POSTPUBERAL CHANGES IN SEMEN PRODUCTION OF CHAROLAIS BULLS EJACULATED AT HIGH FREQUENCY AND THE RELATION BETWEEN TESTICULAR MEASUREMENTS AND SPERM OUTPUT¹,²

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SUMMARY

Twelve Charolais bulls were ejaculated once weekly (1x) and 10 bulls six times weekly (6x) from puberty to 2 years of age to determine the effect of age and ejaculation frequency on semen characteristics, sperm output and testes growth; 6x weekly sperm output at 2 and 3 years of age was determined for each of the 22 bulls. Significant increases as bulls aged were found in sperm concentration, sperm motility, weekly total sperm and total motile sperm output, scrotal circumference and width (P<.01) and ejaculate volume (P<.05). From 1 to 2 years of age, average weekly sperm output for 6x bulls increased 2.4 times from 14.0 x 10⁹ at 53 to 56 weeks to 33.6 x 10⁹ at 101 to 104 weeks of age and for 1x bulls increased 2.2 times from 5.0 x 10⁹ to 11.2 x 10⁹ at the same ages. For 6x as compared to 1x bulls, ejaculate volume was smaller (P<.01) but total sperm and total motile sperm per week were greater (P<.01); differences for sperm motility, sperm concentration and scrotal circumference and width were not significant.

Scrotal circumference and width each increased by 32% from puberty (41 ± 1 weeks) to 2 years of age with slightly over three-fourths of the increase occurring between puberty and 65 weeks of age. Scrotal circumference at 1 and 1.5 years of age was correlated .44 and .52 (P<.05) with 6x weekly sperm output at 2 years of age and .62 and .75 (P<.01) with 6x weekly sperm output at 3 years of age. Testes growth rate, based on the percentage increase in scrotal circumference between 1 and 1.5 years of age, was not significantly correlated (.30) with 6x weekly sperm output at 3 years of age. The correlation increased to .61 (P<.01) when testes growth rate during the first 24 weeks after puberty was used. Thus these relationships accounted for up to 56% of the variation in sperm output. These results and other evidence discussed support the postulate that, except for early detection of bulls with a potential for severe testicular underdevelopment, scrotal measurements of young bulls cannot be used to predict subsequent sperm output precisely.

Ejaculation of Charolais bulls 6x weekly from puberty to 2 years of age was not deleterious to semen traits, weekly sperm output or testes growth and yielded 3.3 times more motile sperm per week than ejaculation 1x weekly.

(Key Words: Charolais Bulls, Ejaculation Frequency, Semen Characteristics, Sperm Output, Testis Growth.)

INTRODUCTION

Greater utilization of genetically superior beef bulls can be achieved if progeny testing is initiated at the earliest possible age. To maximize the reproductive potential of young sires, frequent semen collection should be used to increase the number of sperm available for artificial insemination. While the effect of age and ejaculation frequency on semen characteristics and sperm output of young Angus and Hereford bulls has been reported (Almquist and Cunningham, 1967), such information is not available for Charolais bulls. Puberal characteristics of Charolais bulls and their relationship to...
beef production traits have been previously reported from this center (Almquist and Barber, 1974; Barber and Almquist, 1975).

Testicular size, weight and sperm output of young bulls have been reported to be highly correlated with in situ measurements of scrotal circumference (Willett and Ohms, 1957; Hahn et al., 1969). Although scrotal circumference measurements of young bulls in consecutive years were found to be highly significantly correlated (Hahn et al., 1969), it is not certain that such measurements of young bulls provide an accurate means of predicting sperm output at sexual maturity.

This study was designed to determine: (a) the effect of age and ejaculation frequency on the semen characteristics, sperm output and testes growth of Charolais bulls from puberty to 2 years of age and (b) the relationship between testes-scrotal measurements and sperm output.

EXPERIMENTAL PROCEDURE

Twenty-seven Charolais weanling purebred bull calves donated for this experiment received feeding and management described by Barber and Almquist (1975). At attainment of puberty, bulls were randomly assigned to a high (experimental) or low (control) ejaculation frequency. Two bulls were removed from the experiment at 52 and 62 weeks of age for inability to protrude the penis and serve the artificial vagina. Two bulls were discarded because they had testicular hypoplasia (Almquist and Barber, 1974). One bull was removed at 104 weeks of age because of extremely low semen quality and quantity; necropsy and histological examination revealed degenerative changes in the testes. Age of puberty could not be determined reliably for one bull because of inability to protrude the penis and serve the artificial vagina; by 53 weeks of age, however, the penis had developed sufficiently and semen characteristics were determined for weeks 53 to 104.

For the 22 bulls which completed the experiment, 12 bulls were ejaculated weekly (1x) and 10 bulls six times weekly (6x) consisting of two successive ejaculations on Monday, Wednesday and Friday from puberty to 2 years of age. To maximize sperm output per ejaculate, one false mount, a 2-min restraint period, and two additional false mounts preceded each ejaculation. If ejaculation was not achieved during the fourth mount, the bull was allowed to remount until ejaculation occurred.

Each ejaculum was examined for volume, percentage of progressively motile sperm and sperm concentration. Sperm concentration was determined with a photoelectric colorimeter, except for ejaculates that contained less than \(396 \times 10^6\) sperm/ml which were counted with a hemacytometer. Unless otherwise indicated, sperm output values were not corrected for sperm losses in the semen collection equipment.

Except for initial motility, data for semen traits from 53 to 68, 69 to 88 and 89 to 104 weeks of age were evaluated by analyses of variance. Analyses began with data for 53 weeks of age because this was the first week which included all 22 bulls. Data for initial progressive motility were analyzed for weeks 1 to 20, 21 to 40 and 41 to 60 after puberty; week 1 was the first week after puberty for each bull. This was done because of the wide variation in age at puberty. Log transformations of the data were used for the analyses of ejaculate volume, weekly sperm output and weekly motile sperm output.

In situ measurements of the greatest testes-scrotal circumference and width (hereafter referred to as scrotal circumference and width) were means of measurements by three technicians. Scrotal circumference was measured with a flexible metal tape around the greatest diameter of both testes and the scrotum. Scrotal width was obtained with vernier calipers at the point of maximum dimension of both testes and the scrotum. Measurements were taken at puberty, 52, 65, 78, 91 and 104 weeks of age and those from 52 to 104 weeks of age were evaluated by analyses of variance. At 2 and 3 years of age, each of the 22 bulls was ejaculated at 6x for 4 weeks and data for the last 3 weeks were used to determine mean weekly sperm output. Single scrotal measurements and testes growth rates, based on the percentage increase in scrotal circumference, were correlated with sperm output values to determine the usefulness of scrotal measurements in young bulls for predicting subsequent sperm output.

RESULTS

For the 21 of 22 bulls for which puberty was determined, mean age and body weight at puberty were \(41 \pm 1\) weeks (range 33 to 53 weeks) and \(397 \pm 14\) kg (range 266 to 499 kg). Body weight at 1 and 2 years of age for the 22 bulls averaged \(521 \pm 11\) kg (range 430 to 615
Figure 1. Postpuberal changes in initial sperm motility of semen from Charolais bulls ejaculated 1X or 6X weekly.

kg) and 838 ± 16 kg (range 691 to 1,000 kg).

Only 82 ejaculates, or 2% of the specified number of 4,542, were not obtained between the attainment of puberty and 2 years of age. Ejaculation during a false mount or before the penis could be covered with the artificial vagina accounted for 52 failures while lameness of one bull accounted for 18 failures. The high rate of successful collections is attributed to the practice of changing the stimulus animal and collection site as needed.

Initial Sperm Motility. Changes in mean initial sperm motility during the first 60 weeks after puberty are shown in figure 1. Initial motility increased with age and varied among bulls within frequency (P<.01) but differences attributable to ejaculation frequency were not significant. A separate analysis of the first 20 weeks after puberty showed that the rapid increase (P<.01) in mean initial motility from 34% at 1 to 4 weeks to 56% at 9 to 12 weeks after puberty accounted for most of the improvement in sperm motility as the bulls aged.

Initial motility of second ejaculations from 6X bulls between 1 and 2 years of age averaged 63% and was greater (P<.01) than that of 59% for first ejaculations. For the same period, single ejaculations for 1X bulls averaged 55%.

Ejaculate Volume. Changes in mean semen volume per ejaculate for the 1X and 6X bulls are shown in figure 2. Ejaculate volume varied between ejaculation frequencies and among bulls within frequency (P<.01) as well as among age groups (P<.05). There was a highly significant interaction between age and ejaculation frequency in which semen volume per ejaculate increased with age more for 1X than for 6X bulls. Mean ejaculate volumes for the 1X and 6X bulls at 53 to 68, 69 to 88 and 89 to 104 weeks of age were 4.3, 5.6 and 6.7 ml and 2.8, 3.3 and 5.3 ml, respectively.

Mean ejaculate volume of 5.5 ml for 1X bulls was larger (P<.01) than that of 3.2 for 6X bulls. For 6X bulls between 1 and 2 years of age, the average of 3.3 ml for second ejaculations was greater (P<.05) than that of 3.1 ml for first ejaculations. Since second ejaculations were larger in volume, the lower ejaculate volume for 6X than 1X bulls was due to more frequent semen collections and not to the collection of two successive ejaculations.

Sperm Concentration. Time trends in mean sperm concentration for the 1X and 6X bulls are shown in figure 3. Highly significant differences were associated with age and among bulls within frequency; differences due to ejaculation frequency were not significant. Mean sperm concentration for 53 to 68, 69 to 88 and 89 to 104 weeks of age was 1.2, 1.4 and 1.5 x 10^9 sperm/ml; differences between successive age periods were significant and the difference between the first and third age periods was highly significant.

Between 1 and 2 years of age for 6X bulls, the mean of 1.4 x 10^9 sperm/ml for first ejaculations was larger (P<.01) than the mean of 1.2...
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Figure 4. Changes in weekly motile sperm output for Charolais bulls ejaculated 1X or 6X weekly from puberty to 2 years of age.

2.3- and 2.4-fold increases from 1 to 2 years of age (2.1 and 9.1 x 10⁹ at 53 to 56 weeks and 6.6 and 21.4 x 10⁹ at 101 to 104 weeks of age). Differences attributable to age, ejaculation frequency and bulls within frequency were highly significant. Mean weekly sperm outputs for the three age periods were: 7.5 x 10⁹ at 53 to 68 weeks, 10.0 x 10⁹ at 69 to 88 weeks and 12.4 x 10⁹ at 89 to 104 weeks of age; all differences between means were highly significant. Between puberty and 2 years of age, weekly total motile sperm output for 1X bulls was 70% less than that for 6X bulls.

Scrotal Measurements. Increases in scrotal circumference and width from puberty to 2 years of age are shown in figure 5. Scrotal circumference and width each increased 32% from puberty to 2 years of age; 77% of the increase in circumference and 79% of the increase in width occurred between puberty and 65 weeks of age. Both scrotal measurements varied with age and among bulls within frequency (P<.01); ejaculation frequency had no significant effect on either measurement. The coefficient of variation for each measurement was 11%.

For 6X bulls, scrotal circumference at 52, 65, 78, 91 and 104 weeks of age was correlated .67, .78, .56, .71 and .66 with mean weekly sperm output at 53 to 56, 65 to 68, 77 to 80, 89 to 92 and 101 to 104 weeks of age, respectively. Corresponding correlations between scrotal width and weekly sperm output were .64, .82, .54, .78 and .74. Correlations above .63 and .76 were significant at the 5% and 1% levels of probability, respectively. The over-all correlations between scrotal circumference and width and concurrent weekly sperm output for the five ages studied were .78 and .79 (P<.01). No significant difference was
found between correlations involving scrotal circumference and sperm output and scrotal width and sperm output using Fisher's Z transformation.

Between puberty and 2 years of age, scrotal circumference and width were correlated .98 (P<.01). Scrotal circumference and width at puberty (21 bulls), 52 and 65 weeks of age (22 bulls) were correlated .37 and .38 (P<.05), .80 and .78 (P<.01) and .90 and .92 (P<.01), respectively, with these measurements at 104 weeks of age.

Scrotal circumference and width at puberty (21 bulls), 52, 65 and 78 weeks of age (22 bulls) were correlated .04 and .08, .44 and .32, .43 and .51, .52 and .54, respectively, with 6x weekly sperm output at 2 years of age; values above .42 and .53 were significant at the 5% and 1% levels of probability, respectively. Use of a multiple regression model in which age at puberty (21 bulls) and scrotal circumference at 52 and 65 weeks of age were independent variables, did not significantly increase the percentage of variation explained in the 6x weekly sperm output at 2 years of age. The multiple correlation coefficients between scrotal circumference at 52 and 65 weeks of age and sperm output at 2 years of age were .45 and .48 while the simple correlation values for the 21 bulls were .45 and .46.

Scrotal circumference at 52 and 78 weeks of age was correlated .62 and .75 (P<.01) with 6x weekly sperm output at 3 years of age (22 bulls). When sperm recovered from the collection equipment were included in the data, these correlations were not altered.

Testes growth rate, based on the percentage increase in scrotal circumference between 52 and 78 weeks of age, was not significantly related (.30) with 6x weekly sperm output at 3 years of age (22 bulls). However, the correlation increased to .61 (P<.01) when testes growth rate was based on growth during the first 24 weeks after puberty (21 bulls).

Discussion

The changes in semen characteristics and sperm output with increasing bull age agree generally with those reported by Almquist and Cunningham (1967) for eight Angus and nine Hereford bulls between 1 and 2 years of age and by Thibier and Colchen-Bourlaud (1972) for 57 French Friesian and 16 Normandy bulls between 9 and 15 months of age. Changes in Charolais semen traits and weekly sperm output associated with 1X and 6X ejaculation frequencies were similar to those reported for young Angus and Hereford bulls (Almquist and Cunningham, 1967).

From 1 to 2 years of age, weekly sperm output at 6X for 10 Charolais bulls was greater than that previously reported for 10 Holstein (Almquist and Amann, 1962) or 5 Angus and Hereford bulls (Almquist and Cunningham, 1967) collected at the same frequency at this laboratory (figure 6). The effect of the different feeding and management regimens (Almquist and Cunningham, 1967; Barber and Almquist, 1975) upon these relative breed differences in sperm output is not known.

Scrotal circumference has been shown to be a highly repeatable, easily obtained indicator of testes size which is highly significantly correlated with testes weight in bulls of various ages and with sperm output in young bulls (Willett and Ohms, 1957; Hahn et al., 1969). For older bulls, however, these workers reported that scrotal circumference and sperm output were negatively correlated. Hahn et al. (1969) suggested that this negative relationship indicated a declining efficiency of sperm production with age. Scrotal circumference and width were significantly correlated with concurrent sperm output of young Charolais bulls. Because measurements of scrotal circumference are simpler to obtain and involve less chance for accidental injury to the testes than those for scrotal width, scrotal circumference is the measurement of choice if only one is to be used. High heritability estimates for scrotal circumference indicate...
that bulls selected for this trait should produce more sperm than the average of the population (Coulter et al., 1974).

Scrotal circumference at puberty was not closely related (.37) to scrotal circumference at 2 years of age. However, scrotal circumferences at 52 and 65 weeks of age were good indicators of testes size at 2 years of age (.80 and .90; P<.01). These latter results support the correlation of .91 (P<.01) reported by Hahn et al. (1969) for scrotal circumference measurements of young Holstein bulls in consecutive years.

Preliminary results for eight Holstein bulls reported by Hahn et al. (1969) showed a correlation of .69 (P=0.05) between scrotal circumference measurements taken at 17 to 22 months of age and weekly sperm output 1 year later. In the present study, scrotal circumference at puberty was not significantly related (.04) to 6x weekly sperm output at 2 years of age. Although scrotal circumferences at 52, 65 and 78 weeks of age were significantly correlated .44, .43 and .52 with 6x weekly sperm output at 2 years of age, this relationship accounted for only 18 to 27% of the variation in sperm output. This low relationship was due partly to exclusion of three bulls with severely underdeveloped testes; two were killed at 13 and 14 months of age (Almquist and Barber, 1974) and one at 25 months of age. Although not included in the present study, the latter bull was ejaculated at 6x from puberty to 2 years of age. Scrotal circumference was 27.4 cm at 52 weeks and 30.9 cm at 2 years of age; 6x weekly sperm output at 2 years of age averaged 9.4 x 10⁹ as compared to 34.0 x 10⁹ for the 22 bulls in this study. Inclusion of this bull increased the correlation between scrotal circumference at 52 weeks of age and 6x weekly sperm output at 2 years of age from .44 (P<0.5) to .56 (P<0.1). Future research is needed with a large number of bulls in which early culling of bulls with deficient testes growth is not practiced.

Scrotal circumference at 52 and 78 weeks of age was correlated .62 and .75 (P<.01) with 6x weekly sperm output at 3 years of age. When 6x weekly sperm output data for six Angus and seven Hereford 3-year-old bulls ejaculated in the same manner as the 22 Charolais were included (J. O. Almquist, unpublished data), the composite within breed correlations for 35 bulls were .44 and .71 (P<.01). For the six Angus and seven Hereford bulls, the composite within breed correlation between scrotal circumference at 78 weeks of age and 6x weekly sperm output at 6 years of age was .67 (P<.05). Composite correlations were used because of differences in levels of weekly sperm output among breeds (figure 6). Testes growth rate, expressed as the percentage increase in scrotal-testes circumference between 52 and 78 weeks of age, was not highly correlated (.30) with 6x weekly sperm output at 3 years of age. This low relation may have resulted in part because age at puberty varied from 33 to 53 weeks of age. When rates of testes growth during the first 24 weeks after puberty was used, the relation increased to .61 (P<.01). Thus use of testes growth rate was not superior to a single scrotal measurement for predicting potential sperm output. These results suggest that, except for detection of severe potential testicular underdevelopment, neither a single scrotal measurement at 1 or 1.5 years of age nor postpuberal testes-scrotal growth rate can be used to predict subsequent sperm output precisely.

Factors other than testes size, such as genetic and environmental factors which affect total testicular sperm production, also may limit the value of scrotal measurement for predicting subsequent sperm output. One 6x bull in the present study, for example, showed normal testes growth (scrotal circumference of 38.1 cm at 2 years of age was the same as the mean for 22 bulls) yet was clearly below average in weekly sperm output from puberty to 2 years of age. Testes growth and sperm output plateaued at about 90 weeks of age; 6x weekly sperm output at 3 years of age was 25.3 x 10⁹. Necropsy and histological examination at 3 years of age revealed mild degenerative changes throughout both testes except for severe regression, erosion and denudation of the germinal epithelium in the ventral pole of the right testis. The cause of these changes is unknown.

Testicular hypoplasia is a relative condition that often is difficult to detect and mild cases predispose the bull to testicular degeneration (Roberts, 1971). While severe cases of testicular hypoplasia and degeneration were detected for three of 27 Charolais and three of 27 Angus and Hereford bulls (Almquist and Cunningham, 1967) it is desirable to be able to detect and eliminate at an early age those bulls with less obvious testicular underdevelopment. As more knowledge becomes available about the hormonal control of spermatogenesis, blood levels of steroids and gonadotropins may replace or
be useful adjuncts to scrotal measurements for detection of subnormal testicular development.

LITERATURE CITED