Sperm Production, Output and Urinary Loss in the Rabbit (36910)

W. HOLTZ[1] AND R. H. FOOTE
(Introduced by W. Hansel)
Department of Animal Science, Cornell University, Ithaca, New York 14850

Sperm production in the testis of the rabbit follows a highly organized and regular pattern. Under controlled conditions neither season (1) nor sexual activity (2) seem to alter the spermatogenic cycle. Only 50 to 60% of the spermatozoa produced by the testes are recovered in the collected semen, even when rabbits are ejaculated frequently (3, 4). Therefore the fate of excess spermatozoa especially during sexual rest poses an obvious question. This is of particular significance when tubes are blocked, such as by vasectomy, since the degree of disturbance depends in part on whether excess sperm normally are resorbed or excreted.

Sperm loss through micturition, masturbation or spontaneous seminal emissions has been observed in a variety of species (2, 5–8). However, this has not been observed in the rabbit (9). Consequently, in the rabbit sperm resorption in the excurrent duct system is generally considered to be responsible for the discrepancy between production and output (3, 9, 10), although contradicting evidence has been presented (11–16). The present study was designed to carefully evaluate the urinary pathway as a possible route of sperm excretion.

Materials and Methods. Twelve Dutch Belted male rabbits 16 to 19 mo of age, averaging 2.21 kg in body weight, were kept under controlled laboratory conditions at 20° and a minimum of 12 hr of light/day. They were caged individually in wire mesh cages surrounded by plastic sheeting. In order to avoid potential sperm loss through genital grooming or oral masturbation all animals wore wide plastic collars to prevent oral contact. The hair covering the penis, tail and abdomen was shaved to prevent sperm from adhering to it. Excretia were collected over a screen which separated the feces from the urine. Every other day the inside of the plastic surrounding the cage was sprayed with water and this rinse water was pooled with the accumulated urine. The volume was recorded and a representative sample of 250 ml was taken and centrifuged. The sediment was largely dissolved with 10 ml of concentrated HCl leaving some organic material and the sperm cells. Alternative techniques to separate sperm cells from urinary particles to facilitate sperm counting met with less success. The residue was diluted with water, and four hemocytometer counts were done on each sample to determine the number of sperm cells present. To determine the efficiency of counting sperm recovered in the urine 20×10^6 sperm suspended in urine were sprayed daily for 12 days into 12 of the special cages housing virgin females. Cages were rinsed every other day as before. The recovery rate was $32.2 \pm 11.6\%$ ($\bar{x} \pm$ SD). Further studies revealed that about two-thirds of the sperm were recovered from the cages, but only about half of these were detectable after processing with urine.

All animals were trained to serve an artificial vagina, but were sexually rested for more than 3 mo at the onset of the experiment. The bucks were randomly divided into two groups of six. A changeover design was employed. For 36 days semen was collected from group 1 twice per day every other day ($2\times$/48 hr). Each collection was preceded by two false mounts with a teaser doe. Group 2 bucks were sexually rested. After 36 days the two groups were reversed. Thus each group of bucks contributed information during perods of sexual activity and sexual rest.

The first 12 days of each 36-day period

[1] Present address: Institut für Tierzucht und Haustiergenetik, der Universität Göttingen, Göttingen, Germany.

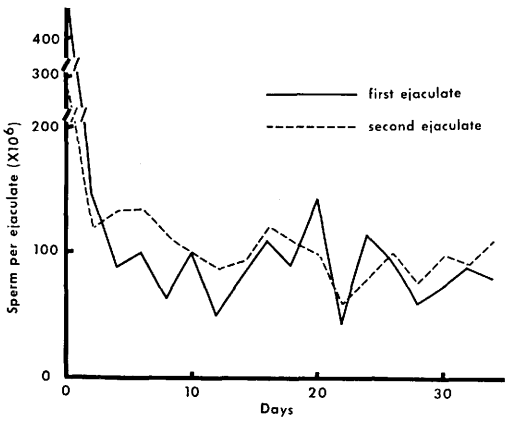

FIG. 1. The average number of sperm in first and second ejaculates of 12 Dutch belted rabbits collected twice every other day for 36 days.

was considered as an adjustment period during which time the daily sperm output had a chance to stabilize (3). No initial adjustment period was necessary for group 2 as these rabbits merely continued to be sexually rested. Sperm output was determined by performing two hemocytometer counts on each ejaculate. Procedural losses, determined on 21 ejaculates from 3 rabbits, amounted to 1.05 $\pm$ 0.76% ($\bar{x} \pm$ SD). These losses were taken into consideration when calculating sperm numbers.

After 72 days all 12 bucks were exsanguinated under anesthesia. Sperm from the sperm containing organs were smeared and stained with nigrosin and eosin for morphology studies. Testis parenchyma, caput, corpus and cauda epididymidis were homogenized and the ductus deferens was flushed. Sperm content was estimated by counting sperm in four aliquots from each source. From the count of the number of spermatids in the testicular homogenate daily testicular sperm production (DSP) was estimated by dividing this number by 3.43, the life-span of the spermatids in days (17).

The DSP by the testis was compared with the daily sperm output (DSO) obtained by frequent semen collection. The difference between the two primarily represents sperm that were voided or resorbed.

Results and Discussion. The two groups of males placed on opposite sequences of sexual activity and rest did not differ in DSP estimated at slaughter to be 205 $\times$ 10^6 and 169 $\times$ 10^6 ($p > 0.1$). Averaged over both groups the estimated sperm produced per gram of testis parenchyma was 40.5 $\times$ 10^6, which is similar to values of 36 to 44 $\times$ 10^6 determined for New Zealand rabbits (3, 4).

There was no difference between groups ($p > 0.1$) in the number of sperm collected during the time each group was ejaculated 2$\times$/48 hr. The average daily sperm output (DSO) during the last 26 days of the 36-day collection period, after sperm reserves had stabilized, amounted to 90.2 $\times$ 10^6 sperm (Fig. 1). This result agrees closely with comparable observations on Dutch Belted rabbits by other workers (1, 4, 12, 13, 18).

It was expected that more sperm might be voided in the urine during periods of sexual rest than during sexual activity. This was true for group 2 rabbits ($p < 0.025$), but unexpectedly there was no difference in this respect for group 1 males (Fig. 2). In both groups of rabbits all interactions for sperm voided in the urine between rabbits, treatments and collection days were statistically significant ($p < 0.05$ in group 1 and $p < 0.025$ or 0.005 in group 2). On days 14, 22 and 32 the sexually rested animals of group

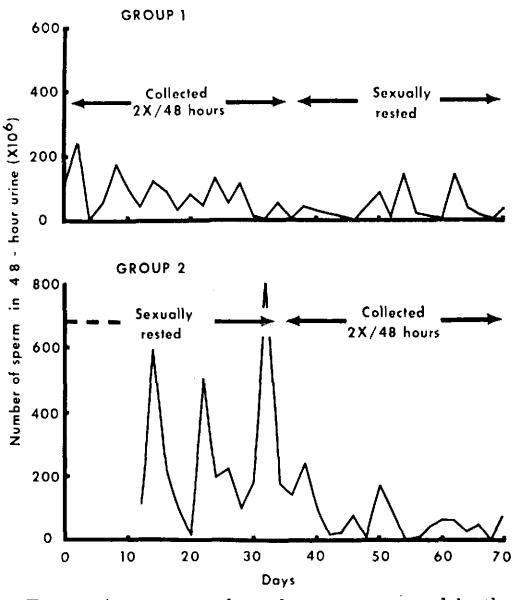

FIG. 2. Average number of sperm recovered in the urine of two groups of 6 male rabbits placed on opposite treatment sequences of sexual activity and rest before sacrificing.

2 expelled particularly large quantities of sperm into the urine (Fig. 2). Possibly fluctuations at similar intervals were starting to develop in the latter part of the resting period of group 1 rabbits. However, no striking increase in number of sperm voided in the urine occurred in this group despite the fact that the 5-wk period of sexual rest should have been more than adequate to restore epididymal reserves. During periods of sexual activity fluctuations appeared less regular, resulting in significant interactions of both treatments and rabbits with collection days ($p < 0.05$). Why all sexually rested rabbits within a group would tend to void sperm in the urine on the same days is uncertain. The fact that each group of bucks was kept in a single battery of cages, or that these batteries were on opposite sides of a room also housing female rabbits suggest that exteroceptive factors emanating from either males or females may have induced a synchronous voiding of sperm. The intervals of 8 to 10 days between peaks is about twice the length of a cycle suggested for the male rabbit by Doggett (19), and Kihlström and Hornstein (20).

The proportion of sperm produced in the testis (DSP) which could be accounted for by sperm collected and/or sperm found in the urine was calculated. No adjustments were made between groups for extragonadal sperm reserves at the time of sacrifice because the value for group 1 ($978 \pm 416 \times 10^6$) did not differ significantly ($p > 0.10$) from group 2 ($1020 \pm 429 \times 10^6$). From 61 to 70% of the DSP were accounted for by ejaculated sperm or sperm in the urine during periods of sexual activity (Table I). In group 2, 82% of the DSP were accounted for in the urine alone during the period of sexual rest. Thus, for the first time substantial numbers of sperm have been shown to be voided in rabbit urine as have been reported for the ram and bull (7, 21–24). The remainder might be accounted for by selective sperm resorption and errors in estimation.

In rabbits sexually active at the time of sacrifice (group 2) stained and tailless sperm decreased from 32.9 ± 16.8 ($\bar{x} \pm$ SD) and 17.0 ± 13.4%, respectively, in the caput epididymidis to 25.2 ± 8.3 and 6.6 ± 2.8% in the corpus epididymidis ($p < 0.005$). This finding suggests that a selective resorption mechanism exists for the elimination of dead and abnormal sperm cells. Such resorption along with procedural errors of estimate could account for the differences of 30 and 39% between DSP and sperm accounted for in semen and urine (Table I). Possibly some sperm adhere to the male, also.

However in sexually rested males of group

TABLE I. Comparison of Daily Sperm Production, Daily Sperm Output and Daily Sperm Loss in the Urine During Different Phases of Sexual Activity.[a]

Group	Daily sperm production (10^6)	Treatment	Daily sperm output (10^6)	Daily sperm loss in urine (10^6)	Percentage of DSP[b] accounted for
1	205 ± 154	Collected after extended period of sexual rest	90 ± 61[c]	34 ± 26[c]	61
2	169 ± 69	Collected after extended period of sexual rest	89 ± 74[c]	29 ± 27[c]	70
1	205 ± 154	Sexually rested after 36 days of regular semen collections	0	21 ± 29	10
2	169 ± 69	Sexually rested after an extended period of sexual rest	0	139 ± 106	82

[a] Tabular values are means $\pm$ SD.

[b] Daily sperm production represented by sperm in ejaculates and/or urine.

[c] Calculated during period of sexual activity after epididymal reserves had been stabilized.

1 a very high rate of resorption would have been required to account for sperm losses since few sperm were voided in the urine. A negative correlation of -0.61 ($p < 0.05$) was found between urinary sperm loss and total epididymal sperm reserves at the end of the experimental period. The correlations of epididymal sperm reserves and testis weight with DSO both were 0.71 ($p < 0.01$).

The following is an attempt to construct a hypothesis explaining the processes of sperm resorption and sperm loss in the male rabbit. A limited number of sperm ooze more or less continuously from the deferent duct into the urethra insuring the constant removal of aged sperm from the distal cauda epididymidis. Random or cyclical emissions also may occur as indicated by dramatic fluctuations in urine content of sperm. In the testis and the proximal part of the epididymis abnormal or degenerating cells are constantly removed from the perpetual flow of newly formed spermatozoa. Normal spermatozoa also may be removed this way, accounting generally for all the spermatozoa produced. The rate at which this process occurs may be subject to feedback from the cauda epididymis and is accelerated as extragonadal sperm reserves build up. Some such drastic change in resorption rate with filling of the epididymal storage is necessary to explain the sperm otherwise unaccounted for in group 1 males during sexual rest. Following intensive semen collection this group appeared to nearly balance sperm production by sperm accumulation and resorption in the excurrent ducts during a period of 36 days. Since the group 2 males, after a much longer period of sexual rest, voided about 82% of the DSP in the urine, it appears that the presumed high resorption following intensive semen collection was a temporary mechanism. Under the conditions of prolonged sexual rest most sperm are voided rather than resorbed.

Summary. Daily sperm production (DSP) in sexually mature rabbits was estimated to be 40.5×10^6/g of testis parenchyma or 187×10^6/animal. The daily sperm output (DSO) during a collection regimen of $2\times$/48 hr was 90.2×10^6 sperm/buck, accounting for 48% of the DSP. Up to 70% of DSP was accounted for by ejaculated sperm plus sperm in the urine. When the same group of males was sexually rested for an extended period 82% of the DSP was accounted for in the urine. The sperm unaccounted for may be resorbed or in part result from small errors of estimation. During the period of sexual rest sperm were voided in the urine in a cyclical pattern.

In another group of males sexually rested for 36 days only 10% of the DSP was found in urine, indicating that a majority of sperm were resorbed. Thus, resorption of sperm in recently filled epididymides may be greater initially than after prolonged periods of sexual rest.

The authors are grateful to Gary Anderson, Dick Cole, Janet Wiebold, Elvira Osirio de Valdivia, Lynne Deuschle and Esther McKay for valuable technical assistance. The senior author was supported by fellowships from the Kellogg Foundation and the Population Council.

1. Gregoire, A. T., Bratton, R. W., and Foote, R. H., J. Anim. Sci. **17**, 243 (1958).

2. Amann, R. P., *in* "The Testis" (A. D. Johnson, W. R. Gomes and N. L. VanDemark, eds.), Vol. 1, p. 433. Academic Press, New York (1970).

3. Lambiase, J. T., and Amann, R. P., J. Anim. Sci. **28**, 542 (1969).

4. Amann, R., Fert. Steril. **21**, 662 (1970).

5. Essenhigh, D. M., Ardan, G. M., Hovell, G. J. R., and Smith, J. C., Brit. J. Urol. **41**, 190 (1969).

6. Fernandez-Collazo, E., Videla, E., and Pereyra, J. C., J. Reprod. Fert. **27**, 145 (1971).

7. Tischner, M., J. Reprod. Fert. **24**, 271 (1971).

8. Vandenbergh, J. G., Biol. Reprod. **4**, 234 (1971).

9. Orgebin-Crist, M. C., J. Reprod. Fert. **15**, 15 (1968).

10. Macmillan, K. L., Desjardins, C., Kirton, K. T., and Hafs, H. D., Fert. Steril. **19**, 982 (1968).

11. Paufler, S. K, and Foote, R. H., J. Reprod. Fert. **17**, 125 (1968).

12. Paufler, S. K., and Foote, R. H., Fert. Steril. **20**, 618 (1969).

13. Paufler, S. K., and Foote, R. H., Proc. Soc. Exp. Biol. Med. **131**, 1179 (1969).

14. Swanson, L. V., and Hafs, H. D., Proc. Soc. Exp. Biol. Med. **131**, 763 (1969).

15. Swanson, L. V., Hafs, H. D., and Peterson, D. E., J. Anim. Sci. **27**, 1197 (1968).

16. Fulka, J., Kopěcny, V., and Koefoed-Johnsen, H. H., Fert. Steril. **22**, 119 (1971).

17. Amann, R. P., and Lambiase, J. T., J. Anim.

Sci. **28**, 369 (1969).

18. Desjardins, C., Kirton, K. T., and Hafs, H. D., J. Reprod. Fert. **15**, 27 (1968).

19. Doggett, V. C., Amer. J. Physiol. **187**, 445 (1956).

20. Kihlström, J. E., and Hornstein, O., Acta Endocrinol. **46**, 597 (1964).

21. Bielanski, W., and Tischner, M., Proc. 6th Int. Congr. Anim. Reprod. A. I. (Paris) **1**, 39 (1968).

22. Bielanski, W., and Wierzbowski, S., 4th Int. Congr. Anim. Reprod. (The Hague) **2**, 274 (1961).

23. Koefoed-Johnsen, H. H., Annu. Rep. The Royal Vet. Agr. College, Copenhagen, 23 (1964).

24. Lino, B. F., Braden, A. W. H., and Turnbull, K. E., Nature (London) **213**, 594 (1967).

Received July 12, 1972. P.S.E.B.M., 1972, Vol. 141.