Semen Output of Rabbits Ejaculated After Varying Sexual Preparation.* (32334)

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That sexual preparation before ejaculation dramatically increases sperm output has been conclusively demonstrated in bulls(1,2). Hafs *et al*(3) showed that sperm output and semen volume responses to sexual preparation were dependent upon the number of false mounts as well as the duration of sexual preparation. Data of another nature obtained from rabbits (4) suggested that this useful experimental animal may also respond to sexual preparation with increased sperm output. These researchers found that second ejaculations contained more sperm than first ejaculations when no attempt was made to impose sexual preparation and suggested that the first ejaculation may have served as a stimulus for increased sperm output in the second. Consequently, the following experiment was designed to study sperm output and semen composition of rabbits ejaculated following different intensities of sexual preparation. A second objective was to determine whether a response to sexual preparation would be altered by duration of the interval between sexual preparation and ejaculation.

Materials and methods. Nine mature rabbits (3 New Zealand White and 6 Dutch Belted) were each ejaculated twice on every second day for 38 days. Before ejaculation, one of the following forms of sexual preparation was imposed: (a) no systematic preparation, (b) one false mount after which the stimulus doe was removed from the buck's cage for 3 minutes (1FM + 3 min), (c) three false mounts and removal of the doe for 30 seconds (3FM + 30 sec), and (d) three false mounts and removal of the doe for 3 minutes (3FM + 3 min). Comparison of treatments c and d was intended to test whether stimuli imposed during active sexual preparation may operate for a brief period after the stimulus doe is removed. A particular sexual preparation treatment was applied to both the first and second ejaculations from a rabbit on any given day and about 30 minutes elapsed between the first and second ejaculations. The sequence of application of sexual preparation treatments was randomized for each buck and each sequence was repeated 5 times. This resulted in a total of 45 first and 45 second ejaculations for each treatment.

Immediately after ejaculation, 5 ml of cold water was flushed through the artificial vagina directly into the semen collection tube to recover any sperm adhering to the latex surface. The volume of the ejaculum (including any gel-mass) was measured and total sperm content was determined hemacytometrically. When a gel-mass was present, it was removed and total sperm content calculated using gel-free volume of the ejaculum. Each semen sample was then gently heated with its gel-mass to liquefy any gel-mass and aliquots of the semen were taken for determining fructose concentration(5), as an index of accessory gland activity.

Data from first ejaculations, second ejaculations and sums of the two ejaculations were considered as separate response criteria. The 3 degrees of freedom and sums of squares for sexual preparation treatments were orthogonally partitioned to compare (a) no preparation *vs.* the 3 preparation treatments, (b) 1FM + 3 min *vs.* the two 3FM treatments, and (c) 3FM + 30 sec *vs.* 3FM + 3 min. Components of variance analyses were also conducted on data for total sperm output (sums of first and second ejaculations) within each treatment to determine the percentage of the total error variance associated with bucks in relation to that associated with replicate observations within bucks.

Results. The average interval between introduction of a stimulus doe into a buck's

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TABLE I. Averages* for Seminal Characteristics of First and Second Ejaculations Following Varied Intensities of Sexual Preparation.

Criteria	Ejaculum	Sexual preparation: None	1 FM† + 3 min‡	3 FM + 30 sec	3 FM + 3 min
Seminal vol (ml)	First	.91	.95	1.15	1.05
	Second	.54	.56	.46	.51
	Sum	1.45	1.52	1.61	1.55
Fructose (mg per ejaculum)	First	.75	.93	1.04	.99
	Second	.55	.54	.49	.53
	Sum	1.30	1.47	1.53	1.52
Total sperm ($\times 10^6$ per ejaculum)	First	37.0	102.9	141.5	140.4
	Second	75.9	98.9	97.4	107.3
	Sum	112.9	201.8	238.9	247.7

* Each mean is the average of 45 first or 45 second ejaculations.
† False mount.
‡ Interval between sexual preparation and ejaculation.

cage and first ejaculation was 30 seconds when no systematic preparation was imposed. When false mounts were imposed, the first false mount occurred after a similar interval, but second and third false mounts occurred within an additional 15 seconds. When a stimulus doe was returned to a buck's cage for the first ejaculation, bucks mounted and ejaculated almost immediately. Similar observations on second ejaculations revealed similar intervals of time between introduction of stimulus does to bucks and false mounts or ejaculation, except that first false mounts occurred within about 20 seconds.

The averages for measured seminal constituents are listed in Table I. The two more intensive methods of sexual preparation (3FM) produced small but non-significant ($P>0.10$) increases in the average seminal volume (including gel-mass) of first ejaculations. However, these increments were at the expense of volume of the second ejaculations because average seminal volume of second ejaculations after more intensive preparation was significantly less than the average after none or 1FM ($P<0.05$). When the volumes of first and second ejaculations were summed, the 4 sexual preparation treatments did not differ significantly ($P>0.10$). Considerable variation in seminal volume, particularly of first ejaculations, was apparently due in large part to unpredictable occurrence of a gel-mass. Six of the nine bucks occasionally produced visible gel-mass, but only in first ejaculations. Of the 45 first ejaculations obtained with each sexual preparation treatment, 15 ejaculations had detectable gel-mass following no preparation, 13 ejaculations following 1FM + 3 min, 18 ejaculations following 3FM + 30 sec, and 13 ejaculations following 3FM + 3 min.

Since the gel-mass does not contain detectable quantities of fructose(6), the effects of sexual preparation on total fructose content of ejaculations should not be complicated by unpredictable occurrence of gel-mass. Averages for fructose content of first ejaculations obtained after sexual preparation were greater than the average after no preparation ($P<0.01$), but no similar trend was apparent in second ejaculations ($P>0.25$). These changes in fructose content approximately paralleled changes in seminal volume.

Sperm output data (Table I) revealed striking changes produced by sexual preparation. First ejaculations obtained after 1FM + 3 min contained 278% of the number of sperm in control first ejaculations ($P<0.01$) and 3 false mounts resulted in an additional 40% increment in sperm numbers when compared to one false mount ($P\simeq0.07$). Although sexual preparation produced a significant increase in the sperm numbers in second ejaculations ($P<0.01$), the increase was not as dramatic as for first ejaculations and 3 false mounts did not produce a significant increase over one false mount ($P>0.10$). However, when the sperm contents of first and second ejaculations were summed, 3 false mounts resulted in approximately 20% more total

TABLE II. Components of Variance and Coefficients of Variation Associated with Average Sperm Output.

Sexual preparation†	Components of variance* σ^2_b	$\sigma^2_{w:b}$	CV‡
None	6.2 (40)§	11.8 (76)	117
1 FM + 3 min	11.9 (31)	22.5 (59)	90
3 FM + 30 sec	12.1 (32)	11.9 (31)	63
3 FM + 3 min	20.2 (40)	20.6 (41)	81

* σ^2_b = buck variance and $\sigma^2_{w:b}$ = within-buck variance ($\times 10^3$).

† Number of false mounts (FM) and interval between sexual preparation and ejaculation.

‡ Coefficient of variation.

§ Contribution of component to CV listed in parentheses.

sperm than one false mount ($P<0.10$).

Differences between bucks were significant in each analysis ($P<0.05$), but none of the buck-by-treatment interactions was significant, indicating that the 9 bucks responded similarly to the sexual preparation treatments. None of the differences between the means for the two treatments which involved 3 false mounts was significant ($P>0.10$).

Variances of total sperm output due to differences between bucks and to differences between observations within bucks are listed in Table II. The coefficient of variation was greatest following no preparation largely because of the high within-buck component relative to total variance. Sexual preparation produced a large reduction in coefficients of variation primarily through reduction of the proportional contribution of the within-buck component to total variance.

Discussion. This experiment demonstrated, for the first time, sperm output response to sexual preparation stimuli in rabbits. The first false mount possesses greater stimulus value than second and third false mounts; a result which is in agreement with the previously reported conclusion that sperm output in bulls varies directly with the logarithm of intensity of sexual preparation(3). Similarly to earlier observations with bulls(7), when no sexual preparation is imposed on rabbits, second ejaculations contain more sperm than first ejaculations, but even minimal sexual preparation reverses this ratio in both species.

Sexual preparation of rabbits in the present experiment resulted in a reduction of the coefficient of variation associated with sperm output. A similar reduction in coefficient of variation was noted when increased sperm output was achieved by increasing frequency of ejaculation(4). Maximum sperm output resulted in that study when 2 ejaculations were obtained from each buck on each of 3 days per week, the maximized output being largely due to significantly greater sperm numbers in second than in first ejaculations. It is interesting to note that the rabbits received no intentional sexual preparation before ejaculation in that research.

While intensive sexual preparation was associated with an increase in absolute value of among-buck variance of sperm output, the relative contribution of among-buck variance to total variation was not appreciably altered by sexual preparation treatment. In contrast, the contribution of within-buck variance to total variance after intensive sexual preparation was considerably smaller than after no preparation. These results suggested that one may reduce error variation and increase the precision of future rabbit sperm output experiments by imposition of adequate and standardized sexual preparation before ejaculation.

Increased intensities of sexual preparation were reported to result in progressively greater seminal volumes of first ejaculations(3,7). The same trend was observed in the present rabbit experiment, despite the erratic and unpredictable occurrence of gel-mass which probably inflated the error variance of seminal volume. The increase in average fructose content of first ejaculations following sexual preparation verified the conclusion that sexual preparation stimuli affected accessory glands as well as epididymides. However, the data (Table I) indicated that the magnitude of the response of accessory glands to sexual preparation stimuli was considerably less than that of the response of epididymides. This limited response of accessory glands to sexual preparation stimuli suggested that available reserves of accessory gland secretions are relatively more limited than are sperm reserves in epididymides.

That there was no significant difference between the two treatments incorporating 3 false mounts, suggested that sexual preparation

stimuli have no latent stimulatory effects on the responses measured. However, that there was no apparent decline in any response during the 3-minute interval of sexual quiescence before ejaculation suggested that the effective longevity of sexual preparation stimuli may be greater than we had formerly believed.

Summary. Three intensities of sexual preparation were compared with none using 9 rabbits which each furnished 2 ejaculations every second day for 38 days. The average sperm content of first ejaculations after one false mount (102.9×10^6) was 178% greater than that following no sexual preparation (37.0×10^6) and 3 false mounts resulted in an additional 40% increment in sperm output over one false mount. Sexual preparation also increased average sperm content of second ejaculations, although the increments were not as large as those obtained in first ejaculations. Analyses of seminal volume and total fructose content revealed that sexual preparation stimuli also affected accessory glands, but the magnitude of effect was proportionately smaller than the magnitude of the effect on epididymides. Sexual preparation also reduced the relative magnitude of the within-buck variance of total sperm output.

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Induction of Cleft Palate in Rabbits by Several Glucocorticoids.* (32335)

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Problems in evaluation of the teratogenic effects of drugs were systematically reviewed by Fraser(1). He cited, from the literature on experimental teratology, the many variables relating to genotype, dose, methods of administering the drug and examining the fetuses. Much of the data supported the impression that drug teratogenicity was sporadic, and fortuitous combinations of the previously mentioned variables were necessary to detect teratogenic potential in any one drug. If this is true, then the possibility of developing general principles or extrapolating results to humans would not be great. However, the sporadic results reported may have been due to a sporadic pattern of drug testing rather than to the lack of basic patterns in the teratogenic potential of drugs.

One approach to this problem is to test a group of closely related compounds in animals of several genotypes. Experiments with glucocorticoids have already revealed cleft palate induction by cortisone, hydrocortisone, dexamethasone, prednisolone and triamcinolone in several strains of mice(2,3,4) and by cortisone in rabbits(5). The present experiment concerns the use of 6 glucocorticoids in two strains of rabbits.

Materials and methods. Female rabbits of the New Zealand White or American Dutch strains were placed with a male of the same strain at 5 P.M. and separated the next morning. Ovulation and fertilization were assumed to have occurred 10 hours after coitus. Drugs were administered intramuscularly, starting 13⅓ days postconception, in the doses shown in Tables I to III or listed below. The rabbits were killed with nembutal and chloroform on the 21st day postconception,

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