JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

THE INFLUENCE OF CAFFEINE ON TURKEY SPERMATOZOA MOTILITY DURING *IN VITRO* CULTIVATION AT 41°C

Tomáš Slanina[*1], *Michal Miškeje*[2], *Ida Petrovičová*[2], *Peter Massányi*[1]

Address(es): MSc. Tomáš Slanina,
[1]Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.
[2]Department of Zoology and Anthropology, Faculty of Natural Sciences, Constantine the Philosopher University in Nitra, Nábrežie mládeže 91, 949 74 Nitra, Slovak Republic.

*Corresponding author: slaninatomas@atlas.sk

ARTICLE INFO

Received 12. 10. 2013
Revised 18. 11. 2013
Accepted 8. 1. 2014
Published 1. 2. 2014

Regular article

OPEN ACCESS

ABSTRACT

The aim of this study was to determine the effect of selected concentration of caffeine on the turkey spermatozoa motility during *in vitro* cultivation at 41°C. Semen samples diluted with physiological solution were used as the control sample – KT and experimental samples constituted semen diluted with caffeine solution - 1.25 mg.ml^{-1} – ET; 0.625 mg.ml^{-1} – FT; 0.313 mg.ml^{-1} – GT; 0.156 mg.ml^{-1} – HT; 0.078 mg.ml^{-1} – IT. Motility parameters were analyzed at six time periods: 0, 1, 2, 3, 4 and 5 hours. Measurement was evaluated by the Computer Assisted Semen Analyser. The highest spermatozoa motility of 70.28 % was detected in the sample ET at the beginning of cultivation. Considerable decrease values were observed after 1 hours of cultivation in all samples (KT – 31.15; ET – 27.15; FT – 15.74; GT – 15.98; HT – 18.78; IT – 15.81%). In all experimental samples higher percentage of motility in comparison to the control at the time 1 was detected. However these differences were not significant. Spermatozoa progressive motility showed similar tendency as spermatozoa motility. Non-significant differences were found in all time periods of *in vitro* cultivation between control sample and samples with the addition of caffeine. The results of our study suggest that the addition of caffeine has no significant positive effect on the motility parameters of turkey spermatozoa during *in vitro* cultivation at 41°C.

Keywords: caffeine, turkey, spermatozoa, motility, CASA

INTRODUCTION

In poultry breeding one of the most important issues is the identification of males with high fertilizing ability. Beside genetic conditionings there are many factors affecting this ability. These include age, season, amount of light, state of health and nutrition, number of copulations or ejaculations per day and sperm competition (**Paluch *et al.*, 2013**). In vitro liquid storage of turkey semen is very important in the management of male turkeys, because artificial insemination is the only possible way to ensure and to keep a large-scale production of turkeys.
Semen of the domestic turkey usually cannot be stored for longer than 6 h without a loss in fertilizing capacity, even when oxygenated and stored with the appropriate diluents at a reduced temperature (**Parkhurst *et al.*, 2000**). The fundamental principle is that chicken or turkey semen is diluted in a medium based on the ionic environment of the male reproductive tract (notably with the main anion as glutamate rather than chloride), although hypertonic compared with seminal plasma, with an added glycolytic substrate and a buffer maintaining a pH of around 7.1 **(Hocking, 2009).**
Recently, there has been increased interest in the use of pharmaceutical substances for potentiation and maintenance of spermatozoa motility during and after semen storage. Methylxanthines, such as caffeine and pentoxifylline, have been shown to increase spermatozoa motility in several mammalian species, including sheep, cats, dogs, and humans (**Parkhurst *et al.*, 2000**). Caffeine is generally considered to be an inhibitor of cyclic nucleotide phosphodiesterase, resulting in an increase in cAMP and inducing spontaneous acrosome reaction (**Funahashi and Nagai, 2001**). Caffeine has a strong stimulating effect on respiration and motility of ejaculated spermatozoa (**El-Gaafary, 1994**). Of course, the effect of caffeine is dependent on the concentration. Caffeine added to the TRIS diluent at a concentration of 10 mM or 20 had positively impact on spermatozoa of bulls and goats (**El-Gaafary *et al.*, 1990; Sinha, 1995**).
Although induction of capacitation and the acrosome reaction by caffeine results in a high sperm-penetration rate, caffeine may also be one of the main reasons for the high incidence of polyspermy (**Mao *et al.*, 2005**). **Tatham *et al.*,** (2003) showed that treatment of buffalo and cattle spermatozoa with caffeine significantly decreased embryo cleavage but also tended to decrease embryo development to the blastocyst stage.
The objective of this research was to study the effects of different concentration of caffeine on the turkey spermatozoa mobility parameters during 5 hours of *in vitro* cultivation at 41 °C.

MATERIAL AND METHODS

Biological material

In this study semen was obtained by penal massaging of the turkeys of the line Big 6 (BUT – British United Turkeys Ltd., Chester, United Kingdom) aged from 35 to 42 weeks. Semen samples were a mixture of several groups of identical individual turkeys.

Sample preparation

Semen was diluted in a ratio of 1 part of semen and 200 parts of physiological solution (Sodium chloride 0.9% Braun, B. Braun Melsungen AG, Melsungen, Germany) – Control sample K. At the same ratio the semen was diluted with four different concentrations of caffeine solution: 1.25 mg.ml^{-1} – ET; 0.625 mg.ml^{-1} – FT; 0.313 mg.ml^{-1} – GT; 0.156 mg.ml^{-1} – HT and 0.078 mg.ml^{-1} – IT diluted in the physiological solution. Samples were cultured at 41°C and recorded at six time periods: 0, 1, 2, 3, 4 and 5 hours. The experiment was realized in 6 replicates.

Analytical method

Each of thus prepared samples was evaluated using a Computer Assisted Semen Analyzer (CASA) system – Sperm Vision (Minitub, Tiefenbach, Germany) equipped with a microscope (Olympus BX 51, Japan) to assess the spermatozoa motility. Each sample was placed into Makler Counting Chamber (depth 10 µm, Sefi–Medical Instruments, Germany). Using the turkey specific set up the

following parameters were evaluated – total motile spermatozoa (MOT), progressively motile spermatozoa (PRO), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) in different time periods.

Statistical analysis

Obtained data were statistically analyzed using PC program Excel and a statistics package SAS 9.1 (SAS Institute Inc., USA) using Student's t–test and Scheffe's test. Statistical significance was indicated by p values of less than 0.05; 0.01 and 0.001.

RESULTS AND DISCUSSION

The highest percentage of total spermatozoa motility (MOT) 70.28 % was detected in the sample ET (with the addition caffeine 1.25 mg.ml-1) at the beginning of *in vitro* cultivation. This value was higher than in control sample – KT (65.84%). In other experimental samples lower values than control at the time 0 were detected. Considerable decreased values were observed after 1 hours of cultivation at 41°C in all samples. The most marked decrease of values was detected in the sample KT – 31.15 % and in the sample ET – 27.15%. The highest differences in spermatozoa motility were observed after one hours of cultivation. In all samples higher percentage of motility (ET – 34.69; FT – 43.13; GT – 47.20; HT – 42.97 and IT 41.96 %) in comparison to the control (34.69 %) were recorded. However these different were not significant. Also non-significant differences were found in other time of *in vitro* cultivation. All results of the spermatozoa motility are shown in Figure 1.

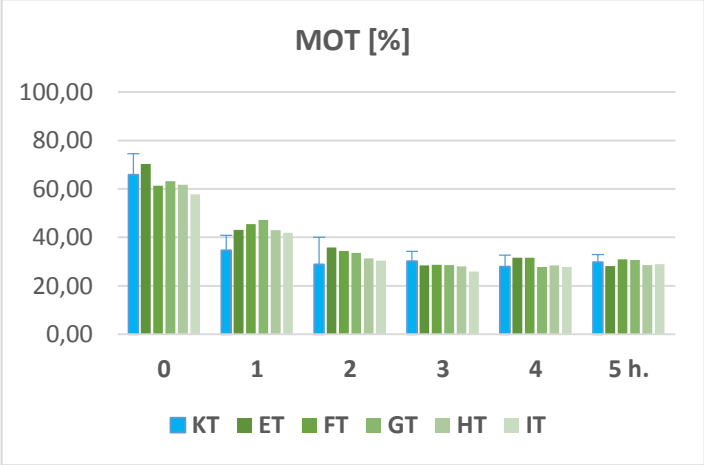

Figure 1 Spermatozoa motility (in %) in samples with different concentrations of caffeine (ET – 1.25 mg.ml^{-1}; FT – 0.625 mg.ml^{-1}; GT – 0.313 mg.ml^{-1}; HT – 0.156 mg.ml^{-1}; IT – 0.078 mg.ml^{-1} and KT – control sample).

Spermatozoa progressive motility (PRO) followed the tendency of spermatozoa motility. Also the highest progressive motility was found in the sample ET (48.93 %) at the time 0 hour (40.97 % in the control – KT). Nevertheless in all analysed samples non-significant differences were found at the beginning of cultivation. When compared the experimental groups to the control sample (12.45 %), higher values were observed after 1 hours of culture in all samples (ET – 18.11; FT – 21.84; GT – 23.30; HT – 17.14 and IT – 16.94 %). Decrease of progressive motility was up to 28.52% in the sample KT and in the analysed samples from 15.21 to 30.82 % after 1 hour of cultivation at 41°C. Very balanced values were detected in all samples after 2, 3, 4 and 5 hours of cultivation. Complete results of this parameter are shown in Figure 2.

The effect of caffeine and pentoxifyline on turkey spermatozoa was studied by **Parkhurst *et al.* (2000).** When caffeine or pentoxifylline was added to semen at 2.5, 5, or 10 mM, no significant effect on spermatozoa mobility was detected, regardless of whether these compounds were added to unstored semen, were present during 6-h storage, or were added following the 6-h storage interval. The mobility of pooled turkey spermatozoa following various storage regimens was assessed by objectively measuring the ability of spermatozoa to penetrate a 2 % Accudenz® solution at 41°C. These results are in accordance with our results, which were obtained by measuring spermatozoa motility parameters using CASA system.

A similar tendency was detected also in other species. **Milani (2010)** evaluated the effect of caffeine on motility of frozen-thawed canine spermatozoa. Semen evaluations were performed using computer-assisted sperm analysis at thawing and during 120 min of incubation at 37°C. The result of his study was that no significant difference between treatments was observed for total motility and progressive motility in any of the incubation periods considered. Progressive and total motility declined in every treatment and in both thawing rates after 30 min of incubation; after that time, a lower decrease of motility was seen at the higher thawing rate rather than at the lower one.

However, results of studies **El-Gaafary *et al.* (1990**) showed a positive effect of caffeine on bull semen quality. Spermatozoa from 4 Friesian bulls were incubated at 37°C for 0, 1, 2, 4 or 6 h in Tris-based or citrate-based diluents supplemented with caffeine at 0 (control), 10, 20, 40 or 80 mM/100 ml diluent. Spermatozoa motility was higher for Tris-based diluents than for citrate-based diluents. Supplementation with caffeine at 10 and 20 mM/100 ml diluent increased ($P<0.005$) the percentage of motile spermatozoa and increased the penetrative ability of spermatozoa into the cervical mucus for both diluents compared with controls.

Positive effect caffeine on boar spermatozoa presented **Yamaguchia *et al.* (2013).** Incubation of frozen-thawed spermatozoa in Modena solution supplemented with 10 mM caffeine for 90 minutes improved ($P<0.05$) percentages of progressive motility, straightness, and linearity of spermatozoa movement compared with no caffeine, without causing damage to plasma or acrosome membranes.

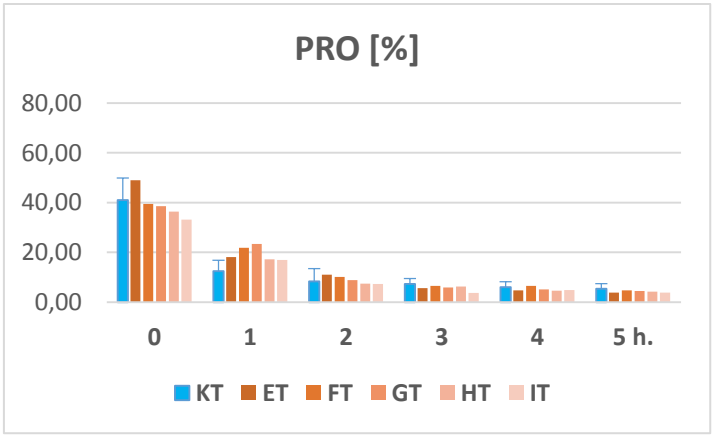

Figure 2 Spermatozoa progressive motility (in %) in samples with different concentrations of caffeine (ET – 1.25 mg.ml^{-1}; FT – 0.625 mg.ml^{-1}; GT – 0.313 mg.ml^{-1}; HT – 0.156 mg.ml^{-1}; IT – 0.078 mg.ml^{-1} and KT – control sample).

CONCLUSION

The aim of this study was to analyse of impact of selected concentration of caffeine on turkey spermatozoa motility parameters. The percentage of spermatozoa motility and progressive motility in all experimental samples were very balanced and without significant differences in comparison to the control sample. The results of this study indicate that the addition of caffeine (1.25; 0.625; 0.313; 0.156 and 0.078 mg.ml^{-1}) has no significant effect on the motility parameters of turkey spermatozoa during *in vitro* cultivation at 41°C.

Acknowledgments: This work was supported by a VEGA project No. 1/0532/11 and by European Community under project no 26220220180: Building Research Centre „AgroBioTech".

REFERENCES

EL-GAAFARY, M.N. 1994. Quality and Fertility of cooled rabbit semen supplemented with cyclic-AMP stimulators. *Animal Reproduction Science*, 34(3-4), 307–313.

EL-GAAFARY, M.N., DAADER, A.H., ZIEDAN, A.E. 1990. Effects of caffeine on bull semen quality and sperm penetration into cervical mucus. *Animal Reproduction Science*, 23(1), 13-19.

FUNAHASHI, H., NAGAI, T. 2001. Regulation of in vitro penetration of frozen-thawed boar spermatozoa by caffeine and adenosine. *Molecular Reproduction and Development*, 58(4), 424-431.

HOCKING, P. M. 2009. Biology of Breeding Poultry. Abingdon : CAB, 2009, 464 p. ISBN 978-1-84593-375-3.

MAO, J., WU, G. M., PRATHER, R. S., SMITH, M. F., CANTLEY, T., RIEKE, A., DIDION, B. A., DAY, B. N. 2005. Effect of methyl-β-cyclodextrin treatment of pig spermatozoa on in vitro fertilization and embryo development in the absence or presence of caffeine. *Theriogenology*, 64(9), 1913-1927.

MILANI, C., FONTBONNE, A., SELLEM, E., STELLETTA, C., GÉRARD, O., ROMAGNOLI. S. 2010. Effect of post-thaw dilution with caffeine, pentoxifylline, 2'-deoxyadenosine and prostatic fluid on motility of frozen-thawed dog semen. *Theriogenology*, 74(1), 153-164.

PALUCH, J., SLANINA, T., MASSÁNYI, P., STAWARZ, R. 2013. The effect of in vitro semen storage temperature and age of males on spermatozoa motility parameters of turkey semen. *Journal of Microbiology, Biotechnology and Food Sciences*, 2(Special issue 1), 1642-1649.

PARKHURST, A. M., KORN, N., THURSTON, R. J. 2000. The Effects of Methylxanthines on the Mobility of Stored Turkey Sperm. *Poultry Science*, 79(12), 1803-1809.

SINHA, M.P. 1995. Effect of methylxanthines on motility and fertility of frozen-thawed goat semen. *Theriogenology*, 44(6), 907-914.

TATHAM, B. G., FEEHAN, T., PASHEN, R. 2003. Buffalo and cattle hybrid embryo development is decreased by caffeine treatment during in vitro fertilization. *Theriogenology*. 59(3-4), 709-717.

YAMAGUCHI, S., SUZUKI, C., NOGUCHI, M., KASA, S., MORI, M., ISOZAKI, Y., UEDA, S., FUNAHASHI, H., KIKUCHI, K., NAGAI, T., YOSHIOKA, K. 2013. Effects of caffeine on sperm characteristics after thawing and inflammatory response in the uterus after artificial insemination with frozen-thawed boar semen. *Theriogenology*. 79(1), 87-93.