ( $P < 0.05$ ) between the sedative drugs used. The xylazine<sup>3</sup> group kept a large proportion of the animal's heads elevated (4 of 10), while the animals in the detomidine and medetomidine<sup>VI</sup> always kept their heads down during the examination (10 of 10). The romifidine group had intermediate values not differing from the others; however 30% of animals evaluated kept their heads in a high position. The electrode type used did not influence this parameter.

A parameter that could be decisive in the choice of sedative is the need of a headstand during the examination. Once all of sedatives showed similar effects the use of head support was required in all groups. The frequency of results according head position during ERG examination regarding electrode and drug applied can be seen in Table 3.

Ideally chemical restraint should produce adequate sedation, muscle relaxation and sufficient anesthesia for the animal to support the implementation of the procedure. (Geiser et al. 1990, Muir et al. 1991, Komáromy et al. 2003, Robertson et al. 2004).

The need for physical restraint was assessed and there were significant differences ( $P < 0.05$ ) between the sedatives. The frequency of results according the need of additional restraint regarding electrode and drug type applied are in Table 3. In the group treated with xylazine<sup>3</sup> 4 of 10 animals required the use of twitch during the test, showing the worst performance when compared with to others groups, but did not differ from group treated with romifidine<sup>IV</sup>. The romifidine<sup>IV</sup> group 3 of 10 animals required the twitch representing an intermediate position between groups.

Group treated with medetomidine did not require additional restraint and presented the best performance for this parameter followed by Detomidine with 10% of animals. Romifidine (30%) had inetrmediate position and Xylazine (40%) with the lower performance between tested groups. Table 3 shows the frequency of results according ease of implementation of ERG exam regarding electrode

Table 3. Frequency of results of evaluated parameters for execution of ERG examination regarding the drug type applied. We use scores from zero (-) to ten cruises (†). Different lower case letters represent statistical ( $P < 0,05$ ).

Parameter	Alpha-2 agonist drugs			
	Xylazine	Romifidine	Detomidine	Medetomidine
Standing position	++++(ab)	++++++(a)	††(b)	++++(ab)
Head position Up	†††(a)	††(ab)	-(b)	-(b)
Ease of implementation	++++(a)	++++++(ab)	++++++(b)	++++++(b)
Additional restraint needed	†††(a)	††(ab)	†(b)	-(b)
Sedation time (min.)	60,9(a)	59,2(a)	66,4(a)	59,0(a)
Cost/exam/100 Kg (R\$)	5,28	4,09	6,80	10,65

and drug type applied. This assessment did not show significant difference between the results regarding electrode type however there were a significant difference in the results ( $P < 0.05$ ) regarding sedative drug applied between groups.

The sedation times were assessed according to the electrode type used and did not show significant differences were found between these groups. Median values for DTL-plus™ and ERG-jet® were 64.95 and 57.80 minutes respectively.

The evaluation of the influence of the electrode on sedation time values came from the assumption of discomfort caused by contact of the electrodes with the horse's cornea could cause enough stress capable to decrease the efficiency of the sedative drugs, which was not confirmed. There were no major interferences in the tests with any of the tested electrodes, which could cause some stimulus to the animal that would lead to a decrease the sedation time. Figure 1 is a graphic representation for the mean of sedation time duration by electrodes.

The use of corneal topical anesthesia probably contributed to reduce the stress by the presence of the electrode but did not influence the value of the parameters recorded. The mean of the time of sedation are shown in Table 3.

Cost effectiveness was evaluated regarding drug price and efficiency for each sedation protocol. The sedative protocol costs showed to be inexpensive. For comparison they are presented in Brazilian Reals (R\$), with United States Dolar (US\$) and European Union Euro (EU€) currency between parentheses. Romifidine showed the lowest cost compared to the others, with a value of R\$ 4.09 (US\$ 2.29 / EU€ 1.84), followed by xylazine R\$ 5.28 (US\$ 3.96 / EU€ 2.38), and detomidine R\$6.80 (US\$ 3.80 / EU€ 3.07), with medetomidine having the highest average value for each 100kg of weight at a cost of R\$10.65 (US\$ 6.98 / EU€ 4.81).

## CONCLUSIONS

Based on results of this experiment, we conclude that there are significant differences between the electrodes types evaluated.

The drugs used for sedation, can be interacting with the electrode used altering the result

Detomidine and medetomidine showed superior performance than romifidine and xylazine.

This study reinforces the need for further research in order to standardize an ERG protocol for horses, regarding sedation drugs and active electrode types.

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