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Stimulation of sperm motility and oxygen consumption of fowl spermatozoa by a low molecular weight fraction of seminal plasma

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Summary. Washed fowl spermatozoa were incubated in a phosphate buffer containing various concentrations of fowl seminal plasma at 41°C, normal body temperature, and the motility and oxygen consumption of spermatozoa were determined. Immediately after the incubation, spermatozoa showed good motility in the various diluents. However, with concentrations of seminal plasma at or below 20%, spermatozoa quickly became immotile. In contrast, at concentrations higher than 40% seminal plasma, spermatozoa were motile even after 15 min. As the concentration of seminal plasma was increased, oxygen consumption of spermatozoa also increased. A filtrate of the seminal plasma, obtained by passing the fluid through an Amicon YM-2 ultrafiltration membrane (M_r < 1000), also stimulated the motility and oxygen consumption of spermatozoa. These results suggest that some low molecular weight factor(s) in fowl seminal plasma stimulated motility and oxygen consumption of fowl spermatozoa at 41°C. A physiological role of this factor(s) may be to assist passage of spermatozoa through the vagina after natural mating.

Introduction

Generally, both vigour of motility and metabolic activity of mammalian spermatozoa increase with rising temperature. Moreover, maximum metabolic activity of spermatozoa occurs between 40 and 47°C (Beck & Salisbury, 1943; Freund, Mixner & Mather, 1959; Salisbury & Lodge, 1962). With fowl spermatozoa, it has been reported that at room temperature most synthetic diluents preserve the vigour of motility but inhibit it at 40–41°C, normal body temperature (Munro, 1938; Nevo & Schindler, 1968; Ashizawa & Nishiyama, 1978; Takeda, 1982a, b). This immobilization is not permanent and movement resumes when the temperature is lowered to room temperature. However, fowl spermatozoa in seminal plasma are not reversibly immobilized at body temperature but maintain vigorous motility (Munro, 1938; Nevo & Schindler, 1968; Ashizawa, Nishiyama & Nagae, 1976; Takeda, 1982a). Seminal plasma also stimulates respiration of spermatozoa (van Tienhoven, 1960; Fewlass, Sexton & Shaffner, 1975; Terada & Watanabe, 1978). It is presumed that motility and respiration stimulating factor(s) are present in fowl seminal plasma.

This study was undertaken to investigate the factor(s) in seminal plasma responsible for the stimulation of fowl sperm motility and respiration and to determine some properties of the factor(s).

Materials and Methods

Animals. White Leghorn roosters (Shaver strain) obtained from Koyu Poultry Farm (Miyazaki, Japan) were used throughout the study. All birds were housed in cages and fed *ad libitum* on a proprietary breeder diet. They were given 14 h light per 24 h.

Treatment of fowl seminal plasma. Semen was collected by the method of Bogdonoff & Shaffner (1954). The spermatozoa were removed by centrifugation (1500 *g*, 20 min), and the supernatant fluid passed through a membrane filter (0·33 μm pore size, PH type, Millipore Corp.) and stored at −80°C until required. The filtrate obtained was considered to be seminal plasma. In Exp. I, seminal plasma was diluted with sodium phosphate buffer, pH 7·2 (Wilcox & Shaffner, 1958) containing 20 mM-glucose to give 0, 10, 20, 40, 60, 80 and 100% seminal plasma. One sample of 100% seminal plasma was placed in a hot water bath at 90°C for 30 min. These solutions were used as diluents in the motility and oxygen consumption assays.

In Exp. II, seminal plasma was subjected to ultrafiltration (Diaflo PM-10, approximate exclusion size of M_r 10 000: Amicon Corporation). The residue was redissolved in phosphate buffer to give the original volume of the seminal plasma and this reconstructed solution and the filtrate were tested.

In Exp. III, a second filtration of the PM-10 filtrate was performed with a YM-2 ultrafiltration membrane (approximate exclusion size M_r 1000: Amicon Corporation). Filtrate and residue were treated as were those of the PM-10 filtration. In Exps II and III, these fluids were also diluted with phosphate buffer (as in Exp. I), and used as diluents in the motility and oxygen consumption assays.

Sperm preparation and measurements of motility and oxygen consumption of spermatozoa. Semen was collected from several roosters by the method of Bogdonoff & Shaffner (1954) and washed twice with phosphate buffer (Wilcox & Shaffner, 1958) containing 20 mM-glucose by centrifuging the suspension at 700 *g* for 15 min. The washed spermatozoa were resuspended in the various diluents and the sperm concentration was adjusted to $0{\cdot}5 \times 10^8$/ml. The sperm suspension for assessment of motility was aerated using a peristaltic pump and poured into a microslide glass cell of rectangular cross-section (Sibata Chemical App. Mfg Co., Ltd, Japan), provided with two side slits to facilitate filling, emptying and washing of the cell. The assessment of motility was carried out by observing spermatozoa from several areas of the cell under light microscopy. Temperature was maintained at 41°C by keeping the cell on a water-jacketed chamber and circulating warm water from a thermostatically-controlled water bath. Sperm motility was evaluated at 0, 5 and 15 min after equilibration of the sperm suspension to 41°C. The motility was classified into four grades: vigorous movement (+++), moderate movement (++), faint movement (+), and motionless (−). The score (±) was also used when very few spermatozoa in the microscopic field were motile.

Oxygen consumption of spermatozoa was determined polarographically with a Clark electrode by the method of Kielley (1963) using a YSI model 53 biological oxygen monitor (Yellow Springs Instrument Co., Inc., Ohio, U.S.A.). Oxygen consumption was measured at 5 min after equilibration of the sperm suspension to 41°C. Sperm concentration was determined by the method of Salisbury, Beck, Elliott & Willett (1943) with a spectrophotometer (Hitachi, Model 100-10, Japan). The rate of oxygen consumption was expressed in terms of μl O_2 consumption/10^8 spermatozoa.h^{-1}.

Results

Experiment I

Table 1 shows the relationship between the motility of the spermatozoa and the various concentrations of seminal plasma. Sperm motility was markedly affected by the concentration of seminal plasma. Immediately after the sperm suspension was equilibrated to 41°C, spermatozoa

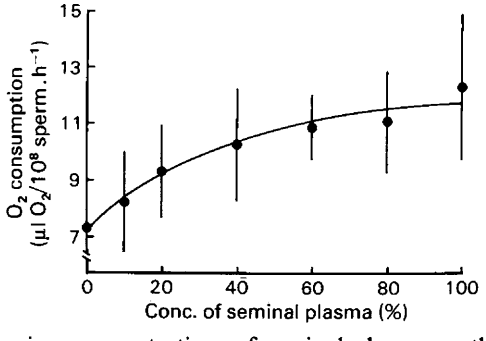
Table 1. Effect of various concentrations of seminal plasma on the motility of fowl spermatozoa incubated at 41°C

	Sperm motility						
	Concentration of seminal plasma (%)						
Time (min)	0	10	20	40	60	80	100
0	+ +	+ +	+ +	+ +	+ +	+ + ~ + + +	+ + +
5	±	± ~ +	+	+ +	+ +	+ + ~ + + +	+ + +
15	±	± ~ +	± ~ +	+ +	+ +	+ +	+ + +

± Very few spermatozoa motile; + faint movement; + + moderate movement; + + + vigorous movement.

showed good motility in the various diluents. However, at seminal plasma concentrations below 20%, spermatozoa quickly lost motility. In contrast, as the concentration of seminal plasma was increased, spermatozoa remained motile even after 15 min. At concentrations higher than 40%, almost no decline of sperm motility was observed.

As the concentration of seminal plasma was raised, the oxygen consumption of spermatozoa increased (Text-fig. 1).



Text-fig. 1. Effect of various concentrations of seminal plasma on the rate of oxygen consumption of fowl spermatozoa incubated at 41°C. Values are mean ± s.e.m. of 3 trials. Oxygen consumption was measured at 5 min after the sperm suspension was equilibrated to 41°C.

Heating of seminal plasma to 90°C did not interfere with the ability of seminal plasma to stimulate the motility (moderate to vigorous movement), and oxygen consumption of spermatozoa (12·84 ± 0·71 and 13·48 ± 0·27 µl $O_2/10^8$ spermatozoa. h^{-1} for untreated and heat-treated seminal plasma respectively: mean ± s.e.m. for 3 trials).

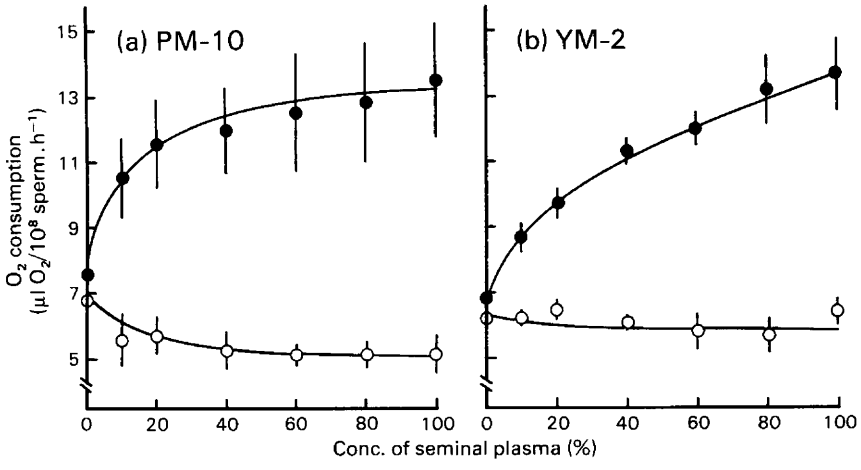
Experiment II

The results are shown in Table 2. At PM-10 filtrate concentrations higher than 40%, spermatozoa maintained good motility throughout the incubation period. A similar stimulating effect was obtained for the oxygen consumption of spermatozoa (Text-fig. 2a). In contrast, in the residue of the ultrafiltration, spermatozoa were almost immotile at 5 min (Table 2) and as the concentration of the residue was raised, the oxygen consumption was slightly decreased (Text-fig. 2a).

Table 2. Effect of various concentrations of filtered seminal plasma on the motility of fowl spermatozoa incubated at 41°C

			Sperm motility						
			Concentration of seminal plasma (%)						
Filter	Fraction	Time (min)	0	10	20	40	60	80	100
PM-10	Filtrate	0	++	++	++	++~+++	++~+++	++~+++	++~+++
		5	±	+	++	++~+++	++~+++	++~+++	++~+++
		15	±	±	±~+	++~+++	++~+++	++~+++	++~+++
	Residue	0	++	++	++	++	++	++	++
		5	±	±	±	±	±	±	±
		15	±	±	±	±	±	±	±
YM-2	Filtrate	0	++	++	++	+++	+++	+++	+++
		5	±	±~+	±~+	+	++	++~+++	++~+++
		15	±	±	±	±	+~++	++	++
	Residue	0	++	++	++	++	++	++	++
		5	±	±	±	±	±	±	±
		15	±	±	±	±	±	±	±

± Very few spermatozoa motile; + faint movement; ++moderate movement; +++ vigorous movement.



Text-fig. 2. Effect of various concentrations of filtered seminal plasma (● filtrate; ○ residue) on the rate of oxygen consumption of fowl spermatozoa incubated at 41°C: (a) PM-10 ultrafiltration; (b) YM-2 ultrafiltration. Values are mean ± s.e.m. of 3 trials. Oxygen consumption was measured at 5 min after the sperm suspension was equilibrated to 41°C.

Experiment III

Sperm motility in the YM-2 filtrate (M_r < 1000) increased with increasing concentrations of the filtrate (Table 2). Likewise, oxygen consumption rate also increased (Text-fig. 2b). However, in the residue, spermatozoa were almost immotile and oxygen consumption slightly decreased with a rise in concentration of the seminal plasma.

Discussion

In most types of synthetic diluents, the motility of fowl spermatozoa, although generally increasing with temperature, is known to decrease as 41°C is reached (Munro, 1938; Nevo & Schindler, 1968; Ashizawa & Nishiyama, 1978; Takeda, 1982a, b). In all cases, reactivation occurred 1–2 min after cooling. Ashizawa & Nishiyama (1978) reported that the rate of oxygen consumption of spermatozoa in Ringer's solution increased with the rise in temperature until a peak at 37°C and was then depressed about 40% at 41°C. Similar results on the effect of Krebs–Ringer–phosphate buffer were obtained by Takeda (1982b). Schindler & Nevo (1962) suggested that the immobilization of fowl spermatozoa at body temperature was due to the lack of oxygen in the medium. However, Takeda (1982a) reported that spermatozoa were immobilized at body temperature even if oxygen was introduced into the medium, suggesting that the immobilization of fowl spermatozoa was not due to the lack of oxygen.

On the other hand, natural fluids such as seminal plasma, blood serum, thin egg white, and shell gland fluid support the motility of fowl spermatozoa even at body temperature (Munro, 1938; Nevo & Schindler, 1968; Ashizawa *et al.*, 1976; Takeda, 1982a). Lindholmer (1974) reported that addition of human seminal plasma or albumin immediately restored progressive motility of human spermatozoa after a wash with an albumin-free buffered salt solution. He postulated that albumin was one of the factors in the seminal fluid responsible for the motility-promoting effect on human spermatozoa. Terada & Watanabe (1978) and Takeda (1982a) suggested that mobilization of fowl spermatozoa at body temperature in blood serum, seminal plasma, egg white, and refined egg albumen may be due to the protective action of macromolecules, especially albumen.

However, one essential serum component for capacitation of hamster spermatozoa, initially identified as a low molecular weight, motility-stimulating factor (Yanagimachi, 1969, 1970; Morton & Bavister, 1974), has been subsequently characterized as taurine (Mrsny, Waxman & Meizel, 1979). Chung (1983) reported that bull seminal plasma contained a motility-stimulating factor for hamster spermatozoa *in vitro*. This factor was also found to be heat stable and to have a small molecular size similar to the sperm motility factor derived from hamster epididymal plasma. In this study, the motility and respiration stimulating factor(s) derived from fowl seminal plasma remained in the filtrate containing components of <1000 molecular weight.

Takeda (1974) reported that living fowl spermatozoa inseminated in the posterior vagina were detected in the uterus, whereas dead spermatozoa inseminated in the cloaca were not detected in the vagina or the uterus. After intrauterine insemination, all living and dead fowl spermatozoa moved rapidly to the upper part of the oviduct. From these observations, Takeda (1974) suggested that the spasmodic contractions of the vagina were not responsible for the sperm movement, but that sperm motility was the primary mechanism for upward transport of spermatozoa from the hen's vagina. Furthermore, Terada (1980) and Terada, Watanabe & Tsutsumi (1984) reported that when spermatozoa from the ductus deferens were inseminated in the upper part of the vagina, high fertilizing ability was retained relative to that of ejaculated spermatozoa in seminal plasma. However, when these spermatozoa were inseminated in the lower part of the vagina, a lower fertilizing ability was obtained compared with controls. Terada (1980) and Terada *et al.* (1984) concluded that the seminal plasma (accessory reproductive organ fluid) which was contaminated with spermatozoa during natural mating played an important role in sperm transport through the hen's vagina.

From the present work, together with the evidence described above, it appears that the stimulating factor(s) in seminal fluid is of low molecular weight ($M_r < 1000$) and that this component may be involved in the upward transport of fowl spermatozoa through the hen's vagina.

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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.

Ashizawa, K., Nishiyama, H. & Nagae, T. (1976) Effects of oviducal cells on the survival and fertilizing ability of fowl spermatozoa. *J. Reprod. Fert.* **47**, 305–311.

Beck, G.H. & Salisbury, G.W. (1943) Rapid methods for estimating the quality of bull semen. *J. Dairy Sci.* **26**, 483–494.

Bogdonoff, P.D., Jr & Shaffner, C.S. (1954) The effect of pH on *in vitro* survival, metabolic activity, and fertilizing capacity of chicken semen. *Poult. Sci.* **33**, 665–669.

Chung, K.S. (1983) Effect of bovine seminal plasma components on motility and fertilizing ability of hamster spermatozoa *in vitro*. *Proc. Vth Wld Conf. Anim. Prod., Tokyo*, Abstr. 81.

Fewlass, T.A., Sexton, T.J. & Shaffner, C.S. (1975) Effect of various levels of egg yolk, milk, seminal plasma or blood serum on the respiration and reproductive efficiency of chicken spermatozoa. *Poult. Sci.* **54**, 346–349.

Freund, M., Mixner, J.P. & Mather, R.E. (1959) Bovine semen metabolism. V. Influence of incubation temperature on certain measures of fructolysis. *J. Dairy Sci.* **42**, 79–82.

Kielley, W.W. (1963) Preparation and assay of phosphorylating submitochondrial particles: sonicated mitochondria. *Methods Enzymol.* **6**, 272–277.

Lindholmer, Ch. (1974) The importance of seminal plasma for human sperm motility. *Biol. Reprod.* **10**, 533–542.

Morton, D.B. & Bavister, B.D. (1974) Fractionation of hamster sperm-capacitating components from human serum by gel filtration. *J. Reprod. Fert.* **40**, 491–493.

Mrsny, R.J., Waxman, L. & Meizel, S. (1979) Taurine maintains and stimulates motility of hamster sperm during capacitation in vitro. *J. exp. Zool.* **210**, 123–128.

Munro, S.S. (1938) Fowl sperm immobilization by a temperature-media interaction and its biological significance. *Q. Jl exp. Physiol.* **27**, 281–291.

Nevo, A.C. & Schindler, H. (1968) Reversible inactivation of cock spermatozoa by temperature. *Proc. 6th Int. Congr. Anim. Reprod. & A.I., Paris*, Vol. II, 1637–1640.

Salisbury, G.W. & Lodge, J.R. (1962) Metabolism of spermatozoa. *Adv. Enzymol.* **24**, 35–104.

Salisbury, G.W., Beck, G.H., Elliott, I. & Willett, E.L. (1943) Rapid methods for estimating the number of spermatozoa in bull semen. *J. Dairy Sci.* **26**, 69–78.

Schindler, H. & Nevo, A. (1962) Reversible inactivation and agglutination of fowl and bull spermatozoa under anaerobic conditions. *J. Reprod. Fert.* **4**, 251–265.

Takeda, A. (1974) The transport of spermatozoa in the vagina of the hen. *Jap. Poult. Sci.* **11**, 45–54.

Takeda, A. (1982a) Studies on reversible inactivation of cock spermatozoa by temperature. I. Effects of several factors on reversible inactivation. *Jap. Poult. Sci.* **19**, 26–36.

Takeda, A. (1982b) Studies on reversible inactivation of cock spermatozoa by temperature. II. Effects of reversible inactivation on O_2 consumption and storage of cock spermatozoa. *Jap. Poult. Sci.* **19**, 135–139.

Terada, T. (1980) Physiological function of avian seminal plasma—a review. *Jap. Poult. Sci.* **17**, 163–174.

Terada, T. & Watanabe, M. (1978) Effects of seminal plasma on the motility and metabolism of cock spermatozoa. *Jap. J. zootech. Sci.* **49**, 47–53.

Terada, T., Watanabe, M. & Tsutsumi, Y. (1984) Possible significance of accessory reproductive fluid in exhibition of fertilizing ability of spermatozoa in the domestic fowl, *Gallus domesticus*. *Jap. J. zootech. Sci.* **55**, 52–58.

van Tienhoven, A. (1960) The metabolism of fowl sperm in different diluents. *J. agric. Sci.* **54**, 67–80.

Wilcox, F.H. & Shaffner, C.S. (1958) The effect of different handling methods and added fructose on the fertilizing ability of chicken spermatozoa after storage. *Poult. Sci.* **37**, 1353–1357.

Yanagimachi, R. (1969) *In vitro* acrosome reaction and capacitation of golden hamster spermatozoa by bovine follicular fluid and its fractions. *J. exp. Zool.* **170**, 269–280.

Yanagimachi, R. (1970) *In vitro* capacitation of golden hamster spermatozoa by homologous and heterologous blood sera. *Biol. Reprod.* **3**, 147–153.

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