Animal 17 (2023) 100713

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Associations between insulin-like factor 3, scrotal circumference and semen characteristics in young Norwegian Red bulls

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ARTICLE INFO

Article history:
Received 23 September 2022
Revised 5 January 2023
Accepted 9 January 2023
Available online 20 January 2023

Keywords:
Bull breeding soundness evaluation
Leydig cells
Performance testing
Puberty
Sperm production

ABSTRACT

With the integration of genomic selection in the cattle artificial insemination **(AI)** industry, bulls are selected for their semen production capacity and fertility at a younger age than previously. Norwegian Red bull calves selected as candidates to become future AI bulls based on their genomic breeding value are kept in a performance testing station from around the age of 3–12 months, allowing for sample collection and analysis of different parameters during their pre- and peripubertal period. Insulin-like factor 3 **(INSL3)** is a small peptide hormone specifically secreted by the mature Leydig cells of the testes. In the foetus, it induces the first phase of testicular descent and is considered to reflect Leydig cell development during puberty; it could therefore be an interesting early indicator of future semen production capacity. The main objective of our study was to evaluate the relationship between INSL3, scrotal circumference **(SC)**, and semen characteristics. This is the first time INSL3 was measured in the Norwegian Red population. We collected blood samples for analysis of INSL3 from 142 Norwegian Red bulls at the performance testing station and measured their SC on the same day. Altogether, measurements were made at four time points: upon arrival at the performance testing station (quarantine **(Q)**: 2–5 months) and later at approximately 6, 9 and 12 months of age. Information on season and place of birth were made available from the database of the breeding company Geno, together with data on semen characteristics from the test station and the AI station. The median SCs for age groups Q, 6, 9, and 12 were 15, 21.5, 29, and 34 cm, respectively. INSL3 was shown to be positively correlated with SC (R = 0.4) but not with any of the semen characteristics. Similarly, we found no correlation between SC and sperm characteristics from data on ejaculates analysed at the performance testing station and AI station. The mean sperm volume for the 31 selected bulls with at least 10 ejaculates produced in the AI station increased from 2.3 ml at the performance testing station to 6.4 ml at the AI station. The corresponding increase in mean sperm concentration was from 497 million/ml to 1 049 million/ml. We conclude that INSL3 exhibits high inter-individual variability in the Norwegian Red bull population, which cannot be explained by the parameters measured in this study. At present, INSL3 cannot be used as a biomarker of sperm production in this breed.

Implications

Cattle breeders require an early indicator of future semen production capacity and potential fertility in bull calves for effective selection of future breeding sires. Insulin-like factor 3, produced by Leydig cells in the testes, could serve as a biomarker of sperm production onset. Although we found a moderate correlation between insulin-like factor 3 levels and scrotal circumference in young Norwegian Red bull calves, there was high individual variation, rendering insulin-like factor 3 problematic as a biomarker of sperm production onset, and thus spermatogenesis, in this breed at present. More work is needed to understand factors influencing the inter-individual variability in insulin-like factor 3 in young bulls of this breed.

Introduction

In the past decade, the introduction of genomic selection in the dairy breeding industry enabled a large number of candidate

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https://doi.org/10.1016/j.animal.2023.100713
1751-7311/

breeding bulls to be evaluated, increased the accuracy of predicted breeding values in young animals and shortened the generation intervals significantly (Taylor et al., 2018). As a consequence, bulls are introduced into semen production at a young age, and the breeding industry faces a challenge in predicting fertility and semen production capacity as early as possible. (Fair and Lonergan, 2018; Taylor et al., 2018; Brito et al., 2021). The shift caused interest in possible indicators that could be used to identify bulls which can produce semen samples at a young age. The reproductive potential of young bulls is limited by the amount of semen they can produce as well as its quality. Identification of breed-specific indicators that can predict a bull's future performance are needed (Byrne et al., 2018; Taylor et al., 2018; Brito et al., 2021).

Bull breeding soundness evaluation **(BBSE)** aims to identify sub-fertile bulls in herds under field conditions (Barth, 2018). There are several existing systems of BBSE around the world, and not all countries are bound to fulfil the requirements listed in these systems (Garcia-Paloma, 2015; Barth, 2018). This BBSE cannot predict fertility but is a simple and repeatable means of assessing breeding potential (Fordyce et al., 2006; Irons et al., 2007). Scrotal circumference **(SC),** sperm morphology and motility assessment are required measurements across all BBSE systems (Barth, 2018; Waite et al., 2019). Scrotal circumference is an important reproductive trait that is easily measureable, with moderate to high heritability (Ferreira et al., 2021). Bulls with larger SC were shown to reach puberty earlier than bulls with a smaller SC, and SC was positively correlated with the proportion of normal sperm, increased sperm output and fertility outcomes. The crucial time for rapid testicular growth in bulls happens during the peripubertal phase. It was shown that age, breed, nutrition and BW can influence SC (Bollwein et al., 2016; Penitente-Filho et al., 2018; Waite et al., 2019).

Under the influence of LH**,** the Leydig cells of the mammalian testis are responsible for the production of androgens, which are essential for the development of the male reproductive system and sperm production. Mature mammalian Leydig cells also specifically secrete a small peptide hormone, insulin-like factor 3 **(INSL3)** (Ivell et al., 2013). INSL3 can also cross the blood-testis barrier, thus being detectable in luminal fluid from seminiferous tubules, rete testis, epididymis and blood (Ivell et al., 2013). Previous findings showed that INSL3 mRNA is exclusively expressed in the testis, and INSL3 production depends on the number and developmental stage of the Leydig cells in the testis. LH influences the differentiation of Leydig cells, consequently influencing INSL3 production (Anand-Ivell et al., 2019). In female mammals, concentrations of INSL3 in peripheral blood are very low or undetectable; the exception is a female carrying a male foetus (Kibushi et al., 2016). Large amounts of INSL3 secreted by the foetal testes under the control of LH induce the first phase of testicular descent (Ivell and Anand-ivell, 2009). For several mammalian species, including bovine, INSL3 was shown to be an accurate measure of Leydig cell development during puberty (Johansen et al., 2014; Anand-Ivell et al., 2019). INSL3 was used as a candidate biomarker for the assessment of puberty in dairy bulls showing the effect of a high plane of nutrition in the first six months on spermatogenesis (Anand-Ivell et al., 2019). The same authors showed that INSL3 is negatively correlated with the timing of puberty and positively correlated with total testis weight at 18 months.

Our hypothesis was that INSL3 could be a potential biomarker of sperm production onset in Norwegian Red **(NR)** bulls. Our main aim was to investigate the relationship between INSL3, scrotal circumference and semen characteristics. We also explored potential factors influencing individual variability in INSL3 concentration in NR bulls during their peripubertal age, such as season and location of birth.

Material and methods

Animals

The breeding organisation, Geno, buys approximately 150 NR bull calves annually for their performance testing programme selected on their genomic breeding value. Six groups of bull calves, aged 2–5 months (median age 16 weeks), arrive at the testing station per year and are quarantined for two weeks upon arrival. During the testing period, bulls are housed in the same group of 10 individuals. Bulls are fed concentrate according to age and grass silage ad libitum. All bulls at the station are tested for temperament, conformation, and sperm quality. At around 12 months of age, they are approved or rejected for the artificial insemination **(AI)** station. Annually, 50–60 bulls are accepted to become AI bulls. This study was performed during a period from September 2020 until December 2021 and included 142 bulls enrolled in the performance testing programme during this period.

Scrotal circumference measurements and blood collection for measurement of insulin-like factor 3

The measurement of SC of NR bulls was done at four time points: upon arrival at the performance testing station (quarantine **(Q)**: age 2–5 months) and later at approximately 6, 9 and 12 months of age. During the procedure, qualified veterinarians used scrotal tape for SC measurement for all bulls while restrained in stocks. Blood samples from the jugular vein were collected into whole blood tubes by a qualified veterinarian on the same day as SC measurements were made. Blood samples were transported to the university laboratory and stored at 4 °C to clot for serum. The next day blood serum was separated and transferred into clean test tubes and aliquoted. The aliquots of serum – 2 × 250 µl per bull were stored in the freezer at −20 °C until analysis. One of these aliquots was sent to the University of Nottingham on dry ice and used for the analysis of INSL3. The number of animals sampled was 96 in quarantine, 111 at 6 months, 137 at 9 months and 123 at 12 months. Due to technical issues, the schedule of the project, and removal of animals from the unit, a few observations in each age group were lost. Overall, samples were collected from 142 NR bulls, with 82 bulls being sampled at all four time points; 34 were accepted to the AI station.

Insulin-like factor 3 assay

INSL3 peptide was measured using a bovine-specific time-resolved fluorescent immunoassay **(TRFIA)**, following the detailed procedure described in Anand-Ivell et al. (2019). Serum samples were diluted threefold in assay buffer, and the dilution factor was used in calculating the actual INSL3 serum concentration. The bovine INSL3 TRFIA assay range was 0.02–16 ng/ml. Each assay plate was run with the same standard range, as well as positive and negative controls. The intra- and inter-plate coefficients of variation were intra: <1% at all concentrations, and inter: 8.0, 3.3 and 4.4% at the lower, middle, and upper range limits, respectively. This assay recognises no other structurally related peptides (Anand-Ivell et al., 2011; 2019). The final INSL3 concentrations calculated for undiluted serum were used for all further data analysis.

Semen characteristics data

Geno made available data from the 142 young NR bulls included in this study. The semen characteristics data included the ejaculate volume (ml) and sperm concentration (million/ml) measured by a photometer (Bovine Photometer n°932, IMV technologies) of the

last ejaculate in sperm quality testing. Semen production data from the AI station included volume and concentration for the 10 first ejaculates from the 34 bulls that became AI bulls. The acceptance criteria for semen samples used for commercial use at the AI station are sperm concentration above 390 million/ml, prefreeze motility >70% and postthaw motility >50%.

Statistical analysis

All statistical analyses were performed with the use of R Studio version 1.4.0 (https://www.r-project.org/). Linear mixed-effects models were fitted and analysed with function lmer from R package lme4 (Bates et al., 2015). We collected information on the postcode of the farm where the bull calves were born, and the date of birth. Norway is divided into nine counties represented by the first number in the postcode. We extracted the county numbers from the postcode and created a 9-level factor variable representing the geographical birthplace of the calves. The number of bulls per county mirrors the distribution of the cattle industry in Norway, with a minimum of one bull calf from county 9 and a maximum of 46 bull calves from county 7. Date of birth was used to create the birth season in four classes as follows: season 1: December 2020 to March 2021 (n = 39); season 2: April to June 2021 (n = 21); season 3: July to September 2021 (n = 55); season 4: October to December 2021 (n = 27). To examine which fixed effects had a significant effect on the response variables, INSL3 and SC, we used the following mixed linear repeatability model with fixed effects of birth county (9 classes), birth season (four classes) and age at measurement (four groups: Q, 6, 9, and 12 months) and random effect of animal:

$$Y_{ijkl} = county_i + season_j + age_k + bull_l + eijk_l$$

where Y_{ijkl} is the observation of INSL3 or SC for bull *l* at the age *k* from county *i* born in season *j*.

Fixed effects of county and season were not significant for any of the variables and were therefore excluded from further analyses. To assess possible associations between the response variables and the semen characteristics, ejaculate volume and sperm concentration of last ejaculate were added to the model as a linear regression term (one at the time).

$$Y_{kl} = age_k + b * semen\ Characteristics + bull_l + e_{kl}$$

Here, b is the regression coefficient for the linear regression of volume or concentration on the response variable. For the 34 bulls accepted to the AI station, additional analyses were performed to examine possible associations to semen production at the AI station. Information on the ejaculate volume and sperm concentration from the first 10 ejaculates at the AI station were used, and the mean value was included as a linear regression term in a model similar to the one above.

Results

Distribution and variability of insulin-like factor 3 and scrotal circumference

The insulin-like factor 3 concentrations (ng/ml) showed large variation between bulls at all time points and exhibited an increasing trend over time (Fig. 1), but mean values for the age groups were not significantly different. The highest variation of INSL3 concentrations (SD = 2.37 ng/ml) was observed at 8–10 months of age. When comparing INSL3 from AI-accepted bulls vs all individuals, we observed larger variation among AI-accepted bulls in all age groups. The greatest difference between the variation of INSL3 of all individuals and AI-accepted bulls was in the 4–5 months group (SD = 2.34–3.13 ng/ml, respectively). The visual distribution of SC for all individuals is presented in Fig. 2. We observed a clear increasing trend in time which is also evident in the summary of the descriptive statistics in Table 1. Mean and median SC values in each age group were very close, a characteristic of symmetric distribution. The standard deviation around the mean was below 3 cm. We found no statistically significant difference in mean SC between bulls accepted to the AI station and rejected ones for any of the age classes. Table 2 provides an overview of sperm characteristic volume (ml) and sperm concentration (million/ml) of individual bulls from the performance testing station and the continuation of their production at the AI station. It also exhibits the proportion of accepted doses as well as prefreeze and postthaw motility of the ejaculates from the AI station. The mean volume for the population of selected 31 bulls with at least 10 ejaculates produced in AI station increased from 2.3 ml at the performance testing station to 6.4 ml at AI station. The corresponding increase in mean concentration was from 497.75 million/ml to 1 049.68 million/ml.

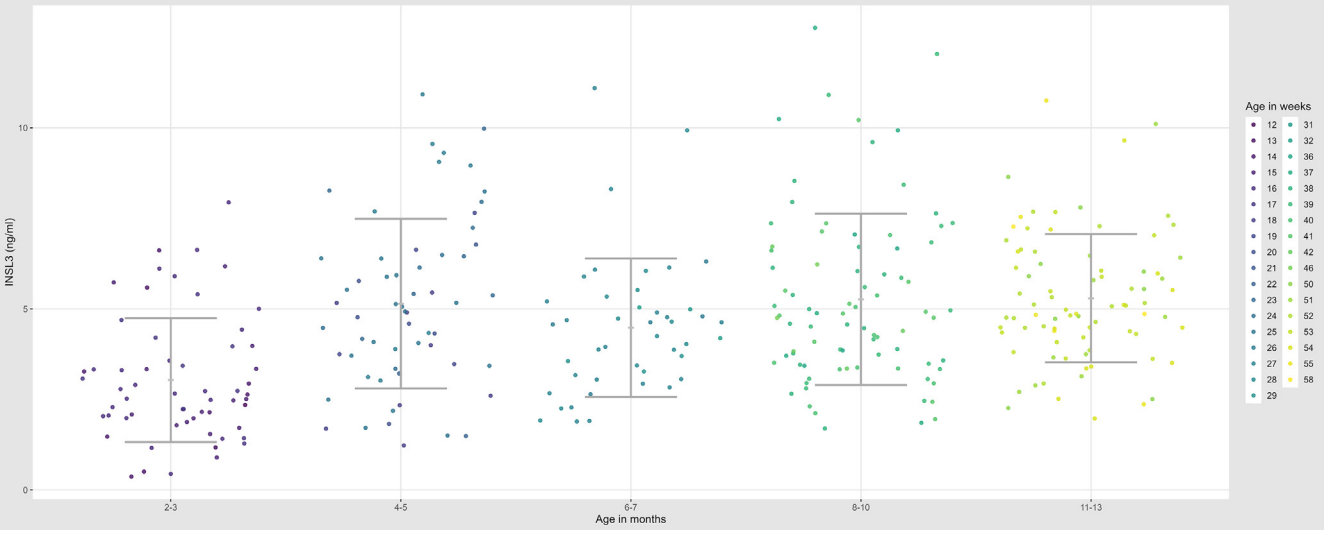

Fig. 1. Distribution of insulin-like factor 3 (INSL3) (ng/ml) concentration in peripheral serum among bulls during the pre- and peripubertal periods according to age in weeks. Data are presented as individual data points in ng/ml and means ± SEM. To clearly show the change over time, age in months for the quarantine group is separated into 2–3 and 4–5 months since it has the highest age range.

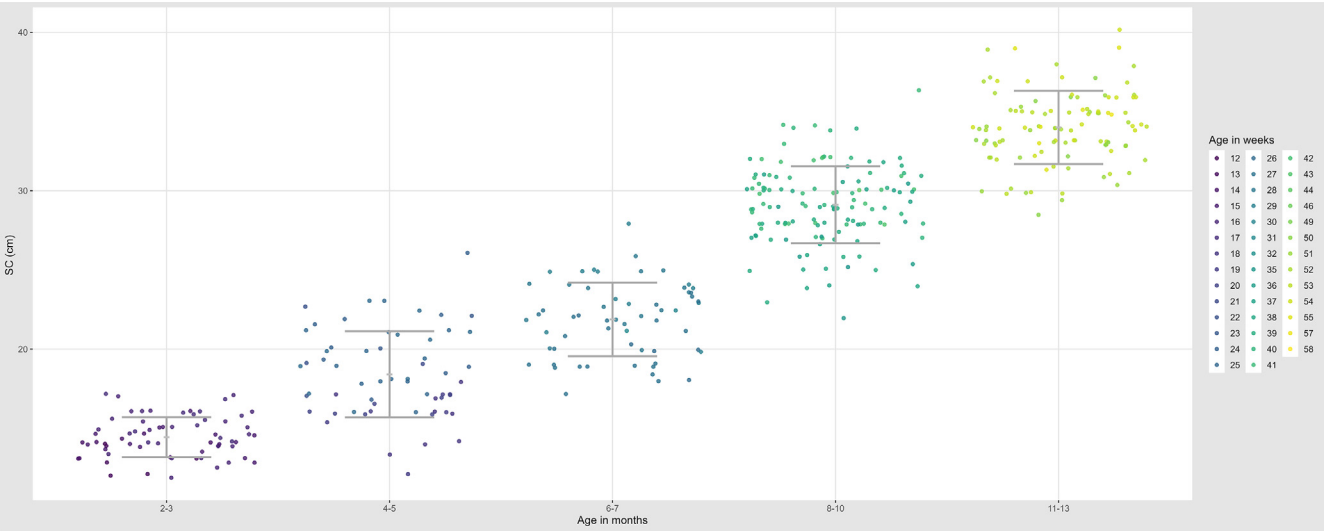

Fig. 2. Distribution of scrotal circumference (SC) (cm) in pre- and peripubertal bulls corresponding to age in weeks. Data are presented as individual data points in cm and means ± SEM. To clearly show the change over time, age in months for the quarantine group is separated into 2–3 and 4–5 months since it has the highest age range.

Table 1
Descriptive statistics for scrotal circumference (cm) of pre- and peripubertal Norwegian Red bulls for all age groups.

Age group	n	Mean ± SD	Median	Min	Max
Q[1]	94	15.0 ± 1.60	15	12	19
6	113	21.4 ± 2.51	21.5	16	28
9	137	29.1 ± 2.45	29	22	36.5
12	122	34.0 ± 2.24	34	28.5	40

Abbreviations: Q = Quarantine, n = Number of analysed bulls.
Samples were collected from 142 Norwegian Red bulls, with 82 bulls being sampled at all four time points; 34 were accepted to the artificial insemination station. Some bulls were only collected 1–3 times.
[1] Quarantine - age 2–5 months.

Relationship between scrotal circumference and insulin-like factor 3

Fig. 3 shows insulin-like factor 3 levels plotted against scrotal circumference measured on the same day. Pearson correlation analyses indicated a moderate positive correlation $R = 0.4$ between INSL3 (ng/ml) levels and SC (cm) with a 95 percent confidence interval of 0.31–0.47.

Mixed model – can we explain part of the variability in insulin-like factor 3 by chosen parameters?

The fixed effect of age at measurement had a significant effect ($P < 0.001$) for both INSL3 and SC. Estimated coefficients for the linear regression of sperm volume and concentration of the last ejaculate from the performance testing station on the response variables INSL3 and SC were not significantly different from 0 (P-value from 0.15 to 0.88). The same was true for mean sperm volume and concentration of the first 10 ejaculates from the AI station (P-value from 0.90 to 0.99).

Discussion

The objective of this study was to investigate the level and variation of insulin-like factor 3 concentration in peripheral serum during the pre- and peripubertal periods of young NR bulls. Our results demonstrated that the concentration of INSL3 (ng/ml) in NR bulls is characterised by high individual variability at all time points. Although these results differ from published studies on young bull calves and Japanese Black beef bull calves, where the population exhibits low variation between individuals (Sakase et al., 2018; Anand-Ivell et al., 2019), the trend of INLS3 levels over time shows a tendency to the same pattern. This resemblance suggests that factors influencing the INSL3 levels might be breed- and population-specific. Interestingly, a similar high inter-individual variability in INSL3 was reported in young men; Anand-Ivell et al. (2021) studied a Swedish cohort of 18-year-old men and showed an approximately 10-fold range of INSL3 values (0.74–8.2 ng/ml). In our NR young bulls at 9 months of age, we observed a similar range of values 1.70–17.49 ng/ml.

By using available information about the bull's birth month and geographical area of birth, we aimed to find if those parameters can explain the high inter-individual variability of INSL3 in our NR population. Aravindakshan et al. (2000) studied differences in the timing of reproductive maturation and developmental pattern of gonadotrophin secretion of Hereford × Charolais bull calves born in spring or autumn. Bull calves born in autumn had lower LH concentrations up to 18 weeks of age which might cause higher variation in the onset of puberty (Aravindakshan et al., 2000). A similar study conducted on Bos indicus Brahman bull calves concluded that bull calves born in spring reached sexual maturity earlier than those born in autumn (Tatman et al., 2004). Since INSL3 is LH-dependent, we hypothesised that we would observe the differences in INSL3 concentration for different birth seasons. Kenny and Byrne (2018) stressed the importance of early life management since the plane of nutrition before 6 months of age determines the age at puberty. The season of birth and geographical area could indirectly affect the plane of nutrition before the introduction to the performance testing station and consequently affect the onset of puberty. Contrary to expectations, our study

Table 2
Descriptive statistics of semen characteristics of the last ejaculate from the performance testing station, the first 10 ejaculates from the artificial insemination (AI) station, and the % of accepted doses from the 31 bulls selected to be AI bulls with at least 10 ejaculates produced.

	Performance testing station – last ejaculate		Artificial insemination station – 10 first ejaculates				
Bull	Volume of last ejaculate (ml)	Concentration of last ejaculate (million/ml)	Volume (ml) mean ± SD	Concentration (million/ml) mean ± SD	% of accepted doses	Prefreeze motility mean ± SD (%)	Postthaw motility (%) mean ± SD
1	2	140	7.73 ± 3.06	578.66 ± 161.37	50	76.66 ± 5.77	28.33 ± 2.89
2	1	920	4.84 ± 1.38	796.6 ± 272.59	50	79 ± 4.18	57 ± 2.74
3	2.5	930	4.35 ± 1.81	1 398.75 ± 481.16	60	78.75 ± 2.5	57.5 ± 5
4	3	850	4.96 ± 0.75	940.83 ± 289.23	60	77.5 ± 4.18	55 ± 7.07
5	3.5	210	5.38 ± 1.25	1 298.6 ± 471.21	60	80 ± 5	51 ± 12.45
6	1	390	4.66 ± 2.16	720.6 ± 172.44	70	79 ± 2.23	50 ± 10.61
7	3.5	770	8.2 ± 1.13	1 078.5 ± 75.66	70	80 ± 0	57.5 ± 3.54
8	3.5	460	4.62 ± 2.29	748 ± 318.25	70	78.57 ± 2.43	56.42 ± 9.88
9	3.5	780	4.75 ± 1.72	1 067.14 ± 414.61	80	75 ± 4.08	51.42 ± 8.02
10	1.5	1 150	4.9 ± 1.96	1 388.6 ± 438.70	80	77 ± 2.73	41 ± 8.94
11	2	400	5.81 ± 2.43	894.87 ± 298.78	80	76.25 ± 4.43	43.75 ± 14.82
12	3	450	11.82 ± 1.22	736 ± 135.88	80	79.28 ± 1.89	44.28 ± 9.32
13	2.5	380	6.27 ± 1.73	1 196.28 ± 348.11	90	78.57 ± 2.43	57.14 ± 5.67
14	3	100	7.4 ± 2.01	1 005 ± 386.46	90	78.75 ± 4.43	58.75 ± 5.18
15	2	640	8.57 ± 2.13	1 025.88 ± 304.55	90	80 ± 4.33	52.77 ± 6.67
16	1.5	500	8.23 ± 2.72	1 423.11 ± 365.40	90	80 ± 3.54	50 ± 5.59
17	4.5	410	5.95 ± 1.30	816.33 ± 423.18	100	79.16 ± 2.04	49.16 ± 12.81
18	2	450	6.77 ± 1.53	1 147.5 ± 360.07	100	78.75 ± 6.29	60 ± 7.07
19	2	360	5.6 ± 1.94	828.44 ± 321.29	100	77.77 ± 2.63	51.11 ± 5.46
20	2	720	4.87 ± 1.90	1 291.37 ± 365.92	100	78.75 ± 3.53	56.25 ± 7.91
21	3.5	510	5 ± 1.66	1 079.22 ± 195.01	100	78.88 ± 2.20	57.77 ± 9.72
22	2.5	400	6.55 ± 1.14	875.8 ± 281.54	100	77 ± 4.21	55.5 ± 11.17
23	1.5	680	4.36 ± 1.30	1 382.3 ± 367.01	100	79 ± 3.94	54 ± 9.07
24	1	800	5.41 ± 1.42	1 077.8 ± 199.05	100	79.5 ± 3.69	60.5 ± 7.25
25	2	680	8.9 ± 2.15	1 389 ± 408.44	100	74 ± 2.24	49 ± 7.42
26	3	770	6.18 ± 2.09	1 332.75 ± 236.38	100	76.25 ± 3.54	40.62 ± 10.50
27	5	440	7.55 ± 1.43	1 137.8 ± 294.38	100	77.5 ± 4.25	55 ± 6.67
28	3.5	960	9.74 ± 2.65	862.4 ± 183.86	100	79.5 ± 4.38	59 ± 9.66
29	0.5	500	7.51 ± 1.76	1 321.4 ± 335.10	100	77 ± 4.83	54.5 ± 7.62
30	3.5	170	8.8 ± 1.10	1 232.44 ± 263.93	100	80 ± 2.5	54.44 ± 5.83
31	4	700	10.28 ± 2.63	1 372.5 ± 458.31	100	78 ± 2.58	49.5 ± 10.39

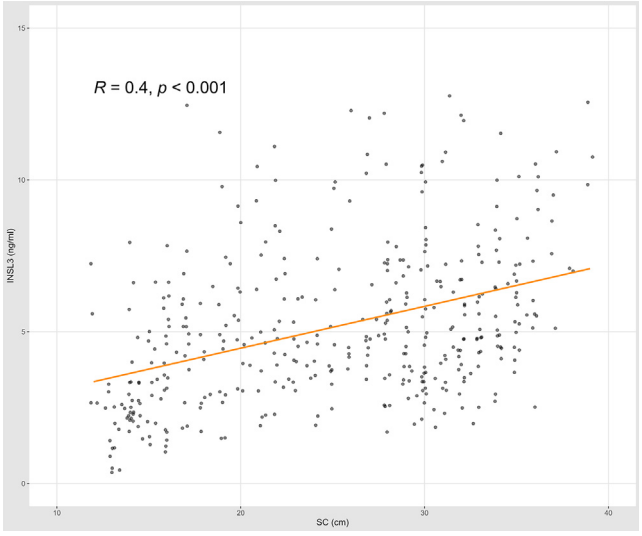

Fig. 3. Relationship between the concentration of insulin-like factor 3 (INSL3) (ng/ml) and scrotal circumference (SC) (cm) measured on the same day in pre- and peripubertal bulls. Regression line shows a moderate positive relationship between INSL3 (ng/ml) and SC (cm) R = 0.4.

did not find any significant effect of birth season or geographical area on INSL3 levels. The other research question was to measure the association between INSL3 and SC for our NR population. Previous studies have demonstrated that the SC of young HF and Japanese Black beef bull calves positively correlated with INSL3 (ng/ml) (Sakase et al., 2018; Anand-Ivell et al., 2019). In a study on young HF bull calves, the authors showed a weak positive correlation R = 0.31 between INSL3 levels at 8 months and SC at 12 months (Anand-Ivell et al., 2019). For Japanese Black beef bull calves, 1–7 months old, a correlation of R = 0.64 was found between SC and INLS3 levels measured on the same days. Our study found a moderate positive correlation R = 0.4 between INSL3 (ng/ml) levels and SC (cm) from NR bull calves from 2 to 12 months of age measured at 4-time points. However, these comparisons should be viewed with caution because of the differences in age and frequency of measurement and the predictive vs descriptive approach. This study aimed to evaluate the association between selected semen characteristics from performance testing and AI station and INSL3 or SC. We found no significant association between these parameters. It is established that young bulls have lower sperm quality and higher variability in volume and concentration between ejaculates (Murphy et al., 2018). This was the reason we also included 10 first ejaculates from the AI station in the analysis. Weerakoon et al. (2018) showed that prepubertal Japanese Black beef bulls with lower sperm motility and a high proportion of morphologically abnormal spermatozoa had decreased INSL3 concentration compared to bulls with normal semen parameters. However, they set up the specific cut-off point for the semen quality parameters and compared INSL3 between two groups, normal and abnormal, which excludes direct comparison. As Anand-Ivell et al. (2011) described, INSL3 is a foetal gender-specific hormone, with concentrations significantly increasing during mid-gestation in cows carrying a male foetus. Anand-Ivell et al. (2011) showed no breed-specific differences in maternal peripheral INSL3 levels for Angus and Brahman cows. However, foetal INSL3 levels ranged between 1 and 5 ng/ml and differed significantly between pure breed and crossbreed foetuses. The impli-

cation is that paternal genetics plays a role in determining INSL3 levels (Anand-Ivell et al., 2011). Moreover, this might be important for further research to explain the high inter-individual variability in the NR population. Research questions that could be studied include the measurement of bovine foetal INSL3 from mid-gestation until the peripubertal period ending at around 12 months to explore the source of variation. Our research showed that the NR breed exhibits a heterogeneous distribution of INSL3 in all age groups. Since the 1970s, Geno introduced broader breeding goals focused on health and fertility ('Norwegian Red breeding program', 2020). In contrast, Holstein Friesian cattle were bred predominantly for milk yield for several decades. Effective population size (a measure of the number of unrelated individuals in a population) for the NR population in the last 5 years was: 239 in 2017, 242 in 2018, 246 in 2019, 250 in 2020 and 253 in 2021. In comparison, the effective population size of Holstein cattle calculated based on the pedigree record from the Canadian Dairy Network was equal to 58 (Makanjuola et al., 2020). These differences between breeds might affect the variability in certain traits, such as INSL3. Several questions remain unanswered, and we propose including genetic parameters in further studies to confirm the paternal effect suggested by Anand-Ivell et al. (2011).

Conclusion

This research extends our knowledge about INSL3 levels in pre- and peripubertal bulls. The results of this investigation show that INSL3 in the Norwegian Red bull population exhibits high inter-individual variability, which cannot be explained by the season and location of birth and might be connected to the large effective population size of the NR cattle population. It was also shown that INSL3 is positively correlated with scrotal circumference. We conclude that high inter-individual variability in INSL3 prevents us from using INSL3 as a biomarker of sperm production onset in this breed at present. Further experimental investigations are needed to identify the source of variation.

Ethical approval

Ethical approval was not required in this study. We worked at the performance testing station under the supervision of a qualified veterinarian employed at the breeding company Geno. The performance testing station fulfils EU requirements for the housing of bulls.

Data and model availability statement

The data/models were not deposited in an official repository. The data/models that support the study findings are available from the authors upon request.

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Author contributions

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Bjørg Heringstad: Validation, Formal analysis, Data Curation, Writing - Review & Editing, Supervision

Jane M. Morrell: Conceptualization, Writing - Review & Editing, Supervision

Elisabeth Kommisrud: Conceptualization, Methodology, Investigation, Funding acquisition, Data Curation, Writing - Review & Editing, Supervision

Declaration of interest

The authors declare no conflict of interest.

Acknowledgements

We would like to thank Hallstein Holen and his team at the performance testing station in Øyer: Jan Tore Rosingholm, Erik Skogli, Sigmund Høibakken and Stein Marius Brumoen for their time and indispensable help with data collection. INSL3 was measured by the Anand-Ivell group, University of Nottingham. The authors thank Michał Maj for valuable ideas and discussions.

Financial support statement

This research received no specific grant from any funding agency, commercial or not-for-profit section.

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