ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS
SBORNÍK MENDELOVY ZEMĚDĚLSKÉ A LESNICKÉ UNIVERZITY V BRNĚ

Ročník LV 8 Číslo 4, 2007

ON THE SPECIFIC CONDUCTIVITY OF STALLION EJACULATE

L. Severa, I. Křivánek, L. Máchal

Received: November 22, 2006

Abstract

SEVERA, L., KŘIVÁNEK, I., MÁCHAL, L.: *On the specific conductivity of stallion ejaculate*. Acta univ. agric. et silvic. Mendel. Brun., 2007, LV, No. 4, pp. 63–68

Ejaculate specific conductivity σ ($S.m^{-1}$) was measured for 10 breeding stallions. The set of measurings was performed three times for native ejaculate and three times for ejaculate stored for 24 hours at 4 °C. The average value of specific conductivity of native ejaculate was found to be 1.22 ± 0.11 $S.m^{-1}$, the same quantity for stored ejaculate was calculated to be 1.11 ± 0.11 $S.m^{-1}$. The values of coefficients of variation have been calculated for several quantities, and qualitative (ejaculate volume, sperm concentration, and sperm motility) and quantitative parameters have been compared by way of phenotype coefficients of correlation. Two dependencies, namely specific conductivity vs. sperm concentration ($r_p = -0.79$; $P < 0.99$) and specific conductivity vs. motility ($r_p = -0.78$; $P < 0.99$), have been evaluated as relevant and enabling supplementary description of stallion ejaculate quality by means of other (physical) quantity.

stallion ejaculate, specific conductivity, sperm concentration, sperm motility

Measuring and especially evaluating and standardizing physical properties of stallion's ejaculate must face one major obstacle – huge variation in ejaculates from different stallions, and even among ejaculates collected from the same stallion (Rousset, 1987 and Katila, 1997). Procedures for correct handling fresh stallion semen and assessing the quality of raw semen is broadly described e.g. in (Malmegren, 1997). Physical properties of semen (as a testing material) change very quickly with time (Shi et al., 2004) and their comparison and evaluating is thus complicated. Livestock ejaculate electric and dielectric properties have been studied for quite a long time (Brandt, 1963; Máchal and Křivánek, 2000) and ejaculate was found to be, in most cases, a substance considerable as imperfect dielectric with some conductivity arising in time variating electric field. This arising is associated with molecule oscillation, deformation, polarization, etc. Measuring of electric and dielectric properties of animal tissues and animal products has been performed by many investigators (e.g. Jain and Woss, 1987; Křivánek and Buchar, 1993; Kent, 1987; Tran and Stuchly, 1987; Welti-Chanes, 2002; and others).

The conductivity values of boar ejaculate were classified by (Kozumplík and Kudláč, 1980). Relation between relative permitivity, conductivity, and qualitative parameters of boar and bull ejaculate were monitored by (Máchal et al., 1997). Following values of phenotype correlation were found for native bull ejaculate: ejaculate volume and observed electric and dielectric properties (r_p ranged from –0.17 to –0.72); sperm concentration and ejaculate electric and dielectric properties (r_p ranged from –0.09 to –0.74); and sperm motility and specific conductivity (r_p ranged from 0.08 to 0.48). Thus negative correlation values were found for ejaculate volume and sperm concentration while positive correlation values for sperm motility. Concerning boar ejaculate, positive values of correlation were calculated in all cases.

The aim of presented paper consists in describing of stallion ejaculate specific conductivity, determining the changes induced by one-day storing, and finding possible relation between qualitative parameters (such as concentration and motility) and specific conductivity.

MATERIAL AND EXPERIMENTAL PROCEDURE

Ejaculate specific conductivity was observed for 10 breeding stallions of different breeds (hereinafter denoted as numbers 1–10) housed in horse-breeding farm in Tlumačov, Czech Republic. The stallions were actively employed in production of insemination doses and were considered to be clinically healthy. The ejaculate collecting was performed on a regular base – two times a week. Monitored samples of ejaculates were collected in April and May in 3 stages (with 2 weeks interval), hereinafter denoted as on site I, on site II, and on site III. Measuring was performed on site (in the laboratory conditions) in compliance with fresh ejaculate handling rules (Katila, 1997). The samples were tested 20 minutes after ejaculation and ejaculate volume and concentration were determined as a complementary biological measuring and factor possibly influencing the physical properties. The sperm concentration was determined by means of Bűrker's cells method. The sperm motility was determined by subjective evaluation method. The temperature of each sample was 38 ± 1 °C. The second day measuring was performed on ejaculate stored at 4 °C in the physical laboratory of Mendel University of Agriculture and Forestry in Brno. The temperature was measured and recorded before each measurement.

Specific conductivity σ was determined by use of bio-impedance spectroscopic analyzer ZO1 designed by OMNI BIO. This apparatus allows measuring conductivity as a function of frequency. The measurements ranged between 10 Hz and 100 kHz. Thus determining of any potential phase shift in the electric signal inside the sample and the capacity component of conductivity was possible.

Special four-contact coaxial probe was used to perform the measurements in order to eliminate the errors induced by transition impedance.

Following relation applies for conductivity probe:

$$\sigma = K \cdot G,$$

where K (m^{-1}) is conductivity parameter of the probe. This parameter is determined by use of normal fluid (20% solution of NaCl with specific conductivity 19.61 $S.m^{-1}$).

The principle of four-contact measuring consists in following arrangement. Two electrodes are designed as feeding ones (the electric signal of given frequency is applied) and two as sensing ones. The sensing electrodes measuring the intensity of electric voltage inside the sample connected to alternating voltmeter with very high input resistance. Thus the influence of transition resistance between sample and sensing contact is eliminated.

The sample's frequency dependence of conductivity was determined by means of apparatus Z01, which is able to shift automatically the frequency in the range of 10Hz–100 kHz with 10-s step.

RESULTS AND DISCUSSION

Immediate determining of breeding stallions' (or generally breeding animals) fertilization capability without necessity of performing special, and often time-consuming, quantitative or qualitative tests, is a big challenge. Since the ejaculate is biological material with highly variable physical properties, the most precise description is needed.

Table I contains data on 3 sets of measuring of ejaculate specific conductivity performed in the farm laboratory (20 minutes after ejaculation) and university laboratory (24 hours later).

I: *Measured values of ejaculate specific conductivity for different stallions and different dates*

	Electric Conductivity σ [Sm^{-1}]					
Sample No.	on site I	2nd day	on site II	2nd day	on site III	2nd day
1	1.15	1.07	1.08	1.01	1.18	1.06
2	1.13	1.13	1.14	1.07	1.12	1.06
3	1.31	1.33	1.28	1.22	1.27	1.22
4	1.12	1.10	1.16	1.01	1.14	1.09
5	0.98	0.96	1.20	1.06	0.99	0.95
6	1.34	1.23	1.40	1.18	1.18	0.91
7	1.28	1.22	1.38	1.21	1.14	0.88
8	1.33	1.28	1.34	1.14	1.29	1.15
9	1.29	1.21	1.32	1.17	1.14	1.08
10	1.23	1.16	1.13	1.01	1.15	0.93

Table data represent the average values of conductivities measured under different frequencies, namely 0.1 kHz, 1 kHz, 10 kHz, and 100 kHz. Such complete measurement set is shown in Table II, namely for experiments performed on site II. Similar data sets are available for all experiments.

II: *Example of detailed on site II conductivity measuring*

	Electric conductivity σ [Sm^{-1}]				
Sample No.	0.1 kHz	1 kHz	10 kHz	100 kHz	$\bar{x}$
1	1.08	1.08	1.09	1.06	1.08
2	1.19	1.20	1.19	1.21	1.20
3	1.26	1.26	1.26	1.34	1.28
4	1.07	1.07	1.07	1.42	1.16
5	1.18	1.18	1.18	1.25	1.20
6	1.36	1.38	1.38	1.48	1.40
7	1.36	1.39	1.39	1.37	1.38
8	1.31	1.32	1.32	1.42	1.34
9	1.31	1.30	1.30	1.38	1.32
10	1.12	1.12	1.12	1.19	1.13

The value of average volume of stallion ejaculate was found to be 41.1 ± 27.0 cm^3 (Table III). This value corresponds with values quoted in the literature (Máchal and Křivánek, 2002). The value of average sperm concentration 0.37 ± 0.28 (10^9.cm^{-3}) was slightly higher than the average value mentioned in literature (Máchal and Křivánek, 2002). The levels of electric conductivity varied in accordance on monitoring time (either on site or 2nd day). The average value of on site ejaculate conductivity was calculated as 1.22 ± 0.11 S.m^{-1} in comparison with 1.11 ± 0.11 measured 2nd day.

III: *The average values of qualitative and quantitative parameters of stallion ejaculate and its specific conductivity*

	n	$\bar{x}$	s_x	V_x [%]
Ejaculate volume [cm^3]	60	41.10	27.0	66
Sperm concentration [10^9.cm^{-3}]	54	0.37	0.28	76
Motility [%]	54	66.50	8.40	13
Temperature [°C]	60	21.70	0.80	3.6
Electric Conductivity on site σ [Sm^{-1}]	27	1.22	0.11	9
Electric Conductivity 2nd day σ [Sm^{-1}]	27	1.11	0.11	10

The average conductivity decrease between on site and 2nd day measurement was 0.11 ± 0.07 Sm^{-1}, which represents 8.9 ± 6.03 %. Also the values of ejaculate conductivity observed for individual stallions and individual comparable measurements (either on site measurement or 2nd day measurement) ranged to a certain extent. To be specific, variation coefficient ranged from 1.0 to 8.3%. This value is much smaller in comparison with variation of sperm concentration value and slightly smaller in comparison with sperm motility variation.

IV: *Calculated values of coefficients of correlation for specific conductivity and selected parameters, and verification test of correlation coefficient*

	Phenotype coefficients of correlation		
	Ejaculate volume [cm^3]	Sperm concentration [$10^9.cm^{-3}$]	Motility [%]
Electric Conductivity measured on site	0.39*	–0.79*	–0.78*

$t_{(n-2)}$ test of correlation coefficient according to Student's distribution

		Tabulate values	
	Calculated value	p 0.95	p 0.99
Ejaculate volume	1.187	1.860	2.896
Sperm concentration	3.719	1.860	2.896
Motility	3.644	1.860	2.896

* $P < 0.01$

Table IV shows the relation between 3 monitored parameters and electric conductivity. The relation was evaluated by means of phenotyte coefficient of correlation. The dependence intensity can be identified (Stávková, 1992) as „strong" in the case of sperm concentration ($r_p = -0.79$) and motility ($r_p = -0.78$), and „weak" in the case of ejaculate volume ($r_p = 0.39$). The sperm concentration and sperm motility exhibited negative values of correlation with electric specific conductivity, while ejaculate volume exhibited positive correlation. The verification test of correlation coefficient proved the dependence relevancy in case of sperm concentration and motility. This finding is in partial agreement with several years ago performed measurings (Máchal and Křivánek, 2000), where similar dependencies were monitored for bull ejaculate. The bull ejaculate exhibited very weak dependence in case of relation between ejaculate volume and motility. The sperm concentration, on the other hand, shown middle strong dependence ($r_p = -0.51$). As compared with these results, stallion ejaculate exhibited clearer dependencies, even in two cases.

CONCLUSIONS

The average specific conductivity of native stallion ejaculate (measured for 10 stallions) was calculated as 1.22 ± 0.11 $S.m^{-1}$ with variation coefficient 9%. The same quantity measured after 24 hours exhibited slight decrease to the value of 1.11 ± 0.11 $S.m^{-1}$ with 10% variation coefficient. Evaluated qualitative parameters – ejaculate volume, sperm concentration, and motility, varied with 66%, 76%, and 13% variation coefficient, respectively. When comparing variation of specific conductivity measured for ejaculate of individual stallions, the value ranged between 1–8%.

Two correlations between qualitative and quantitative parameters were found as evident and possibly promising, namely the dependence between specific conductivity and sperm concentration ($r_p = -0.79$), and specific conductivity and motility ($r_p = -0.78$). The coefficients of correlation were tested for their statistical evidence and have been found to be relevant. When comparing similar measurings performed for bull or boar ejaculate (Máchal and Křivánek, 2000), the dependencies observed for stallion ejaculate are markedly stronger.

It is possible to state that, these results are very promising and described measuring procedures could be used as complementary measuring methods for evaluation of stallion ejaculate properties. But high variability of stallion ejaculate composition and physical properties (see v_x values stated above) is still limiting factor and traditional qualitative methods are necessary for its precise description. More detailed investigation of electric and dielectric properties of individual ejaculate components could lead to more complex understanding of ejaculate characteristics and their possible use in standard practice.

SOUHRN

Měrná vodivost ejakulátu hřebců

Byla měřena a vyhodnocována měrná vodivost σ ($S.m^{-1}$) ejakulátu deseti plemenných hřebců. Měření byla provedena v celkem třech sériích – bezprostředně po odběru a po 24 hodinách, kdy byl ejakulát skladován při 4 °C. Dále byly stanoveny hodnoty objemu ejakulátu, koncentrace spermií a motilita. Průměrná hodnota měrné vodivosti čerstvě odebraného ejakulátu byla vypočtena na 1,22 ± 0,11 $S.m^{-1}$. Průměrná hodnota měrné vodivosti 24 hodin skladovaného ejakulátu měla hodnotu 1,11 ± 0,11 $S.m^{-1}$. Průměrný objem ejakulátu byl 41,1 ± 27 cm^3, koncentrace spermií 0,37 ± 0,28 $10^9.cm^{-3}$ a motilita 66,5 ± 8,4 %. Kvalitativní a kvantitativní parametry byly srovnávány pomocí fenotypového korelačního koeficientu. Dvě závislosti, konkrétně měrná vodivost vs. koncentrace spermií (r_p = –0,79; P < 0,01) a měrná vodivost vs. motilita (r_p = –0,78; P < 0,01), byly vyhodnoceny jako relevantní. V těchto případech je možný doplňkový popis kvality ejakulátu hřebců pomocí další (fyzikální) veličiny.

hřebčí ejakulát, měrná elektrická vodivost, koncentrace spermií, motilita spermií

REFERENCES

BRANDT, A.: Issledovanie dielektrikov na sverkhvysokikh chastotakh, GIMFL, Moscow, 1963

JAIN, R. C. and VOSS, W. A. G.: Dielectric properties of raw and cooked chicken egg at 3 GHz, 23–140 °C, Journal of Microwave Power, 1987, vol. 22, No. 4, p. 221–227

KATILA, T.: Procedures for handling fresh stallion semen, Theriogenology, Volume 48, Issue 7, November 1997, Pages 1217–1227

KŘIVÁNEK, I. and BUCHAR, J.: Dielektrické vlastnosti sýrů, Acta Universitatis Agriculturae et Silviculturae Brunensis, Facultas Agroeconomica, 1993, vol. 26, No. 1, p. 171–185, ISSN 0524-7403

KENT, M.: Electric and dielectric properties of food materials. Essex: Hornbuch, Science and Technology Publishers, 1987, p. 48–51

KOZUMPLÍK, J. and KUDLÁČ, E.: Reprodukce prasat ve velkochovech, SZN Praha, 1980

MÁCHAL, L., KŘIVÁNEK, I., CHLÁDEK, G. and DOLEŽAL, P.: Correlation between the relative permitivity, conductivity and qualitative indicators of bull and boar ejaculates, Journal of Animal and feed Sciences, 6, 1997, 13–22

MÁCHAL, L. and KŘIVÁNEK, I.: The dielectric indicators of the bull ejaculate and semen plasma. Acta univ. agric. et silvic. Mendel. Brun (Brno), 2000, XLVIII, No. 1, pp.: 7–13

MÁCHAL, L. and KŘIVÁNEK, I.: The specific conductivity of the stallion ejaculate and semen plasma, Acta univ. agric. et silvic. Mendel. Brun, 2002, L, No. 5, pp.: 45–52

MALMEGREN, L.: Assessing the quality of raw semen: A review, Theriogenology, Volume 48, Issue 4, September 1997, Pages 523–530

ROUSSET, H, CHANTELOUBE, P. H., MAGISTRINI, M. and PALMER, E.: Assesment of fertility and semen evaluation of stallions, J Reprod Fertil Suppl 1987, 35: 23–31

SHI, Y. D., PAN, L. F., YANG, F. K., WANG, S. Q.: A preliminary study on the rheological properties of human ejaculate and changes during liquefaction, Asian J Androl, 2004 Dec, 6 (4): 299–304

STÁVKOVÁ, J.: Biometrika – university textbook, VŠZ v Brně, 1992

TRAN, V. N. and STUCHLY, S. S.: Dielectric properties of beef liver, chicken and salmon at frequencies from 100 to 2500 MHz, Journal of Microwave power, 1987, vol. 22, No. 1, p. 29–33

WELTI – CHANES, J. et al.: Engineering and Food for the 21st Century, CRC Press LLC 2002, ISBN 1-56676-963-9

Address

Ing. Libor Severa, Ph.D., RNDr. Ivo Křivánek, CSc., Ústav techniky a automobilové dopravy, Prof. Ing. Ladislav Máchal, DrSc., Ústav chovu a šlechtění zvířat, Mendelova zemědělská a lesnická univerzita v Brně, Zemědělská 1, 613 00 Brno, Česká republika