

BRIEF COMMUNICATION

THE MALE RABBIT

I. CHANGES IN SEMEN CHARACTERISTICS AND SPERM OUTPUT BETWEEN PUBERTY AND ONE YEAR OF AGE

R. P. AMANN AND J. T. LAMBIASE, JR

*Dairy Breeding Research Center, Department of Dairy Science,
The Pennsylvania State University, Pennsylvania, U.S.A.*

(Received 15th May 1967)

Although male rabbits are frequently used for research on reproduction, certain questions must be answered to facilitate the design of meaningful experiments. Two of these questions are discussed in the present paper, namely: when is a male sexually mature in terms of sperm production or sperm output?, and what are norms for semen characteristics and daily sperm output (dso)?

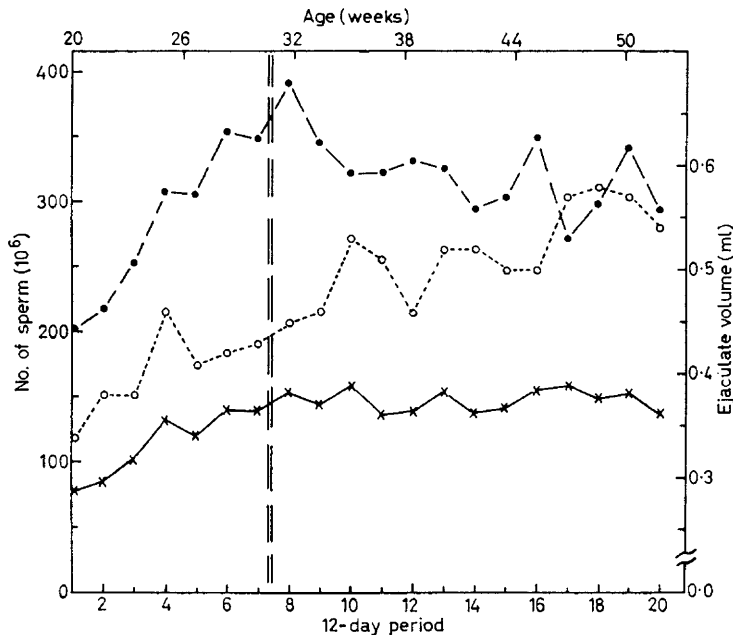
Twelve random-bred New Zealand White bucks, born between 26th June and 2nd July 1965, were purchased at 17 weeks of age. They were exposed to natural daylight and ambient temperature between 0 and 30° C. During postnatal Weeks 18 and 19, rabbits were trained to ejaculate with a Walton-type artificial vagina. Live teaser does were used. From Weeks 20 to 54, two ejaculates were collected 20 to 45 min apart from each male every 48 hr. One false mount preceded each ejaculation. A minimum of 8 min exposure to each of three teaser does was required before a refusal to ejaculate was recorded.

The gel plug was removed if present and ejaculate volume was read directly from the calibrated collection tube. Residual semen in the artificial vagina liner was flushed with about 15 ml of distilled water into a flask. Flushings from ejaculates collected from a rabbit on three consecutive collection days were pooled and counted without further dilution. The two ejaculates from each rabbit were pooled before determination of sperm concentration by haemocytometer. Data were analysed on the basis of twenty 12-day periods. Period 1 started on postnatal Day 141 or 142. The occasional samples contaminated with urine were excluded from calculations of mean ejaculate volume and sperm concentration, but were included when calculating dso. Artificial vagina flushings were included in the dso.

During the experiment, 2880 ejaculates should have been collected, but ninety-one were not obtained. Two males accounted for sixty-six of the ninety-one refusals; only two-thirds of the planned ejaculates were obtained after the twelfth 12-day period. Therefore, the data were excluded from all analyses. Data for the first twelve periods suggested that exclusion of these animals did not bias the results. The remaining ten rabbits refused a total of eight times for both ejaculates and nine times for the second ejaculate. Body weight for

these ten rabbits averaged 3.93 ± 0.10 kg between 32 and 54 weeks of age. At 54 weeks of age when the rabbits were killed the total weight of the two testes averaged 5.84 ± 0.40 g.

Analysis of variance revealed that mean gel-free volumes of first and second ejaculates (0.48 and 0.47 ml) were not significantly different. However, the rabbit \times ejaculate (first versus second) interaction and differences among rabbits and between 12-day periods (Text-fig. 1) were significant ($P < 0.01$).



TEXT-FIG. 1. Age changes in mean gel-free ejaculate volume (ml, ○), sperm concentration (10^6 /ml, ●) and daily sperm output (10^6 /day, ×) for ten New Zealand White rabbits ejaculated twice successively every 48 hr.

During the last thirteen periods, mean ejaculate volume for individual rabbits ranged from 0.05 to 0.81 ml and averaged 0.52 ± 0.07 ml. Gel plugs were found in 75.4% of the first ejaculates, but only in 4.8% of the second ejaculates. Mean sperm concentration for the combined first and second ejaculates differed among rabbits ($P < 0.01$) and 12-day periods ($P < 0.05$). For at least the first three 12-day periods, mean concentration was lowered by the inclusion of data from two rabbits from which sperm were first obtained during the initial 12-day period. During Periods 8 to 20, mean sperm concentration (10^6 sperm/ml) for individual rabbits ranged from 162 to 752 and averaged 322 ± 52 . Daily sperm output (DSO) differed ($P < 0.01$) among rabbits and between 12-day periods and doubled between 20 and 32 weeks of age; after Week 32 (Periods 8 to 20) there was no further increase. Individual rabbits ranged from 79 to 200 and averaged $148 \pm 11 \times 10^6$ sperm/day.

New Zealand White rabbits apparently reach sexual maturity, as evaluated by DSO, at about 32 weeks of age. The linear regression coefficient for DSO during 12-day periods 8 to 20 of -0.005×10^6 sperm/period was not significantly

different from zero. The correlation between paired testes weight and mean DSO during Periods 17 to 20 was $+0.80$ ($P < 0.01$). For twelve bulls, DSO was shown to be correlated with the total weight of the two testes ($+0.82$) and daily sperm production ($+0.87$) (Amann & Almquist, 1962). Only one rabbit, which reached puberty during Period 1, attained sexual maturity later than 32 weeks of age; DSO increased up to about 36 weeks of age.

Although DSO did not change during Periods 8 to 20, mean ejaculate volume increased ($+0.0084$ ml/12-day period, $P < 0.01$). Apparently the accessory sex glands did not reach maximum development as early as the testes and, in fact, increased in secretory activity until at least 1 year of age. As a result, mean sperm concentration declined (-4.6×10^6 sperm/12-day period, $P < 0.05$) during Periods 8 to 20. The decline in sperm concentration after 32 weeks of age in this experiment agrees with the report of Gregoire, Bratton & Foote (1958). They collected ejaculates from four Dutch-type rabbits once weekly for 56 weeks. Sperm concentration (10^6 /ml) averaged 407 during the first 13 weeks, but over the next 43 weeks it averaged only 303.

TABLE 1
SEMEN CHARACTERISTICS OF 1-YEAR-OLD NEW ZEALAND WHITE RABBITS
EJACULATED TWICE SUCCESSIVELY EVERY 48 HR*

	<i>Amann</i> (1966)	<i>Periods</i> 17-20	<i>Mean</i>	95% <i>confidence</i> <i>interval</i>
Ejaculate volume (ml)	0.44	0.56	0.50	0.40-0.60
Sperm concentration (10^6 /ml)	275	301	289	233-345
Daily sperm output (10^6)	125†	150†	138	119-157
No. of animals	9	10	19	19

* One false mount before each of two successive ejaculates collected 15 to 45 min apart.

† Includes spermatozoa recovered by flushing the artificial vagina.

Norms for semen characteristics of 1-year-old New Zealand White rabbits were calculated (see Table 1) from the present data and a previous experiment (Amann, 1966). Direct comparison of these values with others in the literature is invalid. Not only have different ejaculation frequencies or degrees of sexual preparation been used, but some workers have not reported the age or breed of rabbit.

Desjardins, Kirton & Hafs (1965) reported that mean DSO for twelve rabbits ejaculated twice each Monday, Wednesday and Friday was 88×10^6 . Gregoire *et al.* (1958) obtained a mean DSO of about 101×10^6 spermatozoa from four Dutch-type rabbits ejaculated once daily. Similar DSO values for Dutch-type rabbits were reported by Amann (1966). However, the data of McFee, Eblen & Welch (1966) suggest that collection of two successive ejaculates every 48 hr from six Dutch-type rabbits resulted in a mean DSO of only 51×10^6 . Except for the report by Amann (1966), calculated DSO values did not include spermatozoa recovered from the artificial vagina (about 10%).

Authorized for publication on 10th January 1967 as Paper No. 3208 in the Journal Series of the Pennsylvania Agricultural Experiment Station. This investigation was supported by Research Grant No. HD-01356-02 from the National Institute of Child Health and Human Development. Mrs B. R. Billeb provided skilled technical assistance.

REFERENCES

- AMANN, R. P. (1966) Effect of frequency of ejaculation and breed on semen characteristics and sperm output of rabbits. *J. Reprod. Fert.* **11**, 291.
- AMANN, R. P. & ALMQUIST, J. O. (1962) Reproductive capacity of dairy bulls. VIII. Direct and indirect measurement of testicular sperm production. *J. Dairy Sci.* **45**, 774.
- DESJARDINS, C., KIRTON, K. T. & HAPS, H. D. (1965) Rabbit sperm output after varying ejaculation frequencies. (Abstract). *J. Anim. Sci.* **24**, 916.
- GREGOIRE, A. T., BRATTON, R. W. & FOOTE, R. H. (1958) Sperm output and fertility of rabbits ejaculated either once a week or once a day for forty-three weeks. *J. Anim. Sci.* **17**, 243.
- McFEE, A. F., EBLEN, J. R. & WELCH, P. R. (1966) Influence of collection frequency on post-irradiation sperm output by rabbits. *J. Reprod. Fert.* **11**, 287.