

# THE EFFECT OF ADDING TRANSPARENT FLUID TO FOWL SEMEN ON FERTILITY AND HATCHABILITY AFTER 24 H OF STORAGE

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## Summary

The effect of adding transparent fluid (TF) to fowl semen on fertilizing capacity of fowl spermatozoa and on hatchability was studied. Diluted semen and semen containing 15% TF were stored for 24 h at 3-5°C and inseminated at weekly basis for 5 consecutive weeks. No significant differences were observed in fertility, hatchability and embryonic mortality among the treatments. The results suggest that TF is not necessarily detrimental to fowl spermatozoa even when mixed with semen and stored outside the body

(Key Words: Fowl Semen, Transparent Fluid, Storage, Fertility, Hatchability)

## Introduction

The original massage technique for artificial collection of semen from the fowl involves pressure of the phallus in order to milk out the distal vasa deferentia (*receptaculum ductus deferentis*) (Burrows and Quinn, 1935). Depending on the degree and frequency of milking, transparent fluid (TF) is often observed, flowing over the phallus, to be added to semen from the ejaculatory ducts (Berens von Rautenfeld et al., 1976). Modifications of the collection technique of fowl semen have been described by Lake (1957) and Van Wambeke (1976). In these methods, semen is expelled directly from the bulbous ends of the vasa deferentia, being free of TF. Concentration of spermatozoa is, therefore, much higher than that of the milking method. Lake (1957) has found an average of  $7.0 \times 10^6$  sperm/ml of semen from Rhode Island Red males and of  $9.3 \times 10^9$  sperm/ml of semen from commercial broiler breeder males. Such values may differ according to the number of collections per week and/or age of the males.

The TF has been considered to be analogous to the accessory reproductive fluids of male

mammals, which is produced during natural copulation (Nishiyama, 1961; Nishiyama and Ogawa, 1961; Berens von Rautenfeld et al., 1976; Fujihara et al., 1986; Fujihara, 1991, 1992). Lake et al. (1985) diluted fowl semen (2-fold) with either TF or with diluents, and inseminated in the distal extremity of the vagina, resulting in 100% fertility for all treatments. Even when the diluted semen was deposited in the cloacal chamber, reasonable fertility was obtained, much higher than that of undiluted fresh semen, free from TF. These results show that TF is not detrimental to spermatozoa, leading to optimum fertility and hatchability.

The previous report indicates that TF contained in the fowl semen is considered to be harmful to spermatozoa stored *in vitro* (Lake, 1956). It is, however, difficult to compass the results of storage experiments in relation to the effect of TF, since this fluid is either absent or present in different amounts. In this experiment, therefore, the effect of a pre-determined addition of TF to semen on storability and fertilizing capacity of fowl spermatozoa and on hatchability was studied.

## Materials and Methods

### Birds

Heavy broiler breeder males, a commercial strain (Ross 208), were used throughout the present experiment. They were kept in individual

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cages under a 14 h/24 h lighting regimen, and received a breeder diet on a restricted basis (135 g/bird/day). At the start of the experiment, males were 38 weeks of age, and were previously selected for semen quality and quantity. Males producing semen, less than 0.35 ml (an average of two collections per weeks), off-colour or watery semen, or semen contaminated with faeces or urine were removed. Dwarf breeder hens (Ross Pm3), the same age as the males, were kept in cages with two birds per cage. Four treatments consisted of 4 replicates of 16 hens each group. The hens were also fed a breeder diet in a controlled way, and light was provided during 16.30 h/24 h. The experiment was conducted during the months July-August under very hot weather conditions.

#### Collection of semen and TF

Semen, free from TF, was collected from about 25 males according to the method of Van Wambeke (1976). Using the same males as used for semen collection, TF was also obtained by squeezing the tumescent lymphatic folds without contamination of semen shortly after semen collection. In this experiment, however, a slight contamination of TF with semen could not be avoided, therefore spermatozoa were removed by centrifugation at 3,000 rpm for 5 min.

#### Semen preparation

Before the start of this experiment, concentration of spermatozoa was determined by the fluorometric method (Bilgili and Renden, 1984), using a Turner fluorometer, model 112 (California, USA). A value of  $7.5 \times 10^6$  sperm/ml was obtained as an average. The normal concentrations of spermatozoa from the broiler breeder males obtained so far in this laboratory varied between 8 and  $9 \times 10^9$  sperm/ml.

During the present experiments, sperm concentration was determined weekly for 5 consecutive weeks and an average value of  $7.74 \pm 0.56 \times 10^6$  sperm/ml was obtained. Therefore, semen volume and sperm number for insemination was pre-calculated according to the average concentration of spermatozoa, which were measured prior to the experiments. Insemination dose was determined to be 0.08 ml for treatments 1 and 2, 0.097 ml for treatment 3 and 0.025 ml for treatment 4, respectively, but as the result, inse-

minated sperm numbers varied. Sperm numbers inseminated per week were determined following insemination, and the average numbers of inseminated spermatozoa were calculated to be around 310, 217, 250 and  $194 \times 10^6$  sperm/hen/week for treatments 1, 2, 3 and 4, respectively.

#### Treatment 1

Five ml of freshly collected semen was diluted with 5.0 ml of an extender (Van Wambeke, 1972) using an Erlenmeyer flask of 25 ml capacity. The flask was stopped with perforated parafilm and stored for 24 h at 3-5°C.

#### Treatments 2 and 3

The difference between treatments 2 and 3 was insemination dose. The 3.5 ml of semen was mixed with 5.0 ml of the diluent, after which 1.5 ml of TF was added. The storage conditions of semen samples were the same as in the treatment 1.

To investigate the effect of sperm number on fertility and hatchability, insemination dose was increased by 15% in treatment 3.

#### Treatment 4

In the control group (treatment 4), semen with a volume of 0.025 ml/hen/week was inseminated according to the predetermined concentration of spermatozoa.

#### Fertility and hatchability

Inseminations were performed in the afternoon for all experimental groups, after most eggs were laid. Hen with a hard shelled egg in the uterus was inseminated mid-vaginally. Eggs were collected daily and stored at 15°C and 70% relative humidity. All hatchable eggs were incubated weekly.

Although hens were inseminated for 6 consecutive weeks, including preliminary period, the results from the first week of the experiment were excluded due to great variability of fertility among hens for the first week of insemination.

Eggs were candled after 7 days of incubation, and questionable eggs were broken out to investigate early embryonic development. Any questionable eggs were classified as infertile. After 19 days of incubation, the remaining eggs were candled again, and dead embryos were referred to as the second and third week mortality. Hatchability data were recorded after 22 days

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of incubation, recording pipped eggs, dead + culled chicks. Fertility was expressed as the ratio of fertile (F) to total (T) eggs (F/T), and hatchability as the ratio of hatched (H) to total (H/T) and to fertile eggs (H/F). Dead germs were expressed as % of fertile eggs during the first, second and third weeks of incubation (DG 1 W/F, DG 2 W/F, and DG 3 W/F) together with pipped eggs (P/F) and dead + culled chicks (D + C/F).

### Statistical analysis

All experimental data (%) were subjected to the analysis of variance, after transformation to arcsine values, according to the method described by Snedecor and Cochran (1980).

### Results and Discussion

Using the same storage method as described above, the previous experiment has shown that optimum fertility and hatchability after 24 h storage of fowl semen were obtained by weekly inseminations varying from 200 to 300 × 10<sup>6</sup> sperm/hen (Van Wambeke, 1984).

Table 1 shows that fertility and hatchability obtained from both fresh and stored semen were high, and no statistical difference (p > 0.05) were found among the four treatments. These results are in well agreement with those of the previous

studies (Van Wambeke, 1984; Van Wambeke and Mestdagh, 1986). It is also suggested that addition (15%) of TF to semen prior to preservation induced no decrease in the fertilizing capacity of spermatozoa, though the inseminated sperm numbers were less than those of TF-free stored semen (treatment 1) (table 1). Increasing insemination volume (and sperm number) by 15% did not lead to any positive effect on fertility and hatchability. Although slightly suboptimal sperm numbers were inseminated in treatments 1 and 2, any obvious differences (p > 0.05) were not found in fertility and hatchability amongst the four treatments (table 1). Unsatisfactory results of fertility have been improved by inseminating a large amount of semen (Van Wambeke, unpublished data). Therefore, reduced number of inseminated spermatozoa is probably not the cause for slightly lowered fertility in the treatments 2 and 3, since the numbers of 200 to 250 × 10<sup>6</sup> sperm/hen led to optimum fertility result in the previous study (Van Wambeke and Mestdagh, 1986).

Embryonic mortality was higher for the first week than for the 3rd week of incubation, which does not agree with the normal data showing highest embryonic mortality in the latter period of incubation (Abbott, 1975). The number of dead germs of fertile eggs for the first week was high due probably to doubtful eggs classified as

TABLE 1. THE EFFECT OF ADDING TRANSPARENT FLUID (TF) TO FOWL SEMEN ON FERTILITY, HATCHABILITY AND EMBRYONIC MORTALITY AFTER 24 H OF STORAGE (%)

Treatments <sup>1</sup>	Parameters studied							
	F/T <sup>2</sup>	H/T	H/F	DG1W/F	DG2W/F	DG3W/F	P/F	W+D/F
Diluted stored semen	97.2 <sup>5</sup>	88.3	90.8	5.6	0.1	2.9	0.3	0.2
Diluted stored semen + TF <sup>3</sup>	96.0	86.5	90.3	5.6	0.1	3.5	0.3	0.2
Diluted stored semen + TI <sup>4</sup>	96.2	85.9	89.2	7.2	0.4	2.5	0.4	0.2
Undiluted fresh semen	97.6	88.0	90.3	5.2	0.2	3.4	0.6	0.3

<sup>1</sup> The number of eggs set per treatment varied from 1433 to 1477.

<sup>2</sup> For explanation of the abbreviations; see text.

<sup>3</sup> Transparent fluid.

<sup>4</sup> Inseminated sperm number was increased by 15%.

<sup>5</sup> For all parameters studied, no significant differences were found between the treatments (p > 0.05).

infertile in this study. In addition, the hot weather, suboptimal storage conditions of hatching eggs, might also be the cause for increment in the dead germs of fertile eggs.

For all other parameters examined here, no significant differences ( $p > 0.05$ ) were observed among the four treatments.

The TF was first considered to be a retarding factor for the storage of fowl semen, since it contains blood-clotting agents which cause the agglutination of spermatozoa (Lake, 1956). On the other hand, the TF has also been confirmed to have no negative effect on the membrane function of fowl spermatozoa stored *in vitro*, even when added to semen at the rate of 30% of total volume of semen (Fujihara et al., 1992).

Collectively, the present results suggest that TF is not always harmful in the process of storing fowl semen, when contained in the rate of 15% of total volume of semen. However, further work should be continued to investigate the effect of semen extenders and dilution rates on the action of TF.

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#### Literature Cited

- Abbott, U. K. 1975. Identifying causes of problems in hatchability. *Poult. Dig.* 34:446-447.
- Ashizawa, K. and K. Okauchi. 1984. Stimulation of sperm motility and oxygen consumption of fowl spermatozoa by a low molecular weight fraction of seminal plasma. *J. Reprod. Fert.* 71:593-598.
- Berens von Rautenfeld, D., K. D. Budras and R. Glassman. 1976. A morphological study of antibody transport in the transparent fluid flowing from the lymph-folds of the copulatory organ into the cloacal lumen of the cock (*Gallus domesticus*). *Zeit. Mikrosk. Anat. Forsch.* 90:989-1008.
- Bilgili, S. F. and J. A. Renden. 1984. Fluorometric determination of avian sperm viability and concentration. *Poultry Sci.* 63:2275-2277.
- Burrows, W. H. and J. P. Quinn. 1935. A method of obtaining spermatozoa from the domestic fowl. *Poultry Sci.* 43:251-254.
- Fujihara, N. 1991. Comparative physiology of avian semen and spermatozoa. In: *Advances in Reproductive Biology*, pp. 132-155. Editor: Younggong, C. and Cheng, Z., Chin. Acad. Sci. Beijing.
- Fujihara, N. 1992. Accessory reproductive fluids and organs in male domestic birds. *World Poultry Sci. J.* 48:39-56.
- Fujihara, N., H. Nishiyama and O. Koga. 1986. The ejection of transparent fluid into the vagina of the hen. *Japan. Poultry Sci.* 23:101-103.
- Fujihara, N., A. Haegeman and F. Van Wambeke. 1992. Physiological effect of transparent fluid on membrane function of the rooster spermatozoa. *Jpn. Poultry Sci.* 29:177.
- Lake, P. E. 1956. A retarding factor in the problem of fowl semen storage. *Proc. 3rd Intern. Congr. Anim. Reprod.*, Cambridge, 3:104-106.
- Lake, P. E. 1957. Fowl semen as collected by the massage method. *J. Agric. Sci.* 49:120-126.
- Lake, P. E. 1984. The male in reproduction. In: *Physiology and Biochemistry of the Domestic Fowl*, Vol. 5, pp. 381-405. Editor: Freeman, B. M. Academic Press, London.
- Lake, P. E., O. Ravie and D. Waddington. 1985. Some effects of the composition of inseminated semen and the site of its deposition on fertility in *Gallus domesticus*. *Anim. Reprod. Sci.* 9:273-284.
- Nishiyama, H. 1961. On the quality and quantity of the cock semen obtained by different collection methods. *Mem. Fac. Agric., Kagoshima Univ.* 4:43-50.
- Nishiyama, H. and K. Ogawa. 1961. On the function of the vascular body, an accessory reproductive organ of the cock. *Jap. J. Zootech. Sci.* 32:89-98.
- Snedecor, G. W. and W. G. Cochran. 1980. *Statistical Methods* 7th ed. Iowa State University Press, Iowa, pp. 215-237.
- Terada, T., M. Watanabe and Y. Tsutsumi. 1984. Possible significance of accessory reproductive fluid in the exhibition of fertilizing ability of spermatozoa in the domestic fowl, *Gallus domesticus*. *Jpn. J. Zootech. Sci.* 55:52-58.
- Van Wambeke, F. 1972. Fertility and hatchability results with fowl spermatozoa stored in fresh and freeze-dried diluent. *Br. Poultry Sci.* 13:179-183.
- Van Wambeke, F. 1976. The effects of two different methods of semen collection on fertility and hatchability results obtained with stored fowl semen. 5th Europ. Poultry Conf., Malta, 1230-1237.
- Van Wambeke, F. 1984. Effect of semen storage time and number of spermatozoa inseminated on the fertility and hatchability of eggs from dwarf broiler breeder hens. *Br. Poultry Sci.* 25:583-587.
- Van Wambeke, F. and M. Mesdagh. 1986. The storage of fowl semen for 24 and 48 hours. *Proc. 2nd Intern. Poultry Breed. Conf. A. I. Workshop, Ayr.* 85-90.