Relationship of Glycerylphosphorylcholine to Other Constituents of Bull Semen [1]

K. L. MAC MILLAN, C. DESJARDINS, K. T. KIRTON,[2] and H. D. HAFS
Department of Dairy, Michigan State University, East Lansing

Abstract

Glycerylphosphorylcholine (GPC), sperm numbers, fructose, and citric acid were measured in semen samples collected from five mature bulls twice every Monday and twice every Thursday for six weeks. Seminal GPC was significantly correlated with a) the numbers of sperm in first and second ejaculations ($r = 0.72$ and 0.53, respectively), b) the volume of semen of both ejaculations ($r = 0.62$ and 0.49, respectively), and c) the seminal content of fructose ($r = 0.41$ and 0.43, respectively) and citric acid ($r = 0.55$ and 0.33, respectively). Glycerylphosphorylcholine in the semen was 14% greater after a four-day interval between ejaculations than after a three-day interval; none of the other measured constituents differed significantly in that respect. The results suggested that epididymal resorption of sperm was proportionately more rapid than that of GPC, and that measurement of both of these parameters in future experiments may provide simultaneous information on the secretory and resorptive functions of the epididymis.

Dawson et al. (3) demonstrated that glycerylphosphorylcholine (GPC) in bull semen is primarily of epididymal origin. This led Mann (5) to suggest that GPC may be a useful indicator of the epididymal contribution to an ejaculation. Since sperm in transit from the testis reside in the epididymis for several weeks, and because the epididymis is the immediate origin of sperm at ejaculation, particularly at intensive ejaculation frequencies, one might expect the sperm and GPC contents of an ejaculum to be correlated. On the other hand, Amann and Almquist (1) showed that the epididymis is the major site of sperm resorption and that this resorption is inversely proportional to the frequency of ejaculation. Any disparity in the rates of resorption of sperm and GPC would limit the magnitude of any correlation between their seminal levels. If sperm were resorbed preferentially to GPC, an increase in the interval between ejaculations would result in higher GPC : sperm ratios in semen. In this case, epididymal function could be more accurately assessed by measurement of seminal levels of both GPC and sperm numbers.

Our experiment was designed to determine the relationship between the GPC and total sperm contents of semen, and to find whether this relationship can be altered by small differences in the interval between ejaculations. Relationships between GPC and some seminal constituents derived chiefly from accessory glands other than the epididymis were determined as references.

Material and Methods

Five mature bulls (four Holstein and one Guernsey) were each ejaculated twice every Monday and twice every Thursday during a six-week experimental period. Each bull was allowed two false mounts during a 10-min period of active sexual preparation before each ejaculation. The interval between first and second ejaculations varied between 15 and 25 min. Data derived from the first week were considered separately, as the bulls had not been in routine use for one month prior to the experiment. Consequently, the statistical analyses were based on a total of 25 first and 25 second ejaculations for Mondays and for Thursdays.

Ejaculum volume and sperm concentration were determined immediately following ejaculation. Aliquots (0.20 ml) of semen were added to 4.80 ml of 5% trichloroacetic acid (TCA) solution and to 2.00 ml of 4% zinc sulfate septahydrate solution and the mixtures stored at −20 C. The TCA mixture was subsequently centrifuged and the supernatant fluid used to determine the concentrations of citric acid by the method of Saffran and Denstedt (7), and of fructose by the method of Roe (6). Four milliliters of 0.30 N barium hydroxide solution were added to the zinc sulfate mixture and the supernatant fluid after centrifugation was used

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to determine GPC by the method of White (10).

Correlation coefficients between GPC and the other measured constituents were calculated from the sum of cross-products derived from analyses of covariance. The coefficients were thereby corrected for differences between bulls and between Mondays and Thursdays. The correlation coefficients between first and second ejaculations for each constituent were calculated in a similar manner, but were not corrected for differences between Mondays and Thursdays.

Analyses of variance were used to determine the significance of differences between data obtained on Mondays and Thursdays for each constituent in both first and second ejaculations and in the sum of the two ejaculations. A paired "t" test was used to compare the quantities of a constituent in first and second ejaculations.

Results

The averages obtained for first and second ejaculations, separately and combined, are listed in Table 1. The only significant difference between the two days was in total GPC; the average output of 62.2 mg on Mondays was 14% greater than the average output of 54.4 mg on Thursdays ($P < 0.05$). The averages (Table 1) indicated that both first and second ejaculations contained more GPC after a four-day interval than after a three-day interval, but these differences only approached significance ($P \simeq 0.07$). Averages for seminal volume and citric acid of first ejaculations did not differ significantly from the corresponding averages for second ejaculations; while the average fructose content of second ejaculations was significantly greater than that of first ejaculations ($P < 0.05$). In contrast, the average sperm and GPC contents were each greater in first ejaculations than in second ejaculations ($P < 0.01$).

TABLE 1

Average values of seminal characters for bulls ejaculated on Mondays and Thursdays[a]

Seminal character	Ejaculum	Monday	Thursday	Average
Volume *(ml)*	First	7.1	7.0	7.1
	Second	7.5	7.0	7.2
	Total	14.5	14.0	14.3
Total sperm	First	8.2	8.4	8.3
(10^9)	Second	6.0	5.4	5.7
	Total	14.2	13.8	14.0
GPC[b] *(mg)*	First	36.1	31.9	34.0
	Second	26.2	22.5	24.3
	Total	62.2	54.4	58.3
Fructose *(mg)*	First	36.7	35.5	36.1
	Second	41.9	39.6	40.9
	Total	78.6	75.3	77.0
Citric Acid				
(mg)	First	47.0	47.8	47.4
	Second	52.0	49.3	50.6
	Total	99.0	97.1	98.0

[a] Each value is the average of 25 observations.
[b] Glycerylphosphorylcholine.

The correlation coefficients between GPC and other seminal constituents (Table 2) revealed that the relationships between GPC and total sperm were greater than those between GPC and the other constituents, which were taken as indices of seminal vesicular function. However, none of the differences between the correlation coefficients in Table 2 was significant. Correlations between the quantity of each constituent in first and second ejaculations were not large. Nevertheless, GPC content of first ejaculations was negatively related to that in second ejaculations ($r = -0.31$, $P \simeq 0.04$) and sperm content followed a similar pattern ($r = -0.20$, $P \simeq 0.10$). In contrast, fructose content of first ejaculations was positively correlated with that of second ejaculations ($r = 0.37$, $P \simeq 0.01$). Seminal volume and citric acid content of first ejaculations were not measurably related to seminal volume and citric acid content of second ejaculations ($r = 0.05$ and 0.04, respectively).

TABLE 2

Correlations between glycerylphosphorylcholine and some other constituents of bull semen[a]

	Glycerylphosphorylcholine content	
	First ejaculum	Second ejaculum
Seminal volume	0.62	0.49
Total sperm	0.72	0.53
Fructose content	0.41	0.43
Citric acid content	0.55	0.33

[a] $r_{(0.95,44d.f.)} = 0.28$; $r_{(0.99,44d.f.)} = 0.38$.

The limited data from the preliminary week, obtained after one month of sexual rest, were not subjected to statistical analyses. Nevertheless, ejaculations obtained during the first week after one month of sexual rest contained more sperm and more GPC than ejaculations obtained during the following five weeks (Table 1); but the relative decrease in GPC following the first week after sexual rest was greater than the corresponding decrease in sperm numbers, and this was reflected in the greater GPC: total sperm ratio immediately after sexual rest (Monday, Table 3).

TABLE 3

Average sperm numbers and seminal glycerylphosphorylcholine and the ratio between them during the first week after one month's sexual rest

Seminal character	Ejaculum	Monday[a]	Thursday
Total sperm *(10^9)*	First	8.3	9.9
	Second	7.6	8.2
	Total	15.9	18.1
GPC[b] *(mg)*	First	45.1	35.9
	Second	36.2	32.6
	Total	81.3	68.5
GPC/TS[c]	First	5.43	3.63
	Second	4.76	3.98
	Total	5.11	3.78

[a] Monday was the first day of ejaculation after one month of sexual rest.
[b] Glycerylphosphorylcholine.
[c] GPC content (mg) : total sperm (10^9).

Discussion

The significant correlation between total sperm and seminal GPC content was anticipated as Dawson et al. (3) reported that seminal GPC is primarily of epididymal origin. However, the correlation coefficients obtained, although statistically significant, were not sufficiently large to be of great predictive value. Consequently, the sperm and GPC contents of an ejaculum should be used as separate indicators of epididymal functions.

Scott et al. (8) reported that the rate of GPC synthesis in rabbits was greatest in the caput epididymidis and negligible in the testis. Crabo (2) and Wales et al. (9) analyzed bovine epididymal fluid and concluded that GPC was secreted in the caput and corpus epididymidis. Crabo (2) considered that GPC secretion in the cauda epididymidis was minimal. However, Amann and Almquist (1) suggested that the major site of bull sperm resorption was the cauda epididymidis. Results of the present experiment suggested that resorption of GPC if, in fact, GPC was resorbed, was proportionately less than that of sperm; because, while total GPC after a four-day rest (62.2 mg) was significantly greater than the total after a three-day rest (54.4 mg), the total sperm obtained after a four-day rest (14.2×10^9) was not much greater than the total after a three-day rest (13.8×10^9). However, some resorption of GPC may have occurred, because, although there was one-third more time to secrete GPC during a four-day interval than during a three-day interval, the comparable increase in seminal GPC was only 14%. Data obtained after one month of sexual rest also indicated that sperm were resorbed more rapidly than GPC.

The average GPC concentrations for first and second ejaculations were 4.79 and 3.37 mg per ml of semen, respectively. The value for first ejaculations was higher than those reported by Dawson et al. (3) and White (10), but these differences may be due to differences in sexual preparation of the bulls, because Hafs et al. (4) reported that the epididymal contribution to an ejaculation, as measured by total sperm, was considerably increased with more intensive sexual preparation. Neither of the previous researchers described the preejaculation sexual preparation employed. If seminal GPC content reflects the epididymal contribution to semen more accurately than does total sperm content, as suggested by the present data, its measurement may be valuable in future studies on the action of preejaculation sexual preparation on the efficiency of contraction of the epididymis at the time of ejaculation.

None of the correlation coefficients between quantity of a constituent in first ejaculations and quantity of the same constituent in second ejaculations was very large. The compositions of two semen samples taken within a short interval of time were relatively independent. Nevertheless, comparison of the averages for first and second ejaculations (Table 1) indicated that the pools of GPC and of sperm available for ejaculation were relatively much more limited than were the pools of fructose and citric acid.

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