SEMINAL VESICULECTOMY IN BULLS

I. SEMINAL BIOCHEMISTRY

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Summary. Sperm-rich and pre-sperm ejaculates were collected from bulls before and after excision of the seminal vesicles, and the composition of the fluids was analysed. In the pre-sperm fraction, vesiculectomy did not alter pH or levels of chloride, nitrogen and free amino acids. There was little alkaline phosphatase activity in the pre-sperm fraction before surgery, and this low level was further decreased by vesiculectomy, as were fructose, citrate and potassium, while sodium concentration increased. In the sperm-rich fraction, the volume was reduced by vesiculectomy, as were levels of fructose, citrate, potassium and alkaline phosphatase activity. Chloride, glycerylphosphorylcholine and free amino acid levels increased after vesiculectomy. Sodium concentration and pH were unaltered.

The findings indicate that the vesicular glands contribute to the pre-sperm fraction and more significantly to the sperm-rich portion of the ejaculate. The glands that secrete the bulk of the pre-sperm fraction also contribute to the sperm-rich fraction. This investigation clarifies the biochemical contributions of the seminal vesicles to the ejaculate. The glandular contributions to the pre-sperm fraction remain to be defined.

INTRODUCTION

Differences in the chemistry of bovine seminal plasma may be largely attributed to the function of the accessory glands, since these glands are the major sources of secretions that contribute to the fluid volume of semen. Sixty to 90% of the volume of a normal ejaculate comes from the accessory glands, of which the seminal vesicles contribute the largest portion.

The work reported here was performed to add to knowledge of the contribution of the seminal vesicles to the composition of semen.

MATERIALS AND METHODS

Semen collection

Semen was collected by artificial vagina from five Hereford bulls which were 1.5 years of age at the beginning of the study. Pre-sperm fractions were collected

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according to procedures of Faulkner, Masken & Hopwood (1964). Samples of pre-sperm and sperm-rich fractions from each bull were analysed before and after seminal vesiculectomy.

Surgical procedure

Two surgical approaches to the seminal vesicles were investigated. With either approach, food was withheld for 24 hr, and no water was given for 12 hr before the operation. Surgical sites were clipped, scrubbed and swabbed with alcohol and Zephiran.

One approach was through an incision in the left flank. Paravertebral anaesthesia was combined with light general anaesthesia, and the bull was placed in right lateral recumbency.

A simpler approach was suggested by Dr D. D. Delahanty (personal communication). Epidural anaesthesia was used, and the operation was performed with the bull standing. A purse-string suture was used to close the anus. A semicircular incision was begun $\frac{1}{4}$ inch lateral to the hair line opposite the upper quadrant of the anus and extended ventrally to a point opposite the contralateral lower quadrant of the anus. The seminal vesicles were reached by blunt dissection, and their peritoneal investment was stripped away. The vesicles were severed close to the pelvic urethra using an equine ovariectomy knife. One bull (3027) failed to deliver spermatozoa after surgery, indicating that the vasa deferentia were occluded.

Seminal biochemistry

Hydrogen ion activity was measured with a Beckman pH meter using a single-drop hydrogen electrode. The colorimetric method of Roe (1934), as adapted by Mann (1948), was used to determine fructose concentration. Citric acid determination was based on the method of Speck, Moulder & Evans (1946).

Seminal amino acid levels were determined by the procedure of Hopwood & Gassner (1962). Alkaline phosphatase was measured according to the method of Bessey, Lowery & Brock (1946).

Sodium and potassium were determined with a Beckman flame photometer coupled to a Beckman DU spectrophotometer. Chloride was measured by the method of Schales & Schales (1941). Glycerylphosphorylcholine (GPC) was analysed according to the method of White (1959). Total nitrogen was determined by the method of Folin & Wu (1919), as modified by Natelson (1963). The significance of differences between mean values was tested using Student's *t*-test (Steel & Torrie, 1960).

RESULTS

Pre-sperm fraction

Mean values for biochemical components of the pre-sperm fraction before and after seminal vesiculectomy are shown in Table 1. There was no change in pH as a result of vesiculectomy. Fructose, citric acid and potassium were reduced, while the concentration of sodium increased. There was little alkaline phosphatase activity before or after vesiculectomy, but vesiculectomy reduced

TABLE 1
SEMINAL CONSTITUENTS IN THE PRE-SPERM FRACTION BEFORE AND AFTER SEMINAL VESICULECTOMY

	Average no. analyses		Bull 3018		Bull 3021		Bull 3024		Bull 3053		Bull 3027		Average	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
pH	7	12	8.4	8.7	8.9	8.1*	8.7	8.0	8.4	8.6	8.7	8.0	8.5	8.3
Fructose (mg/100 ml)	8	12	0.6	6	47	7*	0	0.3	2	0.1	65	2†	38	3†
Citric acid (mg/100 ml)	8	12	20	15	91	8	30	9	15	0†	281	6*	103	8†
Alkaline phosphatase (Sigma units/ml)	7	9	0.9	0	1.9	0.1	0	0	10.2	0	10.0	0	5	0.2†
Nitrogen (mg/100 ml)	4	4	62	64	83	98	88	85	77	74	88	82	79	79
Chloride (mg/100 ml)	6	11	513	479	423	511	422	514*	490	515	408	495*	478	504
Potassium (mg/100 ml)	6	9	93	91	113	92	43	103	101	91	141	92	110	94*
Sodium (mg/100 ml)	7	9	311	351	315	332	350	354	336	356	329	358	322	349†

* Significant from control at 5% level.

† Significant from control at 1% level.

TABLE 2
SEMINAL CONSTITUENTS IN THE SPERM-RICH FRACTION BEFORE AND AFTER SEMINAL VESICULECTOMY

	Average no. analyses per bull		Bull 3018		Bull 3021		Bull 3024		Bull 3053		Bull 3027		Average	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Volume (ml)	11	11	6.2	3.9†	5.1	4.6	5.1	3.0	6.3	3.3†	4.2	2.8†	5.6	3.4†
pH	11	11	7.0	7.2	7.0	6.7	6.9	6.9	6.9	7.2	7.0	7.3	7.0	7.0
Fructose (mg/100 ml)	11	12	412	26†	347	156†	320	55†	470	23†	353	51†	388	69†
Citric acid (mg/100 ml)	11	11	981	214†	935	627*	1036	392†	898	221†	1063	236†	971	393†
Alkaline phosphatase (Sigma units/ml)	7	10	99	50†	115	117	90	96	74	41	155	22†	103	69†
GPC (mg/100 ml)	4	6	672	1500	1090	1512	—	1118	580	1360*	—	2180	922	1363*
Nitrogen (mg/100 ml)	4	4	1150	988	1346	1137	1388	952†	1408	800†	1462	601	1353	905†
Free amino acids (mg/100 ml)	8	9	116	173	82	92	96	131	97	223†	86	8†	92	150†
Free glutamic acid (mg/100 ml)	8	9	71	81	56	64	60	73	66	90	59	1†	62	73*
Chloride (mg/100 ml)	7	8	101	248†	105	160	122	174	135	254	162	400†	130	349†
Potassium (mg/100 ml)	7	7	227	185	211	179	212	178	280	200	380	173†	269	186†
Sodium (mg/100 ml)	6	8	216	194	227	199	244	196	179	196	160	256†	197	196

* Significant from control at 5% level.

† Significant from control at 1% level.

the low level of activity existing before surgery. Nitrogen and chloride levels were unaltered.

GPC was not detected in the pre-sperm fraction. Levels of free amino acids in the pre-sperm fraction have not previously been documented. The levels in mg/100 ml, which were unaltered after vesiculectomy, were as follows: aspartic acid 0.3 ± 0.2 , glutamic acid 3.7 ± 3.0 , serine 0.3 ± 0.3 , glycine 0.5 ± 0.5 and alanine 0.6 ± 0.5 .

Sperm-rich fraction

Results of biochemical analysis of the sperm-rich fraction before and after vesiculectomy are shown in Table 2. The pH was not affected by seminal vesiculectomy. Differences in volume and the levels of fructose, citric acid, chloride, potassium, GPC, nitrogen, free amino acids and alkaline phosphatase activity were significant. The concentration of sodium was unaltered.

DISCUSSION

The decreases in fructose and citric acid in the sperm-rich fraction were expected after seminal vesiculectomy, since the seminal vesicles are the main source of seminal fructose (Mann, 1946) and of citric acid (Schersten, 1929) in bulls. Residual quantities of these components in semen after vesiculectomy may be due to contributions from the ampullary glands (Mann, 1948) or to incomplete removal of seminal vesicle tissue. The 82% reduction in seminal fructose and 57% reduction in citric acid following vesiculectomy indicate a differential depletion of the major vesicular gland contributions and support the evidence that other accessory glands, in addition to the seminal vesicles, contribute these factors. Decreases in fructose and citric acid in the pre-sperm fraction after vesiculectomy indicate that the seminal vesicles contribute to pre-sperm fluid.

Pre-sperm fractions were devoid of GPC before and after vesiculectomy, supporting the findings of Dawson, Mann & White (1957) that GPC is secreted by the epididymis. Furthermore, GPC concentration increased in the sperm-rich fraction after vesiculectomy. We cannot explain, however, why GPC was present in high concentration in the ejaculate of bull 3027 after the vasa deferentia were presumably blocked.

Seminal vesiculectomy did not alter free amino acid levels in the pre-sperm fraction, but amino acids were increased in the sperm-rich fraction after vesiculectomy. Hess, Ludwick, Martig & Ely (1960) found no significant increase in amino acids after ablation of seminal vesicles. Our results agree with the conclusion of Hopwood & Gassner (1962) that the testes and epididymides are the major sources of seminal amino acids, because levels increased in the sperm-rich ejaculate after seminal vesiculectomy and markedly decreased when the vasa deferentia were occluded in bull 3027.

Decreased alkaline phosphatase activity in the sperm-rich fraction after seminal vesiculectomy and the low level of alkaline phosphatase in the pre-sperm fluid, also decreased by vesiculectomy, further indicate that vesicular gland secretion is present in the pre-sperm fraction.

Rothschild & Barnes (1954) found an inverse relationship between sodium and potassium in seminal plasma, while calcium, chloride and nitrogen were positively correlated. Quinn, White & Wirrick (1965) reported that the concentration of sodium in seminal vesicle fluid was almost twice that in epididymal semen. The concentration of potassium in vesicular gland fluid was about half that of epididymal semen. We found that sodium increased and potassium decreased in the pre-sperm fluid after vesiculectomy. We interpret this to mean that the high sodium content of the bulbo-urethral and prostate gland contributions to pre-sperm fluid was lowered by seminal vesicular secretion, which, richer in potassium, increased the level of this component in the pre-sperm fraction. Levels of sodium, chloride and potassium after vesiculectomy indicate that the bulbo-urethral and prostate glands contribute to the sperm-rich fraction, as proposed by Aalbers (1966). These secretions, high in sodium and chloride but low in potassium, apparently were capable of maintaining the sodium level, increasing chloride and decreasing potassium in the sperm-rich fraction after removal of the seminal vesicles.

Hess *et al.* (1960) found that the pH of pooled, successive ejaculates increased after seminal vesiculectomy. There was no change in the pH of pre-sperm or sperm-rich fractions as a result of vesiculectomy in our investigation. Aalbers (1966) reported that the contribution from the 'urethral glands', relative to the epididymal contribution, increased in the third through to the sixth successive ejaculates. In our study only a single, sperm-rich ejaculate was analysed which may account for the discrepancy.

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