Effect of Oviductal Fluids on Oxidative Phosphorylation in Spermatozoa[1] (36959)

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The oviduct and its secretions provide the physiological environment in which spermatozoa of most mammalian species await the arrival of the ova. During this period, spermatozoa undergo a process called capacitation, a process that spermatozoa must undergo before achieving the capacity to fertilize ova (1, 2). Evidence that capacitation is indeed a necessary step in the fertilization process has been presented for the rabbit, rat, hamster, cat, ewe, frog, pig, cow, mouse and ferret (3–13). Dukelow and Chernoff (14) suggests that primate sperm also undergo capacitation.

Although it has been shown that reproductive tract secretions increase sperm oxygen uptake (15–20) and motility (18), there is no direct evidence that these changes and the acquisition of the ability to fertilize ova are related. Mounib and Chang (16) have suggested, however, that capacitated sperm respire more rapidly and have increased aerobic glycolytic activity over freshly ejaculated sperm. Hamner and Williams (21) also reported in one study none of the eggs were fertilized by slowly respiring freshly ejaculated washed sperm, while 18% of the eggs were fertilized by rapidly respiring spermatozoa which had been incubated *in utero*. Also, it has been suggested by Iritani, Gomes and Van Demark (20) that the increased metabolic rate of spermatozoa incubated in the presence of female reproductive tract secretions is immediately relative to capacitation and irreversible with respect to washing. This suggested that the tract fluids, by increasing the metabolic rate, may have a physiological role of increasing energy production, thus motility; thereby increasing the fertilizing capacity of spermatozoa. The purpose of the present investigation was to investigate the relationship between the increased oxygen uptake by spermatozoa incubated in the presence of female reproductive tract secretions and energy production by measuring oxidative phosphorylation in intact cells.

Materials and Methods. Semen was obtained from boars of proven fertility by the gloved-hand technique. For each series of experiments semen from the same animal was used. Immediately after ejaculation the gelatinous material was removed by straining through cheesecloth. The flask containing the semen was placed in a water-jacketed container and cooled slowly to room temperature and then 4° in order to avoid cold shock. The gel-free semen was centrifuged at 1200*g* for 1.0 to 1.5 min depending upon sperm concentration. The resulting loosely packed pellet was resuspended in an equal volume of 0.25 *M* sucrose in 1.0 m*M* EDTA (Tris-neutralized to pH 7.0). Centrifugation and aspiration were repeated twice, reducing the centrifugation time to 30 and 10 sec, respectively. Sperm concentrations were determined from duplicate counts with a hemocytometer. Final concentrations ranged from 1.6 to 4.5 $\times$ 10^8 sperm/ml.

Oviductal fluid was obtained from mature sows with normal reproductive history by cannulation of a single oviduct following a modification of the method described in Engle, Witherspoon and Foley (22). Fluids ranging in volume from 0.1 ml during diestrus to 5.0 ml during estrus were collected daily and frozen until use. In experiments with heated secretions, the estrous fluid was heated for 2 hr at 60°. For secretions desig-

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TABLE I. Effect of Estrous Oviductal Fluids on Sperm Respiration and Phosphorus Uptake (P/O ratios).

	Control		Oviductal fluid	
Expt	ZO_2[a]	P/O ratio	ZO_2[a]	P/O ratio
1	3.0	1.1	4.5	1.4
2	4.4	0.9	6.1	0.9
3	3.4	1.6	4.0	1.0
4	2.0	1.7	4.0	1.1
5	1.9	1.8	2.1	2.3
	2.9 ± 0.2[b]	1.4 ± 0.2	4.1 ± 0.6	1.3 ± 0.2

[a] Values are μl oxygen taken up/10^8 sperm/hr at 37°.

[b] Mean $\pm$ SE ($p < .01$).

nated as nonestrous, it was necessary to pool collections from Days 6 to 16 because of the small volume of nonestrous fluids. Dialysis of estrous fluid was against two changes of distilled water at 4° for 24 hr.

Oxidative phosphorylation was determined by the method of Morton and Lardy (23) for intact cells. The washed sperm suspension (1.5 ml) was added to prewarmed Warburg flasks and allowed to equilibrate for 10 min. Following equilibration, 1.0 ml of incubation media was tipped from one side-arm giving final concentrations of 40 m*M* KF, 10 m*M* $MgSO_4$, 75 m*M* 2-deoxy-D-glucose, 16 m*M* KH_2PO_4 buffer (pH 7.0), 40 m*M* lactate, 0.5 m*M* ATP (potassium salt), and 0.5 mg hexokinase/ml (Sigma, type IV, yeast). From the other side-arm 0.5 ml of oviductal fluid or 0.5 ml of 0.25 *M* sucrose (control) was tipped in and the flasks were allowed to equilibrate for 5 min before closing the stopcocks. For experiments involving other substrates or ions 0.5 ml of the solution made isotonic with sucrose was added from the side-arm in place of oviductal fluid. Oxygen uptake was measured by the direct method of Warburg (24) with 0.2 ml 14% KOH in the center well. Incubations were from 60 to 90 min at 37°. The oxygen uptake during the 5 min equilibration after adding contents from the side-arms was determined by extrapolation. The reaction was stopped by placing the flask on ice or by adding 0.1 ml 5% TCA. Inorganic phosphorus was determined by the method of Lowery and Lopez (25) as modified by Peel and Loughman (26). Statistical analysis employed the two-tailed *t* test.

Results and Discussion. Values for oxygen uptake by spermatozoa incubated with estrous oviductal fluids were $ZO_2 = 4.1 \pm 0.6$ compared to 2.9 ± 0.6 for controls while P/O ratios were 1.3 ± 0.2 and 1.4 ± 0.2, respectively (Table I). The ZO_2 values reported here are lower than the values of 7.3 and 10.8 reported by Marsh *et al.* (27) and Shelby and Foley (28) for fresh whole semen and 8.69 and 7.2 (without phosphate) observed for fresh washed boar semen by Schul *et al.* (18) and Aalbers, Mann and Polge (29). The reduced oxygen consumption may be due in part to different incubation media used and increased storage time of the sperm prior to incubation. Foley *et al.* (30) reported that a storage period of 72 hr reduced ZO_2 values of extended boar sperm from 8.2 to 4.8. The P/O ratios reported here are slightly lower than those reported by Morton and Lardy (23) for ejaculated bovine spermatozoa and Mohri, Mohri and Ernester (31) for isolated midpieces of bovine spermatozoa. These results confirm the increase in oxygen uptake by porcine spermatozoa exposed to the reproductive tract fluids of the estrous sow (18) and indicate that the increase in oxygen uptake is paralleled by an increase in aerobic energy production. That is, the observed mean increase in oxygen uptake of approximately 29% is paralleled by an equal percentage increase in phosphorus uptake because the P/O ratios are not different.

For spermatozoa incubated in the presence of nonestrous fluids the P/O ratios were significantly ($p < .05$) lower (1.2 ± 0.2) than control values (1.8 ± 0.2). However, an increase in oxygen uptake over controls was noted, 6.2 ± 0.9 vs 4.2 ± 0.2. The lower values for sperm P/O ratios in nonestrous fluids would tend to support existing evidence that the nonestrous female reproductive tract provides a less than optimal environment for spermatozoa (32) whether this lower phosphorus uptake results from an uncoupling of phosphorylation or an increased phosphatase activity in nonestrous oviductal fluids awaits experimentation.

It has been reported that heating or dialy-

TABLE II. Effect of Heated, Dialyzed, and Luteal Phase Oviductal Secretions on Respiration and Phosphorus Uptake.

Experiment	Control		Tract fluids	
	ZO_2	P/O	ZO_2	P/O
Luteal phase	4.2 ± 0.4	1.8 ± 0.2[a]	6.0 ± 0.9[b]	1.2 ± 0.2
Heated fluid	3.1 ± 0.5	1.6 ± 0.3	4.2 ± 0.3[c]	1.5 ± 0.1
Dialyzed fluid	4.3 ± 0.3	1.9 ± 0.2	5.2 ± 0.5[d]	1.8 ± 0.3

[a] Control significantly greater than treatment ($p < .01$).
[b] Mean ± SE for 5 experiments ($p < .05$).
[c] Mean ± SE for 4 experiments.
[d] Mean ± SE for 2 experiments.

sis of oviductal fluids from the rabbit eliminated the sperm stimulating ability by reducing the bicarbonate content (33). Results summarized in Table II indicate that this effect is absent in the porcine species. Similar results have been reported for sheep (20). P/O ratios for spermatozoa incubated in heated or dialyzed estrous fluids were not different from controls.

Aalbers, Mann and Polge (29) showed that a reduction of phosphate concentration increased respiration by boar spermatozoa; however, this is probably not a factor in these experiments since phosphate was added to a final concentration of 16 m*M* and the addition of undialyzed fluid usually resulted in less than a 1 m*M* increase in phosphate concentration.

The variation in oxygen consumption noted throughout these experiments was anticipated from preliminary studies; all possible factors were held constant for each experimental trial. It is felt that this variation was primarily due to the rigorous washing required.

Morton and Lardy (23) reported that phosphorus uptake by bovine epididymal spermatozoa could not be demonstrated in the presence of sodium. In the experiments reported here no such effect was noted with up to 40 m*M* sodium concentration. Dinitrophenol (10^{-4} *M*) prevented phosphorus uptake.

Data reported in Table III show that of the substrates tested, only succinate plus lactate increased oxygen uptake (7.7 ± 3.4) over controls (5.8 ± 3.4) in which lactate was the only exogenous substrate. In experiments in which succinate was the only exogenous substrate P/O ratio of 1.5 to 1.8 were routinely obtained. The addition of bovine serum albumin had no effect on oxygen uptake P/O ratios. Oxygen uptake due to endogenous respiration was negligible in four experiments.

In the presence of calcium, spermatozoa consumed less oxygen and no phosphorus uptake could be demonstrated in freshly washed

TABLE III. The Effect of Various Substrates on Oxygen Uptake by Boar Spermatozoa Incubated in a System for Determining P/O Ratios.

No. of Experiments	Substrate(s) (m*M*)	Control[a]	Experimental
7	Succinate, 10 lactate, 30	5.8 ± 2.0	7.7 ± 3.4[b]
3	Citrate, 10 lactate, 30	8.6 ± 1.1	8.5 ± 0.3
5	Succinate, 40	4.0 ± 1.6	4.1 ± 0.4
4	Malate, 40	1.9 ± 1.1	2.8 ± 0.5
3	Fumarate, 40	3.8 ± 0.5	2.0 ± 0.9

[a] Substrate for control incubations was 40 m*M* lactate.
[b] Significantly greater than control ($p < .05$).

spermatozoa. However, if spermatozoa were washed and stored at 4° overnight in a manner to avoid cold shock, calcium had no effect on phosphorus uptake indicating the loss of a calcium-dependent phosphatase during storage.

Summary. Results are presented that confirm an increase in oxygen uptake by porcine spermatozoa incubated in the presence of estrous oviductal fluids and indicate that increased oxygen uptake is paralleled by a proportional increase in energy production. Spermatozoa incubated in the presence of nonestrous oviductal fluid consumed more oxygen than controls; however, P/O ratios were significantly lower.

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