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An analysis of Selected Aspects of Sperm Quality in Fresh and Cooled-Storage Stallion Semen

Review Article

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ABSTRACT

This review summarises the most significant findings related to the issue of the quantitative and qualitative indicators of stallion ejaculates, its processing when producing chilled insemination doses and the possibility of influencing the individual steps of the entire process. Artificial insemination, with cooled-storage semen, is increasingly used in horse breeding because it offers many advantages over natural breeding. The parameters of sperm quality and, above all, the standardization of conditions in the production of cool-stored insemination doses are still not clearly specified. There is considerable scope for further development and research in this area.

KEY WORDS cooled-stored semen, equine ejaculate, sperm quality, stallion.

INTRODUCTION

Artificial insemination is used more and more in horse breeding, as it offers many advantages over natural breeding. The most important of these is the possibility of choosing the stud from the large number of stallions on offer, lowering the safety risk for both mares and stallions (Janett *et al*. 2003a) and reducing the risk of the transmission of infectious diseases and transport inconvenience (Janett *et al*. 2003a; Aurich, 2012). In addition, when compared with those from natural mating, pregnancy rates have been shown to be equal, or even higher, after artificial insemination with fresh, or cooled-stored semen (Janett *et al*. 2003a; Janett *et al*. 2003b).

Like many other mammals, Equidae directs their reproductive activity into a season, so that it is as advantageous as possible for their young. Seasonal changes in the length of the day are the main cause of the increase and decrease in the sexual activity of horses (Janett *et al*. 2003a; Gamboa *et al*. 2010). Horses are seasonal animals, with maximal reproductive activity in late spring and summer (Aurich, 2016). In order to achieve maximal reproductive performance in stallions, their normal sexual behaviour and libido must be maintained. This ensures that a high quality of ejaculate can be taken (Sieme *et al*. 2004), when the frequency of taking samples, and the intervals between them, are suitable.

How to evaluate the quality of stallion ejaculate?

The qualitative parameters of ejaculate include, without a doubt, the number of spermatozoa, sperm motility and the number of morphologically normal spermatozoa (Colenbrander *et al*. 2003; Love, 2011). These basic parameters usually uncover clear cases of lower fertility, or infertility, in a given individual (Rodriguez-Martinez, 1998). Other parameters, which also definitely belong among the qualitative indicators of ejaculate, are sperm chromatin integrity (Graham, 2001; Rečková *et al*. 2008;

Love, 2011), sperm membrane integrity (Věžník *et al*. 2004; Foster *et al*. 2011; Deichsel *et al*. 2016), the acrosomal integrity of sperm (Gamboa *et al*. 2010; Rečková *et al*. 2015) and mitochondrial activity in the sperm midpiece (Gamboa *et al*. 2010).

To ensure that the number of morphologically normal and motile spermatozoa in a sample is as high as possible, it should be taken from the stallion at regular intervals. These intervals are dependent on each individual stallion because the quality of ejaculate in sexually active stallions is higher than in inactive stallions (Sieme *et al*. 2004). Spermatozoa were taken from the first ejaculates, which are taken at the start of the season, or after long periods without sperm collection, do contain a higher concentration of sperm, but the spermatozoa show a large number of morphological abnormalities, have lower progressive motility and their life capability is shorter (Samper, 2009). The cause of the production of lower quality ejaculate at the start of the season is probably the lower production of gonadotropin during the winter period. However, despite the fact that the seasons affect the quality of stallion ejaculate, the variability of individual stallions (Aurich, 2016) has a much greater influence on the quality of ejaculate. According to Sieme *et al*. (2004), thus far, the effect of the frequency of sample collection, and the intervals between the individual ejaculate collection, on the quality of the semen obtained and on sperm quality (cool-stored or frozen-thawed) has not been totally specified. From a biological point of view, only the spermatozoa that are capable of life are potentially capable of fertilizing an egg, which is why most of the methods used to date are based on evaluating the life capability of spermatozoa (Věžník *et al*. 2004). From the results taken from the *in vivo* environment, it is clear that the best results are achieved by stallions whose ejaculate contains a higher percentage of live and acrosome intact sperm, with high mitochondrial membrane potential (Gamboa *et al*. 2010).

Diluting ejaculate and its later, cooled-stored semen

The actual dilution of ejaculate precedes its macroscopic and microscopic evaluation. The temperature of the diluent and the diluted ejaculate must be the same (±1 ˚C), so that cold shocks are avoided (Ball and Peters, 2004). According to Samper (2009), the percentage of motile spermatozoa is significantly influenced by temperature. It was proved that a temperature of just 22 ˚C can lower the progressive motility of sperm by 25% when compared with a temperature of 37 ˚C. Temperatures of more than 37 ˚C are also harmful to sperm motility, as temperature shocks can occur. During the collection of ejaculate, it is very important to ensure that the ejaculate, and also all the equipment which is in contact with it, is maintained at body temperature.

Sudden changes in temperature can cause heat shocks, which lead to irreparable damage to the sperm (Samper *et al*. 2007). The diluent intended for diluting the selected ejaculate is always added gradually and is constantly mixed. The solvent used must be sterile and pre-heated to the same temperature as the diluted ejaculate (Ball and Peters, 2004).

The correct diluent must have the following characteristics: it must be a source of energy for the sperm, have good buffering abilities, contain a small quantity of electrolytes, ensure the required osmotic pressure, maintain a constant pH level (that corresponds to the requirements of the ejaculate), it cannot be toxic to sperm, and it must be sterile and economically accessible (Louda *et al*. 2001). The composition of each diluent significantly influences the lifespan and fertilization ability of the sperm in the insemination dose (Siddique *et al*. 2006). In practice, a whole range of mostly commercial diluents are used to dilute stallion ejaculates that are intended for the production of insemination doses. They are supplemented by various types and varying amounts of accessories. Restrepo *et al*. (2012) and Janett *et al*. (2012) used Equipro (Minitube International), with the addition of DMF and egg yolk. Morillo Rodríguez *et al*. (2011) and Puglisi *et al*. (2016) used a commercial solvent, GENT (Minitube International), which contains yolk solvent, with glycerol and antibiotics.

Morillo Rodríguez *et al*. (2011) and Janett *et al*. (2012) used INRA 96 (IMV) diluent, with yolk and glycerol. Lorenzoni *et al*. (2011) and Janett *et al*. (2012) used INRA 82, with the addition of glycerol. In the last few years, studies have proved that it is possible to physically remove bacteria from the ejaculate, using the Single Layer Centrifugation method, without needing to use antibiotics (Morrell and Wallgren, 2011; Morrell *et al*. 2014). Morrell and Rodriguez-Martinez (2009) demonstrated an improvement in the quality of sperm could be achieved using the single layer centrifugation method on various types of animals, including stallions. If the ejaculate is kept for longer than 6 hours, it should be placed in cooling equipment, where a constant temperature of 5-8 ˚C is maintained, for a period of 48 hours (Samper, 2009), or 40 hours (Aurich, 2008). The significance of cooling the ejaculate lies in the fact that the metabolism of the spermatozoa is significantly lowered. This lowers the number of metabolic products separated into the diluent in which the sperms are kept (Samper, 2009). Cooling the spermatozoa, from 18 ˚C to 8 ˚C, is a critical step and can cause specific damage to the sperm plasma membrane (Aurich, 2008). The individuality of the stallion, which greatly differs (Janett *et al*. 2003b) between stallions, has a crucial influence on the resulting quality of the cooled-storage insemination dose.

The correct collection and handling of the ejaculate are the keys to maintaining the quality of ejaculate during its processing and chilled storage. The collection of semen should occur after minimal sexual stimulation and ejaculation should occur during the first mounting.

These conditions should lead to the collection of ejaculate that has a higher concentration of sperm, a lower percentage of semen plasma and minimal contamination by bacteria (Aurich, 2008). A higher percentage of semen plasma in the ejaculate has a negative influence on sperm motility (Janett *et al*. 2003b). The processing of stallion ejaculate is far from standardized between the individual AI centers (Heckenbichler *et al*. 2011).

CONCLUSION

The sperm quality parameters for stallions, and the subsequent production technology of cool-stored insemination doses, have not been totally specified. As for the issue of the quality assessment of stallion ejaculate and its processing and storage possibilities, so many questions remain to be answered. In this area, there is great scope for further development and research.

REFERENCES

Aurich C. (2008). Recent advances in cooled-semen technology. *Anim. Reprod. Sci.* **107,** 268-275.

Aurich C. (2016). Seasonal influences on cooled-shipped and frozen-thawed stallion semen. *J. Equine Vet. Sci.* **43,** 1-5.

Aurich J.E. (2012). Artificial insemination in horses – more than a century of practice and research. *J. Equine Vet. Sci.* **32,** 458-463.

Ball P.J.H. and Peters A.R. (2004). Reproduction in Cattle. Blackwell Publication, Ames, Iowa.

Colenbrander B., Gadella B.M., and Stout T.A.E. (2003). The predictive value of semen analysis in the evaluation of stallion fertility. *Reprod. Domest. Anim.* **38,** 305-311.

Deichsel K., Schrammel N., Aurich J. and Aurich C. (2016). Effects of a long-day light program on the motility and membrane integrity of cooled-stored and cryopreserved semen in Shetland pony stallions. *Anim. Reprod. Sci.* **167,** 68-73.

Foster M.L., Varner D.D., Hinrichs K., Teague S., LaCaze K., Blanchard T.L. and Love C.C. (2011). Agreement between measures of total motility and membrane integrity in stallion sperm. *Theriogenology*. **75,** 1499-1505.

Gamboa S., Rodrigues A.S., Henriques L., Batista C. and Ramalho-Santos J. (2010). Seasonal functional relevance of sperm characteristics in equine spermatozoa. *Theriogenology.* **73,** 950-958.

Graham J.K. (2001). Assessment of semen quality: A flow cytometric approach. *Anim. Reprod. Sci.* **68,** 239-247.

Heckenbichler S., Deichsel K., Peters P. and Aurich C. (2011). Quality and fertility of cooled-shipped stallion semen at the time of insemination. *Theriogenology.* **75,** 849-856.

Janett F., Sacher K., Hassig M., and Thun R. (2012). Quality of raw and of cold-stored semen in Icelandic Stallions. *J. Equine Vet. Sci.* **32,** 590-595.

Janett F., Thun R., Niederer K., Burger D. and Hassig M. (2003a). Seasonal changes in semen quality and freezability in the warmblood stallion. *Theriogenology.* **60,** 453-461.

Janett F., Thun R., Bettschen S., Burger D. and Hassig M. (2003b). Seasonal changes in semen quality and freezability in franches–montagnes stallions. *Anim. Reprod. Sci*. **77,** 213-221.

Lorenzoni S.L.G., Arruda N.S. and Rodrigues J.L. (2011). Cryopreservation of equine semen loaded in Cryovials. *Acta Sci. Vet*. **39,** 962-970.

Louda F., Čeřovský J., Ježková A. and Stádník L. (2001). Inseminace hospodářských zvířat se základy biotechnických metod. Pp. 201-211 in Proc. Int. Conf., Praha, Czech Republic.

Love C.C. (2011). Relationship between sperm motility, morphology and the fertility of stallions. *Theriogenology*. **76,** 547-557.

Morillo Rodríguez A., Ortega Ferrusola C., Macías García B., Morrell J.M., Rodríguez Martínez H., Tapia J.A. and Peña F.J. (2011). Freezing stallion semen with the new Cáceres extender improves post thaw sperm quality and diminishes stallion-to-stallion variability. *Anim. Reprod. Sci.* **127,** 78-83.

Morrell J.M. and Rodriguez-Martinez H. (2009). Biomimetic techniques for improving sperm quality in animal breeding: A review. *Open Androl. J.* **1,** 1-9.

Morrell J.M. and Wallgren M. (2011). Colloid centrifugation of boar semen. *Reprod. Domest. Anim*. **46,** 18-22.

Morrell J.M., Klein C., Lundeheim N., Erol E. and Troedsson M. H.T. (2014). Removal of bacteria from stallion semen by colloid centrifugation. *Anim. Reprod. Sci.* **145,** 47-53.

Puglisi R., Bornaghi V., Severgnini A., Vanni R., Balduzzi D. and Galli A. (2016). Cryopreservation of stallion semen: laboratory assessment of sperm injuries after cushioned centrifugation and freezing with conventional and alternative directional freezing methods. *Japanese J. Vet. Res.* **64,** 235-245.

Restrepo G., Duque J.E. and Montoya J.D. (2012). Effect of two protocols of cryopreservation on fertilizing capacity of stallion semen. *Rev. Fac. Nac. Agron. Medellín*. **65,** 6711-6718.

Rečková Z., Machatková M., Rybář R., Horáková J., Hulínská P. and Máchal L. (2008). Evaluation of chromatin integrity of motile bovine spermatozoa capacitated *in vitro*. *Zygote*. **16,** 195-202.

Rečková Z., Machatková M., Máchal L. and Ješeta M. (2015). Relationship between acrosome integrity changes and *in vitro* fertilising ability of bovine spermatozoa. *Vet. Med. Czech.* **60,** 469-475.

Rodriguez-Martinez H. (1998). Optimization of sperm quality in AI bulls. *Reprod. Domest. Anim*. **33,** 233-237.

Samper J.C. (2009). Equine Breeding Management and Artificial Insemination. Saunders Elsevier, St. Louis, Missouri, USA.

Samper J.C., Pycock J.F. and McKinnon A.O. (2007). Current Therapy in Equine Reproduction. Saunders Elsevier, St. Louis,

Missouri, USA.

Siddique M., Ali R. and Raza A. (2006). Effect of buffers on freezing of buffalo bull semen. *J. Agric. Social Sci.* **2,** 117-119.

Sieme H., Katila T. and Klug E. (2004). Effect of semen collection practices on sperm characteristics before and after storage and on fertility of stallions. *Theriogenology.* **61,** 769-784.

Věžník Z., Švecová D., Zajícová A. and Přinosilová P. (2004). Repetitorium Spermatologie a Andrologie a Metodiky Spermatoanalýzy. Veterinary Research Institute, Brno, Czech Republic.