THE IMPORTANCE OF CENTRIFUGAL FORCE IN DETERMINING SEMEN CHARACTERISTICS

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It is the purpose of this study to determine the effect of relative centrifugal force (RCF) $\times g$ on various semen characteristics of the bull and boar.

Entire ejaculates of mature Yorkshire boars were collected and separated from the gel fraction by allowing the semen to run through three layers of cheesecloth into a warm thermos. The ejaculate was then placed in 50-ml centrifuge tubes and centrifuged at 1000 g for 10 or 15 min to remove the spermatozoa. Part of the first supernatant was kept for further analyses, while the remaining semen was centrifuged at forces and times given in Tables 1, 2 and 3. Bull epididymal fluid was collected by flushing through the vas deferens with a 154 mM-KCl, 10 mM-tris buffer at pH 7.2 according to the method of Henle (1938). The sperm concentration in the epididymal fluid was adjusted to a concentration of about 10^8 cells/ml before centrifugation. Bull semen was obtained from American Breeders Service. The bull epididymal fluid and semen were handled as already described for the boar.

All centrifugations, except those at 105,000 g , were in angle heads in 12-ml round-bottomed tubes. Each tube received 8 ml of the sample, the upper 5 ml being removed by pipette at the end of each centrifugation. The temperature was maintained at 15° C, and all RCF $\times g$ values in the Tables represent the maximum force (bottom of centrifuge tube). The centrifugations at 105,000 g , which were run on a Beckman model L with the 40 rotor, represent the force at the middle of the centrifuge tube.

Protein was measured by using the biuret reagent (Layne, 1957), and the nucleotide phosphatase according to the method described by Garbers, Wakabayashi & Reed (1971). Phosphate was measured by adding 1 ml of cold 10% trichloroacetic acid to 1 ml of plasma, centrifuging at 12,000 g for 30 min and measuring phosphate in the supernatant by the method of Sumner (1944). Turbidity was measured at 560 nm (Mann, 1964).

The seminal plasma of Yorkshire boars shows considerable particulate contamination even after centrifuging at 105,000 g for 15 min (Table 1). Comparison of the 1000 g to the 105,000 g force shows a drop of 6 mg in protein/ml, 0.7 in nucleotide phosphatase activity and 0.14 in μ mol phosphate/ml with the higher force.

TABLE 1
THE EFFECT OF CENTRIFUGAL FORCE ON CHARACTERISTICS OF BOAR SEMEN

<i>RCF × g</i>	<i>Centrifuge time (min)</i>	<i>Absorbance λ n = 560</i>	<i>Protein*</i>	<i>Phosphate†</i>	<i>Nucleotide‡ phosphatase</i>
1,000	15	1.40	30.1	1.04	1.78
10,000	15	1.01	30.2	0.97	1.80
30,000	15	0.80	30.1	0.97	1.79
105,000	15	0.18	28.2	0.96	1.51
	60	0.09	24.0	0.90	1.08

* mg protein/ml plasma.

† μmol phosphate/ml plasma.

‡ nmol phosphate hydrolysed from ATP/mg protein/min.

TABLE 2
THE EFFECT OF CENTRIFUGAL FORCE ON CERTAIN CHARACTERISTICS OF BULL EPIDIDYMAL FLUID

<i>RCF × g</i>	<i>Centrifuge time (min)</i>	<i>Absorbance λ = 560</i>	<i>Protein*</i>	<i>Nucleotide† phosphatase</i>
1,000	10	0.32	6.25	5.3
	30	0.09		
4,000	10	0.04	5.50	5.1
	30	0.02		
10,000	10	0.02	5.30	3.6
	30	0.01		
30,000	10	0.005	5.15	2.8
	30	0.000		

* Single values per *RCF × g* represent pooled means for 10- and 30-min time intervals. Protein expressed as mg/ml.

† Activity expressed as μmol phosphate liberated from ATP/mg protein.

TABLE 3
THE EFFECT OF CENTRIFUGAL FORCE ON CERTAIN CHARACTERISTICS OF BULL SEMEN

<i>RCF × g</i>	<i>Centrifuge time (min)</i>	<i>Absorbance λ = 560</i>	<i>Protein*</i>	<i>Nucleotide† phosphatase</i>	<i>Phosphate‡</i>
1,000	15	1.263	74.6	3470	4.8
10,000	15	0.289	73.3	3470	4.8
20,000	15	0.206	72.1	3220	4.8
40,000	15	0.132	71.3	2550	4.6
105,000	60	0.050	61.3	470	4.2

* mg protein/ml plasma.

† nmol phosphate hydrolysed from ATP/mg protein/min.

‡ μmol phosphate/ml plasma.

Bull epididymal fluid appears to contain only slight particulate contamination after centrifuging at 30,000 *g* (Table 2). Between 1000 and 30,000 *g* forces, a drop of over 1 mg protein/ml and of nearly 50 % of the nucleotide phosphatase activity occurs.

Bull semen, like bull epididymal fluid, contains appreciable levels of the magnesium-stimulated nucleotide phosphatase (Tables 3). The activity of this enzyme drops over five-fold when centrifugation is at 105,000 *g*. Protein level declines 13 to 14 mg/ml, and phosphate also declines with the 105,000 *g* centrifugation.

The results of this report definitely support the use of higher centrifuge forces in the study of semen composition. Substances supposedly in soluble form in semen have been shown to be partially pelleted at high centrifugal force. The male accessory glands are the source of particulate elements (Mann, 1964). Garbers *et al.* (1971) demonstrated that a magnesium-stimulated nucleotide phosphatase was associated with a light particulate fraction in epididymal fluid. These results indicate that the nucleotide phosphatase activity is not pelleted until very high centrifugal forces are used.

It is possible that some of the variability attributed to differences in semen composition between animals or between days is due to alterations in the amount of particulate matter being pelleted by lower centrifugal forces. For seminal plasma or epididymal fluid studies, a slow centrifugation to remove spermatozoa followed by a centrifugation at 105,000 *g* appears warranted. With the spermatozoa present, not only would a high centrifugation cause the spermatozoa to be contaminated by the particulate debris, but a considerable loss of sperm constituents would be expected (Mann, 1964).

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REFERENCES

- GARBERS, D. L., WAKABAYASHI, T. & REED, P. W. (1971) Enzyme profile of the cytoplasmic droplet from bovine epididymal spermatozoa. *Biol. Reprod.* (In press).
- HENLE, W. (1938) The specificity of some mammalian spermatozoa. *J. Immunol.* 34, 325.
- LAYNE, E. (1957) *Spectrophotometric and turbidimetric methods for measuring proteins*. In *Methods in Enzymology*, Vol. III, p. 447. Eds. S. P. Colowick and N. O. Kaplan. Academic Press, New York.
- MANN, T. (1964) *The biochemistry of semen and of the male reproductive tract*. John Wiley and Sons, New York.
- SUMNER, J. B. (1944) Scientific apparatus and laboratory methods: A method for the colorimetric determination of phosphorous. *Science, N.Y.* 100, 413.