Reproductive Capacity of Dairy Bulls. XI. Puberal Characteristics and Postpuberal Changes in Production of Semen and Sexual Activity of Holstein Bulls Ejaculated Frequently [1,2]

J. O. ALMQUIST and R. P. AMANN
Dairy Breeding Research Center
Department of Dairy and Animal Science
The Pennsylvania State University
University Park 16802

ABSTRACT

Twenty Holstein bulls attained puberty (50×10^6 sperm/ejaculate; 10% motile sperm) at 39 ± 1 wk of age and 295 ± 4 kg body weight. Starting at puberty, 10 bulls were ejaculated biweekly to 1 yr and then weekly (1×) to 2 yr of age, and 10 bulls were ejaculated three times weekly (3×) to 1 yr and then six times weekly (6×) to 2 yr of age. All characteristics of semen increased through 77 to 88 wk of age. Between 77 to 88 wk and 89 to 104 wk of age, only volume of ejaculate and total sperm per week increased. Motility of sperm increased only during the first 12 wk after puberty. All seminal traits except motility of sperm differed between 1× and 6× bulls. Frequency of ejaculation did not affect the percentage of ejaculates acceptable for freezing or body growth rate. Between 1 and 2 yr of age, 6× bulls yielded 3.3 times more sperm per week and 3.5 times more motile sperm per week than 1× bulls. Within frequency of ejaculation, time of reaction to ejaculation did not change between 1 and 2 yr of age, but an average of 40% more time was needed to obtain an ejaculation on 6× than 1× (9.4 versus 6.7 min). By 13 to 24 wk after puberty, 83% of ejaculates were acceptable for freezing. Thus, Holstein bulls could be sampled in a progeny testing program by 15 mo of age.

Received November 6, 1975.
[1] Authorized for publication as Paper No. 4953 in the journal series of The Pennsylvania Agricultural Experiment Station on October 16, 1975.
[2] Supported in part by Atlantic Breeders Cooperative, Lancaster, PA, Sire Power, Inc., and NEBA, Tunkhannock, PA.

INTRODUCTION

Progeny testing of sires should begin at the earliest possible age. It was reported previously (3, 6, 10) that fertility of Angus and Hereford bulls was sufficiently high to initiate progeny testing immediately at puberty and that ejaculation three or six times weekly rather than once weekly greatly increased output of sperm. With this management, sufficient insemination units for progeny testing can be obtained from many Angus and Hereford bulls by 60 wk of age. Killian and Amann (9) studied changes in seminal characteristics of Holstein bulls ejaculated once weekly during the first 30 wk after puberty and reported rapid increases in seminal characteristics during the first 20 wk after puberty. This report concerns puberal traits and the effect of age and frequent ejaculation on characteristics of semen, output of sperm, and sexual behavior of Holstein bulls between puberty and 2 yr of age.

EXPERIMENTAL PROCEDURE

Twenty Holstein bull calves, born in December, 1959, through February, 1960, were raised from 18 wk of age on a concentrate and hay ration calculated to provide 100% of the upper limit of energy estimated to be required for normal growth of dairy heifers (11). Starting at 24 wk of age, calves were tested every 2 wk for sexual behavior and penile protrusion. When the penis became free of the sheath, collection of semen was attempted weekly with a small artificial vagina. Puberty was defined (14) as the age when the first ejaculate was obtained which contained a minimum of 50×10^6 sperm with at least 10% showing progressive motility.

Based on age and body weight at puberty, bulls were paired and allotted randomly to one of two groups. Seminal characteristics and sexual activity were determined for the control or 1× group which consisted of 10 bulls eja-

culated biweekly (1/2×) from puberty to 1 yr of age and then weekly (1×) from 1 to 2 yr of age, and for the experimental or 6× group containing 10 bulls ejaculated once each Monday, Wednesday, and Friday (3×) from puberty to 1 yr of age and then twice in succession on the 3 days weekly (6×) from 1 to 2 yr of age.

To maximize output of sperm, one false mount, a 2-min restraint period, and two additional false mounts preceded each ejaculation. Stimulus changes and collection of data on sexual behavior were essentially as described (7) except that slight changes of stimulus by movement of the stimulus animal were initiated after 5 min without a mounting response. Procedures for seminal evaluation (7) were augmented by evaluating percentages of unstained or live (14) and normal (10) sperm.

Data for seminal traits but not for sexual activity were evaluated by analyses of variance. Because of the wide variation in age at puberty, data for initial progressive motility also were analyzed for wk 1 to 60 after puberty. Log transformations of the data were used for analyses of seminal volume, spermatozoal concentration, total sperm per ejaculate and per week, and total motile sperm per week.

RESULTS AND DISCUSSION

Puberal characteristics and body weight at 1 and 2 yr of age are summarized in Table 1. Average age at first protrusion of the penis during mounting was 31 wk for Holstein bulls as compared to 33 and 36 wk for Angus and Hereford bulls (14) and 33 wk for Charolais bulls (1). Complete separation of penis and sheath was at 35 wk for Holstein, 37 wk for Angus and Charolais, and 38 wk of age for Hereford bulls. First sperm were detected at a mean age of 37 wk for Holsteins, 36 wk for 20 other Holsteins (9), 38 wk for 24 Charolais (1), 40 wk for 9 Angus, and 42 wk of age for 11 Herefords (14).

TABLE 1. Age, body size, and seminal characteristics at puberty and body weight at 1 and 2 yr of age of Holstein bulls (N = 20).

Item	Mean	SE	Range
Age (wk)			
First penile protrusion	31	1	26 to 41
Penis and sheath separated	35	1	28 to 41
First sperm collected	37	1	30 to 43
First motile sperm collected	37	1	32 to 43
Puberty attained[a]	39	1	35 to 45
Body measurements at puberty			
Weight (kg)	295	4	269 to 333
Wither height (cm)	114	1	106 to 118
Heart girth circumference (cm)	151	1	145 to 163
Characteristics of first ejaculate[a]			
Volume of semen (ml)	2.3	.2	.6 to 4.7
Progressive motility (%)	30	4	10 to 60
Concentration of sperm (10^6/ml)	127	28	32 to 500
Total sperm (10^6)	258	53	54 to 850
Total motile sperm (10^6)	89	26	5 to 425
Live sperm (%)	53	5	19 to 86
Abnormal sperm (%)[b]	31	4	11 to 88
Fructose (mg/ml)	6.6	.4	2.6 to 9.7
Body weight at			
1 yr of age (kg)	383	2	362 to 407
2 yr of age (kg)	660	6	628 to 733

[a]First ejaculate containing a minimum of 50 × 10^6 sperm with at least 10% showing progressive motility.

[b]N = 19.

Mean age and body weight at puberty of 39 wk and 295 kg agree closely with 41 wk and 303 kg for 20 Holstein bulls fed and managed similarly (9). Mean body weight of 383 kg at 1 yr of age is similar to the mean of 389 kg for 381 1-yr-old Holstein bulls (5) and indicates that puberty was not delayed because of insufficient energy intake. Holstein bulls in this study reached puberty 6 wk earlier ($P<.01$) than 20 Angus and Hereford bulls (45 wk), but were not significantly younger than 24 Charolais bulls (41 wk) fed on a high plane of nutrition at this Center (1, 14). Characteristics of the puberal ejaculate were similar to those reported for other Holstein (9) and Charolais (1) bulls, but the concentration of sperm and the total number of sperm in the ejaculate were less than for Angus and Hereford bulls (14). Possibly these differences are a reflection of the difference in age at puberty.

Growth was not affected by ejaculation at high frequency. Body weight for the 1× and 6× bulls averaged 294 and 296 kg at puberty and 660 and 661 kg at 2 yr of age.

The significant increases with age in each semen characteristic (Table 2) follow the trends for Holsteins (4) and beef breeds (2, 3) collected 1×, 3×, or 6× weekly. Except for motility of sperm, characteristics of semen increased ($P<.01$) between 53 to 64 wk and 65 to 76 wk of age. Between 65 to 76 wk and 77 to 88 wk of age, increased ($P<.01$) volume of ejaculate accounted primarily for the higher ($P<.01$) numbers of total spermatozoa per ejaculate and spermatozoa per week. Motility and concentration of sperm also increased ($P<.05$). Most seminal traits plateaued between 77 to 88 and 89 to 104 wk of age which is later than reported in another study (9). Only seminal volume and total sperm per week increased ($P<.05$).

Based on a separate analysis of postpuberal changes, the rapid improvement from 42% at 1 to 4 wk to 62% at 9 to 12 wk after puberty accounted for most of the increase in mean initial spermatozoal motility. This was similar to data from other Holsteins (9) and beef breeds (2, 3). For Normandy bulls, the percentage of motile sperm apparently increased until at least 60 wk of age (12).

Effects of frequency of ejaculation on mean characteristics of semen between 53 and 104 wk of age (Table 2) were similar to those for young dairy (4) and beef bulls (2, 3). However, concentration of sperm for Holsteins in this study was lower ($P<.05$) for 6× than 1× bulls while in beef bulls frequency of ejaculation did not affect concentrations of sperm significantly. There was an interaction ($P<.05$) of age and frequency only for total motile sperm per week. Total motile sperm per week for 1× bulls increased throughout the experiment while that for 6× bulls increased until 77 to 88 wk of age. As illustrated recently (2) mean weekly output of sperm at 6× from 1 to 2 yr of age for these 10 Holstein bulls (20.0×10^9) was lower than that for 10 Charolais (25.5×10^9) but greater than that for 5 Angus and Hereford bulls (12.0×10^9) ejaculated at 6× in the same manner.

As expected (8), more time was required to stimulate sexually and to collect semen from 6× than 1× bulls (Table 2). Time to ejaculation included restraint for 2 min in addition to the time required for three false mounts and the collection mount. It required an average of 40% more time to obtain an ejaculation on 6× than 1×, but the mean time for reaction of 9.4 min is relatively short and a high frequency of ejaculation need not lead to excessively long times for reaction for most bulls. If a faster time of reaction is desired, stimulus pressure must be increased (8). Within each frequency of ejaculation, time of reaction to the first mount and to ejaculation did not change appreciably between 1 and 2 yr of age.

Acceptability of ejaculates for freezing is more important than weekly output of sperm of young bulls if progeny testing is to be completed at the earliest possible age. By 13 to 24 wk after puberty (Table 3), 75% of ejaculates from 1× and 85% of ejaculates from 6× bulls were acceptable for freezing. Differences associated with frequency of ejaculation were not significant. By the same procedures for evaluation of semen, postpuberal improvement in the percentage of ejaculates acceptable for freezing was much more rapid for Holsteins than for Angus and Herefords (6). Values for beef bulls for the same five age periods between 1 and 60 wk after puberty shown in Table 3 were 22, 47, 50, 57, and 66%. For beef bulls (6, 10), 73% of acceptable ejaculates froze satisfactorily (minimum of 10×10^6 motile sperm per ampule after 3-wk storage at −196 C) and fertility was adequate between puberty and 60 wk of age (mean 60- to 90-day nonreturn rate of 69% for 5059 first services from 17 bulls).

TABLE 2. Characteristics of semen and sexual activity of Holstein bulls ejaculated at high (3× to 6×) or low (1/2× to 1×) frequency from puberty to 2 yr of age[a].

Item	Puberty to 52 wk		53 to 64 wk			65 to 76 wk			77 to 88 wk			89 to 104 wk			Mean for 53 to 104 wk	
	1/2×	3×	1×	6×	Mean	1×	6×	Mean	1×	6×	Mean	1×	6×	Mean	1×	6×
Motile sperm (%)	53	57	62	65	63^{b}	62	66	64^{bc}	65	67	66^{c}	65	67	66^{c}	64	66
Volume of ejaculate (ml)	2.7	2.5	3.6	2.5	3.0^{b}	4.3	3.0	3.5^{c}	4.7	3.3	3.9^{d}	5.2	3.4	4.2^{d}	4.4	3.0**
Concentration of sperm (10^9/ml)	.5	.5	1.0	.8	.9^{b}	1.3	1.1	1.2^{c}	1.5	1.2	1.3^{cd}	1.6	1.2	1.4^{d}	1.3	1.1*
Total sperm per ejac. (10^9)	1.4	1.3	3.5	2.0	2.6^{b}	5.7	3.3	4.3^{c}	7.0	4.0	5.3^{d}	8.0	4.0	5.6^{d}	6.1	3.3**
Unstained (live) sperm (%)	69	68	74	77	76^{b}	78	80	79^{c}	78	79	79^{c}	78	80	79^{c}	77	79*
Normal sperm (%)	88	91	89	91	90^{b}	92	94	93^{c}	92	94	93^{c}	93	94	94^{c}	92	94*
Total sperm per week (10^9)	.7	3.8	3.5	12.2	6.3^{b}	5.7	20.0	10.5^{c}	7.0	23.8	12.6^{d}	8.0	24.0	13.8^{d}	6.1	20.0**
Total motile sperm per week (10^9)	.4	2.4	2.1	8.1	5.0^{b}	3.5	13.5	8.4^{c}	4.6	16.2	10.2^{d}	5.2	16.3	10.7^{d}	3.9	13.5**
Time for reaction to:																
First mount (min)	2.0	3.0	2.0	4.3	3.2	2.4	4.1	3.2	3.1	4.8	4.0	2.4	4.1	3.2	2.5	4.3
Ejaculation (min)	9.6	7.7	6.9	9.2	8.0	6.6	8.6	7.6	6.8	10.1	8.4	6.5	9.7	8.1	6.7	9.4

[a]Means for 10 bulls per group. Data for puberty to 52 wk of age were excluded from statistical analyses.

* and ** designate differences ($P<.05$ and $<.01$) between treatment groups and pooled means with different superscripts are different ($P<.01$). Data for sexual activity were not analyzed statistically.

TABLE 3. Postpuberal changes in percentage of ejaculates acceptable for freezing[a].

Weeks after puberty	Ejaculation frequency		
	1/2X to 1X	3X to 6X	Combined
1 to 12	35	35	35
13 to 24	75	85	83
25 to 36	91	96	95
37 to 48	97	96	96
49 to 60	99	96	97
Mean	84	86	86

[a]At least 396 X 10^6 sperm/ml, 40% progressively motile sperm, and 840 X 10^6 total motile sperm per ejaculate.

Thus, Holstein bulls could be sampled in a progeny testing program by 24 wk after puberty or about 15 mo of age. This conclusion is similar to that of Thibier and Colchen-Bourlaud (13) based on studies of Black Pied, Holstein, and Normandy bulls.

Between 1 and 2 yr of age, Holsteins on 6X yielded 3.3 times more sperm per wk and 3.5 times more motile sperm per wk than bulls on 1X (Fig. 1). If 90% of the ejaculates were acceptable for freezing (Table 3) and an insemination unit of 20 X 10^6 motile sperm before freezing was used, the theoretical number of insemination units stored for each bull between 1 and 2 yr of age would be 33,500 for a 6X bull compared to 9500 for a 1X bull.

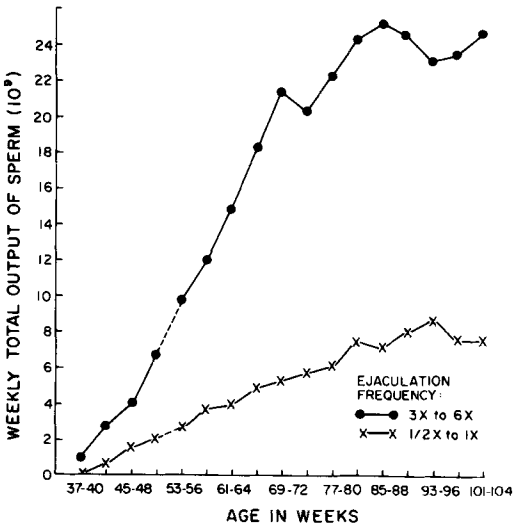

FIG. 1. Changes in weekly total output of sperm for Holstein bulls ejaculated at high or low frequency from puberty to 2 yr of age.

ACKNOWLEDGMENT

Appreciation is expressed to A. Keene, H. Homan, A. Cole, and C. Vener for their assistance.

REFERENCES

1 Almquist, J. O., and K. A. Barber. 1974. Puberal characteristics and early growth of Charolais bulls on high nutrient allowance. J. Anim. Sci. 38:831.
2 Almquist, J. O., R. J. Branas, and K. A. Barber. Postpuberal changes in semen production of Charolais bulls ejaculated at high frequency and the relation between testicular measurements and sperm output. J. Anim. Sci. (In press.)
3 Almquist, J. O., and D. C. Cunningham. 1967. Reproductive capacity of beef bulls. I. Postpuberal changes in semen production at different ejaculation frequencies. J. Anim. Sci. 26:174.
4 Baker, F. N., N. L. VanDemark, and G. W. Salisbury. 1955. The effects of frequency of ejaculation on the semen production, seminal characteristics, and libido of bulls during the first postpuberal year. J. Dairy Sci. 38:1000.
5 Calo, L. L., R. E. McDowell, L. D. Van Vleck, and P. D. Miller. 1973. Parameters of growth of Holstein-Friesian bulls. J. Anim. Sci. 37:417.
6 Cunningham, D. C., J. O. Almquist, R. E. Pearson, and R. C. Martig. 1967. Reproductive capacity of beef bulls. II. Postpuberal relations among ejaculation frequency, sperm freezability and breeding potential. J. Anim. Sci. 26:182.
7 Foster, J., J. O. Almquist, and R. C. Martig. 1970. Reproductive capacity of beef bulls. IV. Changes in sexual behavior and semen characteristics among successive ejaculations. J. Anim. Sci. 30:245.
8 Hale, E. B., and J. O. Almquist. 1960. Relation of sexual behavior to germ cell output in farm animals. J. Dairy Sci. 43(Suppl.):145.
9 Killian, G. J., and R. P. Amann. 1972. Reproduc-

tive capacity of dairy bulls. IX. Changes in reproductive organ weights and semen characteristics of Holstein bulls during the first thirty weeks after puberty. J. Dairy Sci. 55:1631.

10 Martig, R. C., and J. O. Almquist. 1969. Reproductive capacity of beef bulls. III. Postpuberal changes in fertility and sperm morphology at different ejaculation frequencies. J. Anim. Sci. 28:375.

11 Morrison, F. B. 1956. Page 1088 *in* Feeds and Feeding. 22nd ed. Morrison Publ. Co., Ithaca, NY.

12 Thibier, M., and M. A. Colchen-Bourlaud. 1972. Le choix du jeune taurillon sur sa fonction sexuelle (Part 1.). Elevage et Insémination 127:2.

13 Thibier, M., and M. A. Colchen-Bourlaud. 1972. Le choix du jeune taurillon sur sa fonction sexuelle (Part 2.). Elevage et Insémination 128:4.

14 Wolf, F. R., J. O. Almquist, and E. B. Hale. 1965. Prepuberal behavior and puberal characteristics of beef bulls on high nutrient allowance. J. Anim. Sci. 24:761.