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Short communication

EFFECT OF CAFFEINE ON PARAMETERS OF RAM SPERM MOTILITY

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ABSTRACT

The goal of this study was to examine the effect of caffeine on motility parameters of cooling-stored ram sperm – total motility and progressive movement. Caffeine is a cyclic nucleotide phosphodiesterase inhibitor, which can affect sperm motility. The semen was kept at 4˚C for several days. Caffeine was added to fresh ram semen diluted in Triladyl at concentrations of 1, 2 and 4 mmol.l $^{-1}$ every day of sperm storage, shortly before analysis. Sperm motility was analyzed using CASA system after 24, 48, and 72 h of storage. We confirmed the stimulated effect of caffeine given at concentrations of 1, 2, and 4 mmol.l $^{-1}$ on ram sperm total motility and progressive movement. The maximum effect of caffeine was observed 30 min after addition. The most effective concentration of caffeine was 4 mmol.l $^{-1}$. These results indicate that caffeine can maintain motility of ram sperm during long-term cooling-storage

Key words: caffeine; ram sperm; motility; progressive movement

INTRODUCTION

Progress in the use of artificial insemination has been related to search for substances with a potential ability to stabilize sperm membranes, to suppress apoptosis-like changes, to stimulate sperm motility and to enhance fertilizing ability of animal and human spermatozoa. Storage of diluted semen is widely used in artificial insemination (AI) programs. Diluted and cooled ram semen is an alternative to frozen semen when the insemination is done within a short period of time after collection. If the semen is diluted and stored, the practical use of liquid semen under farm conditions may be facilitated.

Comparing to the fresh semen, cooled ram semen suffers from a decrease in motility and alteration of morphology, accompanied by a decline in the survival ability in the female reproductive tract, reduction in fertility and increased embryonic loss. These damages are less pronounced in diluted and chilled than in frozen–thawed ram semen (Aisen *et al*., 2002; Fiser and Fairfull, 1984; Gil *et al.*, 2003). The quality of sperm movement dropped dramatically in cold liquid ram semen stored from 3 to 5 days (Deka and Rao, 1984; O'Hara *et al.*, 2010; Paulenz *et al*., 2002; Pérez *et al*., 1997; Salamon and Maxwell, 2000; Upreti *et al*., 1997).

Caffeine is a cyclic nucleotide phosphodiesterase inhibitor which markedly increased and maintained the respiration and motility of ejaculated bovine spermatozoa (Garbers *et al.*, 1971; Ball and First, 1983; El-Gaafary,

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1994). Caffeine may act directly by affecting cellular metabolism, and its effect depends on the concentration of calcium ions. Synergistic effect of caffeine and heparin on IVF has been reported in cattle (Park *et al*., 1989). It is proved that positive effect of inhibiting phosphodiesterase activity may result in an increase in intracellular cyclic adenosine monophosphate (cAMP), what leads to increase of sperm motility (El-Menoufy *et al.*, 1986).

The aim of the study was to examine the effects of caffeine on selected ram sperm motility parameters following liquid storage under hypothermic conditions.

MATERIAL AND METHODS

Semen collection

All the experiments were carried out with fresh ram spermatozoa. The semen was collected from East-Friesian (EF) and Lacaune (Lc) rams using artificial vagina. The rams were kept at the Institute of Sheep Breeding (Trenčianska Teplá) under uniform nutritional conditions. Volume, concentration and activity were assessed shortly after collection. Volume was determined by ejaculate collector to the nearest 0,1 cm^3; concentration was determined by microcuvette and SpermCue photometer (Minitub, Tiefenbach, Germany); and sperm activity was evaluated subjectively under light microscope (Olympus CH20). Ejaculates from all rams were pooled together to make heterospermia in order to avoid individual variability of rams and were used for the experiment. Ejaculates were diluted in a Triladyl extender (Minitüb, Tiefenbach, Germany) containing egg yolk, lactose and glycerol. The semen was cooled down to 4˚C and transported to the laboratory, where the samples were divided into four groups at 1 ml of ejaculate per tube. Then caffeine (Sigma – Aldrich, Germany) was added to marked tubes at concentrations of 1, 2 or 4 $mmol.l^{-1}$, whereas control group did not contain caffeine (0 $mmol.l^{-1}$). The semen samples were kept in a fridge for several days. Every day aliquots of ejaculates were picked up and placed into separate tubes where caffeine was added shortly before CASA analysis.

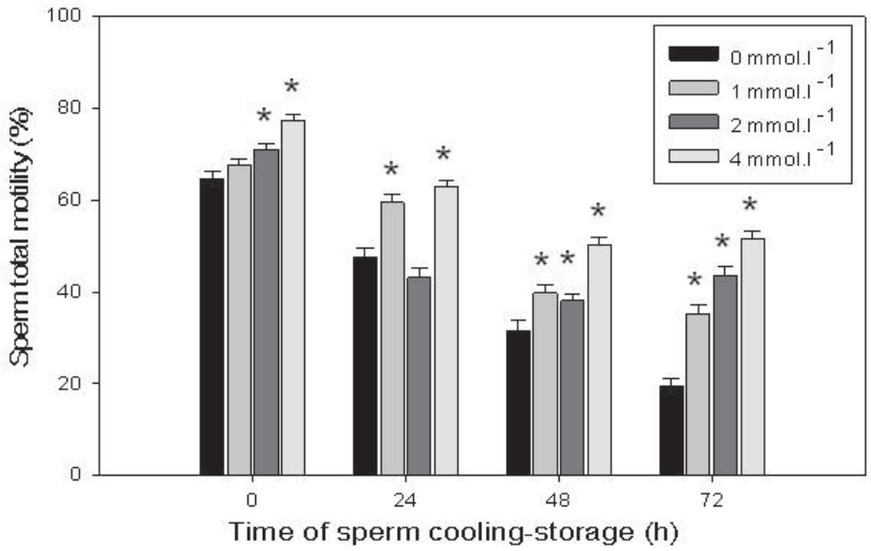
Analysis of sperm motility

Motility parameters of semen at 0, 24, 48 and 72 hours of sperm cooling-storage were analyzed using computer assisted semen analysis (CASA) - Hamilton Thorn motility analyzer (version 7). Caffeine at certain concentration was added to the sperm sample at the day when the analysis was performed. Each sample was analyzed at the time intervals of 0, 0.5 or 2h following caffeine addition. Between these time points the samples were incubated at 37˚C. We analyzed effect of various concentrations of caffeine on ram sperm total motility and progressive movement.

Statistical analysis

The experiments have been done in two replications. In each experimental group 7 view fields were evaluated, so that at least 350 sperm cells per one experiment were counted. Average values were calculated

Figure 1: Effect of caffeine on total sperm motility



* Significant difference compared to control: P< 0.05

from three measurements done at 0, 0.5 or 2h time point during the day. The results were statistically evaluated by two-way ANOVA test and graphically processed using SigmaPlot graphic software (version 9.0 for Windows).

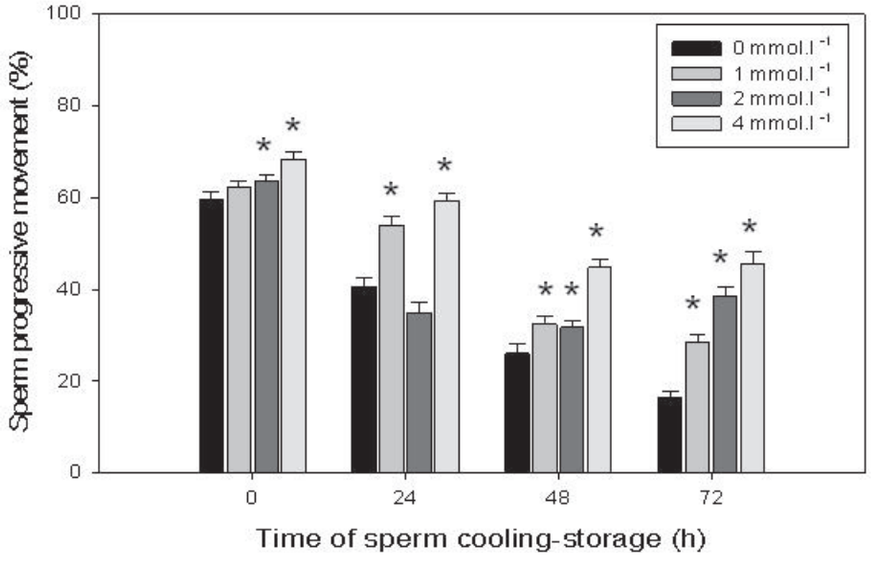
RESULTS

Caffeine affected sperm motility immediately after addition. However, significantly increased motility was recorded only at 2 and 4 mmol.l $^{-1}$ (at the time point 0, Day 1). After 24 h of storage, caffeine at concentration 1 and 4 mmol.l $^{-1}$ positively affected total motility as well as progressive movement of ram sperm measured at all time points (0; 0.5; 2h). Caffeine at 2 mmol.l $^{-1}$ did not alter the parameters of motility.

The positive effect of caffeine on total sperm motility after 48 h cooling-storage was seen at all tested concentrations. The more expressed effect of caffeine after 48 h of storage was observed at the concentration of 4 mmol.l $^{-1}$, where total motility was increased from 31.49% (control group) to 50.30% (Fig. 1). After 72 h storage of sperm, total motility in the control group was 19.58% however immediately after caffeine addition, all tested concentrations significantly increased sperm motility. The highest effect was visible at the concentration of 4 mmol.l $^{-1}$ (51.60%) (Fig. 1).

Effect of caffeine on sperm progressive movement was similar to those for total motility. Values of progressive movement were lower than total sperm motility. Caffeine increased the ratio of progressively moving spermatozoa (Fig. 2).

Figure 2: Effect of caffeine on sperm progressive movement after cooling-storage



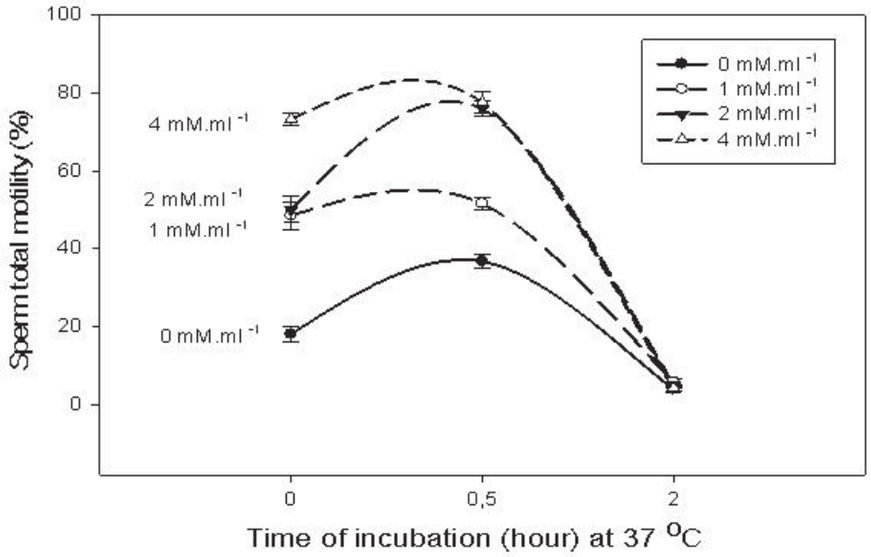
* Significant difference compared to control: P< 0.05

The dynamics of caffeine effect is presented on Fig. 3. Following 72 h of cooling-storage all tested caffeine concentrations affected sperm motility immediately after addition; the greatest effect was noted at 4 mmol.l^{-1}. Sperm motility was the highest after 30 min of caffeine exposure, all concentrations significantly increased sperm motility, but the most expressed effect at this time point was observed at 4 mmol.l $^{-1}$. After 2 h of incubation with caffeine at 37˚C, sperm motility was dramatically decreased. None of the tested concentrations did affect parameters of motility.

DISCUSSION

This study investigated the effect of caffeine on motility of cooling-stored sperm using a CASA system. Caffeine, a phosphodiesterase inhibitor, has been well demonstrated to increase ram sperm motility in a dose-dependent manner. The results of our study demonstrated positive effect of caffeine on sperm motility during cooling-storage at 4˚C. Tested concentrations positively affected sperm total motility and progressive movement immediately after the caffeine addition. However,

Figure 3: Dynamics of caffeine effect on sperm total motility after 72 hours of sperm cooling-storage



* Significant difference compared to control: P< 0.05

the most expressed effect of caffeine was observed at concentration of 4 mmol.l $^{-1}$.

Caffeine was reported to induce an increase in intracellular calcium and an immediate hyperactivation of ram sperm (Colás *et al.*, 2010). The essential role of calcium on sperm capacitation has been proved in several mammalian species (Yanagimachi, 1994), and an increase in intracellular Ca^{2+} concentration has been shown in hyperactivated flagella (Ho and Suarez, 2001).

A number of stimulating effects of caffeine on animal spermatozoa has been reported. A stimulating effect of caffeine on the semen of bulls in a concentration of 2.5 mmol.l $^{-1}$ was confirmed (Bird *et. al*, 1989). Caffeine might increase penetration rate of boar spermatozoa into oocytes by promoting spermatozoa motility and inducing the acrosome reaction of capacitated spermatozoa bound of the *zona pellucida*. Preincubated (capacitated) porcine spermatozoa can not penetrate the oocytes in caffeine-free fertilization medium (Kano *et al*., 1994). Caffeine might promote the capacitation and/or acrosome reaction of boar spermatozoa and, when added to the fertilization medium, caffeine accelerates sperm penetration *in vitro* in pigs (Nagai *et al*., 1993) and mice (Fraser, 1979). Given at 10mmol.l^{-1}, caffeine increased rabbit sperm motility after 24 h of semen refrigeration, whilst lower concentrations (2.5 or 5 mmol.l $^{-1}$) did not affect sperm movement (Lopéz and Alvarino, 2000). Higher concentrations of caffeine (5–10 mmol.l $^{-1}$) did not induce acrosome damages in rabbit sperm (El-Gaafary, 1994). In our study, total and progressive motility of the sperm was increased after 24h of incubation in presence of 1 or 4 mmol.l $^{-1}$ caffeine.

However, along with stimulating effects, a number of negative effects of caffeine have been described. In human *in vitro* fertilization, a higher concentration of caffeine (greater than 2.5 mmol.l $^{-1}$) may adversely affect the sperm fertilization and cleavage of embryos derived from such spermatozoa (Imoedemhe *et al.*, 1992). Similarly, the results of Aitken *et al*. (1983) indicate that caffeine at concentrations of 5 mmol.l $^{-1}$ and above may have potential toxic effect on human spermatozoa.

The fertilizing capacity of the normal human spermatozoon exposed to caffeine did not appear to be enhanced at low concentrations, whereas at the higher concentration (5 mmol.l $^{-1}$) the capacity was adversely affected despite improved motility (Imoedemhe *et al*., 1992). Harrison *et al*. (1980) reported that exposure of spermatozoa to high levels of caffeine may produce some ultrastructural damage in men‘s spermatozoa.

Caffeine at higher concentration (5 mmol.l^{-1}) may cause a reduction of bull sperm motility (Bird *et. al*, 1989), therefore, it is recommended to use caffeine-free fertilization medium for insemination with capacitated spermatozoa in bovine IVF (Momozawa and Fuduka, 2003).

In ram, high concentrations of caffeine caused adverse effects on the sperm motility (Cohen *et al*., 1977; El-Gaafary, 1987). Effect of caffeine on sperm characteristics may be species-specific. Caffeine at concentration of 5 mmol.l^{-1} may cause decrease in fertilization capacity of human and bull sperm, but in rabbits higher concentration (10 mmol.l^{-1}) may cause increased motility. However, this motility increase did not

lead to an improvement of the reproductive parameters. Our results indicate that concentration of 4 mmol.l $^{-1}$ has beneficial effects on ram sperm motility. We observed positive impact of this concentration on sperm motility at all time points. This concentration of caffeine may be a threshold value for stimulating ram sperm.

The study of Lopéz and Alvarino (2000) on rabbit sperm indicates, that the sperm conserved by means of refrigeration over longer periods of time (72–96 h) had lost their capacity to react to added caffeine, due to an exhaustion effect caused by the prolonged periods of conservation.

In contrast, our results show high reaction of ram sperm on added caffeine after 72 h of cooling storage and significant increase in sperm total motility, as well as progressive movement at all tested concentration.

CONCLUSION

Stimulating effect of caffeine on ram sperm motility parameters was confirmed. Caffeine significantly increased motility parameters during sperm long-term cooling-storage at 4°C. Caffeine stimulates sperm motility immediately after adding, but the maximum effect of caffeine was observed after 30 minutes. Caffeine most markedly stimulates sperm motility concentration of 4 mM. However, further analyses, such as penetration test, test of membrane integrity, sperm apoptosis etc. are needed to clarify the influence of caffeine on ram semen parameters and its possible use as an additive to chilled semen.

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REFERENCES

AISEN, E.G. – MEDINA, V.H. – VENTURINO, A. 2002. Cryopreservation and post-thawed fertility of ram semen frozen in different trehalose concentrations. *Theriogenology*, 2002, vol. 57, p. 1801–1808.

AITKEN, R.J. – BEST, F. – RICHARDSON, D.W. – SCHATS, R. – SIMM, G. 1983. Influence of caffeine on movement characteristic, fertilizing capacity and ability to penetrate cervical mucus of human spermatozoa. *J. Reprod Fert.*, 1983, vol. 67, p. 19-27.

BALL, G.D. – FIRST, N.L. 1983. Fertility of ejaculated bull sperm in vitro after exposure to caffeine and cAMP. *J. Anim. Sci.*, 1983, vol. 57, p. 317.

BIRD, J.M. – CAREY, S. – HOUGHTON, J.A. 1989. Motility and acrosomal changes in ionophoretreated bovine spermatozoa and their relationship with in vitro penetration of zona-free hamster oocytes. *Theriogenology*, 1989, vol. 32, no. 2, p. 227-242.

COHEN, M.S. – COLIN, M.J. – GOLIMBU, M. – HOTCHKISS, R.S. 1977. Effects of prostaglandins on sperm motility. *Fertil. Steril*., 1977, vol. 28, p. 78–85.

COLÁS, C. – CEBRIÁN-PÉREZ, J. A. – MUIÑO-BLANCO, T. 2010. Caffeine induces ram sperm hyperactivation independent of cAMP-dependent protein kinase. 2010. *Int. J. Androl.*, 2010, vol. 33, p. 187–197.

DEKA, B.C. – RAO, A.R.1984. Preservation of ram semen. *Indian J. Anim. Sci*. 1984, vol. 54, p. 813–815.

EL-GAAFARY, M.N. 1987. The characteristics of semen from Welsh Mountain and Cambridge rams. PhD Thesis, UCNW, Baangor, 258 pp.

EL-GAAFARY, M.N. 1994. Quality and Fertility of cooled rabbit semen suplemented with cyclic-AMP stimulators. *Anim. Reprod. Sci.*, 1994, vol. 34, p. 307–313.

El-MENOUFY, A.A. – SEIDA, A.A. – FATTOUH, El-S.M. – ABOU-AHMED, M.M. 1986. Effect of caffeine on metabolic activity of ejaculated and epididymal spermatozoa of buffalo. *Reprod. Dom. Anim.*, 1986, vol. 21, no. 4, p. 214-219.

FISER, P.S. – FAIRFULL, R.W. 1984. The effect of glycerol concentration and cooling velocity on cryosurvival of ram spermatozoa frozen in straws. *Cryobiology*, 1984, vol. 21, p. 542–551.

FRASER, L.R. 1979. Accelerated mouse sperm penetration in vitro in presence of caffeine. *J. Reprod. Fertil.*, 1979, vol. 57, p. 337-384.

GARBERS, D.L. – FIRST, N.L. – SULLIVAN, J.J. – LARDY, H.A. 1971. Stimulation and maintenance of ejaculated bovine spermatozoa respiration and motility by caffeine. *Biol. Reprod.*, 1971, vol. 5, p. 336-339.

GIL, J. – LUNDEHEIM, N. – SERQUIST, L. – RODRIGUEZ-MARTINEZ, H. 2003. Influence of extender, temperature, and addition of glycerol on postthaw sperm parameters in ram semen. *Theriogenology*, 2003, vol. 59, p. 1241–1255.

HARRISON, R.F – SHEPPARD, B.L – KALLIZER, M. 1980. Observation on the motility, ultrastructure and elemental composition of human spermatozoa incubated with caffeine. *Andrology*, 2003, vol. 12, p. 34-38.

HO, H.C. – SUAREZ, S.S. 2001. Hyperactivation of mammalian spermatozoa: function and regulation. *Reproduction*, 2001, vol. 122, p. 519–526.

IMOEDEMHE, D.A.G. – SIGUE, A.B. – PACPACO, A. et al. 1992. The effect of caffeine on the ability of spermatozoa to fertilize mature human oocytes. *J. Assist. Reprod. Genet*., 1992, vol. 9, no. 2, p. 155-160.

KANO, K. – MIYANO, T. – MIYAKE, M. – KATO, S. 1994. Effects of caffeine and sperm preincubation on penetration of pig oocytes by ejaculated spermatozoa. *Anim. Sci. Technol.*, 1994, vol. 65, p. 272-276.

LÓPEZ, F.J. –ALVARIÑO, J.M.R. 2000. Effects of added caffeine on results following artificial insemination with fresh and refrigerated rabbit semen. *Anim. Reprod. Sci.*, 2000, vol. 58, p. 147–154.

MOMOZAWA, K. – FUDUKA, Y. 2003. Caffeine in fertilization medium is not essential for bovine IVF by fully capacited spermatozoa. *J. Reprod. Dev*. 2003, vol 49, no. 6, p. 507-512.

NAGAI, T. – MIURA, K. – KIKUCHI, K. – OKAMURA, N. 1993. Effects of caffeine on in vitro fertilization of pig follicular oocytes. *J. Reprod. Dev*. 1993, vol. 39, p. 347-352.

O'HARA, L. – HANRAHAN, J.P. – RICHARDSON, L. – DONOVAN, A. – FAIR, S. – EVANS, A.C.O. – LONERGAN, P. 2010. Effect of storage duration, storage temperature, and diluent on the viability and fertility of fresh ram sperm. *Theriogenology*, 2010, vol. 73, p. 541–549.

PARK, C.K. – OHGODA, O. – NIWA, K. 1989. Penetration of bovine follicular oocytes by frozen-thawed spermatozoa in presence of caffeine and heparin. *J. Reprod. Fertil.* 1989, vol. 86, p. 577-582.

PAULENZ, H. – SODERQUIST, L. – PÉREZ-PÉ, R. – BERG, K.A. 2002. Effect of different extenders and storage temperatures on sperm viability of liquid ram semen. *Theriogenology*, 2002, vol. 57, p. 823–836.

PÉREZ, L.J. – VALCÁRCEL, A. – DE LAS HERAS, M.A. – MOSES, D. –BALDASSARRE, H. 1997.The storage of pure ram semen at room temperature results in capacitation of a subpopulation of spermatozoa. *Theriogenology*, 1997, vol. 47, p. 549–558.

SALAMON, S. – MAXWELL, W.M. 2000. Storage of ram semen. *Anim. Reprod. Sci*., 2000, vol. 62, p. 77–111.

UPRETI, G.C. – JENSEN, K. – OLIVER, J.E. – DUGANZICH, D.M. – MUNDAY, R. – SMITH, J.F. 1997. Motility of ram spermatozoa during storage in a chemicallydefined diluent containing antioxidants. *Anim. Reprod. Sci*., 1997, vol. 48, p. 269–278.

YANAGIMACHI, R. 1994. Mammalian Fertilization, Raven Press, New York , 1994, pp. 189–317.