

PUBERTY IN BEEF BULLS: ACROSOME MORPHOLOGY AND SEMEN QUALITY IN BULLS OF DIFFERENT BREEDS¹

D. D. Lunstra and S. E. Echtenkamp^{2,3}

US Department of Agriculture, Clay Center, NE 68933

Summary

Semen characteristics were evaluated every 2 wk from 7 through 13 mo of age in 31 beef bulls representing six breed groups (Hereford, Angus, Hereford × Angus crossbreds, Angus × Hereford crossbreds, Red Poll and Brown Swiss). Breeds differed in age at puberty, defined as the age at which an ejaculate was first obtained that contained a minimum of 50×10^6 total spermatozoa with at least 10% progressive motility (Hereford, 326 ± 9 d; Angus, 295 ± 4 d; Hereford × Angus, 300 ± 8 d; Angus × Hereford, 296 ± 9 d; Red Poll, 283 ± 9 d and Brown Swiss, 264 ± 9 d). Significant breed differences also were observed in concentration of spermatozoa, progressive motility, seminal protein concentration, abnormal spermatozoa and acrosomal morphology. Considerable variation was observed for the majority of pubertal traits among the 31 bulls, indicating that differences in stage of pubertal development existed among and within breeds of beef bulls between 7 and 13 mo of age. However, adjustment of data to age at puberty negated breed differences ($P > .10$), indicating that the pubertal patterns of change occurring in each semen characteristic were similar for the breeds evaluated. Concentration of spermatozoa, progressive motility, seminal protein concentration, percentage spermatozoa with normal head and tail morphology and percentage spermatozoa

with normal acrosomal morphology increased ($P < .01$) from puberty through 16 wk after puberty in all bulls and all breeds. During the first 6 wk after puberty, rapid increases ($P < .01$) were observed in percentage spermatozoa exhibiting normal head morphology (excluding acrosomes) and progressive motility, and a rapid decrease ($P < .01$) was observed in percentage spermatozoa with proximal cytoplasmic droplets, with values at +6 wk approaching those reported for mature bulls. Percentage spermatozoa with normal acrosomal morphology and concentration of spermatozoa improved more slowly and had not reached mature levels by 16 wk after puberty. Because age at puberty varied by 62 d among breeds and 88 d among bulls and important characteristics of semen quality improved slowly after puberty, careful evaluation of the stage of pubertal development in individual bulls is recommended before selecting young bulls for natural breeding or artificial insemination. Additional investigations are needed to define the patterns of pubertal development through sexual maturity in beef bulls and to establish relationships to fertility. (Key Words: Beef Bulls, Breeds, Semen, Spermatozoa, Acrosomes, Puberty.)

Introduction

Descriptions of pubertal development in beef bulls have been limited to only a few breeds (Wolf et al., 1965; Swanson et al., 1971; Barber and Almquist, 1975), and characterizations of postpubertal changes in semen quality in beef breeds are limited (Almquist and Cunningham, 1967; Almquist et al., 1976). No characterizations of bovine acrosomal morphology around the time of onset of puberty were found in the literature. The use of an increasing number of sire breeds in the beef cattle industry emphasizes the importance of defining differences in the reproductive potential of young sires both among and within

¹The authors gratefully acknowledge the technical assistance of Ms. Shari Ellis, Mr. Ray Sampson, Mr. Steve Furman and Mr. Dave Mitchell with the collection of data.

²Roman L. Hruska US Meat Animal Research Center, ARS.

³Mention of trade name, proprietary product or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

breeds. Because selection and use of superior sires at the youngest possible age is desired for natural mating and artificial insemination (Almquist et al., 1976; Hanson et al., 1980), investigations of semen quality in young beef bulls are needed.

The objectives of this study were to characterize semen quality and age at puberty in breeds of beef bulls between 7 and 13 mo of age and to define breed differences and interrelationships among classifications of sperm morphology and semen quality. Puberty was defined as the age at which the first ejaculate containing a minimum of 50×10^6 spermatozoa with at least 10% progressive motility was obtained from each bull. Information on pubertal age, hormonal concentrations, sexual aggressiveness, body weight and testicular growth of these bulls has been reported (Lunstra et al., 1978).

Materials and Methods

Thirty-one young beef bulls representing six breed groups were evaluated from 7 through 13 mo of age (December through June). The number of bulls per breed group was: five Hereford (H), five Angus (A), five Red Poll (RP), five Brown Swiss (BS), six H \times A crossbred (H \times A) and five A \times H crossbred (A \times H). The bulls representing each breed were selected from large peer groups, and the adjusted weaning weight of each bull approximated the average of the peer group. All bulls had been subjected to the same managerial and environmental conditions from birth, and age of dam was similar for all bulls. Age of bull did not differ among breed groups and averaged 216 ± 1 d at the beginning of evaluation (i.e., 7 mo). Information on diets fed and general characteristics of puberty have been reported (Lunstra et al., 1978).

Semen was collected from each bull once every 2 wk from 7 through 13 mo of age. A sine wave electroejaculator⁴ was used for semen collection (Ball et al., 1974). Stimulus was delivered with a continuous rhythm of increasing stimuli from initiation through erection and ejaculation. Semen collection began when the clear pre-ejaculatory fluids dripping from the

penis began to turn turbid and was terminated when fluids again turned relatively clear. Collection liners and tubes were insulated to maintain semen at 37 C during collection. Concentration of spermatozoa was determined by quadruplicate counts made with a hemacytometer. Progressive motility was determined by duplicate estimates on semen diluted in phosphate buffered saline (pH 7.4); a microscope ($\times 400$ magnification) equipped with a stage warmer (37 C) was used. Live spermatozoa were quantitated by scoring 200 spermatozoa in smears stained differentially with eosin-fast green, prepared according to Hackett and Macpherson (1965). Spermatozoan abnormalities (excluding acrosome status) were evaluated by two direct counts of 100 differentially stained sperm ($\times 400$ magnification) on slides prepared as described for determination of live sperm. Abnormal spermatozoa were categorized into six head and eight tail classifications, based on the descriptions of Herrick and Self (1962) and Saacke (1970). The eight tail classifications were: normal, proximal cytoplasmic droplet, kinked, coiled, abaxial, double, filiform and truncated tails. The six head classifications were: normal, macrocephalic, microcephalic, pear-shaped, decapitated and other irregular heads. Protein concentration in seminal plasma was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard. A .5 ml aliquot of semen from each collection was centrifuged at $10,000 \times g$ for 10 min (4 C) and the supernatant assayed directly for protein content.

For acrosomal evaluation, spermatozoa were fixed with .2% glutaraldehyde in sodium cacodylate buffer (pH 7.4; Johnson et al., 1976), and 20 μ l placed under a 22 \times 22 mm coverslip on a glass slide. The edges of the coverslip were sealed with Permount⁵, immobilizing the coverslip and allowing the use of an oil-immersion microscope objective. Acrosomal integrity was evaluated by two direct counts of 100 fixed spermatozoa using differential-interference-contrast (DIC, $\times 1,000$ magnification) microscopy. Acrosomal integrity was classified only on spermatozoa with normal head morphology. Acrosomal morphology was categorized into three classes: NA = normal acrosomes (characterized by the presence of a tightly adherent, intact acrosome with a smooth surface and periphery and a distinct, uniformly-shaped apical ridge), AA = aberrant acrosomes (characterized by the presence of a tightly

⁴ Lane Manufacturing Co., Denver, CO.

⁵ Fisher Scientific Co., Pittsburgh, PA.

adherent acrosome exhibiting an incomplete or irregularly-shaped apical ridge or lacking a smooth acrosomal surface) and GDA = grossly disrupted acrosomes (characterized by the presence of a swollen, ruffled or vesiculated acrosome or by loss of the anterior acrosomal cap with retention of only the posterior equatorial segment). The NA category was based on descriptions of the intact acrosomal cap of the bovine spermatozoon (Saacke and White, 1972). The AA and GDA categories included acrosomal morphologies described as abnormal (Saacke et al., 1968; Jones, 1975; Cran and Dott, 1976; Coulter et al., 1978; Foster et al., 1980), maturational (Jones, 1975) and degenerative (Saacke and Marshall, 1968; Saacke, 1970; Saacke and White, 1972; Jones, 1975).

Data were analyzed by split-plot analysis of variance for unequal subclass numbers (to adjust for the effect of repetitive measurements on the same bulls) using the least-squares methods of Harvey (1975). Age at puberty, defined as the age at which an ejaculate was first obtained that contained a minimum of 50×10^6 total spermatozoa with at least 10% progressive motility (Lunstra et al., 1978), was used as a covariate. Differences among subgroup means were tested for significance by Duncan's multiple range test (Steel and Torrie, 1960).

Results and Discussion

Breeds differed significantly in age at puberty (H, 326 ± 9 d; A, 295 ± 4 d; H \times A, 300 ± 8 d; A \times H, 296 ± 9 d; RP, 283 ± 9 d and BS, 264 ± 9 d) and pubertal body weight (H, 261 ± 6 kg; A, 273 ± 9 kg; H \times A, 279 ± 8 kg; A \times H, 264 ± 5 kg; RP, 258 ± 8 kg and BS, 295 ± 11 kg; Lunstra et al., 1978). Age at puberty was similar to that reported by Wolf et al. (1965) for larger groups of H (315 d) and A (308 d) bulls. Variation in age at puberty was observed among bulls within each breed group (H, 293 to 339 d; A, 288 to 305 d; H \times A, 262 to 322 d; A \times H, 277 to 324 d; RP, 250 to 300 d and BS, 251 to 291 d), but comparison with breed means indicated that no breed group average was affected unduly by individual bulls reaching puberty at either extreme. It is likely that variation in pubertal characteristics of the bull group representing each breed was reduced by our selection procedures (see Materials and Methods) and that variation within breed would normally be greater in unselected breed populations. For example, range in age at puberty in

larger breed groups has been reported to be 273 to 364 d in H, 273 to 350 d in A, 252 to 343 d in Holstein and 231 to 371 d in Charolais bulls (Wolf et al., 1965; Killian and Amann, 1972; Almquist et al., 1976).

In each breed group, concentration of spermatozoa in ejaculates increased steadily from the time spermatozoa first appeared through 13 mo of age ($P < .01$, table 1). Percentage of spermatozoa exhibiting progressive motility showed a similar linear increase ($P < .01$). Breed groups differed ($P < .05$) in concentration of spermatozoa and progressive motility through the majority of evaluation periods (table 1), and breeds that reached puberty the earliest (BS, RP and A) tended to exhibit higher concentrations of spermatozoa and progressive motility. Concentration of spermatozoa averaged 400 to 700 million/ml of semen at 13 mo of age in the six breed groups (i.e., 10 to 20 wk after puberty was attained). These concentrations are similar to the concentrations of spermatozoa collected biweekly by artificial vagina from H and A bulls (200 to 700 million spermatozoa/ml; Almquist and Cunningham, 1967) and weekly by artificial vagina from Charolais bulls (200 to 1,200 million spermatozoa/ml, Almquist et al., 1976) during the first 12 wk after attaining puberty.

Electroejaculation was selected over the artificial vagina as the method for semen collection in our study, because electroejaculation allows semen to be collected without depending on the sexual aggressiveness of the bull (Hupp et al., 1962; Ball et al., 1974), success rate of semen collection with untrained yearling beef bulls is 98% with electroejaculation and 54% with the artificial vagina (Ball et al., 1974) and total number of spermatozoa, motility of spermatozoa and fertility of semen collected by electroejaculation equals or exceeds that of semen collected by artificial vagina (Hafs and Knisely, 1960; Clarke et al., 1973; Ball et al., 1974). A biweekly ejaculation frequency was utilized in our study, because ejaculation frequency does not affect motility of spermatozoa, morphology of spermatozoa, spermatogenic efficiency or fertility of semen (Amann, 1962; Cunningham et al., 1967; Martig and Almquist, 1969; Almquist et al., 1976). In young Holstein bulls, no differences in concentration of spermatozoa, motility of spermatozoa, percentage of live spermatozoa and percentage of spermatozoa with normal morphology were detected between bulls

TABLE 1. LEAST-SQUARES MEANS FOR SPERM CONCENTRATION, PROGRESSIVE MOTILITY AND PROTEIN CONCENTRATION IN SEMEN OF BEEF BULLS BETWEEN 7 AND 13 MO OF AGE^a

Breed group ^b	Mo of age ^c													All values (mean ± SE)
	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0	12.5	13.0	
Sperm conc., × 10 ⁶ /ml														
H	nd	nd	nd	nd	1d	17d	10d	86d	97de	164d	156d	233d	541e	98 ± 28d
A	nd	nd	nd	.5d	3d	25d	56de	81d	33d	197de	222de	360de	680e	123 ± 28d
H × A	nd	nd	nd	5d	10d	13d	25d	60d	82d	146d	306e	345d	410d	108 ± 25d
A × H	nd	nd	nd	.5d	12d	80d	66de	79d	11.2de	302e	215de	382de	537e	137 ± 27d
RP	nd	nd	4	7d	38d	59d	56de	99d	59d	209de	386e	338d	411d	129 ± 28d
BS	nd	1	20	71e	35d	79d	141e	164e	201e	256e	356e	443e	562e	175 ± 27d
Progressive motility, %														
H	nd	nd	nd	nd	1d	12d	12d	28d	22d	30d	44d	62d	56d	26 ± 4d
A	nd	nd	nd	9de	13de	30e	25de	37de	54e	55e	60e	67d	74e	46 ± 4e
H × A	nd	nd	nd	3d	7de	13d	28de	32d	30d	49e	46de	50d	51d	31 ± 4d
A × H	nd	nd	nd	5de	9de	24de	33e	38de	27d	50e	42d	63d	60de	35 ± 4de
RP	nd	nd	0	6de	20e	15d	32e	50e	51e	44de	60e	50d	60de	39 ± 4de
BS	nd	0	7	18e	16de	35e	60f	58e	43e	46de	56de	50d	69e	45 ± 4e
Protein conc., mg/ml														
H	2d	3d	6d	5d	21de	31e	25d	48de	47e	57ef	46d	46d	55d	30 ± 3d
A	2d	6d	2d	6d	14d	33e	27d	50e	37de	33d	44d	61ef	56d	29 ± 3d
H × A	1d	2d	6d	17e	24de	18d	27d	38d	39de	51e	57d	60e	49d	30 ± 3d
A × H	2d	3d	2d	10d	22de	24de	23d	41de	30d	48e	54d	44d	50d	27 ± 3d
RP	9d	2d	5d	13d	33e	36e	66e	71f	41de	69f	73e	73f	56d	42 ± 3e
BS	2d	3d	6d	16e	18d	23de	29d	36d	28d	42de	49d	54de	53d	28 ± 3d

^aValues are least-squares means after correction for repetitive measurements of bulls.^bH = Hereford, A = Angus, H × A = Hereford × Angus crossbreeds, A × H = Angus × Hereford crossbreeds, RP = Red Poll and BS = Brown Swiss.^cnd = nondetectable.^{d,e,f}Means without a common superscript within a column differ (P < .05).

ejaculated biweekly or three times weekly from puberty through 52 wk of age, and only small differences were noted in semen quality between bulls ejaculated weekly or six times weekly from 53 to 104 wk of age (Almquist and Amann, 1976).

The protein concentration of seminal plasma was measured to monitor, subjectively, development of the gross secretory activity of the epididymis and accessory sex glands in the 31 bulls. In the mature bull, 72% of the seminal plasma protein is derived from the accessory sex glands, primarily from the seminal vesicles (Sexton et al., 1971). The weights of the epididymides and seminal vesicles increase dramatically during the first 30 wk after puberty (Killian and Amann, 1972), and seminal plasma protein concentration increases two- to threefold, but seminal plasma fructose concentration remains constant, during the same period in young Holstein bulls (Killian and Amann, 1974). Therefore, we measured the concentration of seminal plasma protein to provide general information on physiological development of the protein secreting tissues of the pubertal bovine reproductive system.

Protein concentration in seminal plasma increased ($P < .01$) in all bulls and all breed groups between 7 and 13 mo of age (table 1), regardless of the presence or absence of spermatozoa in ejaculates. However, protein concentration did not exceed 20 mg/ml in semen of any bull until a pubertal ejaculate (50×10^6 spermatozoa, 10% motile) was attained. Breed differences in seminal protein concentration were evident ($P < .05$) between 8.5 and 12.5 mo of age (table 1), possibly reflecting breed differences in stage of pubertal development and age at puberty. Protein concentrations in ejaculates from RP bulls were consistently higher ($P < .05$) than in ejaculates from bulls of other breeds during the first 10 to 20 wk after reaching puberty. It is interesting to note that these RP bulls also exhibited the highest concentrations of luteinizing hormone and testosterone in peripheral blood between 7 and 13 mo of age (Lunstra et al., 1978), possibly indicating a relationship between the circulating hormone concentrations and the development of protein secretory activity in the male reproductive organs.

When data were adjusted, using age at puberty as a covariate, and plotted on a scale from 14 wk before through 16 wk after puberty (figure 1), breed differences in sperm concen-

tration and progressive motility became non-significant. The data are presented for all bulls combined. Concentration of spermatozoa increased ($P < .01$) rapidly and linearly from 2 wk before puberty through 16 wk after puberty (figure 1) in a pattern similar to that reported for H and A beef bulls collected biweekly by artificial vagina (Almquist and Cunningham, 1967). Progressive motility also increased ($P < .01$, figure 1) through 16 wk after puberty. The most rapid increase in progressive motility was observed between 2 wk before and 6 wk after puberty (i.e., from $9 \pm 2\%$ to $52 \pm 3\%$ motility), and a slower improvement was noted between 6 and 16 wk after puberty. A rapid increase to a plateau of 50 to 60% progressive motility in samples collected by artificial vagina has been reported to occur within 12 wk after puberty in Charolais bulls (Almquist et al., 1976) and within 17 to 20wk after puberty in H and A bulls (Almquist and Cunningham, 1967). The rapid increase in motility within 6 wk after puberty for all beef breeds in our study is similar to that reported to occur within 10 wk after puberty in Holstein bulls (Killian and Amann, 1972; Almquist and Amann, 1976). The patterns for progressive motility of spermatozoa and protein concentration of seminal plasma were similar (figure 1) and highly correlated ($r = .98$, $P < .01$) through 16 wk after puberty. It remains to be determined whether this relationship is coincidence or a direct effect of protein (or proteins) on the progressive motility of spermatozoa in neopubertal bulls.

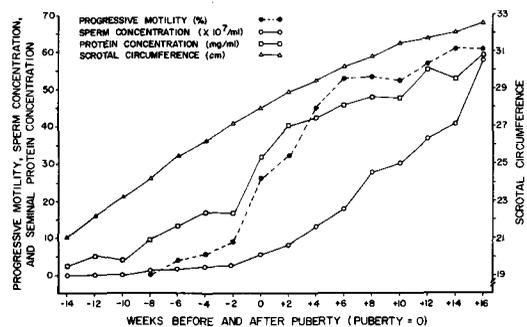


Figure 1. Patterns of change in scrotal circumference (cm), sperm concentration ($\times 10^7$ /ml), progressive motility (%) and seminal protein concentration in semen (mg/ml) of 31 beef bulls during the period from 14 wk before through 16 wk after onset of puberty (50×10^6 sperm, 10% motile). Increases ($P < .01$) occurred in all four traits.

Scrotal circumference increased linearly in all bulls from 14 wk before through 16 wk after puberty (figure 1). Breeds did not differ in scrotal circumference at puberty ($P > .10$; Lunstra et al., 1978), although significant breed differences in age at puberty and body weight at puberty were observed. Scrotal circumference at puberty averaged $27.9 \pm .2$ cm and ranged from 25.9 to 30.1 cm among the 31 bulls evaluated. Average scrotal circumference was negatively correlated ($r = -.65$, $P < .01$) with age at puberty (i.e., bulls with larger scrotal circumference reached puberty earlier, regardless of breed). Simple correlations between scrotal circumference and concentration of spermatozoa ($r = .64$), seminal protein concentration ($r = .80$) and progressive motility ($r = .74$) indicate that scrotal circumference may be useful for describing pubertal status in groups of young bulls, regardless of breed. However, residual correlations (after adjustment for breed and age effects) between scrotal circumference and concentration of spermatozoa ($r = .19$), seminal protein concentration ($r = .07$) and progressive motility ($r = .17$), indicate that scrotal circumference alone would be of little value for predicting the quality of postpuberal ejaculates in individual bulls. With increased frequency of semen collection, scrotal circumference in yearling bulls is correlated with sperm output ($r = .44$ to $.75$; Almquist et al., 1976) and testis weight in mature bulls is highly correlated with sperm output in depletion trials ($r = .79$ to $.94$; Hupp et al., 1962) and with daily sperm production derived from testicular homogenates ($r = .77$; Weisgold and Almquist, 1979), indicating that testis size is related to the sperm producing capacity of beef bulls.

The percentage of abnormal spermatozoa was high among the ejaculates containing first sperm in all breed groups, but declined rapidly ($P < .01$) through 13 mo of age (table 2). Sperm head abnormalities were predominantly pear-shaped and microcephalic heads, and sperm tail abnormalities were predominantly proximal cytoplasmic droplets and kinked and coiled tails. For brevity, only data on normal heads, normal tails and proximal cytoplasmic droplets are presented (table 2). Significant breed differences were present for each of these factors; probably due to breed differences in age at puberty. Progressive linear increases ($P < .01$) were noted in the percentage of spermatozoa with normal heads and normal tails, and a rapid decrease ($P < .01$) occurred in the percentage of

spermatozoa with proximal cytoplasmic droplets, as bull age increased. Breed differences in the percentage of spermatozoa with normal heads had become nonsignificant by 12 mo of age, and breed differences in the percentage of spermatozoa with proximal cytoplasmic droplets disappeared by 12.5 mo of age.

Upon adjustment of sperm morphology data for age at puberty, breed differences became nonsignificant and the patterns of change in normal heads, normal tails and proximal cytoplasmic droplets around puberty became more distinct (figure 2). A rapid linear increase ($P < .01$) in the percentage of spermatozoa with normal heads occurred between -8 and $+6$ wk of puberty, supporting the findings of others (Abdel-Raouf, 1965; Martig and Almquist, 1969; Almquist and Amann, 1976; Hanson et al., 1980). A slower linear increase ($P < .01$) in the percentage of spermatozoa with normal tail morphology occurred concurrently with the improvement in normal sperm heads through 16 wk after puberty. Percentage of sperm alive (unstained) averaged 66 to 74% and did not change ($P > .10$) during the period from 8 wk before through 16 wk after puberty.

The percentage of spermatozoa with proximal cytoplasmic droplets remained at 60 to 70% before puberty, decreased rapidly ($P < .01$) during the first 6 wk after puberty and decreased ($P < .01$) more slowly from 6 through 16 wk after puberty (figure 2). Translocation of the cytoplasmic droplet from proximal to distal positions on the midpiece is indicated of the efficiency of the maturation process in the epididymis (Saacke, 1970), and the translocation and loss of the droplet is dependent on increasing tail motility, initially in the epididymis and subsequently at ejaculation (Dickey, 1965). Comparison of the patterns for progressive motility (figure 1) and proximal cytoplasmic droplets (figure 2) substantiates this negative relationship ($r = -.95$, $P < .01$); i.e., progressive motility increased rapidly from 0 through 6 wk after puberty, and percentage of spermatozoa with proximal cytoplasmic droplets decreased dramatically during this same period. A rapid decrease in the percentage of spermatozoa with proximal cytoplasmic droplets also has been reported to occur in Swedish Red-and-White bulls during the first 2 mo after puberty (Abdel-Raouf, 1965).

Since the acrosomal morphology of spermatozoa in young bulls around the time of puberty has not been previously reported, three general

TABLE 2. LEAST-SQUARES MEANS FOR THE PERCENTAGES OF SPERMATOOZA WITH NORMAL HEADS, NORMAL TAILS AND PROXIMAL CYTOPLASMIC DROPLETS IN SEMEN OF BULLS BETWEEN 7 AND 13 MO OF AGE^a

Breed group ^b	Mo of age										All values (mean ± SE)			
	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5		12.0	12.5	13.0
Normal heads, % ^c														
H	nd	nd	nd	nd	15f	32f	57fg	71fg	71f	76f	87f	90f	90f	68 ± 3fg
A	nd	nd	nd	18fg	25fg	40f	53f	69fg	72f	90g	86f	92f	91f	66 ± 3fg
H X A	nd	nd	nd	16f	34g	54g	55f	63f	76f	82fg	87f	89f	87f	66 ± 2f
A X H	nd	nd	nd	25fg	43h	57g	66gh	76g	72f	88g	87f	92f	91f	74 ± 3g
RP	nd	nd	14	44h	48h	52g	66gh	67fg	74f	82fg	90f	90f	90f	70 ± 3fg
BS	nd	17	23	27g	28g	57g	69h	72fg	70f	76f	80f	89f	92f	66 ± 3fg
Normal tails, % ^d														
H	nd	nd	nd	nd	41g	47g	50fg	55gh	49fg	49f	62f	58f	67fg	55 ± 2fg
A	nd	nd	nd	38fg	46g	57h	58g	63h	56g	61h	63f	68g	69fg	59 ± 2g
H X A	nd	nd	nd	40fg	59h	60h	54fg	60h	54g	57gh	59f	62fg	64f	58 ± 2g
A X H	nd	nd	nd	42g	55h	67i	53fg	61h	54g	61h	58f	63fg	69fg	59 ± 2g
RP	nd	nd	35	35f	34f	36f	48f	42f	43f	49f	62f	59f	62f	50 ± 2f
BS	nd	49	43	44g	41g	54h	56g	49g	50g	51fg	60f	64fg	72g	54 ± 2fg
Proximal CD, % ^e														
H	nd	nd	nd	nd	43g	39h	41h	32h	21g	17g	20g	3f	4f	26 ± 3g
A	nd	nd	nd	44f	25f	32gh	33gh	20g	20g	14fg	11fg	2f	.1f	20 ± 3fg
H X A	nd	nd	nd	37f	42g	42h	25fg	28gh	24g	22g	2f	.7f	.3f	22 ± 3fg
A X H	nd	nd	nd	46f	42g	37gh	23f	11fg	14fg	4f	1f	2f	2f	18 ± 3fg
RP	nd	nd	61	45f	22f	15f	27fg	15fg	8f	4f	7f	.2f	1f	13 ± 3f
BS	nd	76	81	40f	31f	27g	21f	9f	8f	4f	7f	1f	.6f	17 ± 3fg

^aValues are least-squares means after correction for repetitive measurements of bulls. nd = nondetectable.

^bH = Hereford, A = Angus, H X A = Hereford X Angus crossbreeds, A X H = Angus X Hereford crossbreeds, RP = Red Poll and BS = Brown Swiss.

^cPercentage of spermatozoa with normal head morphology, excluding acrosome status.

^dPercentage of spermatozoa with normal tail morphology, excluding proximal cytoplasmic droplets.

^ePercentage of spermatozoa with a cytoplasmic droplet (CD) located proximally on the midpiece.

f,g,h,i. Means without a common superscript within a column differ (P < .05).

classifications of acrosomal morphology (NA, AA and GDA; see Materials and Methods) are presented. The three classifications, progressing from NA through GDA, represent increasing levels of alteration and disruption of the bovine acrosome. Acrosomal morphologies described as abnormal (Saacke et al., 1968; Cran and Dott, 1976; Coulter et al., 1978; Foster et al., 1980), maturational (Jones, 1975) and degenerative or senescent (Saacke and Marshall, 1968; Saacke, 1970; Saacke and White, 1972; Jones, 1975) were included in the AA and GDA classifications, because it was not possible to determine the cause of all acrosomal alterations using DIC microscopy.

Percentage of spermatozoa with an intact acrosome of normal morphology (NA) was essentially zero at appearance of first sperm in each bull and all breed groups (table 3). Significant breed differences existed in percentage NA, percentage AA and percentage GDA through 13 mo of age. Percentage NA was generally higher throughout the study in those breeds that reached puberty at the youngest age (BS, RP and A). Breed differences in acrosomal morphology were due to variation in pubertal age rather than variation in rate of improvement in acrosomal morphology, as adjustment of the data for age at puberty negated breed differences ($P > .10$). A progressive improvement in percentage NA ($P < .01$) was observed within

individual bulls and in each breed group between puberty and 13 mo of age. The AA was the predominant type of acrosome present on spermatozoa collected at the time that bulls attained puberty (9 to 11 mo of age, table 3).

Considerable variation in percentage NA was observed among the 31 bulls at 13 mo of age (range = 43 to 77%, mean = 65%), and individual bull differences were significant ($P < .01$). A large range in the percentage of spermatozoa with intact acrosomes has been reported for H and A bulls at 12 to 14 mo of age (range = 22 to 77%, mean = 50%; Wells et al., 1976). Percentage of spermatozoa with intact acrosomes also exhibits large variation after freezing and thawing of semen collected from H and A bulls at 12 to 15 mo of age (range = 24 to 93%, mean = 62%; Berndtson et al., 1981). Collectively, these data indicate that large variations and high proportions of spermatozoa with other than normal acrosomal morphology are present in semen obtained from beef bulls between puberty and 12 to 15 mo of age.

The patterns of change in bovine acrosomal morphology that occur during pubertal development became more distinct and breed differences became negligible when data were adjusted for age at puberty (figure 3). During the 8 wk period prior to puberty, little change occurred in the proportions of each class of acrosomal morphology (percentage NA averaged $3 \pm 1\%$, the AA class predominated and averaged $85 \pm 6\%$ and percentage GDA averaged $12 \pm 2\%$). From puberty through 16 wk after puberty, percentage NA improved linearly ($P < .01$), while percentage AA and GDA exhibited a linear decline ($P < .01$) during this same period (figure 3). Percentage NA, AA and GDA at 16 wk after puberty was $64 \pm 3\%$, $34 \pm 5\%$ and $2 \pm 1\%$, respectively (mean \pm SE). Because percentage NA averages 80 to 90% in semen from mature bulls of proven fertility (Saacke and White, 1972; Wells et al., 1972) and beef bulls did not achieve a comparable percentage NA by 13 mo of age (table 3) or within 4 mo after reaching puberty (figure 3), further improvement probably occurs in the percentage NA of beef bulls after 13 mo of age. The low percentage of spermatozoa with NA observed in young postpubertal beef bulls implies that inadequate maturation or accelerated degeneration during passage through the epididymis (Saacke and White, 1972; Wells et al., 1972; Jones, 1975) or impaired acrosomal structuring during spermatid transformation in the testis (Saacke, 1970;

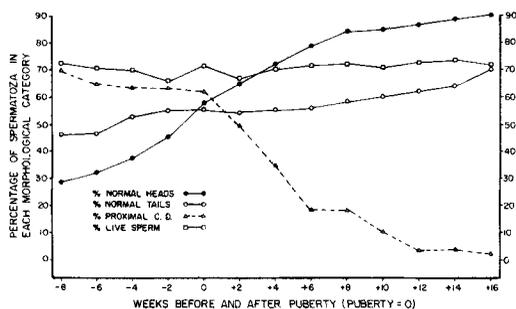


Figure 2. Patterns of change in the percentages of spermatozoa with normal head morphology (excluding acrosomes), normal tail morphology (excluding proximal cytoplasmic droplets) and proximal cytoplasmic droplets and in the percentage of spermatozoa alive (unstained) in semen of 31 beef bulls during the period from 8 wk before through 16 wk after onset of puberty (50×10^6 sperm, 10% motile). Increases ($P < .01$) occurred in percentage normal heads and normal tails, and a marked decrease ($P < .01$) occurred in percentage proximal cytoplasmic droplets during the evaluation period.

TABLE 3. LEAST-SQUARES MEANS FOR ACROSOMAL MORPHOLOGY OF SPERMATOZOA
IN SEMEN OF BULLS BETWEEN 7 AND 13 MO OF AGE^a

Breed group ^b	Mo of age											All values (mean ± SE)		
	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0		12.5	13.0
Normal acrosomes (NA), % ^c														
H	nd	nd	nd	nd	0g	0g	1g	8g	17g	27g	47g	47g	65h	21 ± 2g
A	nd	nd	nd	1g	2g	8h	8h	12gh	19gh	49i	52g	56h	65gh	28 ± 2h
H × A	nd	nd	nd	1g	1g	6h	5gh	11gh	18gh	41i	51g	54h	62g	26 ± 2h
A × H	nd	nd	nd	1g	2g	2gh	9h	14h	23h	34h	47g	55h	62g	25 ± 2gh
RP	nd	nd	0	0g	1g	6h	6gh	12gh	19gh	38hi	49g	54h	68h	25 ± 2gh
BS	nd	0	0	1g	1g	6h	8h	13h	31i	40i	47g	62i	66gh	26 ± 2h
Aberrant acrosomes (AA), % ^{d,e}														
H	nd	nd	nd	nd	81gh	81gh	84g	81g	76h	68i	53h	52i	35gh	71 ± 3g
A	nd	nd	nd	78g	79g	79g	88gh	82g	74h	49g	45g	41gh	32gh	65 ± 3g
H × A	nd	nd	nd	81gh	79g	87h	87gh	85g	74h	55gh	48gh	45h	37h	70 ± 3g
A × H	nd	nd	nd	78g	89h	92h	88gh	80g	67g	62hi	50gh	44h	37h	68 ± 3g
RP	nd	nd	86	87h	92h	92h	90h	83g	78h	59h	47gh	42gh	29g	70 ± 3g
BS	nd	75	85	85gh	87h	85gh	83g	80g	64g	55gh	48gh	37g	32gh	67 ± 3g
Grossly disrupted acrosomes (GDA), % ^{e,f}														
H	nd	nd	nd	nd	19i	19i	15i	10h	8hi	6h	1g	1g	1g	9 ± 1i
A	nd	nd	nd	20hi	20i	12i	4g	6g	6g	2g	3g	2g	1g	6 ± 1h
H × A	nd	nd	nd	18h	21i	7h	7h	4g	8hi	4gh	2g	1g	1g	5 ± 1gh
A × H	nd	nd	nd	21i	8g	6gh	3g	5g	10i	3g	3g	1g	1g	8 ± 1hi
RP	nd	nd	14	13g	7g	3g	4g	5g	3g	2g	3g	2g	2g	3 ± 1g
BS	nd	25	15	14g	12h	9h	8h	6g	6h	4gh	3g	1g	1g	6 ± 1h

^aValues are least-squares means after correction for repetitive measurements of bulls. nd = nondetectable.

^bH = Hereford, A = Angus, H × A = Hereford × Angus crossbreeds, A × H = Angus × Hereford crossbreeds, RP = Red Poll and BS = Brown Swiss.

^cNA = intact acrosome with uniform apical ridge and smooth acrosomal surface.

^dAA = acrosome with irregularly-shaped apical ridge or lacking a smooth acrosomal surface.

^eIncludes acrosomal morphologies described as abnormal, maturational, senescent and degenerative.

^fGDA = disrupted acrosome with swollen, ruffled, vesiculated or missing acrosomal cap.

g,h,i,j Means without a common superscript within a column differ (P < .05).

Jones, 1975) prevailed during the first 4 mo after reaching puberty. Additional investigations are needed to define the patterns and mechanisms affecting acrosomal morphology through sexual maturity in beef bulls.

It is well documented that percentage NA is significantly correlated with the fertility of spermatozoa from mature bulls ($r = .60$ to $.81$, Saacke and White, 1972; $r = .70$, Marshall and Frey, 1976) and with the competitive fertility index of mature bulls used in heterospermic trials ($r = .90$; Saacke et al., 1980). Percentage NA has been reported to be independent of other criteria used to evaluate semen quality (Wells et al., 1972; Berndtson et al., 1981), and the presence of high proportions of spermatozoa with other than normal acrosomal morphology is associated with subfertility and sterility (Saacke et al., 1968). Because the semen of the young beef bulls in our study exhibited relatively low percentage NA, low progressive motility and high percentage abnormal spermatozoa during the first 4 mo after reaching puberty, it could be concluded that unacceptable or significant reductions in fertility would be expected if these bulls or their semen were used for breeding. However, it has been reported that acceptable nonreturn rates (69%) were obtained when semen collected from A and H bulls between puberty and 12 mo of age was

used for artificial insemination (Martig and Almquist, 1969). Their nonreturn rate may have been increased by selecting ejaculates prior to freezing and by utilizing only those ampules that contained at least 10×10^6 motile spermatozoa after freezing and thawing (Cunningham et al., 1967; Martig and Almquist, 1969). Further studies are needed to clarify the relationships between fertility and the characteristics of semen quality in young beef bulls between puberty and sexual maturity.

Literature Cited

- Abdel-Raouf, M. 1965. Sexual behavior and semen picture of bulls of the Swedish Red and White breed between the ages of 9 and 15 months. *Nord. Vet. Med.* 17:318.
- Almquist, J. O. and R. P. Amann. 1976. Reproductive capacity of dairy bulls. XI. Puberal characteristics and postpuberal changes in production of semen and sexual activity of Holstein bulls ejaculated frequently. *J. Dairy Sci.* 59:986.
- Almquist, J. O., R. J. Branas and K. A. Barber. 1976. Postpuberal changes in semen production of Charolais bulls ejaculated at high frequency and the relation between testicular measurements and sperm output. *J. Anim. Sci.* 42:670.
- 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.
- Amann, R. P. 1962. Reproductive capacity of dairy bulls. III. The effect of ejaculation frequency, unilateral vasectomy, and age on spermatogenesis. *Amer. J. Anat.* 110:49.
- Ball, L., L. D. Nelson, J. W. Furman and G. E. Seidel, Jr. 1974. Semen collection and evaluation of bulls for breeding soundness. *Proc. of Annu. Meeting of the Amer. Vet. Soc. for the Study of Breeding Soundness, Columbia, MO.* pp 1-27.
- Barber, K. A. and J. O. Almquist. 1975. Growth and feed efficiency and their relationship to pubertal traits of Charolais bulls. *J. Anim. Sci.* 40:288.
- Berndtson, W. E., T. T. Olar and B. W. Pickett. 1981. Correlation between post-thaw motility and acrosomal integrity of bovine sperm. *J. Dairy Sci.* 64:346.
- Clarke, R. H., R. W. Hewetson and B. J. Thompson. 1973. Comparison of the fertility of bovine semen collected by artificial vagina and electroejaculation from bulls with low libido. *Australian Vet. J.* 49:240.
- Coulter, G. H., R. J. Oko and J. W. Costerton. 1978. Incidence and ultrastructure of "crater" defect of bovine spermatozoa. *Theriogenology* 9:165.
- Cran, D. G. and H. M. Dott. 1976. The ultrastructure of knobbed bull spermatozoa. *J. Reprod. Fertil.* 47:407.
- Cunningham, D. C., J. O. Almquist, R. E. Pearson and R. C. Martig. 1967. Reproductive capacity of beef bulls. II. Postpuberal relations among

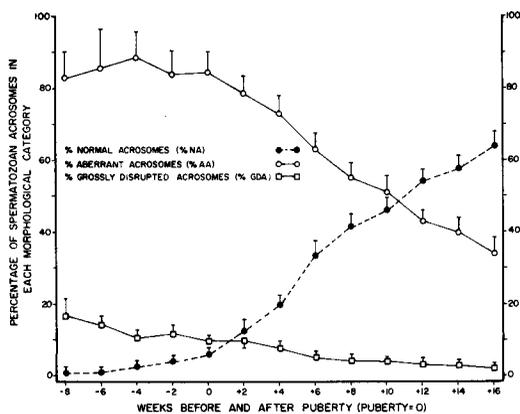


Figure 3. Patterns of change in acrosomal morphology of spermatozoa (mean \pm SE) in semen of 31 beef bulls during the period from 8 wk before through 16 wk after puberty (50×10^6 sperm, 10% motile). The three categories of acrosomal morphology were percentage normal acrosomes (% NA), percentage aberrant acrosomes (% AA) and percentage grossly disrupted acrosomes (% GDA). An increase ($P < .01$) occurred in % NA and decreases ($P < .01$) occurred in % AA and % GDA through 16 wk after puberty.

- ejaculation frequency, sperm freezability and breeding potential. *J. Anim. Sci.* 26:182.
- Dickey, J. F. 1965. An electron microscope study of bovine spermatozoa undergoing maturation in the male reproductive system. Ph.D. Dissertation. Pennsylvania State Univ., University Park.
- Foster, N. M., M. A. Alders, A. J. Luedke and T. E. Walton. 1980. Abnormalities and virus-like particles in spermatozoa from bulls latently infected with bluetongue virus. *Amer. J. Vet. Res.* 41:1045.
- Hackett, A. J. and J. W. Macpherson. 1965. Some staining procedures for spermatozoa: A review. *Canadian Vet. J.* 6:55.
- Hafs, H. D. and R. C. Knisely. 1960. Some norms of sperm output for dairy and beef bulls. *Michigan Quart. Bull.* 43:270.
- Hanson, R. D., J. R. Mitchell and W. N. Fleming. 1980. Abnormal sperm cell patterns in young Holstein sires. *J. Anim. Sci.* 51(Suppl. 1):282.
- Harvey, W. R. 1975. Least squares analysis of data with unequal sub-class numbers. USDA, ARS No. H-4.
- Herrick, J. B. and H. L. Self. 1962. Morphology of spermatozoa. In: J. B. Herrick and H. L. Self (Ed.) *Evaluation of Fertility in the Bull and Boar*. pp 59-66. Iowa State Univ. Press, Ames.
- Hupp, E. W., J. W. Austin, N. R. Parish and R. L. Murphree. 1962. Sperm production of Hereford bulls at different intensities of collection. *J. Anim. Sci.* 21:272.
- Johnson, L., W. E. Berndtson and B. W. Pickett. 1976. An improved method for evaluating acrosomes of bovine spermatozoa. *J. Anim. Sci.* 42:951.
- Jones, R. C. 1975. Fertility and infertility in mammals in relation to sperm structure. In: J. G. Duckett and P. A. Racey (Ed.) *The Biology of the Male Gamete*. pp 343-365. Academic Press, New York.
- Killian, G. J. and R. P. Amann. 1972. Reproductive 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.
- Killian, G. J. and R. P. Amann. 1974. Reproductive capacity of dairy bulls. X. Changes in chemical components and immunoelectrophoretic characteristics of seminal plasma before and after puberty. *J. Dairy Sci.* 57:703.
- Lowry, D. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265.
- Lunstra, D. D., J. J. Ford and S. E. Echternkamp. 1978. Puberty in beef bulls: Hormone concentrations, growth, testicular development, sperm production and sexual aggressiveness in bulls of different breeds. *J. Anim. Sci.* 46:1054.
- Marshall, C. and L. Frey. 1976. Semen evaluation at select sires. *Proc. Sixth Tech. Conf. on Artif. Insem. and Reprod. (NAAB)*. p 91.
- 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.
- Saacke, R. G. 1970. Morphology of the sperm and its relationship to fertility. *Proc. Third Tech. Conf. on Artif. Insem. and Reprod. (NAAB)*. p 17.
- Saacke, R. G., R. P. Amann and C. E. Marshall. 1968. Acrosomal cap abnormalities of sperm from subfertile bulls. *J. Anim. Sci.* 27:1391.
- Saacke, R. G. and C. E. Marshall. 1968. Observations on the acrosomal cap of fixed and unfixed bovine spermatozoa. *J. Reprod. Fertil.* 16:511.
- Saacke, R. G., W. E. Vinson, M. L. O'Connor, J. E. Chandler, J. Mullins, R. P. Amann, C. E. Marshall, R. A. Wallace, W. N. Vincel and H. C. Kellgren. 1980. The relationship of semen quality and fertility: A heterospermic study. *Proc. Eighth Tech. Conf. on Artif. Insem. and Reprod. (NAAB)*. p 71.
- Saacke, R. G. and J. M. White. 1972. Semen quality tests and their relationship to fertility. *Proc. Fourth Tech. Conf. on Artif. Insem. and Reprod. (NAAB)*. p 22.
- Sexton, T. J., R. P. Amann and R. J. Flipse. 1971. Free amino acids and protein in rete testis fluid, vas deferens plasma, accessory sex gland fluid, and seminal plasma of the conscious bull. *J. Dairy Sci.* 54:412.
- Steel, R.G.D. and J. H. Torrie. 1960. *Principles and Procedures of Statistics with Special Reference to the Biological Sciences*. McGraw-Hill Book Co., New York. pp 107-131.
- Swanson, L. V., R. P. Wettemann, N. C. Rawlings, H. D. Hafs and W. T. Magee. 1971. Pubertal relationships of some endocrine and reproductive criteria in Hereford bulls. *J. Anim. Sci.* 33:823.
- Weisgold, A. D. and J. O. Almquist. 1979. Reproductive capacity of beef bulls. VI. Daily spermatozoal production, spermatozoal reserves and dimensions and weight of reproductive organs. *J. Anim. Sci.* 48:351.
- Wells, M. E., O. A. Awa and S. S. Fancy. 1972. Effect of season on acrosome status in the bull. *J. Dairy Sci.* 55:1174.
- Wells, M. E., S. Smith, D. J. Breuer and B. A. Stotts. 1976. Ejaculate characteristics of Angus and Hereford bulls finishing gain test. *Oklahoma Agr. Exp. Sta. Misc. Pub.* 96:134.
- Wolf, F. R., J. O. Almquist and E. B. Hale. 1965. Prepubertal behavior and pubertal characteristics of beef bulls on high nutrient allowance. *J. Anim. Sci.* 24:761.