

THE EFFECT OF CAFFEINE ADMINISTRATION TO DILUTED SEMEN ON THE PROGENY IN RABBIT

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ABSTRACT : The effect of two caffeine preparations was studied on the reproductive parameters (conception rate, litter size and weight, sex ratio of the offspring) in rabbits. In the experiment, AI was carried out on 78 lactating females (divided into 3 groups). Group A was inseminated with diluted semen containing 1 mg *Coffeinum Natrium Benzoicum* per millilitre, group B was inseminated with diluted semen containing 1 mg

Coffeinum Natrium Salicylicum per millilitre, group C was inseminated with caffeine free diluted semen. The caffeine salicylate improved the conception rate (73.08, 100, 71.88 %) ($P < 0.01$). There was no effect of caffeine on the litter size and weight. In the treated groups the rate of new-born male rabbits increased significantly (65.97 and 63.06 vs. 49.26 %) ($P < 0.01$).

RESUME : Effet de la caféine ajoutée à la semence diluée, sur les paramètres de reproduction des lapins.

L'effet sur les paramètres de reproduction chez le lapin (taux de fécondité, taille et poids de la portée, sexe ratio) de deux préparations caféinées de semence a été étudié. 78 femelles allaitantes, divisées en 3 groupes, ont été inséminées artificiellement. Le groupe A a été inséminé avec un semence diluée contenant 1mg de Coffeinum Natrium Benzoicum par millilitre, le groupe B par une semence diluée

contenant 1mg de Coffeinum Natrium Salicylicum par millilitre, le groupe C par une semence diluée sans caféine. Le salicylate de caféine améliore le taux de fécondité (73,08, 100, 71,88% ; $P < 0,01$). La caféine n'a pas d'effet sur la taille et le poids de la portée. Le pourcentage de lapereaux mâles à la naissance augmente significativement ($P < 0,01$) dans les groupes A et B (65,97 et 63,06 vs 49,26 %).

INTRODUCTION

The rate of moving spermatozoa and the intensity of movement often decrease during the storage of semen. The administration of caffeine to the semen can increase the motility (HAESUNGCHARERN and CHULAVATNATOL, 1973). Caffeine is regularly used for the stimulation of the frozen thawed human semen (RONEN and MARCUS, 1978). AMELAR *et al.* (1980) supposed that the caffeine has an influence on the enzymes of glycolysis in the spermatozoa. The results of DAADER *et al.* (1989) seem to confirm this hypothesis because the fructose content of the bull semen reduced after the caffeine administration. In the opinion of MANN and LUTWAK-MANN (1981) the cAMP through a methylxanthine-mediated (e.g. caffeine) action can improve the quality of semen not merely in respect of motility, but also of fertilising capacity, storability and freezability. Some pharmacological agents act as spermistatic inhibitors of enzymes in spermatozoa. If they do not arrest motility permanently, caffeine can reverse the inhibition *in vitro*.

This work attempts to elucidate the effect of caffeine administration to the semen on the conception rate, the litter size, the weight of litter and the sex ratio of the offspring. During our former utilizations of caffeine for stimulation of the semen the alteration of the offspring sex ratio was observed. Two different type of caffeine was used to prove whether the effects are

due to the caffeine or to the other components of the preparations.

MATERIALS AND METHODS

The experiment was carried out on the experimental farm of the college in summer. In the rabbit house the indoor temperature could go up to 25-30 °C during the summer months. The relative humidity was about 60 %. The ventilation and the illumination of the building was natural (through windows). The bucks and the does were fed with a commercial compound feed (crude protein: 17.1 %, crude fibre: 13.2 %), pelleted in 3 mm pellets and provided *ad libitum*. The ration included some hay, too. Drinking water was available from valve type self-drinkers.

Semen was obtained from the bucks with plastic artificial vagina (the liner was made of condom). Each ejaculate was examined under microscope (200x). The percentage of motile spermatozoa was subjectively estimated. Only ejaculates over 50 % motility were pooled and diluted to a final concentration of $5 \cdot 10^6$ spermatozoa/ml. The semen was diluted with the following extender:

- sodium citrate	2.03 g
- D-glucose	1.50 g
- bis distilled water	100.00 ml
- egg yolk	10.00 %
- antibiotic	100000 IU

Table 1 : Effect of caffeine on the conception rate.

GROUP	n	Conception rate (%)
A	26	73.08 a
B	20	100.00 b
C	32	71.88 a

The values followed by different letters are statistically different to each other ($P < 0.01$).

Group A: inseminated with treated semen (*Coffeinum Natrium Benzoicum*)

Group B: inseminated with treated semen (*Coffeinum Natrium Salicylicum*)

Group C: inseminated with untreated semen (caffeine free)

The effects of two preparations of caffeine were studied in the experiment:

1. *Coffeinum Natrium Benzoicum* (inj. 20 %, Biogal, Hungary): CNB,
2. *Coffeinum Natrium Salicylicum* (inj. a.u.v., Chinoin, Hungary): CNS.

78 Bouscat White lactating females were divided and inseminated in the following groups:

1. Group A (n = 26) was inseminated with diluted semen containing 1 mg caffeine (CNB) per millilitre,
2. Group B (n = 20) was inseminated with diluted semen containing 1 mg caffeine (CNS) per millilitre,
3. Group C (n = 32) was inseminated with caffeine free diluted semen.

The females were prepared for AI with an injection of 20 IU of PMSG (Gonadophyl, Phylaxia, Hungary) i.m. 48 hours before insemination. Ovulation was induced with 2 µg of GnRH (Ovurelin, Reanal, Hungary) i.m. injection at the same time with AI. 1 ml diluted semen was used per doe.

The sex of new-borns was determined by the shape of the urogenital region on the day of birth.

Statistical analysis of the results was carried out using the one-way Analysis of Variance (ANOVA) procedure to compare the least square means (STATGRAPHICS, 1991).

RESULTS

Data of fertility, prolificacy, litter weight and offspring sex ratio are shown in the Tables 1-3.

It seems that the CNS has some influence on the fertilising ability (Table 1.) because the conception rate in this group was 100 %, significantly higher than in the other groups. Neither the caffeine preparations cause statistically significant differences neither in the total born pups nor in the pups born alive (Table 2.). The rate of pups born dead is not higher than usually. According to the litter sizes the weight of litters does not present any statistical differences in none of the studied treatments (Table 3.). Both of the caffeine preparations

Table 2 : Effect of caffeine on the litter size.

GROUP	Total born/litter	Alive/litter
A	7.53±4.16	6.53±3.87
B	8.15±1.57	8.10±1.36
C	7.69±2.47	7.41±2.24

Group A: inseminated with treated semen (*Coffeinum Natrium Benzoicum*)

Group B: inseminated with treated semen (*Coffeinum Natrium Salicylicum*)

Group C: inseminated with untreated semen (caffeine free)

cause statistically significant differences ($P < 0.01$) in the sex ratio of the new-born pups. About 15 % more male was born in the treated groups.

DISCUSSION

The in vitro administration of two different caffeine preparations to the rabbit semen caused an increased fertility rate only in the case of CNS. It suggests that maybe the carriers of the CNS preparation (sodium salicylate and salicylic acid) could result the difference in themselves, but it needs further researches to confirm this hypothesis.

The caffeine has no effect on the litter size, and presumably it has no mutagenic or teratogenous effect. It should also be noted that there was no abnormal or deformed pup among the new-borns.

The caffeine has no effect on the litter weight in this way. This differs from data presented by EL-KELAWY *et al.* (1996) who administered the caffeine orally to the doe during the pregnancy period.

The most important difference was found between the sex ratio of the treated groups and the untreated one. The carrier agents were different in the treated groups and the effect was the same, so it seems that the caffeine can cause a statistically significant alteration (about 15 %) in the sex ratio of the offspring in favour of the males. Probably the caffeine is able to modify the sex ratio on the following way. There is natural difference between the velocity of the X- and Y-chromosome bearing spermatozoa (IVÁNCSICS, 1984). The Y-

Table 3 : Effect of caffeine on the weight of the litters and the rate of the new-born males.

GROUP	Weight/litter (g)	Rate of males (%)
A	428.66± 144.47	65.97± 13.69 a
B	467.75± 69.41	63.06± 8.95 a
C	445.25± 137.17	49.26± 11.74 b

The values followed by different letters are statistically different to each other ($P < 0.01$).

Group A: inseminated with treated semen (*Coffeinum Natrium Benzoicum*)

Group B: inseminated with treated semen (*Coffeinum Natrium Salicylicum*)

Group C: inseminated with untreated semen (caffeine free)

chromosome bearing spermatozoa are faster, and maybe the caffeine can increase this difference. When the spermatozoa arrive at the oviduct the relative majority of the Y-chromosome bearing spermatozoa can develop around the ovum.

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