Metabolism of fowl and turkey spermatozoa at low temperatures

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Summary. The rate at which fowl and turkey spermatozoa consumed energy (as ATP) in a glutamate-based medium without glucose decreased with temperature by 75–80% over the range 40 to 5°C. The rate of oxygen utilization of these spermatozoa, as a monitor of their rate of energy production, also decreased by 75–80% over the same range of temperature, although sperm ATP levels remained constant. The rate of glycolysis of fowl spermatozoa decreased by a factor of 20 between 40 and 5°C but remained capable of supporting optimal sperm ATP levels. Turkey sperm glycolysis proceeded extremely slowly and was not capable of supporting optimal ATP levels at any temperature investigated. Turkey, but not fowl spermatozoa, therefore require oxygen to maintain optimal energy levels at all temperatures between 5 and 40°C.

Introduction

The oxidative and glycolytic metabolism of fowl and turkey spermatozoa have been correlated with their ability to conserve both ATP levels and fertilizing capacity *in vitro* at 40°C (Wishart, 1982). Fowl spermatozoa maintain high ATP levels and fertilizing potential for up to 3 h by aerobic or by anaerobic metabolism of glucose. Turkey spermatozoa, however, can only maintain ATP levels and fertilizing potential under aerobic conditions, their glycolytic capacity being considerably lower than that of fowl spermatozoa (Wishart, 1982). These metabolic studies were carried out at the physiological temperature of 40°C whereas practical storage of poultry semen is carried out at low temperature, e.g. 5–10°C (see Lake & Stewart, 1978). Although preliminary results have suggested that aeration can have a beneficial effect on the maintenance of the fertilizing potential of turkey spermatozoa at 10°C (Wishart, 1981), it is not known whether the balance between sperm energy production and energy utilization is maintained at these low temperatures as it is in the physiological ranges.

The present work investigates the rate of energy utilization (as ATP hydrolysis) and the rate of metabolism involved in energy production (as oxygen uptake and lactate production) in fowl and turkey spermatozoa held *in vitro* at a range of temperatures from 40 to 5°C.

Materials and Methods

Animals

Male fowl were from a layer-type control strain obtained from Ross Breeders Ltd, Newbridge, Midlothian, Scotland. Turkey stags were of a medium-weight type obtained from British United Turkeys Ltd, Cheshire, England. All birds were housed in cages, with 14 h light/24 h and fed commercial breeders ration *ad libitum*.

Semen collection and treatment

Semen collection and assessment of sperm numbers were performed as previously described (Wishart, 1982). Semen was diluted in a glutamate-based medium (Wishart, 1982), 4-fold for fowl and 8-fold for turkey spermatozoa, to give approximately equal concentrations of spermatozoa. Aerobic incubations were performed in plastic 25-ml flasks or 10-ml vials in shaking water baths with refrigeration facility. Anaerobic incubations were performed in sealed Eppendorf tubes. Experiments were arranged so that one pooled sample of semen from 6–8 birds could be assessed for a single parameter at each of the 5 temperatures employed.

Biochemical analyses

Sperm oxygen consumption was measured with a Clark-type electrode and standardized by the method of Robinson & Cooper (1970). For a working standard, the oxygen content of 1.2 ml buffer in a 10-mm internal diameter tube, equilibrated at 5, 10, 20, 30 and 40°C was 370 ± 16 ($n = 5$), 320 ± 7 ($n = 6$), 279 ± 9 ($n = 10$), 235 ± 11 ($n = 5$) and 220 ± 4 ($n = 5$) nmol/ml respectively.

Lactate and ATP were measured as previously described (Wishart, 1982).

To estimate the decrease in sperm activity at temperatures ranging from 40 to 5°C, samples were incubated under aerobic conditions for 20–30 min and then exposed to 10^{-6} M-KCN. This arrested sperm oxygen uptake within 2 to 3 sec at the higher temperatures. At 5°C, with a slower electrode response, oxygen uptake was seen to stop 10–15 sec after CN^- addition. Since glycolytic substrates were not present, the fall in sperm ATP concentrations was taken to be a measure of their rate of energy utilization (Text-fig. 1).

Substrates, enzymes and other chemicals were from Sigma (London) Chemical Company Ltd, Poole BH17 7NM, U.K., or from BDH Chemicals Ltd, Poole BB12 4NM, U.K.

Results

The rate of ATP hydrolysis by fowl and turkey spermatozoa at temperatures from 5 to 40°C is shown in Text-fig. 1. Between the two extremes of this temperature range sperm activity varied by 4- to 5-fold. Turkey spermatozoa were significantly more active than fowl spermatozoa at 5 and 10°C, whilst the latter were more active at 30°C. Turkey sperm activity increased as the temperature was raised from 30 to 40°C. However, fowl sperm activity decreased by about 40% under these conditions (Text-fig. 1).

Table 1. ATP concentration in fowl and turkey spermatozoa incubated aerobically and anaerobically at various temperatures

Incubation temp (°C)	ATP conc. (nmol/10 ⁹ spermatozoa)			
	Fowl spermatozoa		Turkey spermatozoa	
	Anaerobic conditions	Aerobic conditions	Anaerobic conditions	Aerobic conditions
5	49 ± 4	44 ± 4	1.9 ± 0.1	48 ± 2
10	49 ± 3	47 ± 3	1.8 ± 0.7	48 ± 4
20	48 ± 3	45 ± 3	1.6 ± 0.6	50 ± 4
30	51 ± 4	48 ± 2	2.2 ± 0.3	57 ± 3
40	49 ± 5	47 ± 3	1.8 ± 0.3	49 ± 2

Each result is mean ± s.e.m. of 4 different samples.

Text-fig. 1. Rate of hydrolysis of ATP by fowl and turkey spermatozoa at various temperatures. Samples were incubated aerobically without glycolytic substrate. Each point represents the mean ATP content of 6 different samples of spermatozoa following the addition of 10^{-6} M-KCN to the medium. Linear regressions were calculated for each temperature. The mean $\pm$ s.e.m. percentage rates of ATP disappearance were: 2.61 ± 0.16 ; 4.55 ± 0.16 ; 9.34 ± 0.40 ; 23.68 ± 0.79 and 13.18 ± 0.79 for fowl spermatozoa and 4.78 ± 0.19 ; 5.91 ± 0.19 ; 11.31 ± 0.47 ; 16.45 ± 0.95 and 21.99 ± 0.95 for turkey spermatozoa, at 5 (Δ), 10 ($\blacktriangle$), 20 ($\circ$), 30 ($\bullet$) and 40°C ($\square$) respectively.

Under conditions of aerobic incubation at temperatures of 5, 10, 20, 30 and 40°C, the ATP concentrations of fowl and turkey spermatozoa did not differ significantly (Table 1). The ATP concentrations before KCN addition in Text-fig. 1 ('100%') would not, therefore, vary with the different incubation temperatures used.

Under conditions of aerobic incubation, sperm utilization of oxygen followed a pattern similar to that of ATPase activity. Thus oxygen uptake of turkey spermatozoa increased 4-fold between 5 and 40°C. Fowl sperm oxygen consumption increased by 8-fold between 5 and 30°C and then decreased between 30 and 40°C (Table 2).

Table 2. Rate of oxygen consumption and lactate production by fowl and turkey spermatozoa held *in vitro* at temperatures between 5 and 40°C

Temp (°C)	Rate of O ₂ consumption ($\mu\text{mol}/10^9$ spermatozoa per min)		Rate of lactate production ($\mu\text{mol}/10^9$ spermatozoa per min)	
	Fowl	Turkey	Fowl	Turkey
5	3.5 ± 0.6 (6)	4.1 ± 0.4 (6)	0.17 ± 0.01 (6)	Negligible
10	5.7 ± 0.6 (6)	5.3 ± 0.9 (6)	0.54 ± 0.04 (6)	Negligible
20	16.1 ± 1.1 (6)	13.3 ± 0.7 (6)	1.25 ± 0.15 (6)	Negligible
30	26.1 ± 1.9 (6)	15.0 ± 0.8 (6)	3.66 ± 0.40 (6)	0.19 ± 0.04 (6)
40	18.2 ± 2.2 (6)	18.4 ± 1.1 (6)	4.13 ± 0.66 (6)	0.15 ± 0.03 (6)

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

Anaerobic production of lactate by fowl spermatozoa varied to a greater degree than did oxygen utilization, by 20-fold over the range of temperatures 5 to 40°C. Lactate production increased at 40°C compared to 30°C (Table 2). However, the rate of anaerobic glycolysis of fowl spermatozoa

was sufficient to support optimal ATP concentrations at all temperatures (Table 1). The rate of anaerobic lactate formation by turkey spermatozoa was extremely low (Table 2). When measured after 1–2 h of anaerobic incubation in the presence of 10 mM-glucose, turkey sperm ATP concentrations were < 5% of the concentration formed following aerobic incubation at each temperature studied between 5 and 40°C (Table 1).

Discussion

When fowl and turkey spermatozoa were incubated aerobically without glucose, the rate of ATP hydrolysis, as a measure of energy utilization, and the rate of oxygen consumption, as a measure of the rate of energy production, decreased by 75–80% as the temperature was lowered from 40 to 5°C. At each temperature stage the percentage decrease in the rate of oxygen consumption was equivalent to the decrease in the rate of ATP hydrolysis, suggesting, since sperm ATP levels remained constant, a coupling of ATP production to oxygen metabolism at all temperatures.

Ashizawa & Nishiyama (1978) found a decrease of a similar magnitude in the rate of oxygen consumption of fowl spermatozoa between 40 and 5°C and Wishart (1981) also reported a similar degree of oxygen utilization of fowl and turkey spermatozoa at 5 and 10°C, respectively. However, Clarke, Sexton & Ottinger (1982) have reported that fowl and turkey spermatozoa ceased to consume oxygen at 5°C. The different findings of the latter study are probably not the result of using different storage media since a glutamate-based buffer was also used. It may be relevant that Clarke *et al.* (1982) used a manometric method to measure oxygen consumption at various temperatures whereas the other studies mentioned above employed the more sensitive polarographic method with a Clark-type oxygen electrode. The motility of fowl spermatozoa, although generally increasing with temperature, is known to decrease as 40°C is reached (Munro, 1938; Nevo & Schindler, 1968) although the mechanism by which this occurs remains obscure. Nevo & Schindler (1968) found no decrease in oxygen consumption as motility was inhibited and suggested that motility of fowl spermatozoa can be uncoupled by temperature from their source of metabolizable energy without affecting the metabolic rate. However, Ashizawa & Nishiyama (1978) found a decrease in oxygen consumption as motility decreased at 40°C. The present work substantiates the latter finding and suggests a relationship between ATP hydrolysis and oxygen consumption and, presumably, motility. It would therefore appear, ignoring compartmental effects, that ATP availability is not a limiting factor in the temperature-dependent decrease of fowl sperm motility.

The rate of ATP hydrolysis and the rate of oxygen consumption of turkey spermatozoa increased between 30 and 40°C, suggesting that the anomalous decrease in motility of fowl spermatozoa at these temperatures may not be a feature of turkey spermatozoa. Indeed, this phenomenon may be relevant only to fowl spermatozoa when incubated under aerobic conditions, since the rate of lactate production of fowl spermatozoa under anaerobic conditions increased between 30 and 40°C, suggesting that an increase, rather than decrease, of sperm activity occurred under these conditions. Unlike the rate of oxygen consumption, which varies 4- to 5-fold between 5 and 40°C, the rate of anaerobic lactate formation increased 20-fold over the same temperature range, again suggesting a different regulation of sperm activity under oxidative and anaerobic conditions. However, despite the lowering of the rate of lactate production by fowl spermatozoa at 5 compared with 40°C, the glycolytic rate appears sufficient to support optimal sperm ATP concentrations at all temperatures investigated. This is not the case with turkey spermatozoa which, under conditions of anaerobic glycolysis at temperatures between 5 and 40°C, contain < 5% of the ATP concentrations that they support under aerobic conditions.

These results therefore show that the patterns of metabolic activity described for fowl and turkey spermatozoa at physiological temperatures (Wishart, 1982) also hold for temperatures down to 5°C. Indeed the rate of oxygen consumption of turkey spermatozoa decreased only by 75%

between 40 and 5°C. For maintenance of turkey spermatozoa in an optimal energy state during storage at low temperatures, it is necessary to aerate the diluted semen.

I thank Mrs F. Ross for skilled assistance and Dr P. E. Lake for helpful discussion.

References

- Ashizawa, K. & Nishiyama, H. (1978) Effects of temperature on the vigour of motility, oxygen consumption and duration of motility of fowl spermatozoa under aerobic conditions. *Jap. Poult. Sci.* **15**, 264–266.
- Clarke, R.N., Sexton, T.J. & Ottinger, M.A. (1982) Effects of holding temperature and storage time on respiratory rate, motility and fertility of chicken and turkey semen. *Poult. Sci.* **61**, 1912–1917.
- Lake, P.E. & Stewart, J.M. (1978) Artificial insemination in poultry. Bull. Min. Ag. Fish. & Food, No. 213. H.M. Stationery Office, London.
- Munro, S.S. (1938) Fowl sperm immobilisation by a temperature media interaction and its biological significance. *Q. Jl exp. Physiol.* **27**, 281–287.
- Nevo, A.C. & Schindler, H. (1968) Reversible inactivation of cock spermatozoa by temperature. *Proc. 6th Int. Congr. Anim. Reprod. & A.I., Paris* **2**, 1637–1640.
- Robinson, J. & Cooper, J.M. (1970) Method of determining oxygen concentrations in biological media, suitable for calibration of the oxygen electrode. *Analyt. Biochem.* **33**, 390–399.
- 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.
- Wishart, G.J. (1982) Maintenance of ATP concentrations in, and fertilizing ability of, fowl and turkey spermatozoa *in vitro*. *J. Reprod. Fert.* **66**, 457–462.

Received 29 April 1983