EFFECT OF BOVINE AND BUBALINE FOLLICULAR FLUID ON THE OXYGEN UPTAKE OF RAM SPERMATOZOA

Sir,

In the recent times reports are appearing concerning the effect of bovine follicular fluid on the spermatozoa. Yanagimachi (4) and Gwatkin and Anderson (1) reported *in vitro* capacitation and fertilisation of spermatozoa treated with the bovine follicular fluid. Yanagimachi (4, 5) has reported an increased activity of the capacitated golden hamster spermatozoa when treated with the heated bovine follicular fluid. Hence the present study was taken up to know if the follicular fluid had any effect on the oxygen uptake of spermatozoa.

The ovaries were collected from the freshly slaughtered cows and buffaloes. Follicular fluids were collected from the mature graffian follicles with the help of sterile hypodermic syringes. The fluids were centrifuged for ten minutes at 2000 RPM and the supernatants were transferred to separate vials. Then they were heated for an hr at 60°C in a hot water bath. The follicular fluid is heated for one hour in order to detoxify it (5). The heated follicular fluids were preserved in the refrigerator at 4°C and used within five days of collection. Semen from a ram was collected with the help of an artificial vagina. Soon after collection, the sperm motility and concentration were noted. In order to ensure uniformity of the test material only such semen samples which showed good motility and concentration were used. The semen samples were diluted with tyrode solution (pH 7.2) 1:30 before they were used in the Warburg's respirometer. One *ml* diluted semen was transferred to each of the two control and four experimental flasks and sperm concentration in each flask was estimated with the help of a haemocytometer at the end of each experiment. For each experiment run, two flasks were set apart for the control purpose and two each for two treatment groups (i.e., bovine follicular fluid 2 flasks and bubaline follicular fluid 2 flasks). Each experiment was run for 2½ hours. Upto first ½ an hr, the follicular fluids were not added to the treatment groups and therefore the treatment flasks also served as controls upto first ½ an hr. Bovine and bubaline follicular fluids were added to the two treatment groups after ½ an hr by tipping the flasks. Half a millilitre of tyrode diluted bovine and bubaline follicular fluids (1:1) were added to each of the two treatment flasks and similar amounts of tyrode solution were added to the two control flasks simultaneously. Pressure changes in the manometers were recorded by noting the change in the broddie's fluid column in the manometers for all flasks every 15 mins. The amount of oxygen consumption in each flask for each group was calculated by using the standard methods of calculation. In all five experiments were carried out. The results are as noted in the Table I.

A perusal of the data indicates that there is an increase in the consumption of oxygen in the treatment groups. An analysis of variance was carried out to see if there were significant difference

between the means of control and treatment groups. As is evident from the analysis of variance tables (Table II), there are no significant differences between the various means.

TABLE I: Showing the mean oxygen consumption in microliters by 10^8 Ram spermatozoa at 37°C.

	½ *hr*	1 *hr*	1½ *hr*	2 *hrs*	2½ *hrs*
Control	9.692 ± 9.585	18.436 ± 13.655	28.44 ± 22.981	37.208 ± 85.024	44.528 ± 121.133
Bubaline follicular fluid	10.87 ± 8.282	21.496 ± 24.918	33.74 ± 51.805	44.456 ± 94.271	52.798 ± 102.54
Bovine follicular fluid	11.528 ± 12.076	22.752 ± 26.689	33.64 ± 72.96	45.704 ± 91.477	53.918 ± 131.956

TABLE II: Analysis of variance tables for various treatment groups.

Sl. No.	*Time duration*	*Source of variation*	*D.F.*	*Sum of squares*	*Mean square*
1.	½ hr	Between samples	2	9.530	4.765
		Within samples	12	119.777	9.98
2.	1 hr	Between samples	2	49.335	24.667
		Within samples	12	261.053	21.754
3.	1½ hrs	Between samples	2	91.90	45.95
		Within samples	12	590.987	49.24
4.	2 hrs	Between samples	2	210.320	105.160
		Within samples	12	1083.173	90.264
5.	2½ hrs	Between samples	2	1263.025	131.512
		Within samples	12	1422.522	181.518

Aerobically, spermatozoa after all fructose has been removed from the semen, remain motile and consume oxygen at the rate of approximately 100-200 microliters 10^9 sperm cells an hour at 37°C. This rate of oxygen consumption can be increased by the addition of substances including fructose, glucose, mannose, lactic acid, pyruvic acid, acetic acid, glycerol and sorbital (3)

In the above experiment there is an increase of oxygen consumption in the treatment groups, however, it is not significantly greater than the controls. Yanagimachi (4,5) observed increased sperm activity (and hence increased oxygen consumption) in the capacitated golden hamster spermatozoa 2½ to 3 hrs after treatment with the heated bovine follicular fluid. Mattner (2) reported that the time required for the capacitation of ram sperm is about 1.5 hrs.

It is difficult to interpret the above results, however, it may be presumed that more time is required for *in virto* capacitation of ram spermatozoa (more than 2½ hrs) or even though capacitation occured but did not result in increased activity. There are no published reports to indicate that the sperm capacitation leads to their increased activity (increased oxygen consumption) in all species.

D. Bhim Reddy, G.P. Sharma, J. Muralidhar Reddy and C. Norman*

Department of Animal Reproduction,
College of Veterinary Science, Rajendranagar, Hyderabad-500030

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*Present Address: Professor of Biology, West Virginia University, Morgantown, W.V., U.S.A.