

DNA-based Paternity Analysis in Multi-bull Breeding Programs on Beef Cattle Operations in Western Canada

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Abstract

A 3-yr study was conducted to evaluate deoxyribonucleic acid (DNA)-based paternity analysis on commercial beef ranches managing multi-bull breeding systems. Five commercial ranches in central Saskatchewan Canada participated in the study with a total of 22 breeding groups. All bulls ($n = 75$) and calves ($n = 2243$) were sampled to determine parentage. Number of calves sired per bull ranged from 1 to 87 (23 ± 15.9). The value of a calculated index of bull prolificacy (BPI) ranged from 0.05 to 3.83. Older bulls had a BPI averaging 1.10, 2-yr old bulls 1.00, and yearling bulls 0.76 ($p > 0.05$). Strong positive ($r = 0.93$, $n = 74$, $p = 0.01$) correlation was observed between total calves born per bull and calves born in the first 21 days BPI, between total calves born per bull and calves born in week-3 BPI ($r = 0.69$, $n = 74$, $p = 0.01$). Bull age was shown to play a significant role when determining prolificacy, with older bulls siring more calves than younger bulls. Bull number per breeding group influenced the number of calves sired. As number of bulls per breeding group increased so did the variation in the number of calves sired by each bull. Conducting DNA parentage testing only on calves born in the first 21 d or in week 3 of the calving season may provide an opportunity to decrease costs and turn-around time for laboratory results and decisions made, prior to the next breeding season.

Keywords: beef cattle, genetic evaluation, on-farm expected progeny difference, paternity, single nucleotide polymorphism

1. Introduction

In western Canada, natural breeding using multi-bull system is a common method of breeding cows (Larson, 2015). In most commercial cow-calf operations genetic variation is introduced into the herd primarily through the purchase of new bulls with the assumption that each bull in a pasture breeding system will sire the same number of calves (Larson, 2013). There has been limited research conducted on the specific traits that cause some bulls to be more prolific breeders than others (Blockey, 1979; Stalhammar, Janson, & Philipsson, 1989; Holroyd et al., 2002; Van Eenennaam et al., 2007). It is known that semen quality and quantity are affected by bull age (Stalhammar et al., 1989). Additionally, scrotal circumference (SC) is highly correlated with bull age; lower fertility rates observed in young bulls are detected in bulls with lower scrotal circumference (Coulter & Kozub, 1989). Spermatological factors have also been shown to play a role in male fertility, and these factors may change with age. There has been limited research evaluating the use of SNP-based DNA parentage testing in commercial cow-calf operations, the numbers of calves sired by each bull, and factors underlying why some bulls sire more calves than others (Van Eenennaam et al., 2007). Van Eenennaam, Weber, and Drake (2014) evaluated two methods of alternate testing when (i) only calves born in the third week or (ii) only calves born in weeks 2, 3, and 4 were sampled and produced sufficiently accurate results. They suggested that producers could possibly sample only a subset of the total calf crop and still be able to accurately determine parentage and make culling decisions (Van Eenennaam et al., 2014). This would allow for producers to get samples back from the lab in a shorter turnaround time and at a lower cost. The objectives of this study were to (1) evaluate paternity test

results as a method of assessing bull contribution to an operation that uses multi-bull breeding systems using two alternate testing methods, when (i) only calves born in the first 21 days or (ii) only calves born in week 3 sampled; (2) determine the association between bull age and other factors affecting progeny number per bull; (3) use paternity test results to calculate bull cost per calf sired and calf income generated per bull as a means of assessing bull economic value to an operation.

2. Materials and Methods

2.1 Collaborating Ranches

Five commercial ranches, defined as Ranch A, B, C, D, and E, collaborated in this study and each operation had different beef breeds and management practices, as described below.

Ranch A. Ranch A has a spring calving herd, and in yr 1, managed 400 cows composed of Angus × Hereford cows and 21 bulls predominantly Red Angus and Beef Booster. The breeding season was 56 to 59 d and managed in four separate breeding groups (A1, A2, A3, and A4) of 3 to 6 bulls and 66 to 130 cows in each pasture. The bull:cow ratios ranged from 1:21 to 1:29. Breeding group A1 (A1; A = ranch, 1 = breeding group) had six bulls and 162 cows with a 1 bull:27 cow ratio and the bulls were placed in the breeding pasture on 14 July and removed 26 September 2015. Breeding group A1 produced 136 calves, with 75 calves (55% of total calves) born in the first 21 d of calving. Group A2 had six bulls and 154 cows with a 1 bull:26 cow ratio, the bulls were placed in the pasture on 14 July and removed 16 September 2015. Group A2 produced 132 calves, with 69 calves (52% of total calves) born in the first 21 d of calving. Group A3 contained three bulls and 70 cows, with a 1 bull:23 cow ratio, the bulls were placed in the pasture on 14 July and pulled 20 September 2015. Group A3 produced 58 calves, with 31 calves (53% of total calves) born in the first 21 d of calving. Group A4 contained four bulls and 85 cows, with a 1 bull:21 cow ratio, the bulls were placed in the pasture on 14 July and pulled 20 September 2015. Group A4 produced 40 calves, with 28 calves (70% of total calves) born in the first 21 d of calving. For yr 2, all cows were included in the study along with 19 Beef Booster bulls managed in four different breeding groups. The calving dates ranged from 11 April to 30 June 2016. In yr 3, all cows and 15 Beef Booster bulls were included in the study and were managed in three different breeding groups (A1, A2, and A3). The breeding season was managed 56 to 59 d in three separate breeding pastures with group sizes of 3 to 6 bulls and 31 to 125 cows per pasture, resulting in bull:cow ratios ranging from 1:10 to 1:12.

Ranch B. Ranch B managed a spring calving program with eight Black Angus bulls and 200 Red and Black Angus × Hereford crossbred cows. The breeding season was 57 d in length, with all 200 cows and eight bulls in one breeding pasture (B5) and a 1 bull:24 cow ratio. Ranch B participated in yr 1 only of the study.

Ranch C. Ranch C in yr 1 had a spring calving herd of 110 crossbred first-calf heifers (C6). The breeding season for this group was 123 d with all heifers in one breeding pasture with eight composite bulls plus a neighbor's bull who invaded the pasture for a portion of the breeding season. The bull:cow ratio was then adjusted to 1 bull:12 cows due to inclusion of the invading bull. For yr 2, this study followed seven bulls and 200 heifers with a 1:28 bull:cow ratio. The bull battery had one black Composite, one red Composite, three Black Angus and two Red Angus breeds. The breeding season was 15 July to 15 September 2015. This breeding group produced 153 calves, with 94 calves (61% of total calves) born in the first 21 d of calving. For yr 3, this study followed seven bulls and 161 heifers with a 1 bull:23 cow ratio.

Ranch D. This study focused on one breeding group (D7) at Ranch D with 101 Black Angus females and four Black Angus bulls for yr 1, all managed in one breeding pasture with a 1 bull:23 cow ratio. The breeding season for this group was 67 d in length with one bull removed after 34 d due to injury and another removed after the breeding season for low BPI. For yr 2, this study followed four Black Angus bulls and 89 cows in one breeding group with a 1 bull:22 cow ratio. Bulls were turned out in the breeding group 2 July and removed 3 September 2015. Calving season started on 3 April and ended 15 June 2016. This group produced 77 calves, with 44 calves (57% of total calves) born in the first 21 d of calving. For yr 3, this study followed four bulls and 56 cows with a 1 bull:14 cow ratio.

Ranch E. Ranch E collaborated in yr 2 and yr 3 of the study. In yr 2, the study followed seven Black Angus bulls and 241 cows in two breeding groups (E8 and E9), with a 1:34 bull:cow ratio. The bulls were placed in breeding fields early July and removed late September. Breeding group E8 had a 1:37 bull:cow ratio and produced 80 calves, with 43 calves (54% of total calves) born in the first 21 d of calving. Breeding group E9 had a 1:31 bull:cow ratio and produced 69 calves, with 40 calves (58% of total calves) born in the first 21 d of calving. In yr 3, the study followed eight bulls and 185 cows with a 1:23 bull:cow ratio. The bull:cow ratios were 1:26 and 1:9 for E8 and E9, respectively.

2.2 Data Collection

2.2.1 Ranch Data

The ranch recorded the breeding season length, number of bulls and cows per pasture, and number of calves born per pasture.

2.2.2 Breeding Soundness Examination (BSE)

Prior to the breeding season start, all bulls underwent BSE exams carried out by licensed veterinarians, as outlined by the Western Canadian Association of Bovine Practitioners (Barth, 2013). Semen was collected from all bulls via either an electro ejaculation technique or massage of the vesicular gland and ampullae. Data collected included percent normal sperm, sperm abnormalities, SC, and bull age. Bulls were examined for any injuries or abnormalities (feet, leg or eye) that may limit their prolificacy or ability to breed, including non-erection during electro ejaculation. Bulls that passed the BSE were allowed into the breeding pasture. An exception was at Ranch C where a neighbor bull and two yearling bulls who did not have a BSE conducted mistakenly had access to the breeding group in yr 1.

2.2.3 DNA Sample Collection from Calves and Bulls

In total, 74 bulls and 2243 calves were sampled for DNA analysis. All bulls were sampled by obtaining ~20 tail hairs, while calf DNA was collected using a tissue sampling applicator. All DNA was extracted and processed commercially (Quantum Genetix Ltd., Saskatoon, Saskatchewan, Canada). All DNA samples were analyzed using SNP technology for sire verification at Quantum Genetix Ltd. (Saskatoon, Saskatchewan, Canada). qRT-PCR genotyping reactions were run as a multiplex to reveal the status of four alleles per reaction (two of one SNP and two of another SNP). Since information from 100 SNPs is used in sire verification, a minimum of 50 multiplex reactions were run per DNA sample. When a correct bull could not be identified using the 100 SNP panel, additional SNPs were tested, and if a bull still could not be determined, the calf was classified as 'no bull match'.

2.2.4 Alternate Sampling

The current study also evaluated the use of parentage testing in calves from cooperating ranches utilizing two different methods: (1) calves born in the first 21 d and (2) calves born in the third week (week 3) of calving to determine if testing only a portion of the calf crop would give similar results to testing the whole calf crop. Calves born in the first 21 d were chosen because the value of having a large portion of a cow herd calve within the first breeding cycle has been well documented (Spratt, 2000; Lardner, Damiran, Hendrick, Larson, & Funston, 2014; Damiran, Larson, Pearce, Erickson, & Lardner, 2018a). It is recommended across western Canada that a management goal is to have 65% of the total cow herd calve within the first 21 d (first cycle) of the calving period. Moreover, Van Eenennaam et al. (2014) suggested that by sampling only the calves born in the third week of the calving period or those born in weeks 2, 3, and 4, producers could, as well, get an accurate idea of which bulls were high and low prolificacy. Thus, if producers can take the DNA sample at birth and keep production records on calving dates, these methods may be an alternative to reduce the cost of testing an entire calf crop.

2.2.5 Economics

Economic value for each bull were calculated for Ranch D. Economic data collected included purchase price (Canadian dollar, CAD) of bulls and where available, the sale prices of calves at weaning. Bull maintenance costs were calculated using a similar procedure as described by Larson (2013, 2015). Weaned calf revenue was calculated as follows:

$$CAD/bull = Calf\ cumulative\ weaning\ BW, kg/bull \times WCP, CAD/kg \quad (1)$$

Where *WCP* denotes weaned 249.4 kg (550 lb) calf prices over the last nine years (2008–2017) in Saskatchewan, Canada, which have averaged CAD3.68/kg (Canfax, 2018). All dollar values are in Canadian dollars (CAD).

2.3 Calculations and Statistical Analyses

A bull prolificacy index (BPI) was calculated using the following equation:

$$BPI = [n/(N/m)] \quad (2)$$

Where *n* equals number of calves sampled that were sired by a specific bull, *N* equals number of calves born from breeding group, *m* equals total number of bulls in the breeding group.

In each breeding pasture calves that were born dead or died shortly after birth (before processing when samples were collected for the rest of herd), also, had DNA samples sent to the Quantum Genetix lab. Since virtually all

calves born were sampled, the number of calves sampled in each herd is closely equivalent to the number of cows exposed \times pregnancy rate. The BPI shows which bulls were siring more than or less than the expected number of progenies based on the overall calving rate of the breeding group of cows and the bull:cow ratio. To interpret the results of the BPI value, a BPI approximately equal to 1 means bulls were siring the same number of calves if they were expected to sire an equivalent number of calves in the pasture. A BPI <1 means bulls were siring less calves than would be expected and a BPI >1 means bulls were siring more calves than expected. In each case, the BPI assumes that each bull has an equal opportunity to breed an equivalent number of cows within a multi-bull pasture. Bulls were divided into three age categories for comparison: yearling bulls, 2-yr old bulls, and mature (>2 yr) bulls. Descriptive statistics analysis was performed using SAS (2003). The correlations between (1) total BPI and 21 d or (2) total BPI and week 3 BPI values, and between SC values and BPI calculations were also analyzed using the PROC CORR of SAS (2003). The correlation coefficients were classified as strong ($r > 0.6$), moderate ($0.6 > r > 0.4$), or weak ($r < 0.4$) (Damiran, Penner, Larson, & Lardner, 2018b). Differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1 Bull Prolificacy and Bull: Cow Ratio

Descriptive statistics on number of bulls, bull:cow ratio, and corresponding range of bull prolificacy index for each breeding group over 3-yr are presented in Table 1. A large variation was observed in the number of calves sired by each bull in a breeding group in the current study. This was consistent with others (Holroyd et al., 2002; Van Eenennaam et al., 2007; Van Eenennaam et al., 2014). In the study by Holroyd et al. (2002), 58% of the 235 bulls used, sired less than 10% of calves in their breeding groups. Further, both Holroyd et al. (2002) and Van Eenennaam et al. (2007) observed bulls in the breeding groups that did not sire any calves. In the current study, all bulls sired at least one calf, and 58% of the bulls used, sired 27% of calves in their breeding groups; most likely due to the lower number of bulls compared to the others ((Holroyd et al., 2002; Van Eenennaam et al., 2007; Van Eenennaam et al., 2014).

Godfrey and Lunstra (1989) tested bulls for serving capacity (SCT) individually. When bulls with high SCT were grouped together and re-tested, they had even higher SCT scores than when they were tested individually. This trend was not observed, however, in bulls with low SCT scores when tested alone, were tested in groups, indicating that bulls with high SCT may attempt to breed more cows in the presence of other bulls (Godfrey & Lunstra, 1989). The SCT is a way of measuring bull libido (Blockey, 1981). Since libido relates to a bull's ability to actually service cows (Coulter & Kozub, 1989), it can be assumed that the bulls siring the maximum number of calves in the individual breeding groups were high-libido bulls. Factors such as injury or poor semen quality, however, could prevent high-libido bulls from siring a high number of calves.

Breeding groups with lower number of bulls in the pasture had less variation (SD) in range of numbers of calves sired compared to those with higher number of bulls (Table 1). Social interaction and aggression between bulls may have played a role in the greater variation in BPI values observed in the current study. Social dominance order and the stability of social dominance are known to affect sexual activity (Blockey, 1979). A study by Price and Wallach (1991) reported that an increase in the number of bulls during libido tests caused an increase in aggression between bulls and reduced overall sexual activity, which suggests an increased opportunity for a dominance hierarchy established when the number of bulls in a breeding group increases. While research by Wagnon, Loy, Rollins and Carroll (1966) established that when greater than 12 bulls are in a breeding group, bulls are no longer able to establish a stable social dominance hierarchy. Since the current study did not have any breeding groups with greater than nine sires, it can be probably assumed there was a linear relationship of social dominance order and that once established, it remained relatively stable. Therefore, it is possible that the bulls in the larger breeding groups were able to establish a social dominance order resulting in an increased variation in the number of calves sired as the number of bulls per pasture increased. Future research is needed to determine if variation in the number of calves sired would rise in breeding groups with greater than 12 bulls or if the variation would plateau as bull number increased.

Investigating low reproductive rates in multi-bull groups, McCosker, Turner, McCool, Post, and Bell (1989) found certain bulls to be responsible, as culling these animals eliminated the problem. As these researchers noticed, a particular breeding pasture had a high percentage of bulls ($>40\%$) over 8-yr of age that contributed to the large observed variation and it was assumed that the older bulls were still dominant preventing other bulls from servicing cows but were unable to successfully breed themselves (McCosker et al., 1989).

There was a large variation among the calculated BPI for all sires, with the lowest value being 0.05 and the highest 3.83 (Table 1). The mean BPI was 0.97 for all data presented in Table 1, with an expected mean of 1 was

not achieved probably due to the error associated with not all calves being assigned a bull. The BPI was further used to compare all factors that were measured both on an individual bull level and on an overall herd level.

Table 1. Descriptive statistics on number of bulls, bull:cow ratio, and corresponding range of bull prolificacy index for each breeding group over 3 yr

Ranch/ Breeding Group	# of Bulls	# of Cows	Bull: Cow Ratio	Mean (\pm SD) calves sired per bull	Maximum calves sired per bull	Minimum calves sired per bull	Range of BPI ¹
Yr 1 (2015)							
A1	8	208	1:26	26 \pm 14	44	10	0.33-1.69
A2	6	128	1:21.3	21 \pm 18	53	5	0.23-2.48
A3	3	66	1:22	21 \pm 8	29	14	0.64-1.32
A4	4	70	1:17.5	18 \pm 5	24	12	0.69-1.37
B5	8	194	1:24.3	23 \pm 16	46	5	0.21-1.90
C6	9	108	1:12	11 \pm 12	34	1	0.08-2.83
D7	4	93	1:23.3	22 \pm 9	30	14	0.60-1.29
Yr 2 (2016)							
A1	6	162	1:27	22 \pm 13	51	9	0.71-2.30
A2	6	156	1:26	22 \pm 11	37	1	0.05-1.41
A3	3	69	1:23	19 \pm 11	33	4	0.20-1.71
A4	4	84	1:21	-	-	-	0.33-3.50
C6	7	196	1:28	21 \pm 28	68	2	0.09-3.05
D7	4	88	1:22	18 \pm 12	35	9	0.47-1.82
E8	4	148	1:37	21 \pm 9	27	10	0.50-1.35
E9	3	93	1:31	23 \pm 7	27	10	0.70-1.26
Yr 3 (2017)							
A1	6	125	1:20.8	21 \pm 17	44	4	0.19-2.11
A2	6	116	1:19.3	19 \pm 22	49	1	0.05-2.53
A3	3	31	1:10.3	10 \pm 7	23	2	0.19-2.22
C6	7	159	1:22.7	23 \pm 11	87	3	0.35-3.83
D7	4	54	1:13.5	14 \pm 10	25	1	0.07-1.85
E8	4	105	1:26.3	26 \pm 8	35	10	0.38-1.33
E9	4	37	1:9.2	10 \pm 8	15	7	0.54-1.62

Note. ¹BPI = bull prolificacy index.

Breeding pasture size was not measured in the current study but may have played a role in number of calves sired by each bull. Fordyce et al. (2002) suggested that in larger pastures, younger and less socially dominant bulls have an opportunity to escape from older, more dominant bulls and are therefore able to sire more calves.

For yr 1 (2015 breeding season), breeding group C6 had a 1 bull:12 cow ratio which was much lower than the other breeding groups (Table 1). If these bulls were in a smaller breeding pasture it would make sense that the less dominant bulls would not be able to escape from the more dominant bulls resulting in the large variation in BPI in that pasture. The previously discussed study by Godfrey and Lunstra (1989) regarding SCT may aide to explain the BPI variation associated with the breeding group C6 with the highest number of bulls (the lowest bull:cow ratio) in yr 1. If the bulls in this pasture that were high-libido bulls to begin with, had increased libido due to the presence of other bulls, it would be probable that the high-libido bulls would sire most of the calves resulting in the rest of the bulls being able to only service a limited number of cows.

Godfrey and Lunstra (1989) further found that when bulls with low SCT were exposed to cows, there was no difference in conception rates in single-bull mating groups compared to high SCT bulls. In contrast, when high and low SCT bulls were combined in a breeding group, there was a difference in number of services (Godfrey & Lunstra, 1989). This suggests that bulls with lower libido may underperform in multi-bull pastures. Additionally, this supports to clarify as to why bulls in group C6 had the largest variation in BPI (0.17-3.24) averaged over 3 yrs (Table 1), even though the bulls were capable of servicing, there was more competition resulting in a lower number of calves sired by some bulls and a much higher sired by others.

As the current study results indicated, lower bull:cow ratios in yr 1 and yr 2 carried higher variations of BPI values, which was not a case in yr 3 (Table 1). This would suggest that those bulls that were exposed to more

cows are more efficient and effective at getting cows serviced and pregnant, and that as bulls are exposed to more cows, there is a greater likelihood of at least one cow being in heat at any particular time. As a result, it is possible that bulls would be spending more time seeking out females in standing heat and less time fighting. In a two-bull breeding group study, Farin, Chenoweth, Mateos, and Pexton (1982) observed that 50% of synchronized heifers were serviced by both bulls, but heifer pregnancy rates did not differ compared to a single-bull pasture. This further suggests that bulls in multi-bull breeding systems with more cows in estrous are allocating their time to breeding those cows thus ignoring other bulls.

Rupp, Ball, Shoop, and Chenoweth (1977) argued that individual bull fertility and libido plays a greater role in overall fertility and pregnancy rates than does a bull:cow ratio. Kasimanickam (2015) stated that a general bull:female ratio for expected cows bred is 15 females per yearling bull, 20-25 per 2-yr old bull, and 25-35 per mature bull. The average bull:cow ratio across western Canada is 1:24 (Larson, 2015), however, the average bull:cow ratio for the operations in the current study was 1:22 with the highest ratio at 1:37 and the lowest at 1:9. More research is needed to reveal the effects of bull:cow ratio and methods to determine an optimal bull:cow ratio on bull prolificacy.

3.2 Prolificacy and Bull Age

Bull age in the current study ranged from 1 to 5-yr old with an average age of 2.64 yr (± 1.27 yr) (data not shown). Bull age had an effect ($p < 0.01$) on the bull's ability to bull calves (Figure 1).

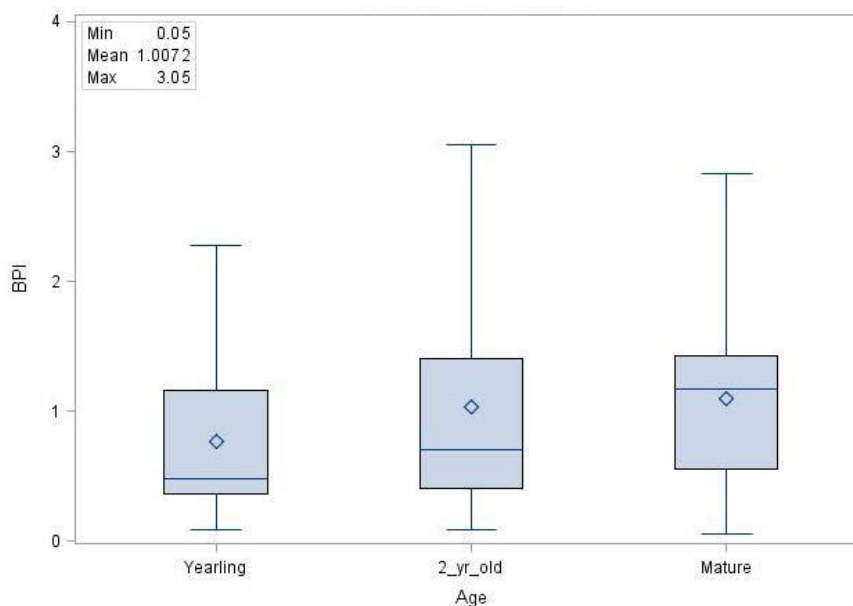


Figure 1. Comparison of bull prolificacy index for yearling, 2-yr old, and mature bulls ($n = 74$)

Note. Greatest variation in BPI was observed among the 2-yr old bulls with a SD of 0.86, 0.64 for mature bulls, and 0.57 for yearling bulls. The older bulls had the highest BPI with an average of 1.10, the 2-yr old bulls had 1.00, and yearling bulls had the lowest, 0.76.

Blocky (1979) revealed that the older, larger bulls that had been on a farm or operation for a longer period tended to be the dominant bulls in the group, which was correlated with sexual activity and number of calves sired. This contradicted their finding that a group of bulls composed of all young bulls (2-yr old) impregnated more females than a group of mixed-age bulls (Blocky, 1979). Many factors are associated with poor prolificacy rates in young bulls, such as reduced semen quality and quantity, lower libido, and reduced social dominance (Blocky, 1979). Nevertheless, these factors have been shown to improve with age, and therefore it is recommended that producers not assume performance of a young bull in its first breeding season as indicative of its future performance (Stalhammar et al., 1989; Carpenter et al., 1992). Further, this would suggest that the technology of DNA parentage testing should not be used as a culling mechanism for young bulls as performance of a yearling or 2-yr old may not be a good indicator of future performance.

In the current study, pooled over 2 yrs (2015 and 2016), the greatest variation in BPI was observed among the 2-yr old bulls (Figure 1) with a SD of 0.86 compared to 0.64 for mature bulls and 0.57 for yearling bulls. The

older bulls had the highest BPI with an average of 1.10, the 2-yr old bulls 1.00, and yearling bulls 0.76. It is important to note that although mature bulls tended to be more prolific in each breeding group, there was one breeding group (A1) where the yearling and 2-yr old bulls sired most of the calves.

Stalhammar et al. (1989) indicated that age is directly correlated with semen quality and quantity. Typically, the young bulls in each breeding group may have sired fewer calves due to reduced semen quality (Amann, Seidel, & Mortime, 2000). In the current study, all bulls, except two yearling and one invading older bull in group C in yr 1, had passed the BSE before being turned out to the breeding groups, so the previously mentioned spermatological defects would almost certainly not have played a role in bull prolificacy. This suggests that the low prolificacy rates of younger bulls may be due to social dominance or reduced libido, and an effect of sexual experience.

3.3 Prolificacy and Scrotal Circumference (SC)

It is well documented that SC is highly correlated with fertility and bull age (Stalhammar et al. 1989; Coe, 1999). In the current study, bull SC ranged from 29 to 46 cm (Figure 2). Only one bull had a SC of 29 cm (a yearling bull incorrectly added to group C6), which was below the required 30 cm SC for yearling bulls to pass the BSE examination (Barth, 2013).

Past research (Holroyd et al., 2002) has suggested that once bulls reach a threshold level SC of 30 cm, selecting bulls for larger SC does not seem to affect fertility. In the current study, no correlation ($r = 0.08$, $p = 0.53$, $n = 65$) was observed between SC values and BPI calculations (Figure 2), since all but one bull reached the pre-defined benchmark for their age and breed as established by Barth (2013).

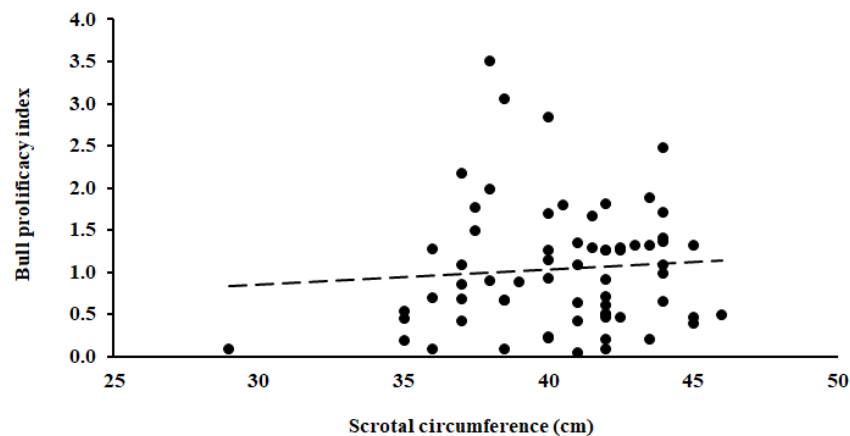


Figure 2. Comparison of bull prolificacy index (BPI) and scrotal circumference (SC) values for all bulls. A weak correlation ($r = 0.08$; $n = 65$) was observed between SC and BPI

An association between SC and BPI has previously been reported by Coulter and Kozub (1989) and Van Eenennaam et al. (2014). It should be noted that the same correlation was not found by Holroyd et al. (2002) possibly due to studying only *Bos indicus* breeds and that all bulls used in the study had acceptable SC values before being turned out to pasture. In contrast, the study by Coulter and Kozub (1989) included bulls that failed to reach industry benchmarks for SC. All bulls in the study conducted by Van Eenennaam et al. (2014) had a SC greater than the minimum threshold value, yet a significant correlation was observed between SC and bull prolificacy when one extreme outlier bull was removed from the data set. This contradicts the current study results and Holroyd et al. (2002) in that once bulls pass a minimum threshold value for SC, selecting for higher SC would not affect fertility. Since SC is known to be affected by multiple factors including age and breed (Barth, 2013), the confounding nature of these other traits may have resulted in the differences observed between the studies. In the current study also, SC was found to be strongly, positively correlated ($r = 0.81$, $p < 0.001$, data not shown) with age of bull. This raises a question of whether SC, age, or a combination of both will affect bull prolificacy signaling for more research needed to determine the effect on fertility.

3.4 Prolificacy and Number of Bulls per Pasture

There was a moderate linear relationship ($r = 0.57$; $p < 0.001$) between the number of bulls in a pasture and the standard deviation of the BPI (Figure 3). This was consistent with Holroyd et al. (2002) who found that when bulls were mated in groups of 8 to 24, there was much greater variation in number of calves sired (ranging from

24 to 94%) compared to bulls that were in groups of 2 to 7 (ranging from 11 to 36%).

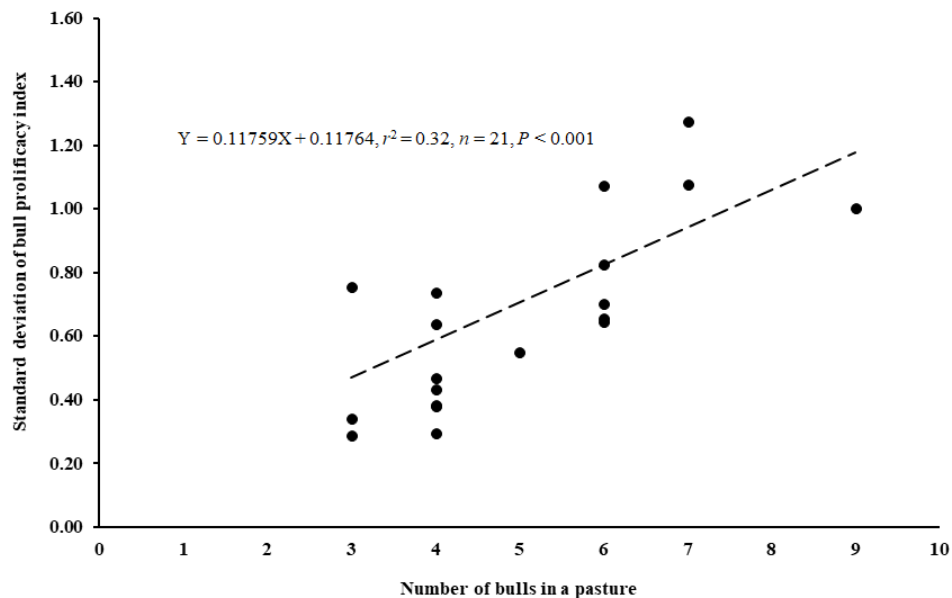


Figure 3. Coefficient of determination between number of bulls per breeding group (X) and the standard deviation (SD) of calculated bull prolificacy index for each group (Y)

Note. There was a moderate linear relationship ($r = 0.57$; $n = 21$, $p < 0.001$) between the number of bulls in a pasture and the SD of the BPI.

Holroyd et al. (2002), also, noticed that bulls in the larger sire groups sired on average more calves than those in the smaller groups. However, as noted previously (Table 1), this trend was not observed in the current study, most likely due to the bull:cow ratio differences between the studies. In the current study, the bulls in the largest breeding groups as well, had the highest bull:cow ratio (Table 1) providing less opportunity for bulls to sire calves as mating activity is known to be dependent on the number of females showing signs of estrus (Rupp et al., 1977).

In yr 1, the relationship between number of bulls per pasture and SD of the BPI may have been amplified, because group C6 (Table 1; Figure 3) had the highest number of bulls and a bull:cow ratio less than industry average (1:12). Theoretically, bulls in this pasture were only expected to sire 12 calves each. In commercial operations, it is expected that bulls be exposed to 24 to 40 cows on average (Fordyce et al., 2002; Larson, 2015), therefore it was not unexpected that bulls in this group would have the greatest variation in their BPI.

3.5 No Bull Match

The DNA parentage results of some breeding groups in the current study had calves that could not be matched to a specific bull, ranging from 1 to 8% of total calves sampled among breeding groups at each ranch. Possible reasons for this may have been due to either laboratory quality control or sampling error in the field. This value was lower than the 14.6% of calves that could not be assigned to a bull in the study by Van Eenennaam et al. (2007) and lower or in the range of 2.7 to 25.6% of calves whose bull could not be detected observed by Holroyd et al. (2002). The difference between the current and previous studies is likely due to the number of DNA markers or SNPs used in each laboratory test. Holroyd et al. (2002) used 12 different DNA markers to achieve the above-mentioned results and found that by testing with additional markers they could assign parentage to 92.5 to 100% of calves in a pasture. Increasing the size of the SNP panel that is used to determine parentage increases the accuracy in which parentage is assigned, however, the larger 1000-5000 SNP panels are costlier when compared to the 100-200 SNP panels (Buchanan et al., 2016). The producer must then decide if the small increase in accuracy justifies the increased cost of the larger SNP panel method. Although the no matching bull rate was relatively low (1 to 8%) in the present study, improving the sampling technique and resubmitting samples when required could potentially help reduce the number in 'no sire matches' category.

3.6 Alternative Sampling

The percentage of the calf crop born each week of the 2015, 2016, and 2017 calving seasons at Ranch A and

Ranch D is presented in Figure 4. Ranches A and D were the only operations able to provide calving dates for all calves and that had all bulls remain in the breeding group for the entire breeding period.

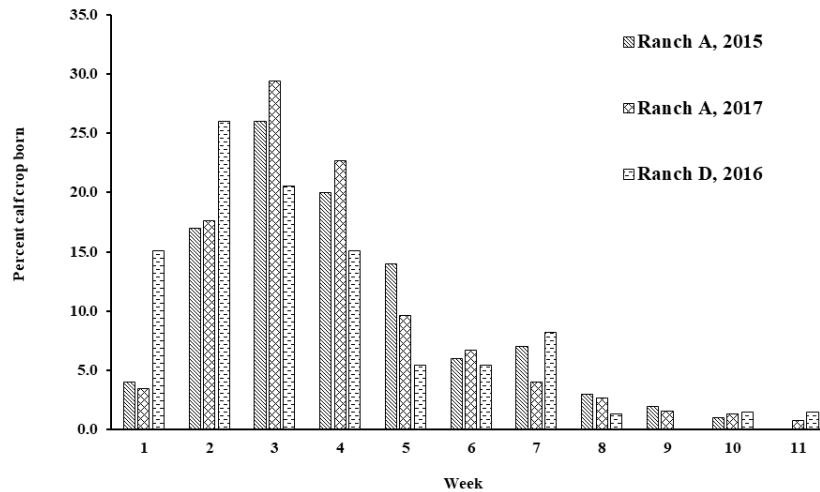


Figure 4. Percent of total calves born each week over 3 yr (Ranch A and D, 2015-2017). Week 3 of the calving period had the highest percentage of calves born (mean = 25.3%) during the calving season

Week 3 of the calving period had the highest percentage of calves born (mean = 25.3%) during the calving season. This suggests that it may be possible to only test a portion of the calf crop and get results back sooner. Only testing calves born in week 3 of the calving season is also suggested by Van Eenennaam et al. (2014) and it would leave 62 d (40 d left in the calving season plus 22 d before resumption of the next breeding season) (Larson, 2013) to receive lab results and make culling decisions about the bull battery. Evaluation of the bulls from the cooperating ranches by calculating breeding prolificacy indexes for bulls using all the calves sampled, only calves born in the first 21 d, and only calves born in week 3 over 3-years is presented in Tables 2 to 5.

Table 2. Bull prolificacy index for calves born in first 21 d, week 3, total calves born, and total calf weaned weight from Ranch A in yr 1

Ranch/breeding group	Bull	BPI ¹			Total weaned weight (kg)
		First 21 d	Week 3	Total	
A1	1108Y	0.78	0.61	0.5	2832
A1	2151Z	0.69	1.06	1.15	6905
A1	256A	0.69	0.00	1.27	7954
A1	493Z	2.59	3.03	1.69	11221
A1	51X	0.26	0.30	0.39	2422
A2	13X	1.36	1.88	1.31	6815
A2	144Y	0.55	0.00	0.94	4294
A2	1496Y	0.55	0.19	0.47	2074
A2	920W	2.73	3.00	2.48	13268
A2	549Z	0.41	0.38	0.23	1086
A2	198A	0.41	0.19	0.52	2753
A3	122Y	1.25	1.76	1.32	6729
A3	212Z	1.17	0.71	0.91	4619
A3	228Z	0.58	0.53	0.64	3200
A4	124Z	1.03	0.50	0.69	–
A4	401A	0.46	0.75	0.86	–
A4	476A	1.26	1.75	1.09	–
A4	9050W	1.26	1.00	1.37	–

Note. ¹BPI = bull prolificacy index.

Table 3. Bull prolificacy index for calves born in the first 21 d, week 3, and total calves born from Ranch A in yr 2

Ranch/breeding group	Bull	BPI ¹		
		First 21 d	Week 3	Total
A1	144Y	1.20	0.67	1.25
A1	549Z	0.96	1.50	0.72
A1	256A	0.64	1.36	0.90
A1	644B	0.48	0.83	0.45
A1	132B	2.24	1.50	2.28
A1	94B	0.48	0.17	0.40
A2	920W	1.04	0.71	1.32
A2	2151Z	0.00	0.00	0.05
A2	493Z	2.17	1.67	1.68
A2	198A	1.22	1.31	1.41
A2	97B	0.00	0.00	0.32
A2	300B	1.57	1.19	1.23
A3	212Z	1.06	0.71	1.09
A3	1496Y	0.19	0.35	0.21
A3	122Y	1.74	1.94	1.71
A4	124Z	0.84	0.46	0.67
A4	21A	2.95	1.15	3.00
A4	401A	1.28	0.00	2.16
A4	175B	0.21	1.38	0.33

Note. ¹BPI = bull prolificacy index.

Table 4. Bull prolificacy index for calves born in the first 21 d, week 3, and total calves born from Ranches C, D, and E in yr 2

Ranch/breeding group	Bull	BPI ¹		
		First 21 d	Week 3	Total
C6	76	2.16	2.36	3.05
C6	83	0.22	0.00	0.18
C6	86	1.49	1.27	1.26
C6	87	0.22	0.18	0.22
C6	92	0.07	0.18	0.09
C6	98	1.86	1.82	1.44
C6	99	0.45	0.18	0.36
D7	30W	1.91	2.67	1.82
D7	58Y	0.64	0.33	0.47
D7	42B	0.91	0.67	0.88
D7	82A	0.36	0.33	0.47
E8	49	0.56	0.50	0.50
E8	58	1.12	1.50	1.35
E8	13Z	0.84	0.25	1.00
E8	59Z	1.49	1.25	1.25
E9	52Z	1.13	2.00	1.09
E9	219U	0.38	0.50	0.70
E9	53Z	1.50	2.50	1.26

Note. ¹BPI = bull prolificacy index.

Table 5. Bull prolificacy index for calves born in the first 21 d, week 3, total calves born, and total calf weaned weight from Ranches A and D in yr 3

Ranch/breeding group	Bull	BPI ¹			Total calf weaned weight (kg)
		First 21 d	Week 3	Total	
A1	132B	1.93	1.71	2.11	13699
A1	167C	0.93	1.37	1.10	6204
A1	198ABB	1.00	0.86	0.72	4037
A1	493Z	1.21	1.20	1.15	7142
A1	649C	0.14	0.34	0.19	787
A1	94B	0.79	0.51	0.72	4171
A2	24C	0.50	0.29	0.36	2160
A2	256A	1.79	1.46	2.17	12695
A2	300B	2.79	1.85	2.53	13867
A2	549Z	0.29	-	0.21	1536
A2	633C	0.57	0.39	0.67	3508
A2	812C	0.07	-	0.05	241
A3	122Y	2.25	1.85	2.23	5925
A3	212Z	0.5	0.69	0.58	852
A3	21A	0.25	0.46	0.19	381
D7	42B	0.89	1.25	0.89	2799
D7	82A	1.00	1.00	1.19	3966
D7	58Y	0.11	-	0.07	253
D7	30W	2.00	0.75	1.85	6199

Note. ¹BPI = bull prolificacy index.

If high prolificacy bulls actually sired most of their progeny after the first 21 d of the breeding season, these calves would have less opportunity for growth before selling at weaning age and therefore be of lower value to the producer. Damiran et al. (2018a) showed that calves born early in the calving season are usually heavier at weaning than those born later. This did not appear to be the case for this dataset as every bull that sired the highest total kg of calf weaned weight in a pasture, also, had the highest BPI in that pasture during all three proposed testing times (Table 5).

Notably, there were some bulls; for example, 256A and 144Y at Ranch A (Table 2) who had a relatively low BPI when testing only calves born in the first 21 d and sired zero calves in week 3. Although both bulls were not as prolific as other bulls at the beginning of the breeding season, they continued to sire a total kg of calves weaned that was in the top half of their breeding group. Overall, as expected, strong correlations were observed (data not shown) between total weaning weight and 21-d BPI ($r = 0.87$, $n = 33$, $p < 0.001$), week-3 BPI ($r = 0.74$, $n = 33$, $p < 0.001$), and total BPI ($r = 0.92$, $n = 33$, $p < 0.001$).

The current study determined that 62 of the 74 (84%) bulls were properly classified as high or low prolificacy bulls based on their 21-d BPI scores (Table 6). However, eight (14%) and four (22%) bulls were incorrectly classified as low and high BPI bulls, respectively, based on testing only calves born in the first 21 d of calving period (Table 6). The results of the week-3 BPI scores indicated that 61 (82%) of the 74 bulls were properly classified as high or low prolificacy bulls. Ten (18%) and three bulls (17.0%) were wrongly classified as low and high BPI bulls, respectively, based on the week-3 portion of calving period (Table 6). Testing only calves born in the first 21 d or only calves born in week 3 created an average of 17% of total number of bulls wrongly classified (12 and 13 bulls in the first 21 d and week 3, respectively), a reasonable number when compared to testing the whole herd (Table 6). Overall, an average of 18% (16 and 19.5% of the high and low BPI bulls, respectively) was wrongly classified when only a portion of calving period was taken for parentage testing.

Table 6. Agreement between number of bulls classified by prolificacy index over 3 yrs¹

Item	High BPI	Low BPI	Properly classified	Wrongly classified
Total number of bulls	56	18	74	-
In first 21 d	48	14	62	12
In week 3	46	15	61	13

Note. ¹Agreement between number of bulls classified as high prolificacy (bull prolificacy index ≥ 0.5) or low prolificacy (bull prolificacy index < 0.5) by parentage testing on calves born in first 21 d and calves born in week 3 vs. total calves. The DNA samples were analyzed using SNP technology for bull verification.

This may suggest, based on the percentage of error, that testing only a portion of calves born may be relatively accurate at identifying high prolificacy bulls rather than low prolificacy bulls. The error percentage observed in the current study was higher than what Van Eenennaam et al. (2014) obtained when they evaluated two methods of alternate testing when only calves born in the third week or only calves born in weeks 2, 3, and 4 were sampled, where less than 3% of low prolificacy bulls were reclassified as high prolificacy.

Using these ranches as an example, if the producer were making culling decisions based on data from the first 21 d, eight bulls that had high BPI, but were incorrectly considered low BPI bulls, would have been wrongly culled (Table 6). If this same ranch were to test all calves born in week 3 of calving alone to base culling decisions on, 10 bulls would be wrongly culled. Moreover, the rate of low total BPI bulls that would be allowed to breed in coming years would be based on their calculated BPI for only the first 21 d or week 3. Overall, a strong correlation was observed between total BPI and 21-d BPI ($r = 0.93, n = 74, p < 0.001$; data not shown), and between total BPI and week-3 BPI ($r = 0.69, n = 74, p < 0.001$; data not shown).

3.7 Economics

The primary source of income for a cow-calf producer is sale of weaned calves, whereby profit is influenced by both weight and total number of calves weaned (Damiran et al., 2018b). A herd bull influences a commercial producer's income as the bull must impregnate females and pass on quality genetics to his progeny (Garrick & Golden, 2009; Van Eenennaam & Drake, 2012). It is therefore critical that these bulls are producing offspring (Van Eenennaam & Drake, 2012), and not burdening the producer with the upkeep of a non-profitable bull. Integrating DNA parentage testing is one way for commercial cow-calf operations using multi-bull breeding systems to verify and evaluate a bull's performance based on progeny and performance of the calves. The cost of maintaining a bull battery is substantial and is borne by the breeding females in a herd, with each bull in a breeding group expected to breed a similar number of females (Larson, 2013). Based on a standard list of assumptions, a bull purchased for CAD6800 and used for four yrs will have annual maintenance costs of CAD2755.87 or CAD102.83 per 25 cows serviced (Larson, 2010). If the number of females serviced is 35 per year, the cost drops to CAD78.74 per cow. If the bull only breeds two cows, the cost per cow increases substantially to CAD1285.43 per cow. Based on the assumptions used for this example, if a bull services fewer than 25 cows, it may be more economical for the producer to use artificial insemination which is estimated to cost CAD94 to CAD125 per female and allows a producer to access proven, genetically superior bulls (Lardner, Damiran, & Larson, 2020). The cost of parentage testing in western Canada varies from CAD12 to CAD20 (plus tax) per animal depending on the laboratory and number of samples being tested. In addition to laboratory costs, there are costs associated with animal identification, labour to collect and submit DNA samples and the time, skill, and potential software cost of interpreting the data and making informed decisions. The benefits of identifying (and culling) under-performing bulls needs to extend beyond one yr to incorporate the performance of progeny into the decision. After 3 yrs, every cooperating ranch had some repeated bulls (bulls used for all three breeding seasons). At Ranch D, there were only two bulls that were used for all three breeding seasons. Additionally, the Ranch D breeding field was 1 of 2 fields in this study that collected and provided wean weights on the calves born in the study. Without basic production data such as date of birth, birth weight, and wean weight, the value extracted from DNA parentage results may be limited.

Figure 5 illustrates the calves sired by each bull in the breeding field at Ranch D, and the total weight kg of calves weaned by each bull. In addition, of the two bulls that were involved for all 3 yrs, one bull (30W) showed repeatability (25+ calves sired each yr) while another bull (58Y) displayed substantially declining rates of prolificacy (siring only one calf in 2017). Thus, current study suggested that the differences between the bulls that can exist in number of calves sired and overall calf kilograms weaned.

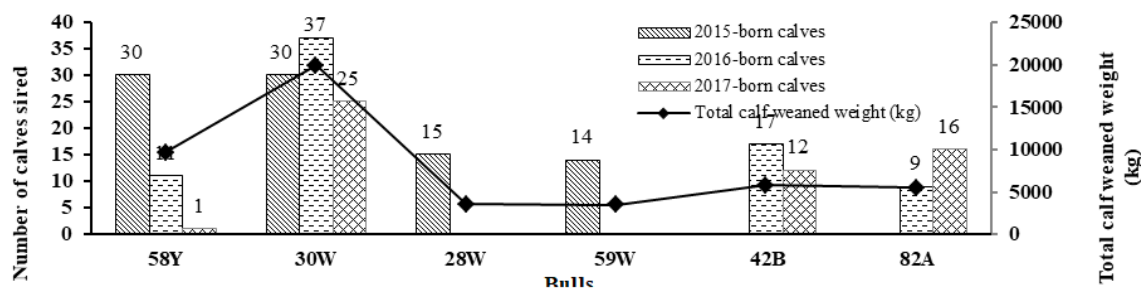


Figure 5. Number of calves sired by each bull and total calf weaned weight (kg) by bull (Ranch D, 2015-2017)

Note. At Ranch D, two bulls (30W and 58Y) were involved for all 3 yrs in the breeding field, two were involved for only yr 1 (28W and 59W were removed after yr 1 and replaced by 42B and 82A), and two (42B and 82A) for yr 2 and yr 3.

Figure 6 shows the estimated market value for the weaned calves from each bull using the average fall-run price for 249.4-kg steers and heifers as reported by Canfax (2018). The two bulls that were used for all 3 yrs have the highest market value for calves weaned, exceeding CAD94,000 for 30W and CAD50,664 for 58Y.

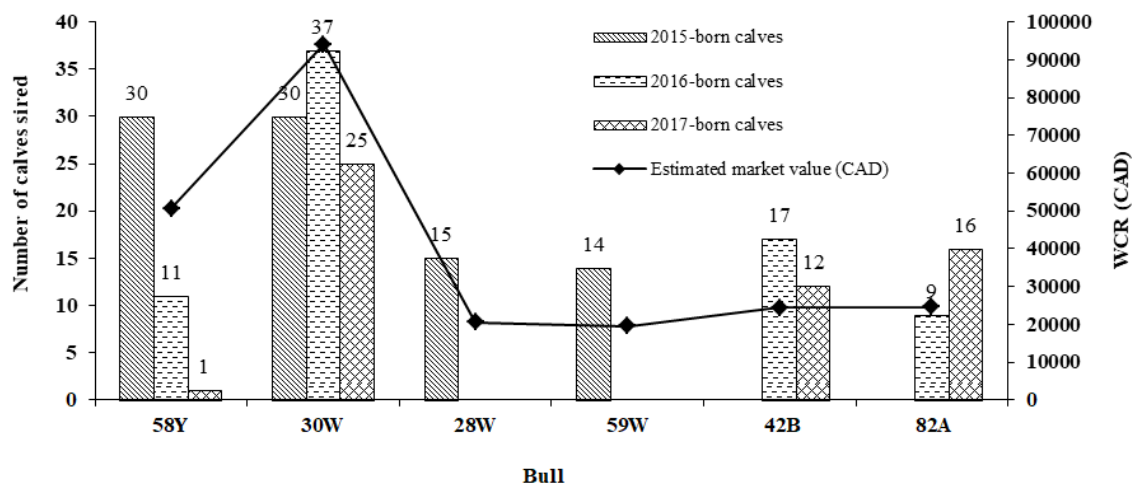


Figure 6. Progeny count and weaned calf revenue (WCR) from each bull (Ranch D, 2015-2017)

Note. At Ranch D, two bulls (30W and 58Y) were involved for all 3 yrs in the breeding field, two were involved only in yr 1 (28W and 59W were removed after yr 1 and replaced by 42B and 82A next yr), and two (42B and 82A) for yr 2 and yr 3

In general, if pregnancy rates are acceptable, a producer may not be concerned with how many calves each bull is siring, as long as his females are pregnant. However, if the progeny are retained for replacement or terminal calves, it could be valuable for a producer to know the bull of each and to see if traits of economic importance were passed on in the progeny. Downstream performance of progeny is an area for future study.

4. Conclusions

The study show that DNA parentage testing allows a producer confidently evaluate prolificacy or culling of the bull. Bull age was shown to play a significant role when determining prolificacy with older bulls siring more calves than younger bulls. The number of bulls in a breeding group, also, influenced the number of calves sired as the number of bulls in a breeding group increased so did the variation in the number of calves sired by each bull. The bull:cow ratio appeared to play a role in the number of calves sired by each bull, but more research is needed to determine if this ratio affects bull prolificacy. Conducting DNA parentage testing only for those calves born in the first 21 d or in week 3 of the calving season may provide producers with a chance to decrease cost and turn-around time for sample collection and lab results prior to the next breeding season. Depending on the age at which bulls are culled on an operation, producers may question the economic advantage of DNA parentage testing progeny to assist with culling decisions. Finally, multi-year studies are needed to evaluate bull prolificacy in changing bull breeding groups which is common in commercial practice to assess the long-term value of DNA parentage testing and use of the results as a tool to cull non-prolific bulls.

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