MOTILITY PARAMETERS OF EQUINE EPIDIDYMAL SPERMATOZOA AFTER 24 HOURS INTRA-EPIDIDYMAL EXPOSURE TO LIDOCAINE USING TWO COMMERCIAL EXTENDERS

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Abstract

Epididymal spermatozoa is the last source for gamete rescue in case of emergency castration or sudden death of a valuable stallion, thus an ideal harvesting and preservation technique should be employed. Routinely, 2% lidocaine intraparenchymatous administration is used to provide analgesia prior to castration, but studies on the effect of lidocaine on epididymal spermatozoa motility parameters are limited. The purpose of this study was to determine the effects of lidocaine on equine epididymal spermatozoa, after 24 hours intraepididymal cool storage using two commercial extenders. We hypothesized that intraepididymal prolonged exposure to lidocaine, might affect motility parameters of epididymal stallion spermatozoa and that different extenders might have an impact. Sperm was collected from 20 epididymides of routinely castrated 3 year old KWPN stallions. 4 stallions received 10 ml 2% lidocaine intraparenchymatous 10 minutes prior to castration and 6 stallions were not medicated. Testicles were transported to an equipped facility and cooled stored for 24 hours. From each sample an aliquot was diluted in a commercial egg yolk based extender, and another in a commercial extender containing defined milk proteins. Motility parameters were registered 30 minutes after dilution, computer assisted. There were no statistical differences between motility parameters of spermatozoa exposed to lidocaine and spermatozoa not exposed, however progressive motility and linearity significantly differed among the two extenders.

Keywords: *stallion, epididymal spermatozoa, lidocaine, commercial extenders, motility parameters*

Introduction

Regional analgesia by means of intraparenchymal 2% lidocaine administration during surgical procedures such as orchidectomy has proven to be effective in reducing pain during routine castration [1,2] or laparoscopic cryptorchidectomy in horses [3]. In one study, administration of 10 ml intraparenchymal 2% lidocaine did not decrease total motility (TM), progressive motility (PM), velocity of the average path (VAP), velocity of the curved line (VCL), linearity (LIN), normal morphology (M) and membrane integrity (MI) of the spermatozoa in vivo, even though relevant concentrations of lidocaine can be detected in epididymal flush, regardless of the blood barrier [4]. This is important in case of emergency castration of valuable stallions, when cryoconservation of cauda epididymis spermatozoa is the last chance to preserve genetic material. Even though viability of spermatozoa significantly decreases after 72 hours, they can be successfully cryopreserved and maintain their fertilization capacity in vitro, after 96 hours of intraepididymal storage at 4 degrees Celsius [5]. Chosing the best extender is crucial when dealing with epidydymal spermatozoa, due to the extremely limited quantity, and extenders can influence the motility parameters of refrigerated epididymal spermatozoa [6]. To the authors knowledge, there is no other study on the

effect of different commercial semen extenders on epidydymal spermatozoa after intraparenchimatous injection of lidocaine.

The purpose of this study was to determine the effects of lidocaine on equine epididymal spermatozoa, after 24 hours intraepididymal cool storage using two commercial extenders. Because lidocaine does cross the blood barrier into the epididymis of the stallion [4], and facilities equipped with the means to extract equine epididymal spermatozoa might be at a a considerable distance, we conducted this study increasing the exposure of spermatozoa to lidocaine during cooled storage. We hypothesized that intraepididymal prolonged exposure to lidocaine, might affect kinematic parameters of epididymal stallion spermatozoa and that different extenders might have an impact.

Materials and method

Animals and castration method

Ten 3 year old KWPN stallions were routinely castrated using a closed technique with the stallions in dorsal recumbency. After a thorough examination of the testicles to detect any gross modifications, each stallion was sedated using xylazine hydrochloride (1.1 mg/kg IV) and butorphanol tartrate (0.03 mg/kg IV). Anesthesia was induced using ketamine hydrochloride (2.2 mg/kg IV) and diazepam (0.07 mg/kg IV). Following aseptic preparation of the scrotal area 10 ml 2% lidocaine hydrochloride (LidoBel, bela-pfarm) was randomly administered to 4 stallions in the parenchyma of the testes using an 18 g 1.5-inch needle. Each testicle was removed using a transfixic ligature (PGA USP 2, SMI, Surgicryl) and a Reimer's emasculator placed approximately 2 cm above the transfixic ligature. Immediately after the removal of the testicle, a hemostatic forceps was placed over the vas deferens in order to prevent leakage and contamination of spermatozoa. Following removal, each testicle was individually packed in a sterile bag, identified and stored at 5 degrees Celsius for transportation, avoiding direct contact of the epididymis with the ice packs. After castration, the stallions were medicated with tetanus toxoid (6000 UI IM) , flunixin meglumine (1.1 mg/kg IV) and a combination of procaine penicillin and streptomycin sulphate (procaine penicillin 4.000 UI/kg and streptomycin sulphate 15 mg/kg IM).

Sperm collection and analysis

After 24 hours intraepididymal cooled storage at 5 degrees Celsius, each cauda epididymis and vas deferens were carefully removed from the testicles after placing a mosquito forceps at the palpable base of the cauda epididymis and dissected free of blood vessels and connective tissue, using an aseptic technique. Spermatozoa was recovered using a retrograde flush technique as previously described [5].

From each stallion, an aliquot was extended to 20 x 10^6 sperm/ml in an extender for chilled semen containing defined milk proteins (EquiPlus, Minitüb, Tiefenbach, Germany; pH 6.8 ± 0.2, 320 ± 20 mOsm/L), and another aliquot was diluted to 20 x 10^6 sperm/ml in an extender for chilled semen containing egg yolk (Gent, Minitüb; pH 6.6-6.8, 310-330 mOsm/L), randomly. Each sample was assessed after 30 minutes maintenance at room temperature for motility parameters using a computer assisted sperm analysis system (SCA® Production, MICROPTIC). Total motility (TM), progressive motility (PM), velocity of the average path (VAP), velocity of the curved line (VCL) and linearity (LIN) were recorded for each sample. Sperm motility was assessed with Sperm Class Analyzer -SCA (Microoptic, Barcelona, Spain) using the following settings: 10x Nikon, negative phase contrast (PC-) optics, calibrate value 0.82µm/pixel, gird distance: 10 µm, box size: 200pixels, VCL/VAP area 4 µm2/min, area:75 µm2/max, static cells threshold <10 µm/s, slow medium 45 µm/s, rapid >90 µm/s, progressive STR >75, VAP points 5 pixels, connectivity 12 pixels. A total of 500 spermatozoa in minimum four fields were assessed using 20 µm Leja slides.

Statistical method

An unpaired t test was used to compare kinematic parameters of lidocaine exposed and non exposed samples using two different semen extenders (GraphPad Prism 6.0). Significance was assessed at $p < 0.05$.

Results

Motility parameters using Equi **Plus Semen extender**

A number of ten pellets were suspended in Equi Plus Semen extender and distributed in one of the two groups: Equi Plus without lidocaine (EP) (n=6) and Equi Plus with lidocaine (EPL) (n=4). Table 1 provides detailed information about the motility parameters analyzed in both EP and EPL groups and the average results. There were no statistical differences between the two groups (fig 1 A-E).

	TM %	PM %	VCL mm/s	VAP mm/s	LIN %
EP 1	99,86	30,52	64,06	35,59	36,68
EP 2	99,74	44,06	74,10	39,67	32,52
EP 3	98,59	13,47	50,43	22,11	29,52
EP 4	97,78	19,70	52,16	23,91	22,67
EP 5	97,67	11,20	42,96	22,07	30,93
EP 6	73,56	16,09	43,20	26,43	41,81
AV	**94,53**	**22,51**	**54,49**	**28,30**	**32,36**
EPL 1	82,03	16,09	46,81	25,39	29,86
EPL 2	72,36	16,91	49,50	27,23	27,82
EPL 3	99,31	34,52	63,49	37,70	39,84
EPL 4	97,71	12,61	45,61	24,34	29,34
AV	**87,85**	**20,03**	**51,35**	**28,67**	**31,72**

Table 1

Motility parameters for Equi Plus group. EP: Equi Plus without lidocaine, EPL: Equi Plus with lidocaine, AV: Average results. Motility parameters TM: total motility, PM: progressive motility, VCL: Velocity of the average curve, VAP: Velocity of the average path, , LIN: Linearity.

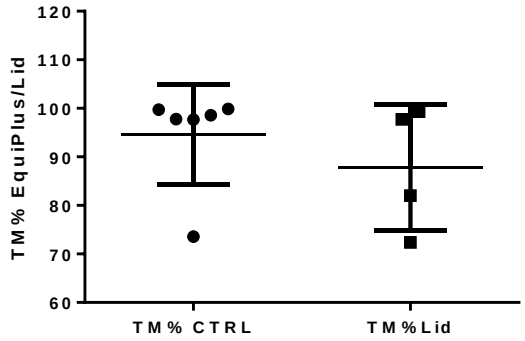

Fig. 1A Total motility for the samples diluted in Equi Plus extender. Lidocaine did not significantly affect TM

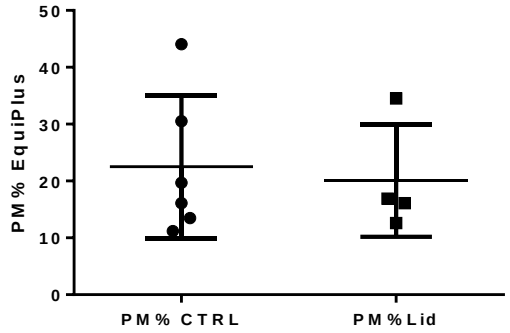

Fig. 1B Progressive motility for the samples diluted in Equi Plus extender. Lidocaine did not significantly affect PM

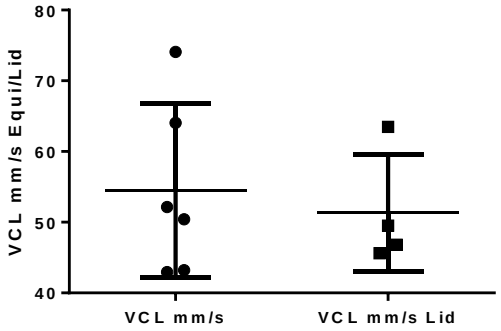

Fig. 1C Velocity of the average curve for the samples diluted in Equi Plus extender. Lidocaine did not significantly affect VCL

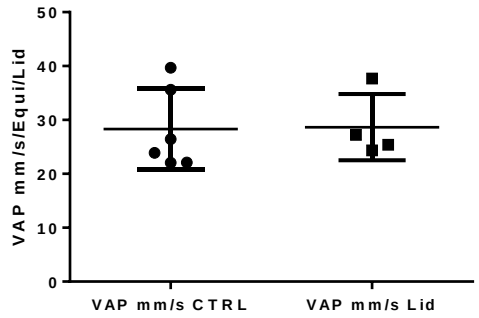

Fig. 1D Velocity of the average path for the samples diluted in Equi Plus extender. Lidocaine did not significantly affect VAP

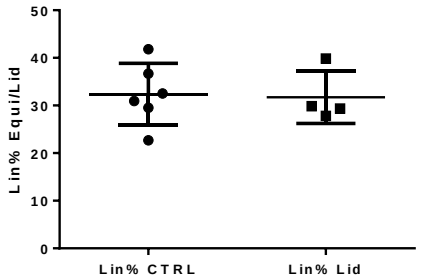

Fig. 1E Linearity for the samples diluted in Equi Plus extender. Lidocaine did not significantly affect Lin

Motility parameters using Gent extender

The remaining samples, extracted from the epididymides of the ipsilateral testicle of the same stallion were suspended in Gent extender and distributed in one of the two groups: Gent without lidocaine (Ge) (n=6) and Gent with lidocaine (GeL) (n=4). Table 2 provides detailed information about the motility parameters analyzed in both Ge and GeL groups and the average results. TM, VCL and VAP showed no statistically significant difference among the two groups (fig 2 A-C) whereas PM and LIN were significantly different among the two groups (fig 2 D, E).

	TM %	PM %	VCL mm/s	VAP mm/s	LIN %
Ge 1	98,54	22,13	52,93	26,60	26,79
Ge 2	99,83	25,36	62,73	30,82	24,63
Ge 3	87,42	7,98	35,90	17,92	25,74
Ge 4	87,35	19,09	49,11	24,71	26,29
Ge 5	99,82	13,82	47,51	30,31	44,01
Ge 6	92,16	8,34	38,11	18,75	26,09
av	**94,19**	**16,12**	**47,72**	**24,85**	**28,93**

GeL 1	85,65	6,61	34,57	17,92	28,00
GeL 2	59,39	15,16	26,68	13,26	26,76
GeL 3	89,29	9,45	35,78	17,80	27,62
GeL 4	96,99	51,81	37,75	20,12	29,90
av	**82,83**	**20,76**	**33,70**	**17,28**	**28,07**

Table 2

Motility parameters for Equi Plus group. EP: Equi Plus without lidocaine, EPL: Equi Plus with lidocaine, AV: Average results. Motility parameters TM: total motility, PM: progressive motility, VCL: Velocity of the average curve, VAP: Velocity of the average path, , LIN: Linearity.

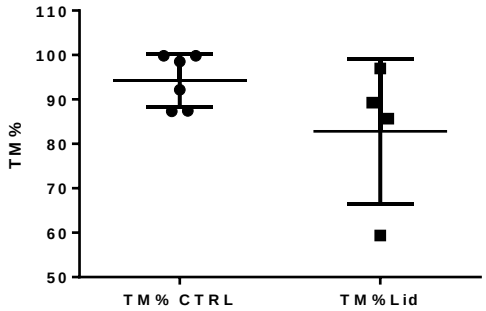

Fig. 2A Total motility for the samples diluted in GENT extender. Lidocaine did not significantly affect TM

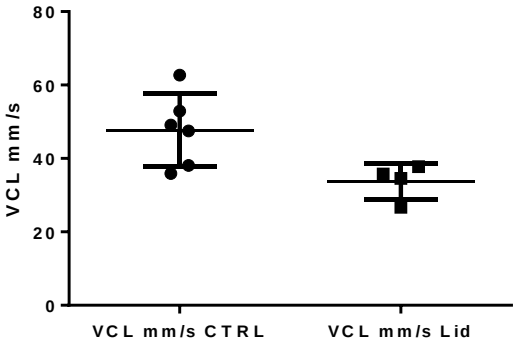

Fig. 2B Velocity of the average curve for the samples diluted with Gent extender. Lidocaine did not significantly affect VCL

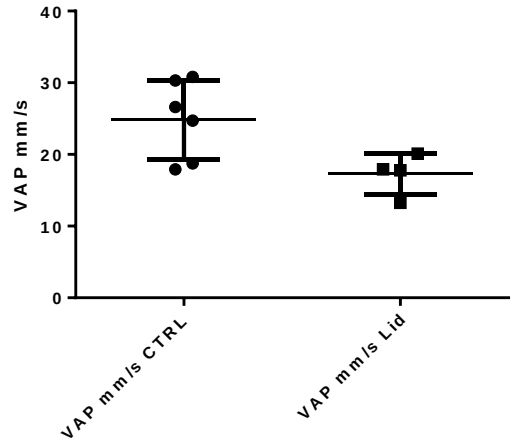

Fig. 2C Velocity of the average path for the samples diluted in Equi Plus extender. Lidocaine did not significantly affect VAP

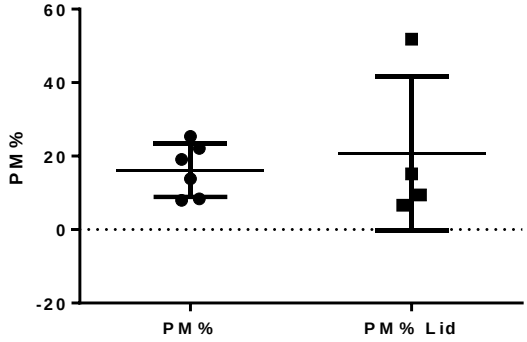

Fig. 2D Progressive motility for the samples diluted in Gent extender. Lidocaine significantly affected PM

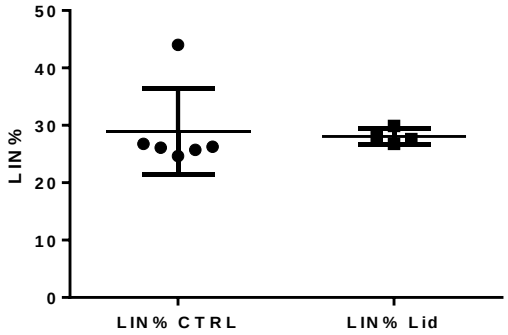

Fig. 2E Linearity for the samples diluted in Gent extender.
Lidocaine significantly affected Lin

Discussion

The present study evaluated motion characteristics of chilled epididymal sperm, after 24 hours intra epididymal exposure to lidocaine, using two commercial extenders. Testing different

semen extenders is not an option with epididymal sperm, due to quantity limitations. Applying a general protocol that works best for a significant number of stallions is the best option. In the current study, the extender for chilled semen containing defined milk proteins produced better results for parameters PM and LIN , compared with the egg yolk extender, in epididymal spermatozoa exposed to lidocaine for 24 hours. This results are similar to other studies, where egg-yolk extenders were proved less efective for both chilled storage of epididymal spermatozoa [7,8], regardless of the lidocaine exposure.

In order to be able to preserve genetic material from deceased or emergency castrated stallions, a method has been described where intraepididymal storage can keep spermatozoa viable for up to 96 hours post castration when epididymides are cooled stored, but motility and most importantly progressive motility decreases time-dependent [9]. This is important when a facility equipped for the procedure is situated at a considerable distance. In the curret study, motility parameters were decreased in all four groups, compared to the Boye study [2], probably due to the prolonged intraepididymal cooled storage.

Lidocaine is routinely used for local analgesia during castration, due to its proven positive effects [10,11]. Similar to another study [2], lidocaine did not seem to affect motility parameters of epididymal spermatozoa even after 24 hours intra-epididymal exposure. The concerns about lidocaine local analgesia were based on the lack of studies regarding the effect of equine spermatozoa exposure to lidocaine, even though results published on human sperm motility showed no negative impact of lidocaine on human spermatozoa, at different concentrations, in vitro [12]. Furthermore, studies on male humans and stallions prove that lidocaine does cross epididymal barrier after being administred in the testicular parenchyma [5, 12].

In the current study, progressive motility and linearity were lower for the samples diluted in Gent chilled extender, regardless of the exposure to lidocaine. Prediction of the fertility of equine sperm prior to cryoconservation remains challenging, and even though latest studies suggest the use of more than one technique in assesing fertility [13], progressive motility is still an important parameter, used to determine the minimum standard requirements for semen for artificial insemination [14] .Interstingly, in the Boye 2019 study, high concentrations of lidocaine did affect the progressive motility and linearity of spermatozoa.

Conclusion

The administration of 10 ml 2% lidocaine intraparenchymatous during routine castration, did not negatively affect motility of epididymal spermatozoa after 24 hours intraepididymal cooled storage, when milk protein chilled semen extender was used. However, when egg-yolk chilled semen extender was used, progressive motility and linearity significantly decreased.

Based on this findings, the injection of lidocaine during routine castration can be used even when epididymal sperm is to be retrieved and milk based extenders seem to improve kinematic parameters of epididymal spermatozoa.

Disclosures

All authors have no conflict of interest to disclosure.

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