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Effect of aeration on the fertilising ability of turkey semen stored for 48 hours at 5 and 15 °C : A study from the 33rd to the 47th week of age

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Summary. Over a 14-week breeding period (33rd to 47th week of age), turkey semen was stored for 24 and 48 h at 5 and 15 °C prior to weekly inseminations. Semen was diluted 4-fold and either held still or agitated in 25-ml conical flasks throughout the storage period. The highest fertility was obtained with semen that was agitated and held at 5 °C. The improved fertility was associated with increased oxygenation during storage of the semen samples caused by agitation. The mean fertility over the 14-week breeding period for semen samples stored under the different conditions at 5 °C were : 92 % (agitated, 24 h) ; 85.6 % (agitated, 48 h) ; 79 % (still, 24 h) ; 63.6 % (still, 48 h) ; 93.8 % (control, freshly diluted). The oxygen content of the diluted samples after 24 h storage at 5 °C were : 370 nmoles/ml (agitated) ; 70 (still).

Introduction.

It is generally recognised that turkey semen is more difficult to store *in vitro* than fowl semen, especially beyond a few hours (see Lake and Ravie, 1982 for a review). Conversely, *in vivo* turkey spermatozoa survive in the female reproductive tract for 6 to 8 weeks compared to the shorter period of 10 to 14 days for fowl spermatozoa. Metabolic differences between spermatozoa of the two species have also been identified (McIndoe and Lake, 1973 ; Sexton, 1974 ; Lake and McIndoe, 1976 ; McIndoe and Mitchell, 1978 ; Wishart, 1981, 1982).

Recently, Wishart (1981) indicated that turkey spermatozoa were sustained better when aerated during storage for 24 h and this was probably due to satisfying their normal metabolic requirements for the maintenance of energy production. Cherms (1981) and Sexton (1981), applying the principle of semen agitation during storage, obtained preliminary evidence of its beneficial effect for storing semen for 6 h. However, from their results it appeared that both the method of agitation and the temperature during storage may be critical factors determining the level of fertility success after longer storage periods.

Greater flexibility could be provided in breeding by artificial insemination if it was possible to store semen for up to 48 h or more at above-zero temperatures ;

apart from being able to create sire farms semen might be transported over longer distances within and between some Countries for selective breeding purposes. In the present study turkey semen was stored on a large scale for up to 48 h under different conditions before each weekly insemination on the Nicholas Turkey Breeding Farms, Sonoma, California. Semen was either aerated, using an orbital shaker, or non-aerated during storage at 5 °C or 15 °C and effects on the fertilising ability of spermatozoa were tested over a 14-week period, commencing with the week before the peak of lay in the turkey flock in their first breeding season.

Materials and methods.

Animals. — Three hundred and sixty hen and 56 male turkeys of a Nicholas Large White strain were hatched simultaneously and used in the experiments when they were between 33 and 47 weeks old. They were housed in pens and fed *ad libitum.* The males from 20 to 22 weeks of age were fed a special breeder diet and the females were subject to a phased feeding programme (Cherms, 1984). The males were given 12 h light/24 h using incandescent light of 5 Lux intensity. The hens were given 14 h light/24 h using cool white fluorescent tubes of 300-500 Lux intensity (Cherms, 1984).

Semen treatment. — Semen was obtained by the conventional massage method and pooled, care being taken to minimise contamination with urine and faeces. The males were on a regular routine of semen collection and were not rested for more than 4 d before semen samples were taken for storage.

Under the field conditions, the density of spermatozoa was not measured each week but from past experience of the strain of turkey it was expected to be on average 12×10^9 spermatozoa/ml semen throughout the entire 14-week period. In the present study it was checked in the 9th week and found to be 12×10^9/ml semen using the haemacytometer method. Knowledge of the precise number of spermatozoa inseminated each week was not considered essential for the purposes of the present experiment because each treatment was being compared weekly on aliquots of the same semen sample for its suitability to maintain spermatozoa during storage.

Semen was diluted 4-fold with a diluent composed as follows : sodium glutamate.H_2O, 1.1 g ; tri-potassium citrate.H_2O, 0.128 g ; magnesium acetate.4 H_2O, 0.08 g ; glucose, 0.36 g ; sodium acetate (anhydrous), 0.146 g ; di-sodium hydrogen phosphate (anhydrous), 0.136 g ; BES (N,N-bis(2-hydroxyethyl)-2-amino ethane sulphonic acid), 3.05 g ; M-NaOH, 5.6 ml dissolved in 100 ml distilled water. The diluent was a modification of that reported previously (Lake and Ravie, 1982) to contain glutamate, sodium, potassium, magnesium and phosphate concentrations equivalent to those found in turkey ductus deferens fluid. It was buffered at pH 7.1 and had an osmotic pressure 402 mosm/kg H_2O.

Semen was collected, pooled and diluted usually at 22 °C and 15 to 20 min elapsed between the collection of semen and the commencement of storage. On each occasion (weekly), the diluted semen was divided for eight different storage treatments and a control (see table 1).

Diluted semen (volumes varying on each occasion between 3 and 8 ml) for storage before use was kept in 25-ml Nalgene plastic conical flasks, loosely stoppered with paper tissue, in cold rooms held at 5 °C or 15 °C. Samples were either kept still or agitated during storage for 24 and 48 h (see table 1 for treatments). The flasks were agitated in air during storage on an orbital shaker (Tekpro Tektator V. Variable Speed Rotator, American Scientific Inc) proscribing a circular motion and rotating at 150 rev./min.

A separate sample of pooled semen from the same males was collected at the end of the experiment period and divided into four 4 ml portions which were then stored for 24 h in 25-ml flasks. Two portions, one held still and the other shaken, were kept at 5 °C and the other two treated similarly at 15 °C. The oxygen content of each of these portions was measured on the farm at Sonoma at intervals of 2, 6, 12 and 24 h with a Clark-type oxygen electrode dipped into the samples. Standardisation of oxygen content was performed by the method of Robinson and Cooper (1970). The 4 ml portions of diluted semen, containing approximately 3×10^9 spermatozoa per ml, was an average volume of the samples stored in 25-ml flasks from week to week for fertility testing.

Insemination procedure. — Before the beginning of the breeding period, 9 groups of hens [40 per treatment ; 2 replicates (pens) of 20 hens] were assigned at random, each to receive diluted semen stored differently or freshly diluted semen (control) from the same males (see table 1). The pens of 20 birds were randomised for position within the same house. The hens were inseminated weekly for 14 weeks commencing with the week before the peak of lay in the turkey flock. They were inseminated twice in the week before saving eggs for week 1, which is commonly practised commercially.

The inseminations were done into a well-everted vagina and performed on each occasion between 13.30 and 15.00 h when most of the hens were estimated not to have hard-shelled eggs in the uterus (shell-gland). The hens were accommodated about 300 yds from the cold rooms where the semen was stored. The ambient temperature varied between 21 and 37 °C during the 14-week period, and for each insemination session the vessels containing the semen were carried to the female house in stainless steel pans containing water at either 5° or 15 °C. The time elapsing from the removal of semen from the cold rooms until the last hen was inseminated with a particular treatment sample was never more than 15 min. The insemination procedure was organised so that each weekly session was started with a different treatment in order to randomise over time.

Each hen received gradually increasing doses of semen until week 6 (0.02-weeks 1 and 2 ; 0.03-week 3 ; 0.05-weeks 4 and 5 ; 0.06 ml-week 6 and subsequently). The resulting numbers of spermatozoa inseminated per dose were approximately 60, 90, 150 and 180 million, respectively. It was planned to give 0.06 ml diluted semen throughout but insufficient semen was given by the males in the early sessions. This does not invalidate the objectives or results because the purpose of the study was to examine any differences between treatments, all of which were administered simultaneously each week.

Fertility differences between treatments were consistent week by week, relative to each other, over the 14-week experimental period. The square roots of

TABLE 1

The fertility of turkey semen stored for 24 and 48 h at 5° or 15 °C and either agitated or kept still during storage. The results are combined for each 2-week period.

Weeks in lay	Still				Agitated				Control (freshly diluted)
	24 h		48 h		24 h		48 h		
	5°	15°	5°	15°	5°	15°	5°	15°	
1/2	(*) 223/298 (74.8)	141/277 (50.9)	144/313 (46.0)	80/247 (32.4)	237/274 (86.5)	226/319 (70.8)	194/267 (72.6)	96/304 (31.6)	266/297 (89.6)
3/4	309/400 (77.2)	266/405 (65.7)	231/421 (54.9)	168/380 (44.2)	369/402 (91.8)	344/432 (79.6)	317/359 (88.3)	190/406 (46.8)	389/415 (93.7)
5/6	214/253 (84.6)	232/332 (69.9)	285/381 (74.8)	112/338 (33.1)	302/330 (91.5)	311/378 (82.3)	299/338 (88.5)	163/374 (43.6)	349/359 (97.2)
7/8	296/352 (84.1)	179/316 (56.6)	273/357 (76.5)	65/354 (18.4)	316/338 (93.5)	232/356 (65.2)	300/339 (88.5)	114/354 (32.2)	389/408 (95.3)
9/10	246/293 (84.0)	201/338 (59.5)	210/289 (72.7)	39/319 (12.2)	318/338 (94.1)	169/320 (52.8)	289/321 (90.0)	101/356 (28.4)	311/329 (94.5)
11/12	221/293 (75.4)	190/305 (62.3)	223/331 (67.4)	94/310 (30.3)	266/278 (95.7)	229/299 (76.6)	272/304 (89.5)	168/336 (50.0)	321/345 (93.0)
13/14	199/273 (72.9)	183/281 (65.1)	151/294 (51.4)	48/310 (15.5)	236/262 (90.1)	206/293 (70.3)	229/292 (78.4)	106/276 (38.4)	282/307 (91.9)
Total	1 708/2 162 (79.0)	1 392/2 254 (61.7)	1 517/2 386 (63.6)	606/2 258 (26.8)	2 044/2 222 (92.0)	1 717/2 397 (71.6)	1 900/2 220 (85.6)	938/2 406 (39.0)	2 307/2 460 (93.8)

(*) Values are total number of eggs fertile/total number of eggs laid with number in parenthesis representing the mean percentage fertility. Residual mean square on the arc sine scale equals 0.0044.

the total percentage of fertile eggs laid over the entire 14 weeks for each group of 20 hens were transformed to arc sine values for the analysis of variance. Thus the data were considered to have arisen from a 2^3 factorial design. Differences between treatment means were compared with their standard errors (Student's t-test).

Results and discussion.

Agitation of the semen samples during storage benefited the retention of the fertilising ability of the spermatozoa (table 1), irrespective of the temperature of storage ($p < 0.001$). The tests were carried out weekly over a 14-week breeding period and confirmed, on a larger scale, the experimental observations of Wishart (1981) who demonstrated that turkey spermatozoa survived better *in vitro* if some degree of aeration occurred during storage. The data in table 2 would indicate that there was greater oxygenation in the agitated samples during storage under the conditions of the present experiment. The apparent differences in oxygen content of agitated semen samples held at 5° and 15 °C are possibly a function of the higher oxygen solubility at the lower temperature (Wishart, 1984). The overall higher (5-fold) oxygen content in the still samples at 5° compared to 15 °C cannot be accounted for by solubility differences alone and may be explained by the lower rate of oxygen uptake by spermatozoa at the lower temperature (Wishart, 1984). The oxygen content of the still flasks held at 5 °C is a balance between the rate of diffusion of oxygen into the medium and the rate of its utilisation by the diluted semen. The former rate is a function of the surface area : volume ratio of the sample. This was variable over the 14-week course of the present work since volumes between 3 and 8 ml were stored (see Materials and methods) depending on the weekly availability of semen. Thus the oxygen content of weekly samples may have been higher or lower than the figures shown in table 2 for 4 ml aliquots of stored semen. It is likely that the average fertility of the still samples (table 1) would have been more depressed if each time the volume was constant at 8 ml. It was noticed that weekly fluctuations in fertility were greater amongst still than agitated samples, which could support this contention.

TABLE 2

The oxygen contents (nmoles/ml) in split portions of a diluted, pooled turkey semen sample, which were either agitated or kept still during storage for 24 h at 5° and 15 °C. See Materials and methods for conditions of storage and details of oxygen measurement.

Interval of storage (h)	5 °C		15 °C	
	Still	Agitated	Still	Agitated
2	89	326	18	276
6	74	333	18	273
12	78	355	10	267
24	70	370	12	300

The low level of oxygen in the still samples held at 5 °C was sufficient to aid the maintenance of a level of fertilising ability ; true anaerobic conditions caused considerable deterioration in fertility of turkey semen after 24 h storage (Wishart, 1981).

It is not possible in the present experiment to compare the significance of differences in oxygen content in agitated samples within each temperature. The apparent high level after 24 h at 15 °C may merely reflect a high mortality of spermatozoa in this case leaving more of the diffused oxygen unused.

Statistically, the fertility from the « 24 h-5°-agitated » semen was significantly less than that from the freshly diluted control ($p < 0.001$). However, the difference over the 14-week breeding period was only 1.8 %, which may be considered to make the routine use of 24-h stored semen a practical proposition. Overall, the semen stored at 5° retained the better fertilising capacity compared with that at 15 °C ($p < 0.001$) and semen after 24 h was better than that stored for 48 h ($p < 0.001$). The difference in fertility between semen stored at 5° as against 15 °C was greater after 48 h compared with 24 h storage in both the agitated and still samples ($p < 0.05$). The cause of the progressive loss of viable spermatozoa is under investigation.

It was evident from an examination of the weekly fertility data that after 48 h at 15 °C, the fertility level of the agitated sample was well below that kept still for 24 h at 15 °C. On the contrary, after 48 h at 5 °C the « agitated » fertility was higher than that kept still for 24 h at 5 °C. This may reflect the creation of an unidentified factor by agitation which causes a greater depressive effect on the fertilising capacity of the total sample with time at the higher temperature.

Clearly, agitation improved the maintenance of the fertilising ability for up to 48 h under the conditions of the present experiment. Agitation may act simply to prevent the sedimentation of spermatozoa, thus avoiding the creation of an adverse microenvironment. However, in view of some recent work showing the benefit of oxygenation on the metabolism of spermatozoa (Wishart, 1982, 1984) it is likely that the beneficial effect of agitation was partly or wholly due to the provision of sufficient oxygenation to sustain the energy requirements for the maintenance of the viability of the spermatozoa. However, from the results of storing at 15 °C it is clear that, given oxygen, other temperature-dependent factors deleterious to the survival of spermatozoa eventually become limiting. Further improvements in storage could result from attempts to identify these factors and nullify their effects.

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Résumé. *Effet de l'aération sur le pouvoir fécondant du sperme de Dindon conservé pendant 48 h à 5 et 15 °C.*

Pendant une période de reproduction de 14 semaines, du sperme de Dindon a été conservé pendant 24 et 48 h à une température de 5 et 15 °C avant les inséminations hebdomadaires. Le sperme a été dilué 4 fois et conservé avec ou sans agitation. La meilleure fertilité a été obtenue avec le sperme qui avait été agité et maintenu à une température de 5 °C. L'amélioration de la fertilité est associée à l'augmentation de l'oxygénation causée par l'agitation. Les taux de fécondation moyens pendant une période de reproduction de 14 semaines pour le sperme conservé à 5 °C ont été les suivants : avec agitation, pendant 24 h : 92 % ; avec agitation pendant 48 h : 85,6 % ; sans agitation pendant 24 h : 79 % ; sans agitation pendant 48 h : 63,6 %. Pour les témoins (sperme fraîchement dilué), le taux de fécondation était de 93,8 %. La teneur en oxygène du sperme dilué conservé à 5 °C pendant 24 h était respectivement de 370 nmoles et 70 nmoles selon que le sperme était agité ou non.

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