ARTICLES

The proportion of beef bulls in western Canada with mature spermiograms at 11 to 15 months of age

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Abstract — A study was conducted to determine the proportion of yearling beef bulls that have mature spermiograms at 11 to 15 months of age, and to evaluate the relationship between semen quality traits, age, and scrotal circumference. Semen samples and data on sperm motility and scrotal circumference measurements were obtained from 1641 bulls of 14 breeds. Criteria for a satisfactory spermiogram included sperm concentration ≥ 400 × 10^6/mL, ≥ 60% progressively motile sperm, and ≥ 70% morphologically normal sperm. The mean scrotal circumference measurements for all bulls combined were 33.4, 34.4, 35.2, 35.8, and 35.3 cm at 11, 12, 13, 14, and 15 months of age, respectively. The percentage of bulls with matures spermiograms at 11, 12, 13, 14, and 15 months of age were 20%, 30%, 51%, 52%, and 61%, respectively. There was a high positive correlation (r = 0.9) between the number of bulls with ≥ 70% normal sperm and scrotal circumference measurement. The main types of morphologic defects observed in immature bulls were proximal droplets and midpiece defects.

Résumé — **Proportion des taureaux de boucherie de l'Ouest du Canada présentant un spermogramme à maturité à l'âge de 11 à 15 mois.** Une étude a été menée afin de déterminer la proportion de taureaux de boucherie d'un an présentant un spermogramme à maturité à l'âge de 11 à 15 mois et d'évaluer la relation entre les critères de qualité de la semence, l'âge et la circonférence scrotale. Les échantillons de sperme et les données sur la motilité des spermatozoïdes et la mesure de la circonférence scrotale ont été obtenus à partir de 1641 taureaux de 14 races. Les critères d'un spermogramme satisfaisant comprenaient la concentration en spermatozoïdes ≥ 400 × 10^6/mL, ≥ 60 % de spermatozoïdes présentant une motilité progressive et ≥ 70 % de spermatozoïdes morphologiquement normaux. Les mesures moyennes de la circonférence scrotale pour tous les taureaux combinés étaient respectivement de 33.4, 34.4, 35.2, 35.8 et 35.3 cm à l'âge de 11, 12, 13, 14 et 15 mois. Les pourcentages de taureaux présentant un spermogramme à maturité à l'âge de 11, 12, 13, 14 et 15 mois étaient de 20 %, 30 %, 51 %, 52 % et 61 % respectivement. Il y avait une forte corrélation positive (r = 0.9) entre le nombre de taureaux possédant ≥ 70 % de spermatozoïdes normaux et la mesure de la circonférence scrotale. Les principales anomalies morphologiques observées chez les taureaux immatures consistaient en gouttelettes proximales et en anomalies de la pièce intermédiaire.

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Introduction

As cows are bred naturally in most beef herds, the selection of herd sires is a critical decision affecting reproductive performance. The use of yearling bulls in a beef breeding herd can increase the profit potential for the producer by reducing bull cost per pregnancy (1). However, yearling bulls must be carefully evaluated before use to ensure that they have reached sexual maturity (1).

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Information obtained from record of performance (ROP) stations indicate that there are approximately 6500 performance-tested yearling bulls offered for sale and available for breeding each year in western Canada. However, many other yearling beef bulls are sold at consignment auctions or by private treaty, but the number is difficult to estimate.

It is well documented that young beef bulls going through puberty exhibit low sperm motility and a high percentage of abnormal spermatozoa (2,3). Qualitative semen traits have been shown to improve with bull age, from 12 to 16 mo in *Bos taurus* bulls (2) and 14 to 18 mo in *Bos indicus* bulls (3). A previous study done approximately 25 y ago indicated that 36% to 41% of yearling bulls completing a performance test had satisfactory semen quality (4). It could be hypothesized that continued selection for larger testicle size since that time has hastened sexual maturation in today's bulls. It would be useful to know the proportion of yearling bulls of various breeds that can be expected to be sexually mature at various ages, to serve as a guide to bull buyers and to offer realistic expectations to those who wish to use yearling bulls for breeding purposes.

Onset of puberty has been defined as the time at which a young bull can produce semen with more than 50×10^6 spermatozoa/mL and in which at least 10% of the sperm are progressively motile (5). The percentage of normal sperm heads, tails, and acrosomes increases in conjunction with a reduction in the number of proximal droplets and an increase in sperm concentration during the first 3 to 4 mo after the onset of puberty (6). Onset of puberty in beef bulls occurs between 8 and 12 mo of age when the scrotal circumference (SC) measurement is about 28 cm (5,7). However, the age at puberty varies by as much 62 d among breeds and 88 d among bulls, regardless of the breed (6). Consequently, there is great variation in the age at which bulls have satisfactory spermiograms. An earlier study showed that 26%, 53%, and 100% of beef bulls produced satisfactory semen quality at 12, 14, and 16 mo of age, respectively (2).

Scrotal circumference measurements are highly correlated to paired testis weight, which is positively correlated to daily sperm production and semen quality (8). It has been shown that SC may be a more accurate predictor of the time of onset of puberty than either age or weight, regardless of breed (6). Furthermore, high correlations were found between breed means for SC in bulls and age at puberty in heifers and fertility of female offspring; that is, the offspring of bulls with a large SC reach puberty at an earlier age than do those with a small SC. (9–11).

The objective of this study was to determine the proportion of yearling beef bulls subjected to breeding soundness evaluation (BSE) with satisfactory spermiograms at 11 to 15 mo of age, and to determine the relationship between semen quality, age, and SC.

Materials and methods

Semen samples from 1641 yearling beef bulls were collected during April and May. Approximately 75% of the samples came from bulls at (ROP) test stations and the remainder from bulls on farms in Alberta, Saskatchewan, and Manitoba. The ages ranged from 11 to 15 mo at the time of the breeding soundness evaluation (BSE), and 14 breeds were represented. The most common breeds were Angus, Charolais, Limousin, and Simmental, the least common breeds included red poll, Hereford, gelbvieh, Blonde d'Aquitaine, braunvieh, salers, shorthorn, red max, and Maine-Anjou.

Semen samples were obtained by electroejaculation and preserved in 0.25 mL of 10% buffered formalin for evaluation in the laboratory. Between 1 and 2 mL of semen were added to the test tube and mixed with the formalin. The exact amount of semen was calculated by subtracting 0.25 mL from the total volume. The percentage of progressively motile sperm and the SC measurements were recorded at the time of the BSE. Sperm motility was scored as poor, fair, good, and very good (0%–39%, 40%–59%, 60%–79%, and ≥80% progressively motile sperm, respectively) according to the standards of the Western Canadian Association of Bovine Practitioners (12).

One smear from each formalin-treated semen sample was stained with eosin-nigrosin and examined at 1000X magnification for assessment of sperm morphology. The percentages of head, midpiece, principal piece, and acrosomal defects, as well as detached heads, teratoids, proximal cytoplasmic droplets, and of normal sperm, were determined. Differential counts of 200 spermatozoa were done for each bull by 2 researchers and were averaged. When the estimates of the 2 researchers for normal spermatozoa differed by more than 10%, or when differences affected the classification of the bull's maturity, both researchers repeated differential counts.

Sperm concentration was measured by spectrophotometer (Spectronic 20; Bausch & Lomb, Rochester, New York, USA). The spectrophotometer was calibrated with semen samples of known concentration (determined by hemocytometer) by diluting semen 1 in 160 with saline (0.9% NaCl), and a simple regression plot was created to determine unknown sperm concentrations (13). Because the amount (0.25 mL) of 10% buffered formalin was known, the amount of semen in the sample was calculated in order to prepare dilutions of 1:160 and estimate sperm concentration; consequently, different volumes from the samples were used to prepare the dilution. The sperm suspension in the formalin-treated semen samples was homogeneous soon after mixing with saline and sperm concentration was estimated shortly after preparing the spectrophotometer sample tubes (14). Sperm concentration was scored as follows: $< 250 \times 10^6$ sperm/mL = poor; 250×10^6 to 390×10^6 sperm/mL = fair; 400×10^6 to 750×10^6 sperm/mL = good; and $> 750 \times 10^6$ sperm/mL = very good.

The criteria used to classify a spermiogram as mature (completion of puberty) was sperm concentration $\geq 400 \times 10^6$ sperm/mL, ≥ 60% progressively motile sperm, and ≥ 70% morphologically normal sperm. This criterion is based on previous work done by Lunstra (7).

Statistical analysis

Assumptions of normality and equality of variances between age group for each sperm defect category were investigated by using an analytical software program (15). The variables of the different sperm defects failed the assumption of a normal distribution. However, data for SC is normally distributed. The data were analyzed by using simple correlation, simple regression plot, and 1-way analysis of variance. The effect of age on each category of defect was tested by using Kruskal-Wallis and protected rank-sum test comparing groups independently in order to compare the medians and determine the significant differences. Because data from the different sperm defects is non-normally distributed and transformation of the data is not possible because of the heterogeneity of the data after transformation (some variables become normal some not), it was not possible

Table 1. Scrotal circumference measurement (mean, *s*) at 11, 12, 13, 14, and 15 mo of age for all breeds combined and for the 4 predominant breeds

Breed	11 months	12 months	13 months	14 months	15 months
All breeds	33.4[a], $s = 2.3$ (95)	34.4[b], $s = 2.0$ (507)	35.2[c], $s = 2.2$ (776)	35.8[d], $s = 2.3$ (250)	35.3, $s = 2.4$ (13)
Angus	33.7[a], $s = 2.4$ (48)	34.3[a], $s = 1.9$ (296)	34.9[b], $s = 2.4$ (411)	35.4[c], $s = 1.7$ (64)	34.5, $s = 1.8$ (9)
Simmental	—	35.6[a], $s = 2.0$ (28)	37.1[ab], $s = 2.3$ (52)	36.8[b], $s = 2.1$ (4)	—
Limousin	30.4[a], $s = 1.1$ (9)	30.8[a], $s = 1.4$ (7)	32.2[a], $s = 1.9$ (35)	33.4[b], $s = 1.9$ (21)	—
Charolais	33.3[a], $s = 1.8$ (15)	34.7[b], $s = 2.1$ (101)	35.9[c], $s = 1.6$ (209)	36.4[c], $s = 2.2$ (105)	37.0, $s = 3.1$ (4)

Numbers in parentheses represent the numbers of bulls
[a,b,c,d]Means in the same row with different superscripts are significantly different ($P = 0.05$)

Table 2. Mean percentages of spermatozoa classified as morphologically normal or abnormal at 11, 12, 13, 14, and 15 mo of age

Morphologic defects of spermatozoa	Age (mo) 11	12	13	14	15[a]
Head	6.24 (5)[b]	5.60 (5)[b]	5.08 (4)[c]	5.16 (4)[c]	4.30
Midpiece	15.09 (13)[b]	16.26 (12)[c]	15.66 (11)[d]	13.33 (10)[e]	14.07
Principal piece	1.47 (1)	2.11 (1)	1.74 (1)	1.04 (1)	1.15
Detached heads	4.60 (4)	5.79 (4)	5.25 (4)	6.96 (4)	2.89
Teratoid	0.88 (0)	0.65 (0)	0.68 (0)	0.65 (0)	0.38
Proximal droplets	17.899 (6)[b]	8.80 (3)[c]	6.69 (3)[c]	5.89 (3)[c]	5.76
Acrosome	1.21 (0)	0.69 (0)	0.619 (0)	0.68 (0)	0.15
Normal	55.67 (63)[b]	63.29 (69)[c]	66.87 (74)[d]	69.57 (75)[e]	71.15

[a]15-month-old bulls were not included in the analysis because the sample size was small, however, the simple means are reported
[b,c,d,e]Means in the same row with different superscripts have significantly different ($P = 0.00$) medians
Numbers in parentheses are median values

Table 3. The percentage of bulls classified as mature for the semen trait categories of sperm morphology, sperm concentration, and motility, and the percentage of bulls classified as mature in all 3 categories

Age (mo)	*n*	≥ 70% morphologically normal sperm	≥ 400 × 10^6 sperm per mL	≥ 60% progressively motile	% mature in all 3 categories
11	95	41.1	34.8	92.7	20
12	507	50	52.3	93.5	30
13	776	59.2	56.4	94.8	51.1
14	250	69.6	64.0	97.2	48
15	13	61.6	84.7	92.4	61.5

to make a direct comparison of means. The relationship between SC and percentage of bulls with ≥ 70% normal sperm was estimated by using simple correlation. A simple regression plot was used to calculate the relationship between sperm concentration and percentage of transmittance. Fifteen-month-old bulls were not included in the statistical analysis, because the sample size ($n = 13$) was too small. However, the mean values for the variables are reported.

Results

Scrotal circumference

The mean SC for all breeds was 33.3, 34.4, 35.2, 35.8, and 35.3 at 11, 12, 13, 14, and 15 mo of age, respectively (Table 1). The means for SC for the predominant breeds at 11, 12, 13, and 14 mo of age were significantly different ($P < 0.05$). The SC of Simmental bulls was greater ($P < 0.05$) than that of Angus, Charolais, and Limousin bulls at 12, 13, and 14 mo of age. There were no 11- and 15-month-old Simmental bulls in this study. The mean daily increment in SC measurement from 11 to 12 mo was 0.033 cm; from 12 to 13 mo, 0.027 cm; and from 13 to 14 mo, 0.020 cm.

The correlation between the size of the SC and the percentage of morphologically normal sperm was low ($r = 0.091$, $P < 0.05$). However, there was a significant positive correlation between size of the SC and the number of bulls with ≥ 70% normal sperm ($r = 0.90$, $P = 0.05$).

The SC increased by 0.7 ± 0.2 cm/mo for all breeds combined from 11 to 14 mo of age. The mean change in SC/d from 11 to 12, 12 to 13, and 13 to 14 mo of age was 0.033, 0.027, and 0.020 cm, respectively.

Sperm morphology

The mean percentages of spermatozoa classified as morphologically normal or abnormal are shown in Table 2. The mean percentage of morphologically normal sperm increased from 11 to 14 mo of age. Significant differences in the medians of morphologically normal sperm ($P = 0.00$) were found between the age groups of 11, 12, 13, and 14 mo. The percentage of sperm with proximal cytoplasmic droplets decreased from 17.8% in 11-month-old bulls to 5.8% in 14-month-old bulls. A significant difference in the medians of sperm with head defects was found between 11- and 13-, 11- and 14-, 12- and 13-, 12- and 14-month-old bulls, but no significant difference was found between 11- and 12-, and 13- and 14-month-old bulls. Significant differences were found for the medians of sperm with midpiece defects among all the age groups ($P = 0.00$). No significant differences were found among age groups in percentages of sperm with acrosome, teratoid, detached head, and principal piece defects ($P = 0.00$). The prevalence of sperm with these defects remained low at all ages in this bull population (Table 2).

The percentage of bulls with ≥ 70% morphologically normal sperm at 11, 12, 13, and 14 mo of age were 41%, 49%, 58.9%, and 69.4%, respectively (Table 3). Over all age groups, only 56.6% of bulls had ≥ 70% normal sperm morphology.

Sperm concentration and motility

Table 3 shows the percentage of semen samples with concentrations < 400 × 10^6 sperm/mL and with < 60% progressively motile sperm. At 11, 12, 13, and 14 mo of age, 65.2%, 47.7%, 43.7%, and 36.0% of semen samples, respectively, contained less than 400 × 10^6 sperm/mL. There was no significant difference in sperm concentration between bulls at 12, 13, and 14 mo of age; however, a significant difference was found between 11-month-old bulls and bulls of the other age groups ($P < 0.05$). The percentages of samples that had < 60% progressively motile sperm were 7.3, 6.5, 5.2, and 2.8 for bulls at 11, 12, 13, and 14 mo of age, respectively. Over all age groups, only 5.4% of the bulls had < 60% progressively motile sperm.

Discussion

Previous studies indicate that SC in yearling bulls is highly correlated with daily sperm production (16), and that 15-month-old bulls with larger testicles have higher epididymal sperm reserves than do those with smaller testicles (17). It has been shown that bulls with larger testicles have an earlier onset of puberty (7) and, as a result, they also have a satisfactory spermiogram earlier than do bulls with smaller testicles (18). In the present study, the mean SC appeared to be larger at any given age for all breeds than were those reported by other authors (2). However, the proportion of bulls with mature sperm traits at 12 and 14 mo of age (Table 3) was not higher than that reported 25 y ago (2). Therefore, the largest SC reported here may not reflect genetic improvement, but rather a tendency for the private practitioners in this study to measure less tightly than did previous researchers. The effect of breed on SC was found to be similar to those of earlier findings (3,7,9,19); that is, Simmental bulls had the largest SC measurement and Limousin the smallest at 12 mo of age (19,20). The growth rate of the SC decreased as age increased from 11 to 14 mo of age, as has been reported previously (7). The growth rate of the SC in this study was slow compared with that of 1.35 cm/mo (0.03 to 0.06 cm/d) reported previously (3,7,9). However, in previous studies, changes in SC/d were studied within individual bulls, rather than between different groups of bulls, and by using measurements from different sources, as in this study. This may account for the lack of agreement between this study and previous studies.

There was a wide variety of sperm abnormalities in all breeds and ages. The percentages of the different types of abnormalities were not correlated to SC. Furthermore, there was no significant relationship between SC and the percentage of normal sperm, when all the bulls were compared. This is probably due to the pubertal changes in semen quality that bulls were undergoing. The diverse relationship of sperm morphology with SC in young beef bulls is in agreement with the findings of another study (17). The high correlation between the number of bulls with ≥ 70% normal sperm morphology and the size of the SC indicates that the larger the SC, the greater the chances of having a bull with ≥ 70% normal sperm morphology. This finding is similar to earlier studies involving yearling bulls (2,7). A similar relationship has also been reported in mature beef bulls, where the probability of having satisfactory semen quality increased as the SC increased to approximately 38 cm (2).

This study showed that the percentage of morphologically normal sperm increased with the increase in the age of the bull from 11 to 15 mo, as has been shown before (21,22). At the onset of puberty, semen has been shown to contain a high percentage of sperm abnormalities, such as pyriform or microcephalic heads, coiled tails, or proximal cytoplasmic droplets. As the age increased from 11 to 14 mo, the SC increased and the percentage of morphologically abnormal sperm decreased (6). Consequently, abnormal sperm morphology in young bulls (11 to 15 mo) of age should not be cause for great concern as regards to the future potential fertility (1). Proximal cytoplasmic droplets on spermatozoa are common in young bulls at the time of puberty and, frequently, high percentages of sperm are affected (6,21,23). In this study, proximal cytoplasmic droplets were the main sperm defect contributing to an immature classification. The effect of proximal cytoplasmic droplets on fertility rates has been studied in vivo (24) and in in vitro models (25). It was demonstrated that fertilization rates were markedly lower for bulls affected with > 30% proximal droplets than for control bulls. Persistence of a high percentage of proximal cytoplasmic droplets in spermatozoa of young bulls over 15 mo of age has been associated with delayed puberty or disturbances in spermatogenesis (26).

In this study, the percentage of sperm affected with midpiece defects appeared to be higher than that reported by others (21). However, the percentages of sperm with head, principal piece, detached heads, teratoids, and acrosome defects were low in all bulls from 11 to 15 mo of age. Other researchers have reported similar findings, where tail, acrosome, head defects,and detached heads were present in less than 5.6% of the sperm (21). Lunstra (6) reported that the percentage of spermatozoa with normal acrosomal morphology improved very slowly and had not reached mature levels by 16 wk after the onset of puberty.

The percentage of bulls with sperm concentrations ≥ 400 × 10^6/mL increased dramatically from 11 to 15 mo of age in this study (34.8% to 84.7%). A previous report indicated that, in some bulls, sperm concentration increased progressively with age but, in others, it decreased or did not change (27). The lowest concentration in 11- to 13-month-old bulls was < 28 × 10^6 sperm/mL, and the highest concentrations in 11-, 12-, and 13-month-old bulls were 496 × 10^6 sperm/mL, 598 × 10^6 sperm/mL, and 713 × 10^6 sperm/mL, respectively (27). Some of the variation in sperm concentrations may have been due to the amount of seminal plasma collected at the time of electroejaculation. In this regard, semen collection by artificial vagina may be more reliable for assessing the capability of young bulls to produce concentrated semen. For this reason, sperm concentration must be interpreted with caution, even though the samples were collected in a standard way, trying to avoid collection of much seminal plasma and only collecting the sperm rich fraction.

The percentage of progressively motile sperm has been reported to increase over a period of 16 wk after the onset of puberty, with a sharp increase occurring 6 wk after the onset of puberty (6). A rapid increase to a plateau of 50% to 60% progressively motile sperm in semen samples collected from Charolais bulls was reported to occur within 12 wk after the onset of puberty (16). In our study, nearly all the bulls from the different age groups had at least 60% motile sperm. This may indicate that there is normally a sharp increase of progressively motile sperm early in the puberal process. Therefore, the percentage of progressively motile sperm was not important in the classification of bull maturity in this study.

The reasons to classify bulls as immature were abnormal sperm morphology and low concentration. The percentages of bulls showing mature spermiograms at the different ages in this study were somewhat similar to those reported nearly 25 years ago (2,3). In the present study, the proportion of bulls that reached maturity changed from 20% at 11 mo of age to 61% at 15 mo of age. Over all ages, only 42% of the bulls were mature and only 57% had ≥ 70% morphological normal sperm. Consequently, the hypothesis that due to genetic improvement beef bulls now mature at an earlier age is not supported.

These results may serve to caution producers regarding the use of young bulls for breeding purposes. Yearling bulls can be as reproductively efficient as 2-year-old bulls (28); however, success in impregnating cycling beef females is highly dependent on the use of bulls that have reached sexual maturity (1). CVJ

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