Effect of pH on the metabolism and fertility of turkey spermatozoa

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Summary. Oxygen uptake during a 4-h incubation period at 37°C, and the motility of the spermatozoa before and after incubation, increased significantly with increasing pH from 6.3 to 8.8. No interaction between buffer and pH was noticed. In a second series of experiments on the aerobic metabolism of turkey spermatozoa, the effect of the pHs 6.8, 7.3 and 7.8 was studied. Fructose was formed from glucose without regard to the pH of the medium. The glucose consumption, i.e. the glucose disappearance minus fructose formation, the lactic acid accumulation, and the oxidation of glucose and of other substances, were higher, although not always statistically, at pH 7.8 than at pH 6.8. The percentage of fertile eggs during the 3rd week of collection after insemination with fresh semen diluted in the pH 7.8 medium was significantly lower than that with semen diluted in the pH 6.8 or 7.3 media. After 4 h of storage at 15°C, the decrease in the fertility of spermatozoa in the high pH medium was apparent from the 1st week of collection.

Introduction

Hydrogen ion concentration is one of the factors which influence motility, viability and metabolism of spermatozoa (Mann, 1964). Few studies have been carried out of the effect of pH on the motility, respiration and fertility of turkey spermatozoa. Brown, Graham & Crabo (1972) reported that the motility of turkey spermatozoa was maintained better at pH values of 7.1-7.5 than at lower or higher ones. Sexton & Giesen (1982) reported that turkey spermatozoa showed higher motility and respiration, and lower fertility, at pH 7.5 than at pH 6.5; the effect of pH was, however, conditioned by the osmolarity of the medium. Lake & Ravie (1982) obtained better fertility of turkey spermatozoa at pH 7.1 than at pH 5.8 or 7.4.

It is possible that the various functions of spermatozoa may respond differently to different buffers or hydrogen ion concentrations and that there may also be interactions between them. The present study deals with the effect of a wide range of buffers and hydrogen ion concentrations and their interactions on the motility, metabolic activity and fertility of turkey spermatozoa.

Materials and Methods

Animals. Broad-Breasted White turkeys (British United Turkeys, T6) were used. The 100 hens and the toms were housed in individual cages at 25°C under continuous 24 h white fluorescent lighting of 90-500 lx and maintained on a commercial breeder's ration fed *ad libitum*. The males, 8 months old at the start of the experimental period, had no previous fertility record and were selected for

their good semen quality. They were kept on a regular routine of semen collection, spaced not more than 5 days apart, to minimize the number of degenerated spermatozoa in the ejaculates (El-Jack & Lake, 1966). The hens were in their first season of egg production. The insemination trials started when the flock reached 50% laying.

Semen collection. Pooled ejaculates were obtained by abdominal massage (Lake & Stewart, 1978). The sperm density, measured with an EEL colorimeter calibrated against haemocytometer counts, ranged from 7.5 to 11×10^9 cells/ml. Only spermatozoa with vigorously swirling motion, assessed by microscopic estimation, were used. The semen was kept at 20°C until the start of the laboratory work, 30–40 min later.

Metabolic tests. The composition of the medium used for the sperm suspensions was: 111 mM-NaCl, 3 mM-KCl, 0.8 mM-CaCl₂, 0.5 mM-MgCl₂, 2.0 mM-NaH₂PO₄, 15 mM-glucose, 7.0 µg vitamins/100 ml, and 1.0 mg amino acids/100 ml (Eagle's Basal Medium without L-glutamine: Bio-Lab Ltd, Jerusalem, Israel), 200 U penicillin/ml, 200 µg streptomycin/ml, 10 µg neomycin/ml and 10 µg kanamycin/ml. The osmolarity of the medium was 330 mosmol, which was maintained at the different pH values by the addition of acid/alkali of the same osmolarity to the buffers used to obtain the desired pH.

To cover a pH range of 6.3–9.3, six different buffers were used at a concentration of 15 mM, having pK values at 20°C around the pH it was desired to maintain. The buffers used were Aces (*N*-[2-acetamido]-2-aminoethane sulphonic acid)—pK 6.9; Hepes (*N*-2-hydroxyethyl piperazine-*N'*-2-ethane sulphonic acid)—pK 7.55; Tricine (*N*-Tris[hydroxymethyl]methyl glycine)—pK 8.15; glycylglycine—pK 8.4; Tris ([hydroxy-methyl]amino methane)—pK 8.3; and glycine—pK 9.2.

In each experiment the effect of 2 or 3 pH values, provided by a particular buffer, was examined. The pH ranges of 2, and sometimes 3, buffers overlapped, and it was therefore possible to examine the effects of the different buffers at the same pH (except for the extreme cases). To remove the seminal plasma, semen was diluted 1:1 with the medium buffered with Hepes pH 7.3 and centrifuged at 1200 *g* for 15 min. The supernatant was discarded and the spermatozoa were resuspended to the original volume of semen with the same medium but buffered at one (the lowest) of the pH values to be tested. The sperm resuspension was then split and further diluted to a concentration of 1×10^9 cells/ml with medium buffered at 2 or 3 pH values.

The oxygen uptake of the spermatozoa was measured at 37°C and at 30-min intervals after a 20-min equilibration time by the direct Warburg technique (Umbreit, Burris & Stauffer, 1957) with 13-ml flasks containing 1.0 ml sperm suspensions (1×10^9 spermatozoa). The glycolytic activity of these spermatozoa was assessed by measuring glucose disappearance (Huggett & Nixon, 1957), lactic acid accumulation (Barker & Britton, 1957), and the formation of fructose in the medium (Mann, 1948). Glucose consumption was calculated by reduction of the accumulated fructose from the glucose which had disappeared. The CO₂ production from glucose was determined by using [U-¹⁴C]glucose (sp. act. 283 mCi/mmol: Radiochemical Centre, Amersham, U.K.), which was added to the medium, and the ¹⁴CO₂ trapped in the KOH of the centre well was assayed (Murdoch, White & Seamark, 1970).

The pH of the sperm suspensions was measured with a combined microelectrode and a pH-meter of a TTT 1C titrator (Radiometer, Copenhagen), before and after incubation. Sperm motility before and during incubation was assessed in the 1×10^9 sperm suspensions by estimation of the percentage of motile spermatozoa (0–100) at $\times 400$ magnification with a differential interference-contrast microscope at room temperature.

Fertility tests. The collected semen was divided into 2 or 3 portions, diluted at a ratio of one part of semen with two parts (1:2 v/v) of medium buffered with Hepes at pH 6.8, 7.3 or 7.8, and used within 20–30 min (fresh) or after storage for 4 h at 15°C. Storage was in 10 mm-diameter vials containing 1 ml sperm suspension and plugged lightly with tissue paper (Lake & Ravie, 1979). The vials were slightly agitated at 30-min intervals to provide a degree of aeration (Wishart, 1981). Sperm motility was scored before and after storage. The insemination dose was 0.06 ml diluted semen and contained $150\text{--}220 \times 10^6$ spermatozoa.

Three series of single inseminations were carried out. At each insemination session the individuals were randomly assigned to treatment groups and the aliquants of the differently treated samples were inseminated consecutively and in rotation. Eggs were collected during Days 2–22 after insemination. Fertility was determined by candling the eggs after 8 days of incubation.

Since time of insemination relative to the time of oviposition has been shown to have a major effect on fertilization (Christensen & Johnston, 1975), inseminations with fresh and stored semen were carried out at the same hour of the day. Therefore, semen designed to be used after storage was collected from one group of randomly chosen males 4 h before the scheduled time of inseminations, whereas semen to be used in the fresh state was collected from another group of males immediately before the inseminations. It was assumed that the differences in the fertility between fresh and stored semen were due to the effect of storage rather than to the differences in the fertility between the different sperm samples, because only semen samples of very high quality were used.

Statistical analysis. The 'net effect' of the pH after its dissociation from the effect of the different buffers on the oxygen uptake and motility of the spermatozoa was calculated by using the least square estimates of the adjusted means according to the General Linear Models procedure (SAS Users Guide, 1979), and the means of each pH value were compared by the *t* test. The results of the aerobic metabolism, which included respiration and glycolysis, were subjected to analysis of variance and to Duncan's Multiple Range Test. The χ^2 test was used to compare the fertilization rates of semen under various treatments.

Results

Metabolic assays

In a first series of experiments the oxygen uptake and motility of the spermatozoa during a 4-h incubation period in different buffers and in different pH values were assessed (Table 1). The effect of the buffer at each given pH was not found to be significant. The calculated 'net effect' of the pH on the O₂ uptake and motility, however, showed that the total O₂ uptake and the motility of the spermatozoa at the end of the incubation period increased significantly with rising pH up to 8.8. At lower pH values sperm motility was depressed even before incubation and spermatozoa were immobile before the end of the incubation period (Table 1).

Table 1. Oxygen uptake ($\mu\text{mol O}_2/10^9$ spermatozoa/4 h) by turkey spermatozoa in different buffers and hydrogen ion concentrations

Buffer	pH*						
	6.3	6.8	7.3	7.8	8.3	8.8	9.3
Aces	2.7 $\pm$ 0.3 (3)	4.7 $\pm$ 0.6 (3)	6.8 $\pm$ 0.6 (3)				
Hepes		5.5 $\pm$ 0.9 (5)	8.1 $\pm$ 0.4 (5)	11.8 $\pm$ 0.5 (9)			
Tricine				9.2 $\pm$ 0.7 (9)	10.6 $\pm$ 0.8 (9)		
Glycylglycine					9.6 $\pm$ 0.6 (9)	12.6 $\pm$ 0.5 (9)	
Tris					10.8 $\pm$ 1.3 (6)	12.6 $\pm$ 0.7 (6)	
Glycine						12.0 $\pm$ 0.5 (6)	11.1 $\pm$ 0.4 (6)
Calculated 'net effect' of pH†							
Oxygen uptake	1.5 $\pm$ 1.8 ^a	3.3 $\pm$ 1.0 ^a	5.8 $\pm$ 0.8 ^b	9.5 $\pm$ 0.6 ^c	10.9 $\pm$ 0.5 ^c	13.5 $\pm$ 0.7 ^d	12.7 $\pm$ 1.3 ^{cd}
Motility: initial-final (% motile spermatozoa)	38-0	45-0	48-27	55-40	84-46	77-51	73-58
Final pH	6.25 $\pm$ 0.15	6.76 $\pm$ 0.09	7.11 $\pm$ 0.07	7.54 $\pm$ 0.05	7.88 $\pm$ 0.05	8.18 $\pm$ 0.05	8.76 $\pm$ 0.11

* Values are mean $\pm$ s.e.m. for the no. of experiments shown in parentheses.

† The results are least square estimates of the adjusted means $\pm$ s.e.m. (see 'Materials and Methods').

Different superscript letters represent significantly ($P < 0.01$) different values (*t* test).

In a second series of experiments the glucose metabolism was measured at three pH levels (6·8, 7·3, 7·8), which could be covered by a single buffer (Hepes) during a 2-h incubation period, i.e., before the motility and viability of the spermatozoa were severely affected by the low pH. By simultaneous measurement of O₂ uptake and the amount of glucose which was oxidized, the rate of oxidation of substances other than glucose could also be calculated, by subtracting the O₂ needed for the oxidation of the glucose from the total O₂ uptake. The results are presented in Table 2. Significantly higher O₂ uptake was observed when spermatozoa were incubated in media with higher pH values. No particular trend was found in the glucose disappearance at the different pHs, but a slight decrease in the formation of fructose with increasing pH resulted in a non-significant increase of glucose consumption. The lactic acid accumulation changes at the different pHs were also non-significant. However, the CO₂ production from glucose was significantly higher at pH 7·8 than at pH 6·8, and more pronounced during the 2nd than during the 1st hour of incubation. Finally, a large proportion of the O₂ uptake was used for the oxidation of substances other than glucose, and more during the 1st than the 2nd hour of incubation. Moreover, during the first hour of incubation a significant increase in this oxidation was apparent at the highest pH medium.

Table 2. Aerobic metabolism of turkey spermatozoa at different pH levels (μmol/10⁹ spermatozoa)

Length of incubation (min)	pH	Glucose disappearance	Fructose formation	Glucose consumption	Lactic acid accumulation
0–60	6·8	1·77 ± 0·28	1·17 ± 0·08	0·60 ± 0·28	0·26 ± 0·07
	7·3	1·69 ± 0·22	1·03 ± 0·13	0·66 ± 0·28	0·18 ± 0·03
	7·8	1·74 ± 0·23	0·99 ± 0·16	0·75 ± 0·24	0·32 ± 0·06
60–120	6·8	1·46 ± 0·21	1·09 ± 0·16	0·37 ± 0·15	0·12 ± 0·04
	7·3	1·85 ± 0·35	0·97 ± 0·16	0·89 ± 0·25	0·15 ± 0·03
	7·8	1·63 ± 0·22	0·90 ± 0·10	0·73 ± 0·19	0·29 ± 0·12

Length of incubation (min)	pH	CO ₂ production from glucose	O ₂ uptake	O ₂ used for other than glucose oxidations (calculated)
0–60	6·8	0·78 ± 0·16 ^A	2·67 ± 0·22 ^A	1·89 ± 0·16 ^A
	7·3	1·44 ± 0·14 ^B	3·61 ± 0·05 ^B	2·17 ± 0·15 ^A
	7·8	1·36 ± 0·17 ^B	4·17 ± 0·30 ^B	2·81 ± 0·24 ^B
60–120	6·8	1·12 ± 0·16 ^a	2·31 ± 0·24 ^a	1·19 ± 0·15
	7·3	1·78 ± 0·36 ^{ab}	3·16 ± 0·17 ^b	1·38 ± 0·39
	7·8	2·53 ± 0·25 ^b	3·75 ± 0·29 ^b	1·22 ± 0·22

Values are mean ± s.e.m. for 5 replicates.

Comparisons for the significance of the results were made vertically and separately for 0–60 and 60–120 min of incubation. Different superscript letters indicate statistically significant differences ($P < 0·05$) (Duncan's Multiple Range Test).

Fertility tests

The results (Table 3) show that the fertilizing ability of turkey spermatozoa is preserved better in media with low than with high pH values. With fresh semen, high and similar fertilization rates were obtained at all pH levels during the 1st and 2nd weeks of egg collection. During the 3rd week of collection the decrease in the fertilization rate was statistically significant with the semen diluted at the highest pH (7·8). When stored semen was used, the decline in the fertility at pH 7·8 was already apparent from the 1st week of egg collection.

Table 3. Percentages of fertile eggs after single inseminations with fresh or stored (for 4 h) semen suspended in media at different pHs

Semen type	pH*	Days after insemination†		
		2-8	9-15	16-22
Fresh	6.8 (27)	89.0 (100) ^a	84.0 (100) ^a	80.0 (60) ^{a,b}
	7.3 (31)	85.5 (110) ^a	77.6 (94) ^{a,b}	71.9 (57) ^{b,c}
	7.8 (23)	82.7 (52) ^a	75.9 (54) ^a	55.0 (40) ^c
Stored	6.8 (31)	79.5 (88) ^d	72.6 (95) ^{d,e}	60.0 (85) ^e
	7.3 (22)	86.1 (72) ^{a,d}	77.9 (68) ^{a,d,e}	62.8 (43) ^{c,e}
	7.8 (35)	51.1 (90) ^f	44.2 (95) ^{f,g}	37.2 (86) ^a

* In parentheses, no. of hens.

† In parentheses, no. of eggs.

Different superscript letters indicate significant differences at $P < 0.05$ (χ^2 test). Comparisons to be made within vertical columns of the semen type, between fresh and stored semen for each pH and between the different periods after insemination for each pH value.

Discussion

The formation of fructose from glucose has so far been reported for fowl spermatozoa only (Lorenz, 1958; Scott, White & Annison, 1962). The present study revealed that the same process occurs also during incubation of turkey spermatozoa. The mechanism and the physiological purpose of this formation are not known.

The viability and metabolic rate of the spermatozoa were depressed at the lower pH values. The O_2 consumption increased significantly with increasing pH. Sexton & Giesen (1982) found that at an osmolarity of 280 mosmol, but not at 350 mosmol, respiration rate was significantly higher at pH 7.5 than at pH 6.5. Opposite results were obtained with cock spermatozoa, i.e. an adverse effect of increasing pH on the O_2 consumption (Lardy & Phillips, 1943; Schindler & Lehrer, 1969), indicating, perhaps, species differences.

Although the glucose consumption and lactic acid accumulation by the spermatozoa did not increase significantly at the higher pH values, the significant increase in the O_2 uptake and in the amount of glucose oxidized to CO_2 at these pHs suggests an enhancing effect of the higher pHs on the aerobic metabolism of turkey spermatozoa.

The amounts of glucose which were converted to lactic acid and those oxidized to CO_2 were less than could be accounted for by the amount of hexose consumption. The difference is explained by the formation from glucose of metabolites other than lactic acid (Mann & Lutwak-Mann, 1981). On the other hand, only part of the total O_2 uptake could be accounted for by the sugar oxidation, ~35% in the 1st hour and ~60% in the 2nd hour of incubation. The rest must have been used for the oxidation of extracellular and intracellular substances other than glucose. This metabolic pathway is more pronounced during the 1st than during the 2nd hour of incubation. It seems that turkey spermatozoa during the 2nd hour of incubation resort to oxidation of glucose after a partial depletion of other sources of energy during the 1st hour of incubation, in contrast to mammalian sperm cells which use the glycolytic mechanism as the predominant source of energy (Mann & Lutwak-Mann, 1981).

The results show that, in a wide range of hydrogen ion concentrations of the medium, the metabolic and physical activity of turkey spermatozoa increased, whereas the ability to preserve fertilizing ability decreased with increasing pH. It seems that higher metabolic rates *in vitro* in a certain medium at 37°C do not necessarily indicate a greater capacity of the spermatozoa to

maintain their fertilizing ability in the oviduct of the hen or to preserve it at 15°C. Increased metabolic rates may even advance the senescence of the spermatozoa and the loss of their fertility.

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References

- Barker, J.N. & Britton, H.G.** (1957) The enzymatic estimation of L(+) lactic acid. *J. Physiol., Lond.* **138**, 3P.
- Brown, K.I., Graham, E.F. & Crabo, B.G.** (1972) Effect of some hydrogen ion buffers on storage and freezing of turkey spermatozoa. *Poult. Sci.* **51**, 840-849.
- Christensen, V.L. & Johnston, N.P.** (1975) The effect of time of day of insemination and oviposition on the fertility of turkey hens. *Poult. Sci.* **54**, 1209-1214.
- El-Jack, M.E. & Lake, P.E.** (1966) The effect of resting roosters from ejaculation on the quality of spermatozoa in semen. *J. Reprod. Fert.* **11**, 489-491.
- Huggett, A.S.G. & Nixon, D.A.** (1957) Enzymic determination of blood glucose. *Biochem. J.* **66**, 12P.
- Lake, P.E. & Ravie, O.** (1979) Effect on fertility of storing fowl semen for 24 h at 5°C in fluids of different pH. *J. Reprod. Fert.* **57**, 149-155.
- Lake, P.E. & Ravie, O.** (1982) Effect on fertility of storing turkey semen for 24 hours at 10°C in fluids of different pH. *Br. Poult. Sci.* **23**, 41-47.
- Lake, P.E. & Stewart, J.M.** (1978) Artificial insemination in poultry. *Bull. Min. Agric., Fish. Fd* No. 213.
- Lardy, H.A. & Phillips, P.H.** (1943) Effect of pH and certain electrolytes on the metabolism of ejaculated spermatozoa. *Am. J. Physiol.* **38**, 741-746.
- Lorenz, F.W.** (1958) Carbohydrate metabolism of cock spermatozoa. *Nature, Lond.* **182**, 397-398.
- Mann, T.** (1948) Fructose content and fructolysis in semen. Practical application in the evaluation of semen quality. *J. agric. Sci., Camb.* **38**, 323-331.
- Mann, T.** (1964) *The Biochemistry of Semen and of the Male Reproductive Tract*. Methuen, London.
- Mann, T. & Lutwak-Mann, C.** (1981) *Male Reproductive Function and Semen*. Springer-Verlag, Berlin.
- Murdoch, R.N., White, I.G. & Seamark, R.F.** (1970) Oxidative and glycolytic activity of ejaculated ram spermatozoa in the presence of steroid hormones. *Acta endocr., Copenh.* **64**, 557-568.
- SAS Users Guide** (1979) Eds J. T. Helwig & K. A. Council. SAS Institute Inc., Cary, NC.
- Schindler, H. & Lehrer, A.R.** (1969) The effect of egg albumen and of sperm immobilization on the rate of oxygen uptake by fowl spermatozoa. *Poult. Sci.* **48**, 36-40.
- Scott, T.W., White, I.G. & Annison, E.F.** (1962) Glucose and acetate metabolism by ram, bull, dog and fowl spermatozoa. *Biochem. J.* **82**, 398-404.
- Sexton, T.J. & Giesen, A.F.** (1982) Beltsville poultry semen extender. 6. Holding turkey semen for 6 hours at 15°C. *Poult. Sci.* **61**, 1202-1208.
- Umbreit, W.W., Burris, R.H. & Stauffer, J.F.** (1957) *Manometric Techniques*. Burgess Publishing Co., Minneapolis.
- Wishart, G.J.** (1981) The effect of continuous aeration on the fertility of fowl and turkey semen stored above 0°C. *Br. Poult. Sci.* **22**, 445-450.

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