

## RESPIRATORY STUDIES OF BOVINE SPERMATOZOA AND ENDOMETRIAL PREPARATIONS

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**Summary.** Bovine spermatozoa, washed or in seminal plasma, were incubated alone or in combination with oestrogenic and progestational endometrial preparations; oxygen consumption was determined for 1 hr.

Oxygen uptake by combinations of washed spermatozoa and endometrial preparations could be predicted by addition of the respiratory activity of the isolated tissues. When diluted whole semen was substituted for washed spermatozoa, oxygen uptake by the combined tissues was significantly less than that predicted by addition. The factor in seminal plasma responsible for the depression of respiratory activity was not identified.

### INTRODUCTION

An increase in the respiration of bovine semen incubated in the presence of oviduct fluid and follicular fluid, but a decrease in respiration when incubated with uterine and cervical secretions, has been reported (Olds & VanDemark, 1957b). Hamner & Williams (1963) showed that the respiration of washed rabbit spermatozoa was stimulated by contact with secretions of the female reproductive tract. This stimulation was later attributed to an increased bicarbonate level in the secretions (Hamner & Williams, 1964).

Wales & Restall (1966) reported that the respiration of washed ram spermatozoa was stimulated by incubation in cervico-vaginal, uterine, tubal and follicular fluids when compared with that of control spermatozoa incubated in saline. This advantage was no longer apparent when glucose was added to the control medium and they concluded that any increase was due to the presence of a substrate in the fluid. There was no difference due to the presence or absence of bicarbonate. Restall & Wales (1966) incubated spermatozoa in fluids from the oviduct of the sheep and concluded that there were no differences in respiration which could be attributed to the different stages of the oestrous cycle.

To define the role played by hormones in the respiration of spermatozoa, Mounib (1964) studied the metabolism of washed bull spermatozoa to which various steroid and gonadotrophic hormones had been added. Progesterone, 17- $\beta$ -oestradiol and testosterone all depressed respiration when 1-mg quantities

were added to 4 ml of sperm suspension. The depression was marked with testosterone and progesterone but less so with oestrogen. Gonadotrophic hormones had no effect on respiration. Baker, Schultze & Davis (1949) and Gassner & Hopwood (1955) also showed that sperm respiration was depressed after the addition of testosterone to diluted semen and associated this effect with the androgenicity of the steroid used.

Mather & Dale (1969) showed an increase in the respiration of bovine endometrium during pro-oestrus and oestrus. The present study was conducted to determine if spermatozoa respond to this cyclic increase in respiration exhibited by bovine endometrium.

### MATERIALS AND METHODS

Bulls used for teaching purposes served as donors for the collection of semen; all bulls had been examined and evaluated by the procedure outlined by Carroll, Ball & Scott (1963) and had been classified as satisfactory potential breeding animals. This examination involved determination of sperm concentration and motility, an estimate of the percentage of live cells, and an evaluation of the morphological characteristics of the cells. The semen was collected by electro-ejaculation. The pre-ejaculatory emissions were discarded and only the sperm-rich portion was used in the experiments.

In those experiments utilizing washed spermatozoa, the ejaculate was centrifuged at 40 g for 3 min, at 170 g for 3 min, and finally at 375 g for 2 min. The supernatant was removed and the spermatozoa resuspended in the calcium-free Krebs-Ringer phosphate solution. Each sample was centrifuged three times and washed twice, after which the final volume was adjusted with Krebs-Ringer phosphate to a volume adequate for each experiment. In those experiments using whole semen, equal parts of Krebs-Ringer phosphate buffer solution were similarly used to dilute the ejaculate and bring the volume to that necessary for each experiment.

Respiratory studies were conducted in a Warburg constant volume manometric apparatus. All flasks were paired and contained in addition to the various tissues: 0.1 ml 1.0% glucose, 0.1 ml 2.5% streptomycin sulphate, and 0.2 ml 5 N-KOH in the centre well. Following a 15-min equilibration period, all flasks were incubated at 37° C with a shaking rate of 70 strokes/min for 3 hr. If the system exhibited consistent oxygen utilization for 3 hr, the results were considered valid and measurements for the 1st hr were taken as representative. The number of spermatozoa per ml was determined by use of a haemocytometer.

Endometrial tissue was obtained from a nearby abattoir. The cattle used were of mixed breeding and all were sexually mature as indicated by the size of the animal and gross appearance of the reproductive tract. The reproductive tracts were retrieved as quickly as possible, put into plastic bags and submerged in ice for transport to the laboratory. Criteria for the selection of oestrogenic uteri and progestational uteri were based on the ovarian state as described by Zemjanis (1962) and cervical secretory state as described by Alliston, Patterson & Ulberg (1958).

The endometrium was stripped from the myometrium and passed through a tissue press which had a pore size of 0.1 mm. This procedure reduced the tissue to a particle size small enough to be weighed accurately into 1-g aliquots. Nitrogen determinations on the incubated tissue revealed no significant difference between oestrogenic and progestational tissue (Vanselow, 1940).

## RESULTS

The oxygen consumed by  $1 \times 10^8$  spermatozoa varied from 1.08  $\mu\text{l}$  to 4.84  $\mu\text{l/hr}$  in ten experiments utilizing washed spermatozoa and from 1.25  $\mu\text{l}$  to 7.16  $\mu\text{l/hr}$  in seven experiments utilizing whole diluted semen. The corresponding mean values were 2.73 and 4.36  $\mu\text{l}$  of oxygen consumed per hr. These lower-than-normal values may in part be explained by the suppression of oxygen uptake by phosphates (Bishop & Salisbury, 1955).

Concentration of spermatozoa per ml varied from  $1.60 \times 10^8$  to  $4.72 \times 10^8$  in the washed preparations and averaged  $2.98 \times 10^8$ . The diluted whole semen concentrations varied from  $1.81 \times 10^8$  to  $11.48 \times 10^8$  spermatozoa/ml and had a mean of  $7.81 \times 10^8$  spermatozoa/ml. The lower concentration of spermatozoa in the final washed preparation resulted from the inability to obtain complete separation of the cells from the seminal plasma and the loss of cells while removing the seminal plasma. Longer periods of centrifugation at higher speeds resulted in better separation and loss of fewer cells, but manipulation associated with repeated handling appeared to alter the cells as gauged by a greater number of detached heads. There was no correlation between concentration of spermatozoa within these ranges and oxygen consumption per cell.

Because of variations both in oxygen consumption per cell and sperm concentration in the different samples, there were variations in the oxygen consumption per flask. Mean flask values were 8.89  $\mu\text{l/hr}$  for the ten washed spermatozoa experiments and 32.38  $\mu\text{l/hr}$  for the seven diluted whole semen experiments. Mean oxygen consumption of the seventeen progestational endometrial preparations was 16.46  $\mu\text{l/g/hr}$ , whereas that for the seventeen oestrogenic endometrial preparation was 21.17  $\mu\text{l/hr}$ .

The above data from spermatozoa and endometrium treated in separate flasks were used as a basis for comparison with the results obtained using spermatozoa incubated in combination with the endometrial preparations. Data are presented in four parts: washed spermatozoa and oestrogenic endometrium; washed spermatozoa and progestational endometrium; diluted whole semen and oestrogenic endometrium; and diluted whole semen and progestational endometrium (Table 1). In each case, the predicted mean column represents the results which might be expected by simple addition of  $\text{O}_2$  consumption of isolated spermatozoa and isolated endometrium; the observed mean column presents the results which were observed when the spermatozoa and tissue were combined in a common flask. Spermatozoa used in any particular experiment were collected from the same animal at the same time and endometrium used in each comparison was prepared in the same manner from the same source.

The paired sample technique and the two-tailed 't' test were used to evaluate

these two treatments. Neither group involving washed spermatozoa showed a significant difference between predicted and observed results for spermatozoa combined with endometrium.

In the case of whole semen incubated with the two endometrial preparations, there was suppression of respiration. The expected results, predicted by summing the respective oxygen consumptions of the isolated tissues, were significantly higher than the results which were observed when the tissues were incubated in combination ( $P < 0.05$ ).

A test for heterogeneity of variance substantiated that neither the oxygen consumption of washed spermatozoa nor that of diluted whole semen was influenced by the hormonal state of the endometrium.

TABLE 1  
OXYGEN CONSUMPTION IN  $\mu\text{L}/\text{HR}$  OF OESTROGENIC ENDOMETRIUM (OE) AND PROGESTATIONAL ENDOMETRIUM (PE) INCUBATED WITH WASHED SPERMATOZOEA (WS) AND UNWASHED WHOLE SEMEN (US)

	<i>Predicted mean</i>	<i>Observed mean</i>	<i>Mean difference</i>	<i>S.E. of mean difference</i>
WS and PE	26.33	25.98	0.35	0.31
WS and OE	31.71	29.51	2.20	1.77
US and PE	47.34	36.25	11.09	4.35
US and OE	51.20	36.02	15.18	5.08

## DISCUSSION

Even though fertilization occurs in the oviduct, the uterus does have an influence on the process; the influence is exhibited in the capacitation of spermatozoa in certain species (Chang, 1951; Austin, 1951). For example, Adams & Chang (1962) indicated that rabbit spermatozoa can be capacitated in 6 hr in the uterus whereas the process requires 10 hr in the oviduct. There remains much to be learned about uterine influence on bovine spermatozoa, and there are conflicting opinions concerning their capacitation. Mahajan & Menge (1966) report that incubation of spermatozoa in the uteri of oestrous heifers had no effect on the fertilizing capacity of bull spermatozoa; they, therefore, question the need for capacitation of bovine spermatozoa as a prerequisite to fertilization and feel that, if capacitation is necessary, it is of very short duration. Conversely, a fluorometric method, developed by Ericsson (1967) for the measurement of sperm capacitation in the rabbit, applied to bull spermatozoa indicated that the spermatozoa do undergo capacitation in the oestrous uteri of cows. Several workers have implied that changes in respiration of spermatozoa are involved in this process (Hamner & Williams, 1963, 1964; Mounib, 1964; Mounib & Chang, 1964; Wales & Restall, 1966).

The higher respiration of oestrous endometrium compared to di-oestrous endometrium, which has been reported in the bovine (Mather & Dale, 1969) and similar findings in other species (Carroll, 1942), leaves little doubt that there is a hormonal involvement in uterine respiration. The observed change in

oxygen consumption is presumptively a cellular response; the secretion alone has been reported to be devoid of respiratory activity in the bovine.

The important role of exogenous carbohydrates in sperm respiration has been demonstrated in the metabolism of ram spermatozoa by Restall & Wales (1966) and Black, Crowley, Duby & Spilman (1968), and Olds & VanDenmark (1957a) have shown that the carbohydrate content of bovine uterine secretions varies in the various stages of the oestrous cycle.

Complete agreement does not exist as to the rôle played by bicarbonates in sperm metabolism within the uterus. As Salisbury & Lodge (1963) and Foley & Williams (1967) have pointed out, respiration in the presence of bicarbonate may be significantly higher than in its absence and, when KOH is used in a Warburg apparatus to remove the carbon dioxide, the results may be correspondingly altered. Until more is known about bicarbonate and glucose levels in the luminal secretions of the bovine uterus, theories as to their influence on sperm metabolism must be speculative.

Endometrial preparations, fluids and cells, used in the present investigation, did respire. When washed spermatozoa were incubated with these preparations, the resultant respiratory activity did not differ significantly from that expected from a summation of the relative oxygen consumptions of the isolated tissues. The implication is that the metabolism of washed spermatozoa is not depressed by endometrial preparations, either oestrogenic or progestational. When, however, whole semen was incubated with these same endometrial preparations, there was a significant depression of the combined respiratory activity. These observations suggest that endometrial metabolism is depressed by seminal plasma, an effect not previously considered as a mechanism involved in capacitation.

The presence of seminal fluid has proved to be significant in *in vitro* experiments. Since it is not known how long seminal fluids remain influential in the female tract, their importance *in vivo* is entirely a matter of speculation.

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