Norwegian University of Life Sciences
Faculty of Biosciences
Department of Animal and Aquacultural Sciences (IHA)

Philosophiae Doctor (PhD)
Thesis 2021:62

Genetic analyses of semen characteristics in Norwegian Red

Genetiske analyser av sædkvalitets-egenskaper hos Norsk Rødt Fe

Henriette Berg Olsen

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Ås (2021)

Thesis number 2021:62
ISSN 1894-6402
ISBN 978-82-575-1836-3

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Forord

Denne studien er en del av prosjektet «Reproductive sustainability in Norwegian Red» (Prosjektnr: 255097/E50) som er finansiert av Forskningsmidlene for jordbruk og matindustri (FFL/JA). Prosjektet har som mål å bedre Storfefruktbarheten ved hjelp av avl og genetikk. Norsk Rødt Fe (NRF) utmerker seg internasjonalt som en rase med god kufruktbarhet grunnet omfattende registrering av slike egenskaper, tidlig inkludering av egenskapene i avlsmålet med betydelig vekting, samt et bredt avsmål som sikrer en bærekraftig rase med som utmerker seg på flere områder enn bare melkeproduksjon. Denne studien tar for seg oksefruktbarheten i NRF, nærmere bestemt sædkvalitet. I Norge er vi flinke til å registrere mye og arkivere det meste. Dette gjør at vi har store mengder med data, langt tilbake i tid, fra andrologitesten på Øyer teststasjon og fra kvalitetsundersøkelse av sæden foretatt på seminstasjonen på Store Ree. Disse dataene ønsket vi å benytte for å øke det kunnskapsgrunnlaget til genetikken bak oksefruktbarhet i vår populasjon, og bidra til mer litteratur på feltet internasjonalt.

Det har vært både givende og utfordrende å jobbe med egenskaper som er lite undersøkt i et genetisk perspektiv, særlig når estimatene for de ulike egenskapene peker i alle retninger avhengig av studie. En årsak til de sprikende resultatene kan være raseforskjeller og det var dermed viktig å få undersøkt disse egenskapene i vår populasjon.

Jeg ønsker å takke mine fantastiske veiledere Gunnar Klemetsdal og Bjørg Heringstad for all hjelp og støtte gjennom dette prosjektet. Takk for at dere har motivert når ting har vært vanskelig og gitt meg en push når det trengs. Takk for at dere har vært tilgjengelig for alle spørsmål, både dumme og mindre dumme. Takk til Gunnar for alle samtalene vi har hatt på kveldstid på IHA når alle andre har gått hjem, jeg har lært så mye av deg! Takk til Bjørg for det flotte og tålmodige menneske du er. Din stemme som sier «Er dette

egentlig relevant?» eller «dette er overflødig» kommer til å følge meg for alltid og har gjort meg til en bedre artikkelforfatter.

Takk til Geno for tilgang til data og en spesiell takk til Eiliv Kummen ved Øyer teststasjon og Simon Reisvaag, Ragnhild Gjeset og Sigbjørn Karlsen ved seminstasjonen på Store Ree for faglig input rundt seminuttaket og egenskapene som registreres.

Takk til alle kolleger på IHA, fra kantinedama til Instituttlederen. Takk spesielt til de andre stipendiatene som jeg både kunne le og være frustrert sammen med. Takk til Nini for alle de gode samtalene vi har hatt og takk til mine nye kolleger ved NSG som gir meg utfordringer og muligheter til å utvikle meg videre som forsker.

Til slutt vil jeg takke familien min, samboeren min, vennene mine, og ikke minst de firbeinte: Pelspølsa og Mr. Loffesen. Dere står ved min side i tykt og tynt og gjør meg til et bedre og lykkeligere menneske. Takk!

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1 Abbreviations and definitions

AI: Artificial insemination
BBSE: Bull breeding soundness evaluation
BW: Body weight
Bw_150d: Body weight when 150 days old
Bw_330d: Body weight when 330 days old
CASA: Computer assisted sperm analysis
Conc: Sperm concentration
Conc1: Sperm concentration categorized into 10 categories
Conc2: Sperm concentration recorded and given on a continuous scale
Dwg: Average daily weight gain between 150 and 330 days of age
EBV: Estimated breeding value
GEBV: Genomic breeding value
GS: Genomic selection.
GWAS: Genome-wide association study
Mot0h: Motility in fresh semen
Mot24h: Motility after storing in a refrigerator for 24 hours
Mot48h: Motility after storing the semen in a refrigerator for 48 hours
Mot_pre: Motility score before cryopreservation
Mot_post: Motility score after cryopreservation
Mot%pre: Percentage motility before cryopreservation
Mot%post: Percentage motility after cryopreservation
Mot%change: Difference between mot%pre and mot%post
NR: Norwegian Red
N_straw: Number of accepted straws from the semen collection
ROH: Runs of homozygosity
SNP: Single nucleotide polymorphism
QTL: Quantitative trait locus

2 List of papers

Paper I.
Olsen, H. B., Heringstad, B., & Klemetsdal, G. (2020a). Genetic analysis of semen characteristic traits in young Norwegian Red bulls. Journal of Dairy Science, 103(1), 545–555. https://doi.org/10.3168/jds.2019-17291.

Paper II:
Olsen, H. B., Heringstad, B., & Klemetsdal, G. (2020b). Genetic correlations between body weight, daily weight gain, and semen characteristic traits in young Norwegian Red bulls. Journal of Dairy Science, 103(7), 6311–6317. https://doi.org/10.3168/jds.2019-18116.

Paper III:
Olsen, H. B., Heringstad, B., & Klemetsdal, G. (2021). Genetic analysis of semen characteristic traits in Norwegian Red bulls at the artificial insemination center. Journal of Dairy Science (in press). https://doi.org/10.3168/jds.2020-19294.

3 Abstract

The research on the genetics of bull fertility is limited, many studies are old, estimates are often based on few observations or few bulls, and with large or lacking standard errors. With more than 20 years of data on semen characteristics available from Norwegian Red (NR) bulls at the performance test station and the AI center, an opportunity existed to increase our knowledge of these traits both in a local and global perspective. With cow fertility improving in many cattle populations, further progress in cattle fertility might be achieved on the bull side. Furthermore, new technologies such as sexed semen is becoming increasingly popular, and bulls that produce a larger amount of semen with the higher quality required for such methods might be more desired by breeding companies in the future.

In the NR population, bull fertility is assessed first in a bull breeding soundness evaluation at the test station and later during quality assessment of semen at the artificial insemination (AI) center. During performance testing, the bulls body weight at 150 and 330 days and average growth were also recorded. Using these data, the main aims of this project were: 1) To estimate genetic parameters and genetic trends for semen characteristics from NR bulls at the performance test station, 2) To estimate genetic correlations between semen characteristics and body weights and growth measured on NR bulls at the test station, and 3) To estimate genetic parameters for semen characteristics of bulls at the AI center, and to estimate genetic correlations between these traits and corresponding semen characteristics measured at the performance test station.

The semen characteristics data from the test station consisted of 14,972 ejaculates from 3,927 young Norwegian Red bulls. The traits recorded were semen volume, sperm concentration, motility in fresh samples, and after storing the semen in a refrigerator for 24 and 48 hours, and sperm defects. Body weight and growth traits were available for 2,034 of these bulls. From

the AI center, data from 137,919 ejaculates from 3,145 bulls were analysed including records on semen weight, sperm concentration, motility before and after cryopreservation, motility change during cryopreservation, and number of accepted straws made from the semen collection.

Results show that all the semen characteristic traits are heritable with moderate heritability for amount of semen (volume or weight), sperm concentration and number of straws produced from each collection (0.14 to 0.22), and low heritability for motility traits (0.02 to 0.08) and sperm defects (0.02). Heritabilities for traits measured on bulls at the AI center were generally higher than the corresponding traits measured on more immature and unexperienced bulls at the test station. Most semen characteristic traits were favorably genetically correlated, or had a genetic correlation close to zero, except for a negative genetic correlation between semen weight and sperm concentration for bulls at the AI center. All test station traits showed a slightly unfavorable genetic trend between 1994 and 2016 implying that the andrology testing and the minimum semen quality requirements before selection of AI bulls have not been sufficient to genetically improve semen characteristics over time. Body weight was favorably genetically correlated with all semen characteristic traits, while average daily weight gain was unfavorably genetically correlated with sperm concentration (-0.25 ± 0,15) and motility in fresh samples (-0.53 ± 0.34). Genetic correlations between corresponding traits at the test station and AI center were high for semen volume/weight (0.83) and concentration (0.78), and somewhat lower for motility in fresh samples (0.49).

To reverse the unfavorable genetic trends and breed for bulls with better sperm quality we suggest including semen characteristics traits in the routine genetic evaluation of NR. Genomic breeding values for these traits can be used for selection of bull calves and will also provide a means for monitoring bull fertility in the population. Estimating genetic correlations between semen characteristics and other traits in the total merit index of Norwegian Red should be prioritized. Such correlations are largely lacking in the literature and might explain the negative trends observed.

4 Sammendrag

Genetiske studier av oksefruktbarhet er i fåtall og de studiene som finnes er ofte gamle, estimatene er basert på få observasjoner eller få dyr, eller standardfeilen på estimatene er store eller mangler. Med mer enn 20 år med sædkvalitetsdata, innhentet fra test- og seminstasjon til Norsk Rødt Fe (NRF), så vi en mulighet for å øke kunnskapsgrunnlaget for disse egenskapene både i et lokalt og globalt perspektiv. Nå som kufruktbarheten bedres i storfepopulasjoner verden rundt, kan en økning i oksefruktbarhet ha større betydning. Videre øker interessen for nye teknologier og metoder slik som kjønnsseparert sæd. Dette kan gi ytterligere behov for okser som produserer en god mengde sæd med god kvalitet i fremtiden.

I NRF populasjonen undersøkes sædkvalitet først ved en andrologitest på teststasjonen og senere ved kvalitetssjekk av sæden på seminstasjonen. I tillegg til andrologitesten, veies oksene på teststasjonen ved 150 og 330 dager og den gjennomsnittlige tilveksten registreres. Ved hjelp av disse dataene ønsket vi og: 1) Estimere genetiske parametere og genetiske trender for sædkvalitetsegenskaper målt på okser på teststasjonen, 2) Estimere genetiske korrelasjoner mellom sædkvalitetsegenskaper og vekt og tilvekst målt på okser på teststasjonen og 3) Estimere genetiske parametere for sædkvalitetsegenskaper målt på okser på seminstasjonen, samt estimere genetiske korrelasjoner mellom disse egenskapene og korresponderende egenskaper fra teststasjonen.

Sædkvalitetsdata fra teststasjonen bestod av 14,972 ejakulater fra 3,927 okser. Egenskapene som ble målt var sædvolum, sædkonsentrasjon, motilitet i fersk prøve, samt motilitet etter lagring i kjøleskap i henholdsvis 24 og 48 timer, og spermiedefekter. Registreringer av kroppsvekt og gjennomsnittlig tilvekst var tilgjengelig for 2,034 av disse oksene. Fra seminstasjonen hadde vi data fra 137,919 ejakulater fra 3,145 okser. Disse inkluderte registreringer av sædvekt, sædkonsentrasjon, motilitet før og

etter kryopreservering, endring i motilitet under kryopreservering og antall aksepterte sædstrå fra uttaket.

Resultatene viser at sædkvalitetsegenskapene er arvelige med moderate arvegrader for mengde (volum og vekt), konsentrasjon og antall aksepterte strå (0.14 til 0.22), og lav for motilitet (0.02 til 0.08) og spermiedefekter (0.02). Arvegradene for egenskapene målt på seminstasjonen var generelt høyere enn for korresponderende egenskaper målt på de mer umodne og uerfarne oksene på teststasjonen. De fleste sædkvalitetsegenskapene var fordelaktig genetisk korrelert eller hadde en genetisk korrelasjon nær null. Unntaket var en negativ genetisk korrelasjon mellom sædvekt og konsentrasjon for okser på seminstasjonen. Alle sædkvalitetsegenskapene målt på teststasjonen har hatt en uønsket genetisk trend mellom 1994 og 2016. Dette indikerer at andrologitesten og minimumskravene til sædkvalitet før seleksjon av seminokser ikke har vært tilstrekkelig for å øke oksefruktbarheten over tid. Kroppsvekt var positivt korrelert med sædkvalitet, mens gjennomsnittlig tilvekst hadde en uønsket, genetisk korrelasjon til sædkonsentrasjon (-0.25 ± 0,15) og motilitet i fersk prøve (-0.53 ± 0.34). Genetiske korrelasjoner mellom korresponderende egenskaper på test- og seminstasjonen var høy for volum/vekt (0.83) og konsentrasjon (0.78) og noe lavere for motilitet i fersk prøve (0.49).

Vi foreslår å inkludere sædkvalitetsegenskaper i den rutinemessige genetiske evalueringen av NRF for å kunne reversere de uønskede genetiske trendene og avle for okser med bedre sædkvalitet. Genomiske avlsverdier for disse egenskapene kan brukes for å selektere oksekalver, i tillegg til å kunne brukes for å overvåke oksefruktbarheten i populasjonen fremover. Det bør prioriteres å estimere genetiske korrelasjoner mellom sædkvalitet og andre egenskaper i det overordnede avlsmålet til NRF. Slike korrelasjoner mangler i litteraturen og kan være noe av forklaringen for de negative genetiske trendene som her ble observert for sædkvalitetsegenskaper.

5 Synopsis

5.1 Introduction

5.1.1 Fertility in dairy cattle

The biological definition of fertility is the ability to produce offspring, an ability that is fundamental to breeding and continued animal production. A successful outcome of a mating or insemination depends on the fertility of both the cow and the bull, and fertility traits are used to describe the reproductive ability of either gender. Much research has been performed on the genetics of cow fertility (Berry et al., 2014), and cow fertility traits are now included in the total merit index of many dairy populations. The genetics of bull fertility, however, has received considerably less attention. Through artificial insemination (AI) a few high-ranking bulls can sire many calves each. These bulls must produce functional sperm cells with good movement (motility) that can swim through the female reproductive tract and fertilize the egg. Cryopreservation is used to prolong the durability of the semen; hence, the sperm cells must tolerate freezing. To ensure that future AI sires have sufficient semen quality, many breeding organizations perform a bull breeding soundness evaluation (BBSE) on the potential candidates which includes an assessment of the bull's libido as well as examination of their semen. To maximize the chance of a successful insemination, quality testing of the semen is also performed at the AI center. Two sources of semen characteristic measurements can thereby be available for research, and below is a description of the most commonly measured traits, and how they are recorded:

- **Semen amount (volume)**: Usually measured directly from the measurement cup and given in mL. Alternatively, the semen is weighed, and the amount is given in grams.
- **Sperm concentration**: Measured by a spectrophotometer and given in number of spermatozoa in a mL.
- **Sperm motility**: Motility is a measurement of movement or swimming ability of the sperm cells, and progressive motility is the fraction of sperm cells that moves in a relatively straight line. Motility is commonly measured by visual inspection using a microscope and given either as a percentage of the sperm cells that

swims, or as a score on a scale, e.g. from 1 to 5 where 1 is poor and 5 is excellent. The range and increments of the scale differ among studies. In addition to subjective microscope evaluation, motility can also be measured by computer assisted sperm analysis (CASA). CASA systems capture multitude images of the sperm cells at 50 to 60 frames per second to provide detailed, unbiased evaluation of the swimming ability of the sperm (Amann and Waberski, 2014).

- **Sperm defects**: Sperm defects include morphological abnormalities such as loose heads, tail defects, abnormal intermediate part, or proximal and distal droplets (Kealey et al., 2006; Druet et al., 2009). Some studies categorize sperm abnormalities, while others only measure total defects. Sperm defects are commonly measured under a phase contrast microscope and usually given as the percentage of sperm cells with a defect (or percentage that is considered normal). Sperm defects can also be measured by CASA.

Since dairy cattle breeding organizations use frozen semen for prolonged durability during storing and transportation, some studies have also examined semen characteristics after cryopreservation (Ducrocq and Humblot, 1995; Karoui et al., 2011; Berry et al., 2019). Sperm motility and defects are typically examined after freezing and thawing to ensure the sperm's ability to tolerate the procedure.

Semen characteristics have been found to be affected by several environmental and management factors. Below is a list of the most important factors that have been shown to influence semen characteristics:

- **Age of the bull:** The effect of age on various semen characteristics have been explored in many studies, and findings generally show increased semen volume with increasing age (Karoui et al., 2011; Al-Kanaan et al., 2015; Berry et al., 2019) as semen volume will increase with increasing size of the scrotum. Semen concentration and motility also increases with age, but reaches an maximum earlier, when the bulls around 20-25 months (Al-Kanaan et al., 2015; Berry et al., 2019;Mathevon et al., 1998b).
- **Season of collection:** Season can include many factors such as temperature, length of daylight, humidity, and feed quality. How, and if, the semen characteristics are affected will therefore depend on the location. This may be why Everett et al. (1978) found sperm output to be highest during spring and lowest in the winter, while Mathevon et al. (1998b) generally found higher production during winter, and Brito et al. (2002) did not find a significant difference.

- **Management:** Management includes several factors that can significantly affect semen characteristics such as bull handler and bull preparation routines (Chenoweth, 1983), lab technicians, and equipment used. Furthermore, the ejaculate number and interval between collections have been found to influence the semen characteristics. Increasing semen volume with longer interval between collections is well documented; Mathevon et al. (1998a)Fuerst-Waltl et al. (2006), and Al-Kanaan et al. (2015) all reported the highest volume with the longest interval between collections.

5.1.2 Heritability of semen characteristics

The data used in studies that have estimated genetic parameters for semen characteristics are typically collected from either the BBSE or quality testing at the AI center, and trait definitions and how the traits are recorded vary among breeding organizations and studies. The heritability of semen characteristics varies considerably both between and within traits because of differences in population and breed, maturity of the bulls, how the traits are recorded and defined, and statistical modelling and sample size.

Table 1 aims to review heritability estimates of the most common semen characteristic traits, the age of bulls in each study as well as the number of observations and number of bulls in the data.

Table 1. Heritability estimates with standard error (SE) or confidence interval [CI] of semen volume, sperm concentration, motility and sperm defects (alternatively fraction of normal spermatozoa) as found in the literature. Authors, number of records[1], number of bulls[1], and the bulls age and breed for each study is also provided.

Authors	No. records	No. bulls	Age	Breed	Heritability (SE) or [CI]			
					Volume	Concentration	Motility	Defects
Ducrocq and Humblot (1995)[2]	2,387	1,957	12–15 mo	Normande	0.65 (0.09)	0.37 (0.09)	0.23 (0.08)	0.19 (0.07)
Mathevon et al. (1998a)	6,656	602	10–30 mo	Montbéliard	0.08	0.32		
Mathevon et al. (1998a)	9,726	241	13–84 mo	Montbéliard	0.49	0.08		
Mathevon et al. (1998b)	5,644	137	< 30 mo	Holstein	0.24	0.52	0.31	
Mathevon et al. (1998b)	2,023	61	4–6 yr	Holstein	0.44	0.36	0.01	
Kealey et al. (2006)	841	841	Mean 446 d	Hereford	0.09 (0.08)	0.16 (0.08)	0.22 (0.09)	0.35 (0.10)
Gredler et al. (2007)	12,746	301	Mean 3.4 yr	Simmental	0.18 (0.02)	0.14 (0.04)	0.04 (0.01)	
Druet et al. (2009)	4,686	515	12–18 mo	Holstein	0.22 (0.05)	0.19 (0.05)	0.43 (0.08)	
Karoui et al. (2011)	42,284	502	12–138 mo	Holstein	0.22 [0.13, 0.32]	0.19 [0.07, 0.28]	0.09 [0.03, 0.18]	0.25 (0.10)
Al-Kanaan et al. (2015)[3]	10,341	562	10-136 mo	Holstein	~0.05 – 0.16	~0.11 – 0.25	~0.06 - ~0.30	
Sarakul et al. (2018)	13,535	131	18–108 mo	Holstein crossbred	0.12 (0.05)		0.24 (0.08)	
Berry et al. (2019)	36,127	794	10–168 mo	Several	0.20 (0.04)	0.20 (0.07)	0.37 (0.03)	

[1] Numbers of records and bulls might vary depending on trait within the study.
[2] Mean of 11 semen collections used for estimation of heritability.
[3] Heritabilities measured over a heat-stress gradient.

Table 1 shows that the heritability of semen characteristics varied largely both within and between traits. The number of bulls included in the studies ranged from 61 to 1,957, and the mean age of the bulls ranged from 12 months to 6 years. Some of the heritability estimates lack information about standard error or confidence interval.

Berry et al. (2014) performed a meta-analysis of the most commonly used reproductive traits and found that sperm volume and concentration were moderately heritable (0.20 and 0.17, respectively), while the heritability of motility was low (0.05). A few studies have estimated the heritability of motility after cryopreservation and obtained estimates ranging from 0.13 to 0.24 (Ducrocq and Humblot, 1995; Karoui et al., 2011; Berry et al., 2019).

5.1.3 Genetic correlations between semen characteristics

A few studies have estimated genetic correlations among semen characteristics, shown in Table 2. Similar to the heritability estimates, genetic correlations vary largely between studies and traits.

Table 2. Genetic correlation estimates, with standard error in parenthesis (SE) or confidence interval in brackets [CI], between the semen characteristics; volume, concentration, motility before and after cryopreservation, and sperm defects (or percentage of normal sperm).

	Concentration	Motility pre	Motility post	Defects	Reference
Volume	-0.43 (0.15)	-0.17 (0.21)	-0.26 (0.20)	-0.26 (0.24)	Ducrocq and Humblot (1995)
		-0.38		-0.32[1]	Kealey et al. (2006)
	0.06 (0.13)	0.21 (0.17)			Gredler et al. (2007)
	-0.55 (0.18)	-0.20 (0.19)		0.23 (0.26)	Druet et al. (2009)
	-0.13 [-0.47, 0.18]	0.07 [-0.41, 0.53]	0.13 [-0.24, 0.50]		Karoui et al. (2011)
		0.04 (0.38)			Sarakul et al. (2018)
	-0.40 (0.20)	0.07 (0.06)	-0.30 (0.25)		Berry et al. (2019)
Concentration		0.67 (0.13)	0.55 (0.16)	-0.27 (0.24)	Ducrocq and Humblot (1995)
		0.81		-0.36[1]	Kealey et al. (2006)
		0.48 (0.17)			Gredler et al. (2007)
		0.12 (0.20)		-0.34 (0.24)	Druet et al. (2009)
		0.54 [0.17, 0.89]	0.38 [0.02, 0.78]		Karoui et al. (2011)
		0.29 (0.04)	0.56 (0.25)		Berry et al. (2019)
Motility pre			0.81 (0.09)	-0.68 (0.16)	Ducrocq and Humblot (1995)
				-0.51[1]	Kealey et al. (2006)
				-0.55 (0.19)	Druet et al. (2009)
			0.87 [0.74, 0.97]		Karoui et al. (2011)
			0.92 (0.03)		Berry et al. (2019)
Motility post				-0.43 (0.22)	Ducrocq and Humblot (1995)

[1] The sign has been reversed in the table because fraction of normal sperm cells was considered in the analysis.

Overall, the genetic correlation between volume and concentration were negative, while the genetic correlation estimates between concentration and motility (before and after cryopreservation) were moderately to highly positive. The genetic correlations between volume and motility, however, were close to zero, while sperm defects appear to be favorably genetically correlated with all semen characteristic traits. Motility before and after cryopreservation were highly genetically correlated.

The meta-analysis by Berry et al. (2014) also found that greater sperm concentration was genetically associated with higher sperm motility, and only weakly associated with volume. In that study, percentage of normal sperm (an alternative to sperm defects) was also favorably genetically correlated with sperm concentration and motility.

5.1.4 From Norwegian Red bull calf to AI bull

Around 8,000 of the most promising Norwegian Red bull calves that are born each year are genotyped and gets genomic breeding values. Of these, the 150 calves with the best total merit index and pedigree are bought by Geno and brought to the performance test station where their conformation, growth, temperament, and bull fertility are assessed. After testing, 50 to 60 of the bulls are each year selected for semen production at the AI center. Today, this selection is based predominantly on their total merit index, and the performance test station is now mainly a place to keep bulls until they are old enough for semen production and to train them in the procedure. Nevertheless, the testing gives an assessment of the bull calves general health, temperament etc., and bulls can be excluded if not meeting certain criteria with regards to these traits. Furthermore, the assessment of libido and semen characteristics are still used to detect bulls that are not suitable for breeding because of poor fertility, but to a lower extent than before introducing genomic selection.

Before the introduction of genomic selection in 2013, more bulls (~ 300 per year) were selected for performance testing based on pedigree and breeding values. The dam's conformation, health history, and milk production were also taken into account when selecting. Results from the phenotypic evaluation were used to select the top ~ 120 of the bulls to be used as test bulls in progeny testing. Of these, ~ 10 to 12 were selected and used as elite bulls.

5.1.5 Purpose, hypotheses and aims of study

The research on the genetics of bull fertility is limited, many studies are old, and the estimates of genetic parameters were based on few observations or bulls, and standard errors were often large or lacking. Before this current project and thesis, genetic parameters for bull fertility in Norwegian Red had not been published. With a large amount of data, going back to 1994, there was a great opportunity to estimate precise genetic parameters for semen characteristics in the Norwegian Red population, and add knowledge to the limited literature. The data from the performance test station was particularly interesting since the bulls were not selected for semen characteristics prior to testing. Based on the available literature, we hypothesized that the semen characteristics were heritable, and genetically correlated, but to a variable extent. Genetic correlations between semen characteristics and other important traits are generally lacking in the literature, and a good place to start was to hypothesize the existence of a genetic correlation between the semen characteristic traits and the bulls body weight and daily weight gain, also measured at the performance test station. In addition to estimating these genetic parameters, also of interest was to examine whether the BBSE, carried out on the performance test station, was a good indicator for the semen production at the AI center; more precisely, it was tested whether the traits measured were genetically the same at the two stations. More than 20 years of test-bull data also made

it possible to estimate genetic trends for semen characteristics to test whether they were significantly different from zero.

To summarize, the specific aims were:

1. To estimate genetic parameters and genetic trend for semen characteristics in young Norwegian Red bulls at the performance test station.
2. To estimate genetic correlations among body weight traits, daily weight gain, and semen characteristic traits for young Norwegian Red bulls.
3. To estimate genetic parameters for semen characteristics of AI bulls, estimate genetic correlations between these traits and andrology traits measured at the performance test station, and to calculate genetic change of bull fertility for Norwegian Red bulls in semen production.

5.2 Data Material

Data on semen characteristics from both the performance test station at Øyer and the AI center at Store Ree was provided by Geno. The first and second paper used data from the performance test station, and the third used data from the performance test station and the AI center. A description of the data and the traits analyzed are given in Table 3. The number of observations and number of bulls varied among traits, and the table shows the range.

Table 3. Description of data used in papers I, II and III.

Data source	Performance test station	Performance test station	AI center
Traits	**Semen characteristics;** Semen volume, sperm concentration, motility in fresh semen, motility after storing for 24 and 48 hours, and sperm defects.	**Weight and growth traits;** Body weights at 150 and 330 days of age and average daily weight in between.	**Semen characteristics;** Semen weight, sperm concentration, motility score before cryopreservation, percent motility before cryopreservation, motility score after cryopreservation, percent motility after cryopreservation, and number of accepted straws.
No. records	2,844 - 14,972	3,209	84,246 - 137,772
No. bulls	899 - 3,972	3,209	3,104 - 3,143
Years	1994 – 2016	2002 – 2012	1994 - 2020
Used in	Papers I, II and III	Paper II	Paper III

5.3 Papers

Paper I

Olsen, H. B., Heringstad, B., & Klemetsdal, G. (2020a)

Genetic analysis of semen characteristic traits in young Norwegian Red bulls

Journal of Dairy Science, 103(1), 545–555.

J. Dairy Sci. 103:545–555
https://doi.org/10.3168/jds.2019-17291

Genetic analysis of semen characteristic traits in young Norwegian Red bulls

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ABSTRACT

The aim of this study was to estimate genetic parameters and genetic trends for male fertility in Norwegian Red bulls. We analyzed data on semen characteristics traits collected at the performance test station of young bulls from 1994 to 2016, in an andrology test used to ensure acceptable semen quality before being selected as an artificial insemination bull. Traits included were volume, concentration, and motility (percentage of moving sperm cells) in fresh samples and after storing for 24 and 48 h, and sperm defects. The data consisted of 14,972 ejaculates from 3,927 young (11–15 mo) Norwegian Red bulls. Genetic parameters were estimated using bivariate linear animal models that included age in months, group-year, and collection-group (main effect of the interaction between ejaculate number and interval between collections) as fixed effects, and test-day and additive genetic and permanent environment effect of the bull as random effects. Considerable genetic coefficients of variation were found for concentration and volume, with lower values for motility. Estimated heritabilities ranged from 0.02 and 0.03 (for sperm defects and motility in fresh samples) to 0.14 (volume and concentration measured on a continuous scale). All estimated genetic correlations were favorable, but the genetic correlations between volume and concentration and volume and sperm defects were not significantly different from zero. The genetic correlations between concentration and motility traits ranged from 0.53 to 0.83, and those between volume and the motility traits were between 0.24 and 0.57. All traits showed a slightly unfavorable genetic trend. Our results indicate that selection of bulls with better sperm quality is possible.
Key words: andrology, genetic variation, heritability, genetic correlation, genetic trend

INTRODUCTION

In dairy, most focus has been given to female fertility, whereas male fertility has received much less attention. Male fertility refers to the behavior and libido of the bull, such as their eagerness to mount, as well as traits that describe the amount and quality of the semen they produce. Semen volume is made up of the sperm cells and the liquid that surrounds them. This liquid contains sugars and proteins and is an energy source for sperm cells on their journey through the female reproductive tract. To ensure gestation, millions of spermatozoa are released during ejaculation to ensure that one will reach and fertilize the egg, giving an advantage to high-quality semen: ejaculates with a high concentration of sperm cells without defects and good overall motility (movement).

Heritability estimates of semen characteristics vary considerably both between and within traits. The varying results within traits may be due to factors such as differences in sample size, statistical modeling, population or breed, age and maturity of bulls, as well as how the traits are recorded and defined. Berry et al. (2014) performed a meta-analysis of male reproductive performance in dairy and beef cattle using results from 25 studies. Heritability estimates in the review ranged from 0.04 to 0.65 for volume, 0.10 to 0.56 for concentration, 0.01 to 0.51 for motility, and 0.07 to 0.35 for sperm abnormalities. The pooled mean heritability estimates obtained in the meta-analysis were moderate for volume (0.197), concentration (0.169), and sperm abnormalities (0.194), and low for motility (0.054). Later, Al-Kanaan et al. (2015) used data from an AI station (562 Holstein bulls, 10,341 records) to estimate genetic parameters for semen characteristic traits along a temperature and humidity gradient using a linear random regression model and obtained the following maximum heritability estimates: 0.18 for volume, 0.27 for concentration, and 0.29 for motility. With a similar data basis (787 bulls from 16 different breeds, 35,573 records), Berry et al. (2019) obtained heritability estimates of 0.20 for volume and concentration and 0.37 for motility.

Received July 18, 2019.
Accepted August 27, 2019.
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Berry et al. (2014) also reviewed genetic correlation estimates between semen characteristics, but references were few. The pooled mean estimates (standard errors, SE) between volume and concentration, volume and motility, and concentration and motility were −0.16 (0.10), 0.06 (0.13), and 0.61 (0.10), respectively. Further, motility correlated highly positive with a larger number of normal sperm cells in the sample: 0.87 (0.08).

For Norwegian Red, sperm quality is assessed for the first time at the performance test station when the bulls are around 12 mo old. Here, the libido of the young bulls is tested (mounting, propulsion, and protrusion), and several andrology traits are assessed to ensure acceptable semen quality. By achieving at least 3 out of 5 in an overall grade based on results from the andrology traits, bulls pass this test and may become an AI bull. Andrology test data from 1994 onward were available from the performance test station, and our aim was to use these data to estimate genetic parameters and genetic trend for semen characteristics in young Norwegian Red bulls.

MATERIALS AND METHODS

Data and Traits

Data on 6 semen characteristics traits (volume, concentration, motility, motility after storing for 24 and 48 h, and sperm defects), which were routinely collected at the performance test station of young Norwegian Red bulls from 1994 to 2016, were made available by Geno Breeding and AI Association (Hamar, Norway). After a performance test on growth and conformation, promising bulls had their semen inspected to ensure acceptable semen quality before being selected as an AI bull (Geno, 2014).

Andrology testing was initiated by bulls being taken to another pen with other bulls to mount and become aroused. When a bull mounted within the pen, he was considered ready for semen collection. A phantom was tried first, while another bull was made available if this was not successful. Semen was collected in an artificial sheath when the bull had a satisfying erection. Average age at this test was 12 mo.

The collected ejaculates were stored at 38°C and analyzed within 2 h by trained technicians. Semen volume was measured directly from a scaled tube, and concentration was measured by using a spectrophotometer. Morphology of the sample was determined by visual inspection under a phase contrast microscope (magnification 100 to 400×). Morphological abnormalities such as loose heads, tail defects, abnormal intermediate part, proximal droplets, and distal droplets were recorded if more than approximately 10% of a particular defect was detected in a sample. Because the frequency of collections with a recorded defect was low, we defined a binary trait as 1 if any defect was registered and 0 otherwise.

The phase contrast microscope was also used to assess the motility of the sample, a measurement of a sperm cell's ability to swim. Motility was assessed subjectively and given as the percentage of moving sperm cells, with 10% increments starting at 0. After first inspection, samples were stored in a refrigerator until they were reactivated (heated to 38°C in 5 min) and evaluated for motility again after 24 h and 48 h. Because of the workload of the technicians, not all fresh samples were reevaluated after 24 or 48 h.

Some changes in the management routines and laboratory equipment occurred during the period of data collection. Before the year 2000, bulls were kept in individual stalls instead of pens of 12 to 18 animals. Further, the spectrophotometer was replaced in March 2013. Until this date, the photometer used could not register concentrations $<390 \times 10^6$ spermatozoa (**spz**)/mL. Therefore, in this period, the concentration was set to 390×10^6 if the photometer showed 0 but sperm cells were found during microscope evaluation. Consequently, concentration was defined as 2 traits, before and after March 2013 (**conc1** and **conc2**); conc2 was as recorded with the higher-resolution photometer, and conc1 was categorized into 10 classes: 0, 1–390, then in intervals of 200, and finally $>1{,}790 \times 10^6$ spz/mL.

The raw data included 16,780 semen collections. We required volume to be >0 to consider the other traits possible to score. Ejaculates with volume >12 mL or concentration $>3{,}000 \times 10^6$ spz/mL were considered erroneous and removed (mean plus 6 and 8 times the standard deviation for volume and concentration, respectively). Further, 1,169 observations were duplicates and therefore removed.

Only bulls with information on group number and group year were kept. Group number and group year define the group and the year that bulls were sent off the station (either for slaughter or to the AI center). Finally, the analysis was carried out with bulls aged 320 to 473 d (10.5–15.5 mo) on the day of testing.

The final data set contained 14,972 semen collections from 3,927 bulls, with information on one or more of the following traits: volume, conc1, conc2, motility in fresh samples (**mot0h**), motility after storing for 24 h (**mot24h**), and 48 h (**mot48h**), and sperm defects. The pedigree was traced back 4 generations and included 27,437 animals.

The number of andrology-tested bulls varied over time as shown in Figure 1A, with an overall average of 171 per year. The number of observations per bulls ranged from 1 to 11, and the mean varied over time

as shown in Figure 1B. The number of observations per trait is given together with descriptive statistics in Table 1.

Models

Initially, the GLM procedure in SAS (version 9.4; SAS Institute Inc., Cary NC) was used to test whether group-year (1, ..., 131), age in months (10, ..., 15), and collection_n-interval (a fixed effect of combinations of ejaculate number (1 = first semen collection to 6 = sixth or later collection) and number of days since previous collection (1 = 1–4 d, 2 = 5–10 d, and 3 = >10 d) had a significant effect ($P \leq 0.05$) on andrology traits. Group-year and age were significant for all traits, whereas collection_n-interval affected all traits except mot48h. Collection_n-interval was therefore not included in the model when estimating variance components for mot48h.

Estimation of (co)variance components were performed running bivariate linear animal models in DMU using the average information (AI)REML procedure (Madsen and Jensen, 2013). Standard errors of heritability and repeatability were calculated based on Taylor series expansion. Results from bivariate analyses (volume and each of the other traits) were used to estimate heritability and repeatability (formulas in Table 2 and 3), and bivariate models for each trait combination were used to estimate correlations between the semen characteristic traits. The following model was used:

$$\begin{bmatrix}\mathbf{y}_1\\ \mathbf{y}_2\end{bmatrix}=\begin{bmatrix}\mathbf{X}_1 & 0\\ 0 & \mathbf{X}_2\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\ \mathbf{b}_2\end{bmatrix}+\begin{bmatrix}\mathbf{Zt}_1 & 0\\ 0 & \mathbf{Zt}_2\end{bmatrix}\begin{bmatrix}\mathbf{t}_1\\ \mathbf{t}_2\end{bmatrix}+\begin{bmatrix}\mathbf{Za}_1 & 0\\ 0 & \mathbf{Za}_1\end{bmatrix}\begin{bmatrix}\mathbf{a}_1\\ \mathbf{a}_2\end{bmatrix}+\begin{bmatrix}\mathbf{Zp}_1 & 0\\ 0 & \mathbf{Zp}_2\end{bmatrix}\begin{bmatrix}\mathbf{p}_1\\ \mathbf{p}_2\end{bmatrix}+\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix},$$

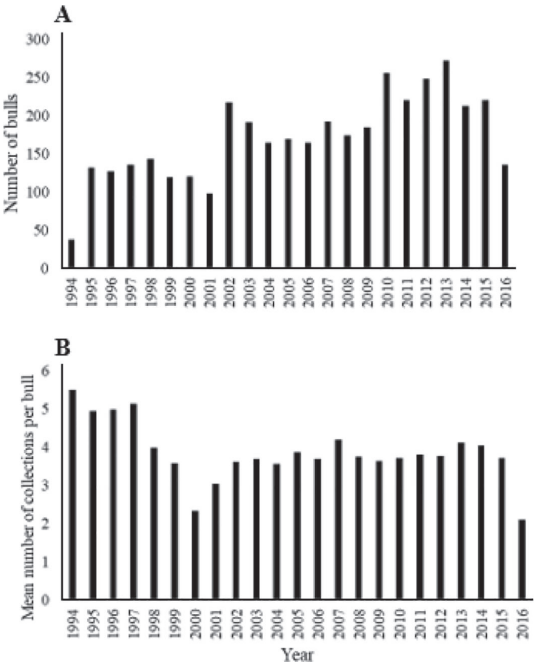

Figure 1. (A) Number of andrology tested bulls per year, and (B) average number of semen collections per bull from 1994 to 2016. First year of testing was used if the bulls were tested over 2 yr.

where $\mathbf{y}_1$ and $\mathbf{y}_2$ are vectors of observations for the 2 semen characteristic traits; $\mathbf{b}_1$ and $\mathbf{b}_2$ are vectors of the fixed effects for the 2 traits, including group-year, age, and collection_n_interval (the latter being excluded in the model for mot48h); $\mathbf{t}_1$ and $\mathbf{t}_2$ are vectors of the

Table 1. Descriptive statistics for the 7 semen characteristics traits

Item	n	Mean	SD	Median	Minimum	Maximum
Volume (mL)	14,963	2.6	1.5	2.5	0.5	12
Conc1[1]	12,108	2.9	1.8	3	0	9
Conc2[2]	2,844	475.3	306	440	0	1,745
Mot0h[3] (%)	14,563	64.9	21.3	70	0	80
Mot24h[3] (%)	10,035	61.2	19	70	0	80
Mot48h[3] (%)	4,024	55.5	20.3	60	0	80
Defects[4]	14,972	0.04	0.18	0	0	1

[1]Concentration recorded before March 2013 and categorized into 10 classes [0, 1–390, then increments of 200, and finally >1,790 × 10^6 spermatozoa (spz)/mL].

[2]Concentration recorded after March 2013 given as 10^6 spz/mL.

[3]Motility in fresh samples (0 h) and after storing for 24 and 48 h.

[4]Binary trait: scored as 1 if >10% of a particular sperm defect was present in the sample, and 0 otherwise. Defects were loose heads, abnormalities in the tail or the intermediate part, distal droplets, and proximal droplets.

Table 2. Additive genetic $\left(\sigma_a^2\right)$, permanent environmental $\left(\sigma_{pe}^2\right)$, test-day $\left(\sigma_{test\text{-}day}^2\right)$, and residual $\left(\sigma_e^2\right)$ variance components of the 7 semen characteristics traits together with their repeatability[1] (c^2)

	Variance component (SE in parentheses)				
Trait	σ_a^2	σ_{pe}^2	$\sigma_{test\text{-}day}^2$	σ_e^2	c^2
Volume (mL)	0.27 (0.05)	0.29 (0.04)	0.05 (0.01)	1.29 (0.02)	0.29 (0.01)
Conc1[2]	0.19 (0.06)	0.71 (0.06)	0.10 (0.01)	1.84 (0.03)	0.32 (0.01)
Conc2[3]	12,573 (5748)	26,682 (5168)	2,383 (675)	50,188 (1629)	0.43 (0.02)
Mot0h[4] (%)	11.8 (4.6)	75.7 (5.6)	9.1 (1.7)	312.0 (4.4)	0.21 (0.01)
Mot24h[4] (%)	19.9 (5.9)	42.5 (6.1)	15.1 (2.3)	270.7 (4.8)	0.18 (0.01)
Mot48h[4] (%)	41.3 (15)	60 (15.3)	22.6 (4.7)	272.6 (9.7)	0.26 (0.02)
Defect[5]	0.0005 (0.0003)	0.0093 (0.0004)	0.0001 (0.0001)	0.0211 (0.0003)	0.32 (0.01)

[1]Repeatability: $c^2 = \sigma_a^2 + \sigma_{pe}^2 / \sigma_a^2 + \sigma_{pe}^2 + \sigma_{test\text{-}day}^2 + \sigma_e^2$.

[2]Concentration recorded before March 2013 and categorized into 10 classes [0, 1–390, then increments of 200, and finally >1,790 × 10^6 spermatozoa (spz)/mL].

[3]Concentration recorded after March 2013 given as 10^6 spz/mL.

[4]Motility in fresh samples (0 h) and after storing for 24 and 48 h.

[5]Binary trait: scored as 1 if >10% of a particular sperm defect was present in the sample, and 0 otherwise. Defects were loose heads, abnormalities in the tail or the intermediate part, distal droplets, and proximal droplets.

random test-day effects for the 2 traits; $\mathbf{a}_1$ and $\mathbf{a}_2$ are vectors of the random additive genetic effects of animal for the 2 traits; $\mathbf{p}_1$ and $\mathbf{p}_2$ are vectors of the random permanent environmental effects of bull for the 2 traits; $\mathbf{e}_1$ and $\mathbf{e}_2$ are vectors of the random residual effects for the 2 traits; and $\mathbf{X}$, $\mathbf{Zt}$, $\mathbf{Za}$, and $\mathbf{Zp}$ are known incidence matrices connecting the observations to the corresponding fixed and random effects. The following assumptions were made for distribution of random effects:

$$\begin{bmatrix}\mathbf{t}_1\\ \mathbf{t}_2\end{bmatrix} \sim N\left[\begin{pmatrix}0\\0\end{pmatrix}, \mathbf{I}\otimes\begin{pmatrix}\sigma_{t_1}^2 & \sigma_{t_1t_2}\\ \sigma_{t_1t_2} & \sigma_{t_2}^2\end{pmatrix}\right],$$

$$\begin{bmatrix}\mathbf{a}_1\\ \mathbf{a}_2\end{bmatrix} \sim N\left[\begin{pmatrix}0\\0\end{pmatrix}, \mathbf{A}\otimes\begin{pmatrix}\sigma_{a_1}^2 & \sigma_{a_1a_2}\\ \sigma_{a_1a_2} & \sigma_{a_2}^2\end{pmatrix}\right],$$

$$\begin{bmatrix}\mathbf{p}_1\\ \mathbf{p}_2\end{bmatrix} \sim N\left[\begin{pmatrix}0\\0\end{pmatrix}, \mathbf{I}\otimes\begin{pmatrix}\sigma_{p_1}^2 & \sigma_{p_1p_2}\\ \sigma_{p_1p_2} & \sigma_{p_2}^2\end{pmatrix}\right],$$

and

$$\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix} \sim N\left[\begin{pmatrix}0\\0\end{pmatrix}, \mathbf{I}\otimes\begin{pmatrix}\sigma_{e_1}^2 & \sigma_{e_1e_2}\\ \sigma_{e_1e_2} & \sigma_{e_2}^2\end{pmatrix}\right],$$

where $\sigma_{t_1}^2$, $\sigma_{t_2}^2$, and $\sigma_{t_1t_2}$ are the test-day variance for the 2 traits and the test-day covariance between the 2 traits, respectively; $\sigma_{a_1}^2$, $\sigma_{a_2}^2$, and $\sigma_{a_1a_2}$ are the additive genetic variance for the 2 traits and the covariance between the 2 traits; $\sigma_{p_1}^2$, $\sigma_{p_2}^2$, and $\sigma_{p_1p_2}$ are the permanent environmental variance for the 2 traits and the covariance between the 2 traits; $\sigma_{e_1}^2$, $\sigma_{e_2}^2$, and $\sigma_{e_1e_2}$ are the residual variance for trait and the residual covariance between the 2 traits; $\mathbf{A}$ is the relationship matrix based on the 27,437 animals in the pedigree; and $\mathbf{I}$ is an identity matrix.

Breeding values were regressed on test-year (simple linear regression) to assess genetic time trends. The slopes were considered significantly different from 0 if the level of significance (P) was $\leq$0.05. To make it possible to compare the rate of genetic trends across traits, a measure of the relative change per trait was calculated as the ratio of the estimated slope of the regression line to the standard phenotypic deviation of the trait.

Because the data set included information on whether bulls were selected for AI afterward, and thus had passed the andrology test (or not), genetic trends were also estimated for approved and not-approved bulls.

RESULTS

Descriptive Statistics and Distributions

Volume ranged from 0.5 to 12 mL with a mean of 2.6 (Table 1), and the distribution was right skewed as shown in Figure 2A. About 70% of the samples were $\leq$3 mL, and 95% of the collections contained $\leq$5 mL. For the distribution of motility, most observations were

Table 3. Heritability[1] of the 7 semen traits on the diagonal and genetic correlations below (SE in parentheses)

Item	Volume	Conc1	Conc2	Mot0h	Mot24h	Mot48h	Defects
Volume (mL)	0.14 (0.02)						
Conc1[2]	0.04 (0.17)	0.07 (0.02)					
Conc2[3]	0.30 (0.24)	—[4]	0.14 (0.06)				
Mot0h[5] (%)	0.57 (0.15)	0.71 (0.16)	0.65 (0.25)	0.03 (0.01)			
Mot24h[5] (%)	0.24 (0.15)	0.66 (0.15)	0.83 (0.16)	0.96 (0.03)	0.06 (0.02)		
Mot48h[5] (%)	0.40 (0.15)	0.53 (0.21)	0.59 (0.30)	—	—	0.10 (0.04)	
Defects[6]	−0.04 (0.24)	−0.90 (0.27)	—	−0.79 (0.23)	−0.78 (0.25)	—	0.02 (0.01)

[1]Heritability: $h^2 = \sigma_a^2 / \sigma_a^2 + \sigma_{pe}^2 + \sigma_{test\text{-}day}^2 + \sigma_e^2$, where variance components are additive genetic $\left(\sigma_a^2\right)$, permanent environmental $\left(\sigma_{pe}^2\right)$, test-day $\left(\sigma_{test\text{-}day}^2\right)$, and residual $\left(\sigma_e^2\right)$.

[2]Concentration recorded before March 2013 and categorized into 10 classes [0, 1–390, then increments of 200, and finally >1,790 × 10^6 spermatozoa (spz)/mL].

[3]Concentration recorded after March 2013 given as 10^6 spz/mL.

[4]Analysis did not converge.

[5]Motility in fresh samples (0 h) and after storing for 24 and 48 h.

[6]Binary trait: scored as 1 if >10% of a particular sperm defect was present in the sample, and 0 otherwise. Defects were loose heads, abnormalities in the tail or the intermediate part, distal droplets, and proximal droplets.

on the right-hand side of the graph (Figure 2D). Table 1 shows that the average motility decreased from 65% when fresh to 55% after storing for 48 h. At either time point, 80% was the highest motility measured, but considerably more of the fresh samples had this high level of spermatozoa movement (Figure 2D). Figures 2B and 2C show the distribution of the 2 concentration measures, both with most observations on the left side. Only 5% of the samples evaluated for conc2 had concentrations >10^9 spz/mL.

Fixed Effects

Generally, increasing age had a favorable effect on all traits (results not shown). From the solutions for group-year, a negative environmental trend was indicated for volume and conc1 (Figure 3A and B), among others. Further, volume increased with both ejaculate number and, in particular, length of interval between collections, whereas ejaculate number had an enlarging effect on conc1, conc2, mot0h, and mot24h (results not shown).

Heritabilities and Repeatabilities

Generally, the variance components were larger for the permanent environmental effect than for the additive genetic effect, which again was correspondingly larger than the variance component for day of testing (Table 2). The additive genetic standard deviation was 0.5 mL for volume and 112 × 10^6 spz/mL for conc2. The additive genetic standard deviation for motility increased from 3.4% in fresh samples to 4.5 and 6.4% after storing the sample for 24 and 48 h, respectively. These estimates correspond with genetic coefficients of variation of 20% for volume, 23.6% for conc2, and 5.3, 7.3, and 11.6% for mot0h, mot24h, and mot48h, respectively.

Estimated repeatabilities were low to moderate (Table 2), with the subjectively evaluated motility traits being the lowest (0.18–0.26). The Conc2 trait had the highest repeatability of all traits with 0.43, whereas conc1, categorized into 10 classes, had somewhat lower repeatability (0.32), being equal to that of the defect trait. The repeatability of volume was 0.29.

The estimated heritabilities ranged from low to moderate (Table 2). Volume and conc2 had the highest heritabilities of 0.14. Similar to the repeatability estimates, conc1 had lower heritability than conc2 (0.07 and 0.14, respectively). The estimated heritability of motility increased from 0.03 in fresh semen to 0.06 and 0.10 after storing for 24 and 48 h, respectively. Defects had the lowest heritability of all semen traits (0.02).

Genetic Correlations

All estimated genetic correlations were favorable (Table 3). Volume had the strongest genetic correlation with mot0h (0.57), whereas correlations with conc1 and defects were not significantly different from 0. Overall, concentration had moderate or high genetic correlations with motility; the highest correlation was 0.83 between conc2 and mot24h. Further, concentration was negatively genetically correlated with defects (−0.90), meaning that a higher concentration was genetically associated with fewer defects. A higher motility (mot0h) also correlated genetically with fewer defects (−0.79), and the motility in fresh samples was genetically very similar to motility after storing the semen for 24 h, with a genetic correlation of 0.96.

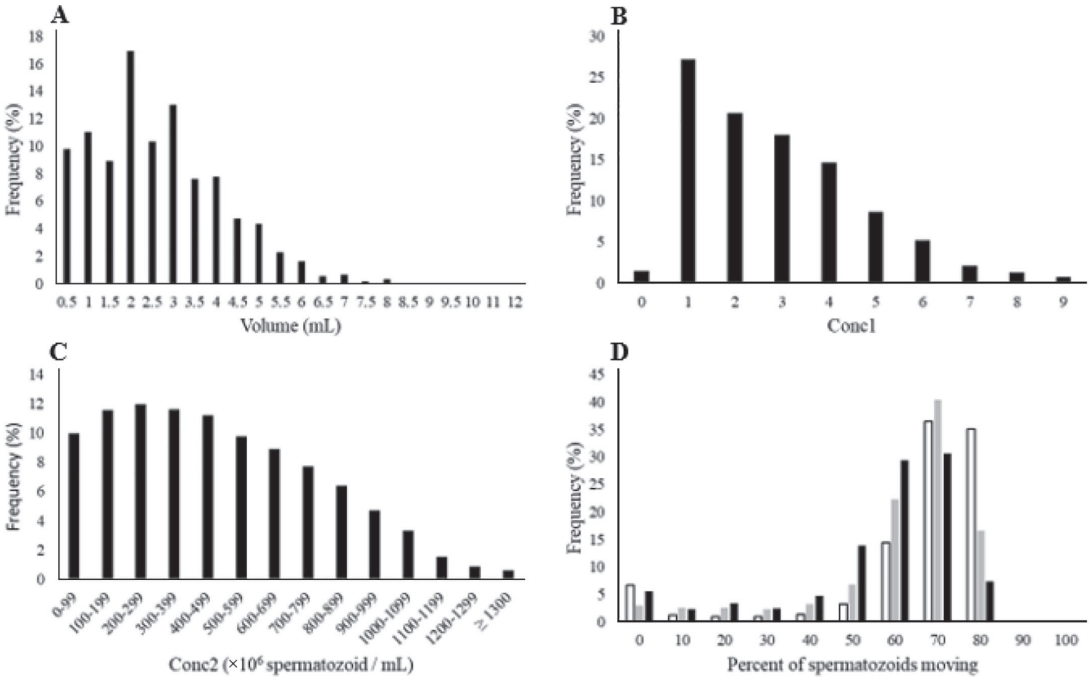

Figure 2. Distributions of (A) volume (mL), (B) concentration recorded before March 2013 and categorized into 10 classes [0, 1–390, then increments of 200, and finally >1,790 × 10^6 spermatozoa (spz)/mL], (C) concentration recorded after March 2013 given as 10^6 spz/mL, and (D) motility in fresh samples (white bars), after storing for 24 h (gray) and 48 h (black).

Genetic Trends

All traits showed a significant ($P < 0.05$) slightly unfavorable genetic trend between 1994 and 2016 (Table 4). For the traits with phenotypes recorded for >20 yr, relative change (slope/SD), was largest for conc1 and mot24h and smallest for mot0h; defects were least affected.

DISCUSSION

The data set used in this analysis (14,972 observations from 3,927 bulls samples over more than 20 yr) is one of the largest analyzed for genetic parameters on sperm quality traits in dairy cattle. Generally, the heritabilities found in this study were in the lower range of those reported in the review by Berry et al. (2014). One reason for the low heritability estimates in our study might have been that the bulls were young, 10.5 to 15.5 mo old, and some of the youngest might have been prepubertal. In cattle, a commonly used definition of puberty is "the age at which an ejaculate contains 50 million spermatozoa with a minimum of 10% motility," because such an ejaculate can lead to pregnancy (Evans et al., 1996; Thundathil et al., 2016). In addition, bulls must have adequate sexual behavior and genital development to copulate and ejaculate. By following the above definition, all bulls in our data set fulfilled the second requirement because only records with volume >0 were kept; however, no requirement was set for the ejaculate to contain the minimum values required to meet the definition for puberty. It is important to note that Killian and Amann (1972) detected the first sperm when the bulls were 9 mo ± 3 wk, whereas they found puberty to occur as late as 10.25 ± 1 mo. Because one goal of andrology testing at the performance test station is to ensure that bulls sent to the AI center have adequate semen quality, knowing that the bulls have reached puberty before testing is essential to measure their true potential. This should therefore be a requirement before testing. Further, Chenoweth (1983) reviewed sexual behavior in bulls and concluded that techniques for sexual preparation such as restraint and false mounts can influence the semen characteristics

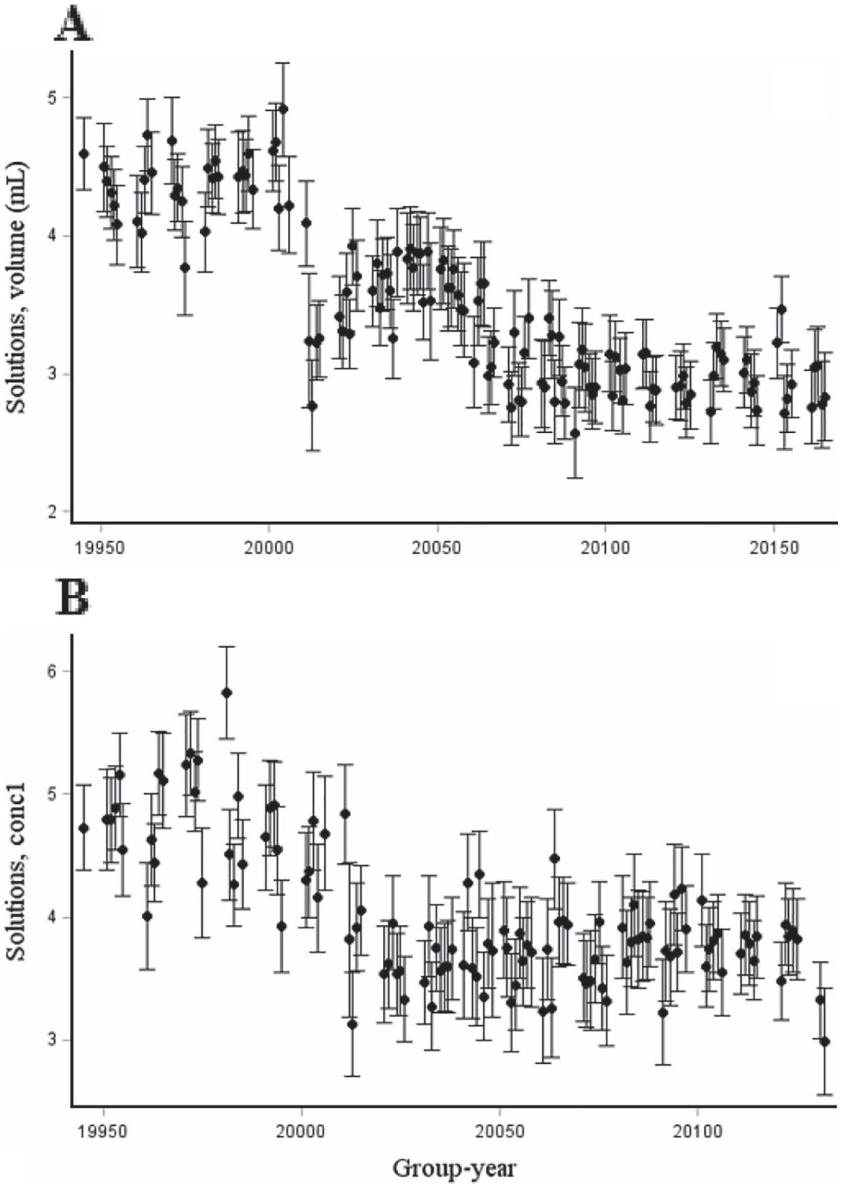

Figure 3. Fixed effect solutions for group-year (containing μ) from bivariate analysis of (A) volume (mL) and (B) conc1 [concentration recorded before March 2013 and categorized into 10 classes: 0, 1–390, then increments of 200, and finally >1,790 × 10^6 spermatozoa (spz)/mL]. Standard errors are plotted as whiskers.

Table 4. Estimated genetic trend (1994–2016) for the 7 semen characteristics traits from regressing EBV on year, and the slope's level of significance (P) of being different from 0

Trait	Intercept	Slope	P-value	Slope/SD[1]
Volume (mL)	0.164	−0.002	0.0124	−0.0013
Conc1[2]	0.068	−0.004	<0.0001	−0.0022
Conc2[3]	5.215	−0.599	<0.0001	−0.0020
Mot0h[4] (%)	0.857	−0.008	0.0464	−0.0004
Mot24h[4] (%)	2.103	−0.047	<0.0001	−0.0025
Mot48h[4] (%)	2.428	−0.025	0.0003	−0.0012
Defect[5]	−0.003	0.00002	<0.0001	0.0001

[1]Slope/phenotypic standard deviation of trait (from Table 1).

[2]Concentration recorded before March 2013 and categorized into 10 classes [0, 1–390, then increments of 200, and finally >1,790 × 10^6 spermatozoa (spz)/mL].

[3]Concentration recorded after March 2013 given as 10^6 spz/mL.

[4]Motility in fresh samples (0 h) and after storing for 24 and 48 h.

[5]Binary trait: scored as 1 if >10% of a particular sperm defect was present in the sample, and 0 otherwise. Defects were loose heads, abnormalities in the tail or the intermediate part, distal droplets, and proximal droplets.

of the sample in dairy bulls. An improved protocol should consider a standardized preparation protocol for the bull rather than the current situation, in which bulls with insufficient erection are allowed several false mounts before semen collection. These changes, as well as measuring all traits on all semen collections, would likely enhance the value of these data for genetic means.

In our data, the mean volume and concentration (Table 1) were generally lower than results obtained by others (Ducrocq and Humblot, 1995; Mathevon et al., 1998b; Al-Kanaan et al., 2015; Berry et al., 2019). One cause for the low mean values was the change of housing of bulls in the year 2000, from tie stalls to group pens with 10 to 15 bulls in each. The effect of this change can be seen in the size of the group-year solutions that correct for changes in the station's management and routines over the years. Figure 3A shows the effect of group-year on volume. Although the effect varied among years, it tended to drop after the year 2000, and this tendency was similar for conc1 (Figure 3B). Pens with several bulls kept together allow them to mount each other and complete a full ejaculation within the pen. (E. Kummen, former head veterinary at Geno's performance test station, Øyer, Norway; personal communication, May 10, 2019). Moreover, sperm production is highly dependent on age of collection (Killian and Amann, 1972; Mathevon et al., 1998a; Al-Kanaan et al., 2015) and, as expected with young bulls that are still maturing, we found that increasing age had a favorable effect on all semen traits measured. After the onset of puberty, semen volume increases as scrotum and testicle size increase (Brito et al., 2002). In contrast to our results, Brito et al. (2002) did not find a significant effect of age on concentration or motility. The bulls in their study, however, were considerably older than ours, with the youngest group consisting of bulls up to 36 mo of age. Mathevon et al. (1998a), however, found that concentration and motility increased up to approximately 22 mo of age, a finding supported by Al-Kanaan et al. (2015). In addition to group-year and age, we estimated the joint effect of ejaculate number and days since last collection. Volume increased with increasing ejaculate number, and even more so with a longer interval between collections. This is in agreement with Mathevon et al. (1998a), Fuerst-Waltl et al. (2006), and Al-Kanaan et al. (2015). They all reported the highest volume with the longest interval between collections. For concentration and motility (except mot48h), increased ejaculate number enhanced all variables, whereas interval was less important. In contrast to our findings, Karoui et al. (2011) and Fuerst-Waltl et al. (2006) found a higher concentration in the first ejaculate, whereas motility was nearly unaffected or lower in the first ejaculate. Note that in those studies "ejaculate number" refers to the number of ejaculates taken on the same day, whereas in our study, it was the number of ejaculates that a bull has ever given, and therefore includes the effect of the bulls' increased experience and familiarity with the test. A lack of consensus exists on whether the interval length affects motility. Everett et al. (1978), Mathevon et al. (1998a), and Berry et al. (2019) all found that interval length was statistically significant for motility, but whereas the latter authors could not find a clear trend, Mathevon et al. (1998a) found that the shortest interval (2 d) gave the highest percentage of motility in the samples. In contrast, Al-Kanaan et al. (2015) did not find a significant effect of interval on motility, whereas Fuerst-Waltl et al. (2006) found a significant effect on progressive motility, but not on motility score.

As mentioned earlier, the heritability estimates obtained from our data (Table 2) were somewhat lower than the pooled mean heritability estimates from the meta-analysis performed by Berry et al. (2014), but all estimates, except for sperm defects, were within the large range of heritability estimates found in the literature. The heritability of volume in our study was estimated to be 0.14, which is lower than the meta-analysis estimate of 0.20 (Berry et al., 2014), but between the heritability estimates found in 2 studies conducted by Mathevon et al. (1998a,b) analyzing performance test-station data for Holstein and French Montbéliarde bulls, respectively (0.24 and 0.08). We estimated the heritability of conc2 and motility in fresh samples to be 0.14 and 0.03, respectively, which is close to the findings of Berry et al. (2014; 0.17 and 0.054). The heritability of conc2 was larger than that for conc1 (0.07), likely because some information was lost due to the

categorization of conc1, and conc1 being less accurately measured because of the lower-resolution photometer used, especially for values between 0 and 390 $\times$ 10^6 spz/mL. In contrast to volume and concentration, which were recorded objectively, motility was recorded subjectively. This less accurate measurement is likely to result in lower heritability. Interestingly, storage of semen resulted in higher heritability for motility compared with that of fresh samples. In the literature, however, motility after thawing is the commonly examined challenge trait. Because frozen semen is usually used in Norwegian Red, and we do not know whether semen that tolerates storing also tolerates freezing, the importance of the storage challenge is difficult to evaluate at the current time. Motility in the literature may refer to motility score (from bad to good) or the percent of cells that move as measured by trained technicians or by computer-assisted sperm analysis (CASA). In addition to differences in statistical models, populations, and breeds, different trait definitions can explain some of the variation in heritability estimates found for motility in the literature. Among the 7 semen characteristic traits analyzed in this study, sperm defect contained the least amount of genetic information, with an estimated heritability of 0.02. This is lower than heritability estimates for sperm abnormality found in the literature, which ranged from 0.07 to 0.35 (Ducrocq and Humblot, 1995; Kealey et al., 2006; Corbet et al., 2013). In those studies, sperm abnormalities were measured on a continuous scale as the percent of sperm with a defect in a sample. In our study, the trait was treated as binary and the frequency of collections registered with a defect was low, which resulted in the low heritability estimate. We chose to analyze all traits using linear models, although a threshold model would have been theoretically more appropriate for sperm defects, being a binary trait.

All genetic correlations were favorable, in the sense that a genetic improvement in any of the traits would also improve the others as a correlated selection response. We were not able to estimate the genetic correlation between conc1 and conc2, because very few bulls had information on both traits, and the analyses did not converge. However, the genetic correlations between these traits and either of the remaining ones were similar and little affected by the categorization of conc1. The genetic correlation between mot0h and mot24h was close to 1, meaning that motility in fresh and storage-challenged samples can be considered genetically much the same trait. The genetic correlations between concentration and motility in fresh samples were high and favorable (0.65–0.71), which is in agreement with Berry et al. (2014) and Karoui et al. (2011), who found estimates of 0.61 and 0.54, respectively. The genetic correlations between volume and the 3 motility traits ranged from 0.24 to 0.57, being highest in fresh samples and larger than those reported by Berry et al. (2014) and Karoui et al. (2011) (0.06–0.13). Further, we found volume and conc1 and conc2 to have little or no genetic correlation (0.04 and 0.30, respectively), whereas most studies have found a negative genetic correlation between these 2 traits (Karoui et al., 2011; Berry et al., 2014, 2019). Volume, concentration, and motility were found to be negatively correlated with sperm defects (-0.04 to -0.90), which is favorable and means that a genetic increase in the traits mentioned results in fewer defects in the sample as a correlated response.

Over the period from 1994 to 2016, all traits showed a slightly unfavorable genetic trend (Table 4), with a slope of the linear regression being significantly ($P < 0.05$) different from zero. The relative largest genetic changes for traits recorded >20 yr were found for conc1 and mot24h, whereas mot0h and defects seemed to be least affected. The EBV for volume plotted in Figure 4 show the regression line for the selected bulls to generally lie above that for unselected bulls, but the difference is very small. Corresponding results were obtained for conc1 and mot0h. The small difference between selected and unselected bulls is likely due the low heritability of the 7 traits, but particularly the low selection intensity that can be practiced for these traits at the performance test. Thus, andrology testing and the minimum semen quality requirement before selection of AI bulls were not sufficient to genetically improve semen volume or quality over time. Likely, the unfavorable genetic trends were correlated selection responses caused by selection on traits that are genetically correlated with andrology. The genetic relationship between andrology and other traits is not well known (Berry et al., 2014). Thus, there is a need to examine the genetic association between male fertility and production traits, health traits, and cow fertility in Norwegian Red and other populations.

This study demonstrated the need to intensify selection for improved semen quality in the future. One obvious solution is to base selection on genomic breeding values that are already available when recruiting bull calves and updated throughout the performance testing. This should have the potential to achieve a positive selection differential in semen traits. For this to be possible, andrology testing must continue, preferably with the recommendations stated previously with standardized preparations of bulls known to have reached puberty, as well as measuring all traits on all semen collections. Moreover, research should examine the genetic relationship between the andrology traits in the performance test and at the AI center. A special focus should

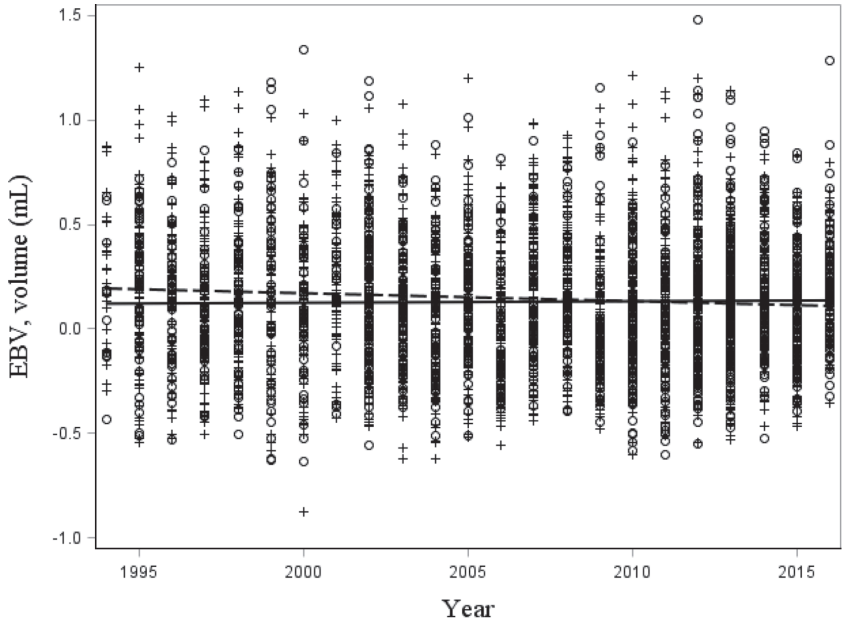

Figure 4. Estimated breeding values of volume for the bulls that were approved for AI production (+) and those that were not (○). Both group's EBV were regressed on year to estimate genetic trend for selected (solid line) and unselected (dashed line) bulls.

be given to determine the genetic association between motility after thawing and storage-challenged motility at the performance test. Following these guidelines, it should be possible to select AI bulls with better semen quality in the future.

CONCLUSIONS

Using a large data set sampled over ≥20 yr with 14,472 records on almost 4,000 bulls that were unselected with respect to andrology, genetic variation was estimated for all 7 examined traits. The genetic coefficient of variation was largest for concentration (23.6%), followed by volume (20%), and was lower for motility in fresh samples (5.3%). Heritability estimates were low to moderate (0.02–0.14) and in the same order as that for genetic variance. The size of the data set allowed for precise estimates of genetic correlations between traits, all of which were found to be favorable. The genetic trends were slightly unfavorable for all traits, which implies that phenotypic selection with the current intake regimen to the AI station does not ensure a positive genetic trend of andrology traits in Norwegian Red bulls. The lack of antagonistic relationship between traits and the amount of genetic variance within traits indicate that selection for these traits is possible. Using genomic breeding values for the traits when buying bulls for the performance test station is recommended to reverse the unfavorable genetic trends found in this study.

ACKNOWLEDGMENTS

Thanks to Geno (Hamar, Norway) for giving access to data, and to The Research Council of Norway, Oslo, for funding (project number: NRF-255097/E50).

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Paper II

Olsen, H. B., Heringstad, B., & Klemetsdal, G. (2020b)

Genetic correlations between body weight, daily weight gain, and semen characteristic traits in young Norwegian Red bulls

Journal of Dairy Science, 103(7), 6311–6317

J. Dairy Sci. 103:6311–6317
https://doi.org/10.3168/jds.2019-18116

Genetic correlations between body weight, daily weight gain, and semen characteristic traits in young Norwegian Red bulls

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ABSTRACT

The aim of this study was to estimate genetic parameters for body weight (BW) at 150 d (Bw_150d), and 330 d (Bw_330d) of age and average daily weight gain (Dwg), and to estimate genetic correlations between these traits and semen characteristic traits: volume; concentration (Conc); motility in fresh, 24-h, and 48-h samples (Mot0h, Mot24h, Mot48h); and sperm defects. Data were collected at the performance test station of young Norwegian Red bulls from 2002 to 2012, before selection of bulls for artificial insemination. The weight and growth data consisted of observations for 3,209 bulls, and andrology information was available for up to 2,034 of these bulls. Genetic parameters were estimated using linear animal models. Models for BW and growth traits included the group and year the bull left the station and the pen they occupied during weighing (group-year-pen) and parity of their dam as fixed effects. Models for andrology traits had group-year, age in months (11 to 15), and the interaction between ejaculate number and days since previous collection included as fixed effects. Estimated heritability was 0.14 for Bw_150d, 0.26 for Bw_330d, and 0.34 for Dwg; the estimated genetic correlations among these traits were all favorable. Both BW traits correlated favorably with all the semen characteristic traits (0.20 to 0.76), whereas Dwg was favorably correlated with volume, Mot24h, Mot48h, and sperm defects, and unfavorably correlated with Conc (−0.25) and Mot0h (−0.53). Our results indicate that the genetic correlations between weight and growth traits and semen characteristics depend on the age of the bulls. Although most genetic correlations were favorable, selection for higher daily weight gain between 150 and 330 d might explain the slight negative genetic trends observed for semen characteristics in young Norwegian Red bulls.

Key words: heritability, genetic parameter, andrology

INTRODUCTION

Olsen et al. (2020) found that semen characteristic traits of young Norwegian Red bulls showed a slightly unfavorable genetic trend between 1994 and 2016. Thus, phenotypic selection on semen characteristics practiced at the performance test station has not been sufficient to genetically improve semen volume or quality over time. We hypothesized that the unfavorable genetic trends were caused by selection for other traits that have unfavorable genetic correlations with andrology traits. In contrast to the many studies estimating genetic parameters for cow fertility based on very large data sets (e.g., Andersen-Ranberg et al., 2005; Tiezzi et al., 2012; Carthy et al., 2015), genetic studies on bull fertility are few and based on a relatively small number of animals. Further, genetic correlations between sperm quality and performance traits are largely lacking for both dairy and beef cattle (Berry et al., 2014; Thundathil et al., 2016). Regarding BW, growth traits, and semen characteristics, only 2 studies have been published, both of which used data from beef cattle. In these studies, the genetic correlations between BW (weaning weight and yearling weight) traits and semen characteristics (concentration, motility, and percent of normal sperm cells) ranged from −0.36 to 0.75 (Knights et al., 1984; Smith et al., 1989), and genetic correlations between average daily weight gain and motility and percent of normal sperm were −0.36 and 0.34, respectively (Smith et al., 1989).

Norwegian Red is a dual-purpose breed in which growth is an important trait. Slaughter weight and carcass classification are included in the total merit index (Geno, 2018). Average daily weight gain measured at the performance test station was, until 2013, used as one of the criteria for selection of bulls for AI. Data from the performance test station can therefore be used to meet the objective of this paper; namely, to estimate genetic correlations among BW traits, daily

Received December 23, 2019.
Accepted February 27, 2020.
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weight gain, and semen characteristic traits for young Norwegian Red bulls.

MATERIALS AND METHODS

Data

Data from the performance test station for young Norwegian Red bulls from 2002 to 2012 were made available by Geno (Hamar, Norway), the breeding organization for Norwegian Red cattle. Each year, around 250 of the most promising Norwegian Red bull calves were performance tested. Bulls arrived at the station at 4 to 5 mo of age and were placed in pens of 12 to 18 animals by age. Concentrates were given according to age, and grass silage was available ad libitum. Conformation and temperament were assessed during the stay. Further, bulls were weighed, and BW at 150 (±5) d and 330 (±5) d were used to compute average daily weight gain. At the end of the stay, when the bulls were around 12 mo old, several andrology traits were measured and used to ensure that only bulls with acceptable semen quality were selected and sent to the AI center.

Routine registrations of bulls' BW (kg) at age 150 d (**Bw_150d**) and at 330 d (**Bw_330d**) and average daily weight gain (**Dwg**; g/d) from 150 to 330 d from yr 2002 to 2012 were provided by Geno. Records of Bw_330d <299 or >500 kg were considered erroneous and removed. The same was done for observations of Dwg <500 or above >2,000 g/d. Bulls were transferred from the station in groups (either for slaughter or to the AI center), and all bulls were assigned with a group number and a group year, as well as the pen number they occupied during weighing. Finally, the data contained information on whether the bull was the dam's first calf.

Andrology data were available for 2,034 of the 3,209 bulls with weight and growth information, and included the following traits:

(1) Volume (mL).
(2) Concentration recorded by photometer (**Conc**). The photometer could not register measurements $<390 \times 10^6$; therefore, concentration was set to 390×10^6 if the photometer read zero but sperm cells were found during microscopic evaluation. Because of this, we categorized Conc into 10 classes: 0, 1–390, thereafter in intervals of 200, and finally $>1{,}790 \times 10^6$ spermatozoa/mL.
(3) Motility measured in fresh samples by subjective inspection under a phase contrast microscope (given as percentage of moving sperm cells, with 10-percentage-unit increments starting at 0; **Mot0h**). After first inspection, samples were stored in a refrigerator until they were reactivated (heated to 38°C in 5 min) and evaluated for motility again after 24 h (**Mot24h**) and 48 h (**Mot48h**), measured in the same way as Mot0h.
(4) Sperm defects—a binary trait scored as 1 if more than 10% of a particular spermatozoa (**spz**) defect was present in the sample, or >20% defects in total, and 0 otherwise. Defects were loose heads, abnormalities in the tail or the intermediate part, distal droplets, and proximal droplets.

Only semen collections with volume >0 mL were kept for analyses. Samples with volume >12 mL or Conc $>3{,}000 \times 10^6$ spz/mL were considered erroneous and removed. Bulls had to be between 320 and 472 d (10.5–15.5 mo) old at the test-day, and only bulls that had been assigned group number and group year were kept. See Olsen et al. (2020) for further details on semen collection and editing of data.

Descriptive statistics for the andrology, BW, and growth traits are summarized in Table 1. Figure 1 shows Dwg plotted against Bw_150d and Bw_330d. The pedigree of the bulls was traced back as far as possible and included 41,356 animals in the additive genetic relationship matrix.

Models

Initially, univariate, linear animal models were run in DMU using the average information (AI)REML procedure (Madsen and Jensen, 2013) to estimate variance components for the body weight traits and Dwg. The following model was fitted:

$$\mathrm{Y}_{ijk} = \mathrm{gyp}_i + \mathrm{pod}_j + \mathrm{a}_k + \mathrm{e}_{ijk},$$

where Y_{ijk} is an observation of Bw_150d, Bw_330d, or Dwg on the kth bull; gyp_i is the fixed effect of the ith group-year-pen ($i = 1, \ldots, 261$); pod_j is the fixed effect of the parity of the dam in 2 classes ($j = 1$: dam's first calf, or 2: second or later calf); a_k is the random additive genetic effect of the kth bull $N \sim \left(0, \mathbf{A}\sigma_a^2\right)$, with $\mathbf{A}$ being the additive genetic relationship matrix and σ_a^2 the additive genetic variance; and e_{ijk} is the random residual $N \sim \left(0, \mathbf{I}\sigma_e^2\right)$, where $\mathbf{I}$ is an identity matrix and σ_e^2 is the residual variance.

For andrology traits, the following linear animal repeatability model was fitted:

$$\mathrm{Y}_{ijklmo} = \mathrm{age}_i + \text{group-year}_j + \text{collection\_n-interval}_k + td_l + a_m + pe_m + e_{ijklmo},$$

Table 1. Descriptive statistics of semen characteristics and BW and growth traits measured on Norwegian Red bulls at the performance test station

Trait	n	Samples (n)	Mean	SD	Minimum	Maximum
Volume (mL)	7,634	2,034	2.4	1.4	0.5	12
Conc[1]	7,635	2,034	2.5	1.6	0	9
Mot0h[2] (%)	7,364	2,014	63.8	21.9	0	80
Mot24h[2] (%)	4,934	1,591	60.4	20.9	0	80
Mot48h[2] (%)	1,165	899	52.3	25.5	0	80
Sperm defects[3]	7,640	2,034	0.05	0.21	0	1
Bw_150d[4] (kg)	3,209	3,209	163.6	21.5	92.9	250
Bw_330d[4] (kg)	3,209	3,209	411.6	29.5	299	500
Dwg[4] (g/d)	3,209	3,209	1,377.7	118.7	850	1,811

[1]Concentration categorized into 10 classes: 0, 1–390, thereafter increments of 200, and finally >1,790 × 10^6 spermatozoa/mL.

[2]Motility in fresh samples (Mot0h) and after storing for 24 h (Mot24h) and 48 h (Mot48h).

[3]Binary trait; scored as 1 if >10% of the sperm in the sample had a particular defect or >20% defects in total, 0 otherwise. Defects were loose heads, abnormalities in the tail or the intermediate part, distal droplets, and proximal droplets.

[4]BW of bulls when 150 d (Bw_150d) and 330 d (Bw_330d) old, and average daily weight gain (Dwg) in between.

where Y_{ijklmo} is the oth observation on 1 of 6 andrology traits; age_i is the fixed effect of the ith age class in months ($i = 11, \ldots, 15$); group-$year_j$ is the fixed effect of the jth group and year the bull left the test station ($j = 1, \ldots, 74$); collection_n-$interval_k$ is the fixed effect of kth class of ejaculate number (from 1 = first semen collection to 6 = the sixth or more collection) and interval in days since previous collection (1 = 1 to 4 d, 2 = 5 to 10 d, and 3 = >10 d), $k = 1, \ldots, 16$ (not used in the model for Mot48h because the variable was not significant at $P < 0.05$ in a proc GLM in SAS). Test-day (td), additive genetic (a), permanent environmental (pe), and residual (e) effects were assumed random; see below for their distributions.

Bivariate linear animal models were used to estimate genetic correlations among the weight and growth traits and between weight and growth traits and semen characteristic traits. The following assumptions were made for the distribution of the random test-day (td), additive genetic (a), permanent environmental (pe), and residual (e) effects in the bivariate models:

$$\left[td_{andro}\right] \sim \left(0, \mathbf{I} \times \sigma^2_{td_2}\right),$$

$$\begin{bmatrix} a_{weight\ or\ growth} \\ a_{weight,growth,or\ andro} \end{bmatrix} \sim N\left(\begin{pmatrix}0\\0\end{pmatrix}, \mathbf{A} \otimes \begin{pmatrix} \sigma^2_{a_1} & \sigma_{a_1a_2} \\ \sigma_{a_1a_2} & \sigma^2_{a_2} \end{pmatrix}\right),$$

$$\left[pe_{andro}\right] \sim \left(0, \mathbf{I} \times \sigma^2_{pe_2}\right),$$

$$\begin{bmatrix} e_{weight\ or\ growth} \\ e_{weight,growth,or\ andro} \end{bmatrix} \sim N\left(\begin{pmatrix}0\\0\end{pmatrix}, \mathbf{I} \otimes \begin{pmatrix} \sigma^2_{e_1} & \sigma_{e_1e_2} \\ \sigma_{e_1e_2} & \sigma^2_{e_2} \end{pmatrix}\right),$$

where $\mathbf{A}$ is the additive genetic relationship matrix. The test-day and permanent environmental effects were only included in the model for the andrology traits ($andro$), where $\sigma^2_{td_2}$ is the test-day variance and $\sigma^2_{pe_2}$ is the permanent environmental variance, and $\mathbf{I}$ are identity matrices. The (co)variance matrices for additive genetic and residual effects contained variances on the diagonal and covariances on the off-diagonal. In the bivariate analysis of weight or growth and andrology traits, the residual covariance was restricted to zero because the measurements differed in both time and space.

Results from the univariate analyses were used to estimate the heritability (h^2) of weight and growth traits, and results from bivariate models between Dwg and semen characteristic traits were used for the andrology traits. The formulas were

$$h^2_{weight} = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_e},$$

$$h^2_{andrology} = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{pe} + \sigma^2_{td} + \sigma^2_e}.$$

Standard errors were computed using Taylor series approximation.

RESULTS AND DISCUSSION

BW and Growth Traits

Figure 1a shows that there was little or no phenotypic association between Bw_150d and Dwg, probably

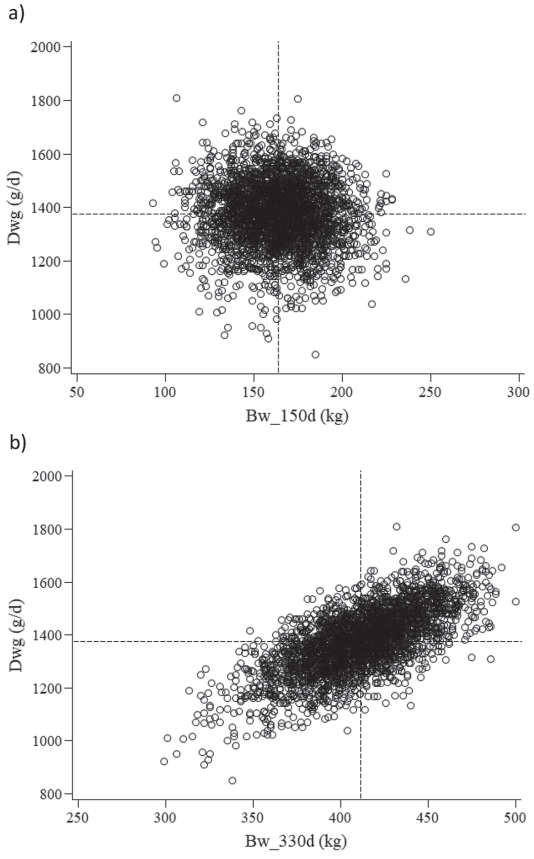

Figure 1. Average daily weight gain (Dwg; g/d) plotted against (a) BW at age 150 d (Bw_150d) and (b) BW at age 330 d (Bw_330d) for Norwegian Red bulls (n = 3,209) from 2002 to 2012. Dashed lines show the mean for each trait.

because Bw_150d was mainly affected by the environment before arriving at the test station. However, Bw_330d was phenotypically correlated strongly with Dwg (Figure 1b). Despite the clear positive phenotypic association, the figure also shows that some bulls with below-average weight could grow well above average and vice versa.

Estimated heritability was 0.14 for Bw_150d, 0.26 for Bw_330d, and 0.34 for Dwg (Table 2). The lower heritability estimate for Bw_150d was likely caused by noise from the prestation environment compared with BW measured at the later stage and daily weight gain recorded during the performance test. In a previous study, also using data from the performance test station for Norwegian Red (Aass, 1996), the heritabilities (SE) of Bw_330d and Dwg (from 90 to 330 d) were estimated to be 0.33 (0.15) and 0.30 (0.14), respectively, which corresponds with our results. Přibyl et al. (2008) estimated genetic parameters for live BW in dual-purpose Czech Fleckvieh bulls at various ages (60 to 400 d) and found that heritability decreased slightly from 100 d to about 180 d of age and increased thereafter. They estimated the heritability of live weight at 150 and 330 d to be 0.20 and 0.35, respectively, which is somewhat higher than our estimates but shows the same overall picture of increased heritability with age. The heritability estimates of Bw_330d and Dwg were also similar to estimates by Smith et al. (1989), although they found yearling weight to have a higher heritability than average daily weight gain (0.33 and 0.25, respectively) in Hereford, Angus, and Red Angus. Knights et al. (1984), however, estimated the heritability (SE) of yearling weight in Angus to be 0.49 (0.05). However, large differences in management, production system, and breed make comparison between the latter 2 studies on beef bulls and Norwegian Red difficult. All genetic correlations among the BW and growth traits were positive (Table 2), although the genetic correlation between Bw_150d and Dwg was not significantly different from zero. The genetic correlation was 0.64 between Bw_150d and Bw_330d, and 0.83 between Bw_330d and Dwg. This is in accordance with Smith et al. (1989), who found a strong genetic correlation between average daily weight gain and yearling weight (0.92). Further, Přibyl et al. (2008) estimated a genetic correlation between BW at 150 d and BW at 330 d of 0.77.

BW, Growth Traits, and Semen Characteristics

Both BW traits were favorably genetically correlated with all the semen characteristic traits (Table 3), but with high standard errors for correlations with motility traits and sperm defects. A large amount of data is needed to estimate precise genetic correlations, particularly for traits with low heritability that contain a smaller amount of genetic information. For Bw_150d and Bw_330d, the highest genetic correlations were with Mot0h (0.76) and Mot48h (0.66), respectively. In contrast to the BW traits, Dwg had negative genetic correlations with Conc (−0.25) and Mot0h (−0.53), although correlations were favorable with the remaining traits. Smith et al. (1989) also found a negative genetic correlation between average daily weight gain and motility (−0.36) for beef bulls and a negative genetic correlation between motility and yearling weight (−0.36).

Table 2. Estimated variance components and heritability for BW of Norwegian Red bulls when 150 d (Bw_150d) and 330 d (Bw_330d) old, and for average daily weight gain (Dwg) from 150 to 330 d, as well as genetic correlations between traits (SE in parentheses)

	Variance component[1]			Genetic correlation	
Trait	σ_a^2	σ_e^2	Heritability	Bw_330d	Dwg
Bw_150d (kg)	58.5 (17.1)	369.7 (16.5)	0.14 (0.04)	0.64 (0.11)	0.11 (0.18)
Bw_330d (kg)	183.5 (42.3)	526.3 (34.6)	0.26 (0.06)		0.83 (0.06)
Dwg (g/d)	3,479.2 (727.3)	6,812.6 (563.0)	0.34 (0.06)		

[1]Where σ_a^2 is the additive genetic variance and σ_e^2 is residual variance.

In their study, both birth weight and weaning weight correlated genetically with motility (0.36 and 0.13, respectively). Thus, the genetic correlation between motility and BW was positive for weight measured early in the bull's life and got weaker with increasing age, which corresponds to our results. Knights et al. (1984) also estimated the genetic correlations between BW (weaning and yearling weight) and subjectively scored semen concentration and motility for Angus bulls. In contrast to Smith et al. (1989) and our results, they found the genetic correlation between these semen quality traits and BW traits to increase from weaning until the bulls were 1 yr old (0.30 and 0.75, respectively). Furthermore, they estimated the genetic correlation between objectively measured concentration (spz/mL) and yearling weight to be 0.31, which agrees with our result for the genetic correlation between concentration and Bw_330d of 0.32. We estimated the genetic correlations between sperm defects and Bw_150d, Bw_330d, and Dwg to be −0.41, −0.52, and −0.28, respectively; hence, higher BW and daily weight gain are associated with fewer sperm defects. This corresponds to Smith et al. (1989), who found positive genetic correlations between percent of normal sperm and weight traits of 0.20, 0.26, and 0.34 for weaning weight, yearling weight, and daily weight gain, respectively. Because of the lack of literature on these genetic correlations in dairy and dual-purpose cattle, we consider these measures in beef to be relevant. It should be noted, however, that all standard errors were high in Smith et al. (1989), and no standard errors were given in Knights et al. (1984).

Our results indicate that the genetic correlations between weight and growth traits and semen characteristics depend on the age of the bulls; that is, they might be different for young and adult bulls. A greater BW at a given age has been associated with lower age of puberty and maturity; in particular, nutrition before 6 mo is known to affect the onset of puberty (Brito et al., 2012). This means that the bulls in our data set with a high BW at 150 d might be more sexually mature during the andrology test taken at approximately 12 mo age than bulls that started out at a lower weight. Similarly, a bull with a low BW at 150 d can have a high average weight gain but an increased age of pu-

Table 3. Heritability (h^2) of semen characteristics[1] and genetic correlations between semen characteristics and BW of Norwegian Red bulls when 150 d (Bw_150d) and 330 d (Bw_330d) old, and with average daily weight gain (Dwg) in between (SE in parentheses)

	Trait			
Characteristic	Bw_150d (kg)	Bw_330d (kg)	Dwg (g/d)	h^2
Volume (mL)	0.53 (0.11)	0.46 (0.09)	0.17 (0.08)	0.17 (0.04)
Conc[2]	0.50 (0.13)	0.32 (0.14)	−0.25 (0.15)	0.05 (0.02)
Mot0h[3] (%)	0.76 (0.45)	0.38 (0.43)	−0.53 (0.34)	0.01 (0.01)
Mot24h[3] (%)	0.20 (0.19)	0.26 (0.16)	0.16 (0.15)	0.05 (0.02)
Mot48h[3] (%)	0.48 (0.27)	0.66 (0.22)	0.50 (0.19)	0.12 (0.06)
Defects[4]	−0.41 (0.26)	−0.52 (0.25)	−0.28 (0.25)	0.02 (0.01)

[1]From bivariate analyses with Dwg.

[2]Concentration categorized into 10 classes: 0, 1–390, thereafter increments of 200, and finally >1,790 × 10^6 spermatozoa/mL.

[3]Motility in fresh samples (Mot0h) and after storing for 24 h (Mot24h) and 48 h (Mot48h).

[4]Binary trait; scored as 1 if >10% of the sperm in the sample had a particular defect or >20% defects in total, 0 otherwise. Defects were loose heads, abnormalities in the tail or the intermediate part, distal droplets, and proximal droplet.

berty. Olsen et al. (2020) suggested that some of the bulls could be sexually immature at the andrology test, which might explain the favorable genetic correlation between the BW traits and all semen characteristic traits, and might explain why the genetic correlations were stronger between Bw_150d and volume, Conc, and Mot0h than between Bw_330d and the aforementioned andrology traits. Furthermore, the negative genetic correlations between Dwg and Conc and Mot0h could reflect the immaturity of bulls at testing for andrology traits.

In addition to our recommendation of making sure bulls are sexually mature and prepared in the same way (Olsen et al., 2020), it would be useful to measure all traits on all semen collections, because Mot24h and especially Mot48h registrations were lacking for many samples in the current data. It is tempting to explain the considerable difference in the genetic correlation for Dwg with Mot0h (−0.53) and Mot48h (0.50), respectively, by different genes affecting the 2 traits. However, another explanation might be differences in the recording of the 2 traits; for example, that only "successful" semen collections, in terms of volume, Conc, or Mot0h, were tested after 24 and 48 h. The strong genetic correlation between Mot0h and Mot24h of 0.96 (Olsen et al., 2020) indicates that the genes affecting the 2 traits are similar and thus points to a difference in recording practice. Consequently, the estimated genetic correlations between Dwg and Mot24h and Mot48h might be closer to expected for properly prepared and sexually mature bulls, but the recording practice might also mean that the phenotypically best bulls receive a measurement of motility after storage. Results from analyses of Mot24h and Mot48h should therefore be interpreted with caution.

Consequences for Selection Response in Semen Characteristics

Our results suggest that selection on Dwg at the performance test station might have affected sperm Conc and Mot0h negatively, perhaps explaining the slight genetic decline in semen characteristics reported by Olsen et al. (2020). Among volume, Conc and Mot0h, they found the largest relative genetic decline for concentration and the smallest relative genetic change for Mot0h. The attained selection responses are the result of the traits selected for in the performance test, the traits' true genetic parameters, and correlated responses to other traits in the breeding goal. Although selection for Dwg was based on breeding values, selection for semen characteristics has been performed on a phenotypic level by combining the results from the semen collection into an overall score from 0 to 5, where ≥3 implied that the bull was approved.

CONCLUSIONS

Our results indicate that the genetic correlations for BW and growth traits with semen characteristics depend on the age of the bulls. Although the majority of genetic correlations were favorable, we found unfavorable genetic correlations between Dwg and Conc and Dwg and Mot0h. Because all genetic correlations among the semen characteristics have been estimated to be favorable, selection for Dwg at the performance test might explain the slight negative genetic trend observed for semen characteristics in young Norwegian Red bulls.

ACKNOWLEDGMENTS

The authors are grateful to Geno (Hamar, Norway) for access to data, and to the Research Council of Norway (Oslo) for funding (project number: NFR-255097/E50). The authors have not stated any conflicts of interest.

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Paper III

Olsen, H. B., Heringstad, B., & Klemetsdal, G. (2021)

Genetic analysis of semen characteristic traits in Norwegian Red bulls at the artificial insemination center

Journal of Dairy Science, in press

J. Dairy Sci. 104
https://doi.org/10.3168/jds.2020-19294

Genetic analysis of semen characteristic traits in Norwegian Red bulls at the artificial insemination center

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ABSTRACT

Compared with cow fertility, genetic analyses of bull fertility are limited and based on relatively few animals. The aim of the present study was to estimate genetic parameters for semen characteristics of Norwegian Red bulls at the artificial insemination (AI) center (Geno AI station, Stange, Norway) and to estimate genetic correlations between some of these traits and andrology traits measured at the performance test station. The data from the AI center consisted of records from 137,919 semen collections from 3,145 bulls with information on semen weight, sperm concentration, motility before and after cryopreservation, motility change during cryopreservation, and number of accepted straws made. Data from the performance test station included 12,522 observations from 3,219 bulls on semen volume, concentration, and motility (%) when fresh and after storing for 24 and 48 h. Genetic parameters were estimated using linear animal repeatability models that included fixed effects of year-month of observation, age of bull, interaction between semen collection number, and interval between collections for all traits and type of diluter for postcryopreservation traits. The random effects included test-day, permanent environmental, and additive genetic effects of the bull. Based on records from the AI center, we found that semen weight, sperm concentration, and number of straws were moderately heritable (0.18–0.20), whereas motility had a lower heritability (0.02–0.08). Heritability of motility (%) was higher after cryopreservation than before. Genetic correlations among the semen characteristics ranged from unfavorable (−0.35) to favorable (0.93), with standard errors ranging from 0.02 to 0.22. Among the most precise genetic correlation estimates, number of straws made from a batch correlated favorably with semen weight (0.62 ± 0.06) and sperm concentration (0.44 ± 0.08), whereas sperm concentration was negatively correlated with weight (−0.33 ± 0.09). The genetic correlation between motility (%) before and after cryopreservation was 0.64 ± 0.14, and motility change during cryopreservation had a strong favorable genetic correlation with motility after cryopreservation (−0.93 ± 0.02). The estimated genetic correlation (standard error) between the traits volume, concentration, and motility when fresh measured at the performance test station and their respective corresponding traits at the AI center were 0.83 (0.05), 0.78 (0.09), and 0.49 (0.31). The final product at the AI center (number of accepted straws) correlated genetically favorably with all semen characteristic traits recorded at the performance test station (ranging from 0.51 to 0.67). Our results show that the andrology testing done at the performance test station is a resource to identify the genetically best bulls for AI production.

Key words: andrology, bull fertility, genetic parameter

INTRODUCTION

Extensive research has been done to evaluate female fertility traits and to estimate their heritability (Berry et al., 2014), and cow fertility is now included in the total merit index of many dairy cattle populations (Pryce et al., 2014). Even though one bull can be used on thousands of females with frozen semen and AI, genetic studies of bull fertility have received much less attention than female fertility, and the studies performed have generally been based on relatively small data sets. Sufficient semen quality is required for the sperm cells to fertilize the egg and can thereby serve as indicator traits for field fertility, and favorable genetic correlations have been documented between semen characteristics and female reproductive performance in cattle (Johnston et al., 2014; Hagiya et al., 2018). Heritability tends to be larger for semen characteristics than for female fertility traits (Berry et al., 2014) but varies considerably both between and within traits because of differences in population and breed, maturity of the bulls, how the traits are recorded and defined, and statistical modeling and sample size. Berry et al. (2014)

Received July 15, 2020.
Accepted January 25, 2021.
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performed a meta-analysis of the most commonly used reproductive traits and found that sperm volume and concentration were moderately heritable (0.20 and 0.17, respectively), whereas the heritability of motility was low (0.05). More recent studies have estimated the heritability of volume to be between 0.12 and 0.28, the heritability of concentration to be between 0.14 and 0.27, and the heritability of motility to be between 0.03 and 0.37 (Al-Kanaan et al., 2015; Sarakul et al., 2018; Berry et al., 2019; Olsen et al., 2020a). Heritability of motility after cryopreservation has been estimated to range between 0.13 and 0.24 (Ducrocq and Humblot, 1995; Karoui et al., 2011; Berry et al., 2019).

Similar to the heritability estimates, genetic correlations among semen characteristic traits vary between studies. The meta-analysis by Berry et al. (2014) found that greater sperm concentration was genetically associated with higher sperm motility but only weakly associated with volume, which was supported by our analysis of data for young bulls at the performance test station (Olsen et al., 2020a). Other studies have estimated the genetic correlation between sperm volume and concentration to be strongly negative (Ducrocq and Humblot, 1995; Berry et al., 2019). Further, motility after freezing and thawing has been found to be weakly or negatively genetically correlated with volume and positively correlated with concentration and to have a strongly positive genetic correlation with motility before freezing (Karoui et al., 2011; Berry et al., 2019).

Olsen et al. (2020a) found that all semen characteristic traits measured on young bulls at the test station showed a slightly unfavorable genetic trend between 1994 and 2016. Although the genetic decline raises concern, we do not know the genetic associations between these traits and semen characteristics measured on Norwegian Red (**NR**) bulls in semen production at the AI center. A genetic study of semen characteristics for NR AI bulls has so far not been performed. The aim of this study was therefore to estimate genetic parameters for semen characteristics of AI bulls and to estimate genetic correlations between these traits and andrology traits measured at the test station.

MATERIALS AND METHODS

Data

Data from a total of 144,095 semen collections from 3,150 NR bulls routinely collected from 1994 to January 2020 at the Geno AI station (Stange, Norway) were available. The routine for semen collection was initiated by bulls being taken to the collection area and allowed 2 false mounts to become aroused before being tied up. Ten minutes later the bulls were allowed at least 1 false mount, and semen was collected. After another 10 min the procedure was repeated, giving 2 ejaculates in total per semen collection (S. Reisvaag, Geno AI center, Stange, Norway; personal communication). The general rule was that the ejaculates were not mixed if more than 20 min had passed between semen collections.

To ensure optimal fertility results from semen used for insemination, Geno controls all ejaculates when fresh and after freezing and thawing. Initially, collected semen was visually inspected, and the ejaculate was discarded if abnormalities such as discoloration, blood, or visible clusters of any material were noted. The 2 ejaculates were mixed and weighed before sperm concentration was measured with a photometer. Samples with $<390 \times 10^6$ spermatozoa/mL were discarded. The weight and sperm concentration of the sample determined the amount of diluter, and ejaculates with <1 g of semen or <10 g of total weight (ejaculate + diluter) were discarded. Subjective analysis with a phase contrast microscope was used to assess percentage of motile sperm cells (**mot%pre**), starting at 0 with 5% increments; progressive motility score on a scale of 1 to 4 (**mot_pre**); and sperm defects such as loose heads or abnormalities in the tail or intermediate part as well as proximal and distal droplets. Samples with <70% motile sperm cells, motility score <3, >10% of a particular sperm defect, or >17% defects in total were discarded. After samples were cooled to 5 ± 3°C, diluted a second time, and properly mixed, they were ready to be filled into straws. The straws were frozen following IMV Technologies' standard freezing curve for bull semen and stored in a container with liquid nitrogen until evaluation of semen quality following cryopreservation. One straw per batch was reactivated (heated to 35 ± 3°C), and percent motility (**mot%post**), motility score (**mot_post**), and percentages of sperm defects were measured in the same way as when fresh, but the acceptable threshold level for motility was lower. Now, samples with <50% motile sperm cells, motility score <3, >10% of a particular sperm defect, or >17% defects in total were discarded. The change in motility during cryopreservation (**mot%change**) was calculated as the difference between mot%pre and mot%post. If the sample was rejected before cryopreservation, it would not be measured after cryopreservation; however, this concerned few records (Table 1). If the semen collection was approved at all levels of assessment, the number of straws made from that batch was recorded (**n_straw**).

For genetic analyses, only records with semen weight >0 were kept. Observations with weight >35, sperm concentration $<250 \times 10^6$ or $>2{,}800 \times 10^6$, n_straw <20 or >3,000, or mot%change <0 were considered erroneous and omitted (n = 10,575 observations). Furthermore, records were excluded if the bull was younger

than 13 mo or older than 100 mo at the time of collection. Age class of the bull at the time of collection was defined as age in months from 13 to 39 and thereafter grouped in intervals of 10. The data contained a total of 284 year-month (**yr_mo**) classes after excluding observations from yr_mo 200504, 200505, and 200506, containing only 1 observation each. Due to few semen collections performed in July each year, recordings during this month were combined with June registrations. Semen collection number per bull was categorized as 1, . . . , 10 and thereafter in intervals of 10 up to ≥100. Interval between semen collections varied and was defined as 0 (twice on the same day), 2 (1 or 2 d), 3, . . . , 7, and ≥8 d between semen collections. Semen collection number and interval between collections were combined to a joint fixed effect (**ncol_interval**) with 151 levels, where, for example, 4_3 means the bull's fourth semen collection with 3 d since the previous collection. Because of few observations in ncol_interval 3_2 and 4_2, those were combined with 3_3 and 4_3, respectively. Type of diluter was changed during the period of data collection from milk to Biladyl (Minitube), and SpermVital (Kommisrud et al., 2008) was used for 2.7% of the ejaculates. Effect of diluter was included only when analyzing the postcryopreservation traits. Test day was included as a random effect because the number of observations per subclass was small.

After edits, the data set had a total of 137,919 observations on 3,145 NR bulls, with descriptive statistics given in Table 1. Before grouping, the mean interval between semen collection was 7 d and the median was 4. Bulls had on average 51 semen collections, and their mean age at day of collection was 27 mo.

Also available were andrology data from the bull breeding soundness evaluation at the performance test station, where the most promising NR bull calves were tested each year. The calves arrived at the station at 4 to 5 mo of age, and growth, conformation, and temperament were assessed during the stay. At the end of the stay, when the bulls were around 12 mo old, several andrology traits were measured and used to ensure that only bulls with acceptable semen quality were selected and sent to the AI center. The andrology data from the bull breeding soundness evaluation were analyzed in a previous study (Olsen et al., 2020a), and we used 12,522 observations on 3,219 bulls measured from 1994 to 2016. Here, sperm quantity (referred to here as "volume") was measured (in mL) directly from the measurement cup, and concentration was recorded by a photometer. The photometer was replaced in March 2013; up until this date, the photometer used could not measure concentrations $<390 \times 10^6$ spermatozoa/mL, and concentration was set to 390×10^6 if the photometer showed a value of 0 but sperm cells were found during microscope evaluation. Consequently, concentration was defined as 2 traits, before (**conc1**) and after (**conc2**) March 2013; conc2 was as recorded with the higher-resolution photometer, and conc1 was categorized into 10 classes (0, 1–390, then in intervals of 200, and finally $>1{,}790 \times 10^6$ spermatozoa/mL). Motility was measured subjectively under a phase contrast microscope at 3 time points: when fresh and after storing for 24 and 48 h. Only semen collections with volume >0 mL were kept for analyses. Samples with volume >12 mL or sperm concentration $>3{,}000 \times 10^6$ spermatozoa/mL were considered erroneous and removed. Bulls had to be between 10.5 and 15.5 mo old at the test day, and only bulls that had been assigned a group number and group year (the group and the year bulls were transferred from the station) were kept. Similar to the data from the AI station, we included an interaction between semen collection number (1 = first semen collection to 6 = sixth or later collection) and number of days since previous collection (1 = 1–4 d, 2 = 5–10 d, and 3 = >10 d) as a fixed effect in addition to group-year and age of the bulls in months. See Olsen et al. (2020a) for further details on semen collection, editing of data, and descriptive statistics.

Table 1. Descriptive statistics of Norwegian Red bulls from the Geno AI station (Stange, Norway)

Trait[1]	Records, no	Bulls, no	Mean	SD	Minimum	Maximum
weight	137,772	3,143	8.67	3.10	0.1	35
conc	136,470	3,134	1,184.29	378.83	250	2,800
mot_pre	135,064	3,134	3.97	0.18	1	4
mot%pre	85,368	3,107	77.96	4.97	0	90
mot_post	135,811	3,133	3.94	0.27	1	4
mot%post	133,460	3,123	55.26	6.12	0	80
mot%change	84,246	3,104	23.15	7.11	0	85
n_straw	128,251	3,110	590.3	263.7	20	2,967

[1]weight = semen weight (g); conc = sperm concentration (10^6 spermatozoa/mL); mot_pre = motility score (1 to 4) before cryopreservation; mot%pre = percentage motility before cryopreservation; mot_post = motility score (1 to 4) after cryopreservation; mot%post = percentage motility after cryopreservation; mot%change = motility change during cryopreservation; n_straw = number of accepted straws.

Models

To estimate variance components for the traits recorded at the AI center we used univariate, linear animal repeatability models in DMU (Madsen and Jensen, 2013). The following model was defined:

$$Y_{ijklmno} = \mu + age_i + yr\_mo_j + ncol\_interval_k + diluter_l + testday_m + a_n + pe_n + e_{ijklmno},$$

where $Y_{ijklmno}$ is the oth observation on one of the semen characteristics; μ is the mean; age_i is the fixed effect of the ith age class in month $i = 13, \ldots, 39$, thereafter in intervals of 10 up to 100 (33 classes); yr_mo_j is the fixed effect of the jth month and year, $j = 1, \ldots, 284$; $ncol\_interval_k$ is the fixed effect of the kth group of semen collection number and interval between collections, $k = 1, \ldots, 151$; $diluter_l$ is the fixed effect of the lth diluter, l = milk, Biladyl, or SpermVital (included only for the postcryopreservation traits); $testday_m$ is the random effect of the mth test day $\sim N\left(0, \mathbf{I}\sigma^2_{td}\right)$, where $\mathbf{I}$ is an identity matrix and σ^2_{td} is the test-day variance; a_n is the random genetic effect of the nth bull $\sim N\left(0, \mathbf{A}\sigma^2_a\right)$, with σ^2_a being the additive genetic variance; pe_n is the random permanent environment effect of the bull $\sim N\left(0, \mathbf{I}\sigma^2_{pe}\right)$, with σ^2_{pe} being the permanent environmental variance; and $e_{ijklmno}$ is the random residual $\sim N\left(0, \mathbf{I}\sigma^2_e\right)$, with σ^2_e being the residual variance. The pedigree of the bulls was traced back as far as possible, up to 8 generations, and the additive genetic relationship matrix $\mathbf{A}$ included 32,078 animals.

Bivariate linear animal models were used to estimate genetic correlations among the semen characteristics recorded at the AI center and with the traits recorded at the performance test station. The effects included in the model used for the AI traits were as described above, whereas the following model was used for the traits recorded at the performance test station:

$$Y_{ijklmo} = \mu + age_i + group\text{-}year_j + collection\_n\text{-}interval_k + testday_l + a_m + pe_m + e_{ijklmo},$$

where Y_{ijklmo} is the oth observation on one of the 6 andrology traits; μ is the mean; age_i is the fixed effect of the ith age in months, $i = 11, \ldots, 15$; $group\text{-}year_j$ is the fixed effect of the jth group and year the bull left the test station, $j = 1, \ldots, 131$; $collection\_n\text{-}interval_k$ is the fixed effect of the kth group of ejaculate number (1 = first semen collection to 6 = sixth or more collection) and interval in days since previous collection (1 = 1–4 d, 2 = 5–10 d, and 3 = >10 d), $k = 1, \ldots, 16$; $testday_l$ is the random effect of the lth test day; a_m is the random genetic effect of the mth bull; pe_m is the random permanent environment effect of the bull; and e_{ijklmo} is the random residual.

The following assumptions were made for the distribution of the random test day (**td**), permanent environmental (**pe**), additive genetic (**a**), and residual (**e**) effects included in the models, where the subscript numbers refer to location (1 = AI center and 2 = AI center or test station):

$$\begin{bmatrix}\mathbf{td}_1\\ \mathbf{td}_2\end{bmatrix} \sim N\left[\begin{pmatrix}0\\0\end{pmatrix}, \mathbf{I} \otimes \begin{pmatrix}\sigma^2_{td_1} & \sigma_{td_1td_2}\\ \sigma_{td_1td_2} & \sigma^2_{td_2}\end{pmatrix}\right],$$

$$\begin{bmatrix}\mathbf{pe}_1\\ \mathbf{pe}_2\end{bmatrix} \sim N\left[\begin{pmatrix}0\\0\end{pmatrix}, \mathbf{I} \otimes \begin{pmatrix}\sigma^2_{pe_1} & \sigma_{pe_1pe_2}\\ \sigma_{pe_1pe_2} & \sigma^2_{pe_2}\end{pmatrix}\right],$$

$$\begin{bmatrix}\mathbf{a}_1\\ \mathbf{a}_2\end{bmatrix} \sim N\left[\begin{pmatrix}0\\0\end{pmatrix}, \mathbf{A} \otimes \begin{pmatrix}\sigma^2_{a_1} & \sigma_{a_1a_2}\\ \sigma_{a_1a_2} & \sigma^2_{a_2}\end{pmatrix}\right],$$

$$\begin{bmatrix}\mathbf{e}_1\\ \mathbf{e}_2\end{bmatrix} \sim N\left[\begin{pmatrix}0\\0\end{pmatrix}, \mathbf{I} \otimes \begin{pmatrix}\sigma^2_{e_1} & \sigma_{e_1e_2}\\ \sigma_{e_1e_2} & \sigma^2_{e_2}\end{pmatrix}\right],$$

where $\mathbf{I}$ is an identity matrix and $\mathbf{A}$ is the additive genetic relationship matrix, including 32,078 animals if location for trait 2 was the AI center and 46,635 animals if trait 2 was from the test station. The covariance matrices show variances on the diagonal and covariances on the off-diagonal. For analyses of one trait recorded at the AI center and the other recorded at the performance test station, the residual covariances, $\sigma_{e_1e_2}$, were set to 0 because the measurements differed in time and place.

The heritability (h^2) and repeatability (c^2) were calculated using the following formulas, where variables are as defined previously:

$$h^2 = \frac{\sigma^2_a}{\sigma^2_{td} + \sigma^2_a + \sigma^2_{pe} + \sigma^2_e},$$

$$c^2 = \frac{\sigma^2_a + \sigma^2_{pe}}{\sigma^2_{td} + \sigma^2_a + \sigma^2_{pe} + \sigma^2_e}.$$

RESULTS AND DISCUSSION

Fixed Effects: AI Center

Figure 1 shows the effect of the bull's age on semen weight, sperm concentration, motility change during cryopreservation, and number of straws made from one semen collection. The effect of age on various semen characteristics has been explored in many studies, and similar to our results for semen weight, findings generally show increased semen volume with increasing age (Karoui et al., 2011; Al-Kanaan et al., 2015; Berry et al., 2019). Also consistent with our results, Karoui et al. (2011) and Berry et al. (2019) observed the most rapid increase in semen volume before 2 yr of age, and from the age of 50 mo onward the amount of semen did not change much (Figure 1). The age effect for n_straw generally followed the same pattern, indicating that the number of straws strongly depended on semen weight. Similar to Berry et al. (2019), we found that semen concentration increased rapidly until 20 mo of age, reaching a maximum around 30 mo and decreasing thereafter. From 60 mo onward, concentration seems to have plateaued for the bulls in our data. Bulls from 20 to 30 mo of age had numerically the smallest change in motility during cryopreservation, but the difference between the most extreme solutions was small and standard errors were large and overlapping. Solutions for the effect of ncol_interval (results not shown) revealed that the bull's first semen collection generally had higher weight and lower sperm concentration, whereas later semen collections did not differ much in terms of amount or quality. Regarding interval between collections, both semen weight and n_straw increased with longer interval. Increasing semen volume with a longer interval between collections has been well documented in other studies; Mathevon et al. (1998), Fuerst-Waltl et al. (2006), and Al-Kanaan et al. (2015) all reported the highest volume with the longest interval between collections. Consecutive semen collections on the same day had a negative effect on change in motility during cryopreservation, and a longer interval in days gave a smaller loss in motility, but standard errors were large. The fixed effects for yr_mo (results not shown) indicated strong fluctuations over the years and variation between seasons for semen characteristics traits.

Variance Components and Parameters: AI Center

Studies considering bull fertility have typically been based on relatively few animals. Among the most recent studies, for example, Al-Kanaan et al. (2015) considered 562 bulls, Sarakul et al. (2018) included 131 bulls, and Berry et al. (2019) estimated genetic parameters based

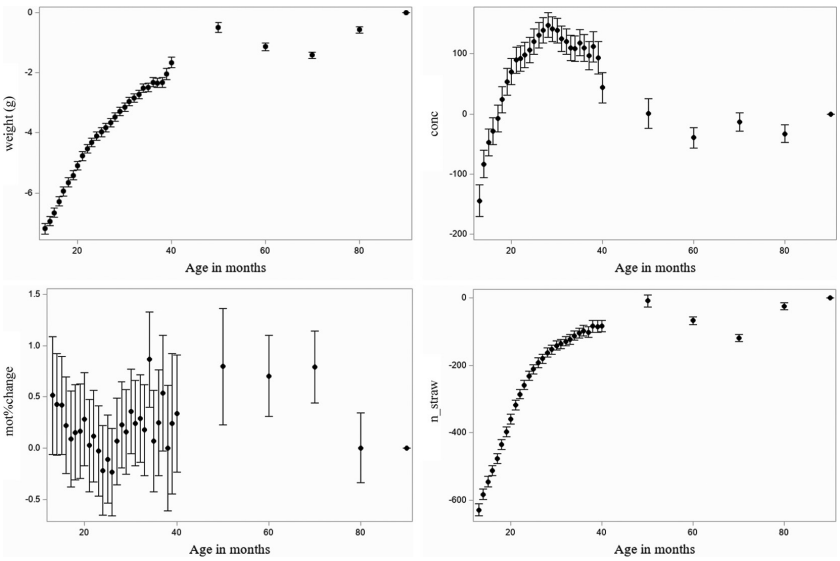

Figure 1. Solutions for fixed effect of age in months estimated with univariate models for semen weight (weight), sperm concentration (conc), motility change during cryopreservation (mot%change), and number of straws (n_straw) made from the semen collected at the AI center of Norwegian Red bulls (Geno AI station, Stange, Norway). Age classes >40 mo are merged in groups of 10. Error bars show ±1 SE.

Table 2. Estimated variance components, heritability (h^2), and repeatability (c^2) (SE in parentheses) of Norwegian Red bulls from the Geno AI station (Stange, Norway)

	Variance component[2]					
Trait[1]	σ^2_a	σ^2_{pe}	σ^2_{td}	σ^2_e	h^2	c^2
weight	1.52 (0.19)	1.44 (0.14)	0.32 (0.01)	3.77 (0.01)	0.22 (0.024)	0.42 (0.008)
conc	32,132 (4,890)	51,614 (3,880)	4,372 (171)	71,629 (282)	0.20 (0.029)	0.52 (0.008)
mot_pre	0.0011 (0.0003)	0.0053 (0.0003)	0.0022 (0.0001)	0.0255 (0.0001)	0.03 (0.009)	0.19 (0.006)
mot%pre	0.40 (0.16)	3.22 (0.19)	1.52 (0.07)	19.15 (0.10)	0.02 (0.006)	0.15 (0.005)
mot_post	0.0012 (0.0004)	0.0058 (0.0004)	0.0054 (0.0002)	0.0526 (0.0002)	0.02 (0.006)	0.11 (0.004)
mot%post	2.88 (0.60)	6.45 (0.51)	2.25 (0.08)	26.39 (0.11)	0.08 (0.016)	0.25 (0.007)
mot%change	2.20 (0.50)	4.49 (0.43)	3.06 (0.13)	37.31 (0.19)	0.05 (0.011)	0.14 (0.005)
n_straw	11,021 (1,494)	13,902 (1,155)	3,221 (118)	32,482 (133)	0.18 (0.022)	0.41 (0.008)

[1]weight = semen weight (g); conc = sperm concentration (10^6 spermatozoa/mL); mot_pre = motility score (1 to 4) before cryopreservation; mot%pre = percentage motility before cryopreservation; mot_post = motility score (1 to 4) after cryopreservation; mot%post = percentage motility after cryopreservation; mot%change = motility change during cryopreservation; n_straw = number of accepted straws.

[2]Additive genetic $\left(\sigma^2_a\right)$, permanent environmental $\left(\sigma^2_{pe}\right)$, test day $\left(\sigma^2_{td}\right)$, and residual $\left(\sigma^2_e\right)$ variance components were obtained from univariate models, but bivariate models gave very similar estimates.

on 787 bulls from 16 breeds. More than 3,000 bulls in our study permitted precise heritability estimates and genetic correlations.

Estimated variance components for the semen production traits are given in Table 2. The genetic coefficient of variation was largest for n_straw (18%), followed by sperm concentration (15%) and semen weight (14%). Among the motility traits, mot%change varied genetically the most (6.4%), followed by mot%post (3%), whereas mot%pre had the lowest genetic coefficient of variation (0.8%). Semen weight, sperm concentration, and number of straws were moderately heritable (0.18–0.22), whereas among the motility traits mot%post had the highest heritability (0.08). The heritability estimates of semen weight (0.22 ± 0.024), sperm concentration (0.20 ± 0.029), and mot_pre (0.03 ± 0.009) agreed with the meta-analysis performed by Berry et al. (2014) in which heritability of volume, concentration, and motility was estimated to be 0.20, 0.17, and 0.054, respectively. Some studies obtained considerably higher heritability estimates for motility before cryopreservation, such as 0.22 in Kealey et al. (2006) and 0.37 in Berry et al. (2019). In addition to the traits previously discussed, Berry et al. (2019) estimated genetic parameters for postcryopreservation traits. They obtained a heritability estimate for motility score after cryopreservation of 0.13, which was larger than our estimates for mot_post of 0.02 and mot%post of 0.08 (Table 2). Ducrocq and Humblot (1995) and Karoui et al. (2011) estimated even higher heritability for this trait (0.24 and 0.22, respectively). Similar to Karoui et al. (2011), we found the largest heritability of motility after cryopreservation, whereas Berry et al. (2019) found the largest heritability estimate before cryopreservation, and Ducrocq and Humblot (1995) estimated similar heritability estimates for motility score before and after cryopreservation. Regarding motility change during cryopreservation, Berry et al. (2019) obtained a heritability of 0.21 for this trait, which is considerably higher than our heritability estimate of 0.05.

In our previous study of 3,972 bulls at the NR performance test station (Olsen et al., 2020a), the heritability for both volume and concentration was 0.14, which is lower than the estimates in the present study. The heritability of motility in fresh samples, however, was the same based on date from the performance test station and from the AI center (0.03), but the genetic coefficient of variation was considerably lower for the adult bulls (0.8 vs. 5.3%), as was the phenotypic coefficient of variation (6.3 vs. 31.1%).

One problem with estimating variance components on semen characteristics from the AI station is the potential bias arising from preselection of these traits after the performance test station. We calculated the selection intensity for the performance test station traits volume, concentration, and motility in fresh samples and after storing for 24 and 48 h (measured from 1994 to 2016) and found that the selection on these traits has been almost nonexistent (ranging from 0.036 for volume to 0.10 for concentration).

The repeatability (Table 2) was highest for concentration (0.52), followed by semen weight (0.42) and n_straw (0.41). The repeatability for the motility traits ranged from 0.11 to 0.25. The repeatability of semen weight and concentration were higher for bulls in AI production than for the test bulls (Olsen et al., 2020a). The increase in both heritability and repeatability is likely a result of increased age and sexual maturity resulting in more consistent semen collections.

Table 3. Estimated genetic correlation (SE in parentheses) among semen characteristics of Norwegian Red bulls from the Geno AI station (Stange, Norway)

Trait[1]	conc	mot_pre	mot%pre	mot_post	mot%post	mot%change	n_straw
weight	−0.33 (0.09)	0.05 (0.14)	0.23 (0.16)	0.01 (0.14)	0.15 (0.11)	−0.05 (0.12)	0.62 (0.06)
conc		−0.06 (0.15)	−0.35 (0.18)	−0.33 (0.14)	−0.22 (0.12)	0.13 (0.13)	0.44 (0.08)
mot_pre			0.48 (0.18)	0.59 (0.15)	0.09 (0.17)	0.12 (0.18)	0.01 (0.14)
mot%pre				0.39 (0.22)	0.64 (0.14)	−0.34 (0.21)	−0.05 (0.18)
mot_post					0.74 (0.11)	−0.68 (0.13)	−0.20 (0.15)
mot%post						−0.93 (0.02)	−0.05 (0.12)
mot%change							0.12 (0.13)

[1]weight = semen weight (g); conc = sperm concentration (10^6 spermatozoa/mL); mot_pre = motility score (1 to 4) before cryopreservation; mot%pre = percentage motility before cryopreservation; mot_post = motility score (1 to 4) after cryopreservation; mot%post = percentage motility after cryopreservation; mot%change = motility change during cryopreservation; n_straw = number of accepted straws.

Genetic Correlations: AI Center

Estimated genetic correlations among the semen characteristic traits measured at the AI station are given in Table 3 and ranged from being unfavorable (−0.35) to strongly favorable (0.96). Some of the estimates had large standard errors (ranging from 0.02 to 0.21), and in the following we focus on the most precise. Both semen weight and sperm concentration had favorable genetic correlation with n_straw (0.62 and 0.44, respectively), which is not surprising because concentration and particularly semen weight determine the number of straws that can be made from a sample. We estimated a negative genetic correlation between sperm concentration and semen weight (−0.33 ± 0.09). Berry et al. (2014) and Karoui et al. (2011) also reported a negative genetic correlation between semen weight and concentration, but their estimates were borderline significant or not significant, whereas Berry et al. (2019) obtained an estimate of −0.40 ± 0.20 which agrees with the results in the present study. Furthermore, Karoui et al. (2011), Berry et al. (2014), and Berry et al. (2019) obtained positive genetic correlations between sperm concentration and motility in fresh samples of 0.73, 0.61, and 0.29, respectively, whereas our corresponding estimate was negative but with a high standard error (−0.35 ± 0.18). Consistently, the same negative genetic correlations were estimated between sperm concentration and motility after cryopreservation (−0.33 ± 0.14 for mot_post and −0.22 ± 0.12 for mot%post). When measured on the same scale, motility before cryopreservation correlated favorably with motility after cryopreservation (0.59 ± 0.15 for motility score and 0.64 ± 0.14 for percentage motility). These correlation estimates were weaker than in other studies, where estimates ranged from 0.81 to 0.92 (Ducrocq and Humblot, 1995; Karoui et al., 2011; Berry et al., 2019). Furthermore, the estimated genetic correlation between the 2 measures of motility (mot_pre vs. mot%pre and mot_post vs. mot%post) were positive both before and after cryopreservation (0.48 and 0.74, respectively). Motility change during cryopreservation showed strong favorable genetic correlations with mot%post (−0.93 ± 0.02) and mot_post (−0.68 ± 0.13). In contrast, Berry et al. (2019) estimated a genetic correlation of −0.19 between motility change and motility after cryopreservation.

The estimated permanent environmental correlations (Supplemental Table S1, https://figshare.com/articles/online_resource/supplementary_tables_JDS19294_docx/14627529; Olsen, 2021) and test-day correlations (Supplemental Table S2, https://figshare.com/articles/online_resource/supplementary_tables_JDS19294_docx/14627529; Olsen, 2021) are given in supplementary tables. Overall, these correlations had low standard errors.

Genetic Correlations Between Traits Measured at the AI Center and Performance Test Station

Table 4 shows the number of bulls with registrations from both the performance test station and the AI center for all trait combinations. The estimated genetic correlations between these traits can be found in Table 5. A strong, favorable genetic correlation was estimated between semen volume measured at the test station and semen weight recorded at the AI center (0.83 ± 0.05). Similarly, sperm concentrations measured at the test station and in the AI center were strongly genetically correlated (0.78 ± 0.09 and 0.59 ± 0.20 for conc1 and conc2, respectively). Note that conc1 was categorized into 10 categories. Table 4 shows that only 377 bulls were assessed for both conc2 and concentration at the AI center, but the standard error was still reasonably small, likely due to the traits being recorded similarly. We estimated a favorable genetic correlation between motility in fresh samples measured at the test station and mot%pre (0.49 ± 0.31); that is, with a high standard error. The variable motility after storing for 24 h correlated genetically favorably with both mot%pre and mot%post (0.59 ± 0.23 and 0.58 ± 0.15, respec-

Table 4. Number of bulls with data for the trait combinations of semen characteristics measured at the performance test station[1] and traits measured at the Geno AI station (Stange, Norway)[2] of Norwegian Red bulls

Trait	weight	conc	mot_pre	mot%pre	mot_post	mot%post	mot%change	n_straw
volume	2,311	2,303	2,303	2,280	2,303	2,297	2,297	2,289
conc1	1,935	1,930	1,931	1,908	1,930	1,924	1,907	1,917
conc2	380	377	376	376	377	377	376	376
mot0h	2,309	2,301	2,301	2,278	2,301	2,295	2,277	2,287
mot24h	1,989	1,982	1,982	1,973	1,982	1,980	1,972	1,972
mot48h	1,385	1,383	1,383	1,377	1,384	1,382	1,376	1,376

[1]volume = semen volume (mL); conc1 = sperm concentration recorded before March 2013 and categorized into 10 classes (0, 1–390, then increments of 200, and finally >1,790 × 10^6 spermatozoa/mL); conc2 = sperm concentration recorded after March 2013 (given as 10^6 spermatozoa/mL); mot0h, mot24h, and mot48h = percentage motility in fresh samples and after storing for 24 and 48 h, respectively.

[2]weight = semen weight (g); conc = sperm concentration (10^6 spermatozoa/mL); mot_pre = motility score (1 to 4) before cryopreservation; mot%pre = percentage motility before cryopreservation; mot_post = motility score (1 to 4) after cryopreservation; mot%post = percentage motility after cryopreservation; mot%change = motility change during cryopreservation; n_straw = number of accepted straws.

tively). However, results involving motility after storing for 24 and 48 h should be interpreted with caution because our previous work has indicated a selection bias for these variables, with more bulls with high-quality semen having their motility inspected after storing (Olsen et al., 2020a,b). Finally, except for conc2, all test station traits explored in this study were found to have high genetic correlations to n_straw at the AI center, with estimated genetic correlations ranging from 0.51 ± 0.13 to 0.67 ± 0.16.

Overall Discussion

With a large data set consisting of 137,919 records on 3,143 bulls, and close to zero preselection for the semen traits after the performance test, genetic parameters for semen characteristics could be estimated with good precision. The heritability estimates for both semen weight and sperm concentration were somewhat larger than those found for corresponding traits at the performance test, whereas the heritability estimated for all motility traits was low in size. The low heritability estimates for motility traits may be a result of imprecise recording. The large standard errors found for the genetic correlations involving motility variables suggest the same. Thus, there is a need to reconsider the definition of the traits recorded. Sperm motility should ideally be measured objectively (e.g., with computer-assisted sperm analysis). In addition to n_straw being a product of semen weight and sperm concentration, the trait also includes a quality aspect as only semen collections with minimum motility and normal sperm cells are approved. The heritability of n_straw was close to that of semen weight and concentration, and with positive genetic correlations with both traits it could be an interesting alternative to explore. Under genomic selection, bulls are becoming younger than the average AI bulls in this study and therefore more comparable with the bulls at the performance test station. At this age, all the semen traits have been estimated with favorable genetic correlations (Olsen et al., 2020a), likely because the traits then are affected by early maturity. Still, high genetic correlations were estimated between corresponding traits in the 2 environments.

Table 5. Genetic correlations (SE in parentheses) between traits measured at the performance test station[1] and traits measured at the Geno AI station (Stange, Norway)[2] of Norwegian Red bulls

Trait	weight	conc	mot_pre	mot%pre	mot_post	mot%post	mot%change	n_straw
volume	0.83 (0.05)	−0.21 (0.12)	0.11 (0.17)	−0.11 (0.20)	−0.12 (0.18)	−0.17 (0.15)	0.23 (0.15)	0.58 (0.10)
conc1	−0.28 (0.16)	0.78 (0.09)	0.04 (0.22)	0.00 (0.28)	−0.15 (0.25)	−0.02 (0.21)	0.12 (0.21)	0.51 (0.13)
conc2	−0.06 (0.26)	0.59 (0.20)	0.53 (0.31)	0.28 (0.41)	0.07 (0.13)	−0.16 (0.30)	0.30 (0.29)	0.25 (0.24)
mot0h	0.32 (0.21)	—[3]	0.16 (0.27)	0.49 (0.31)	0.05 (0.31)	0.28 (0.26)	−0.14 (0.27)	0.61 (0.18)
mot24h	0.37 (0.15)	—	0.19 (0.22)	0.58 (0.23)	0.23 (0.23)	0.59 (0.15)	−0.37 (0.18)	0.56 (0.12)
mot48h	0.38 (0.20)	—	0.27 (0.26)	0.70 (0.26)	−0.17 (0.31)	0.14 (0.25)	0.31 (0.26)	0.67 (0.16)

[1]volume = semen volume (mL); conc1 = sperm concentration recorded before March 2013 and categorized into 10 classes (0, 1–390, then increments of 200, and finally >1,790 × 10^6 spermatozoa/mL); conc2 = sperm concentration recorded after March 2013 (given as 10^6 spermatozoa/mL); mot0h, mot24h, and mot48h = percentage motility in fresh samples and after storing for 24 and 48 h, respectively.

[2]weight = semen weight (g); conc = sperm concentration (10^6 spermatozoa/mL); mot_pre = motility score (1 to 4) before cryopreservation; mot%pre = percentage motility before cryopreservation; mot_post = motility score (1 to 4) after cryopreservation; mot%post = percentage motility after cryopreservation; mot%change = motility change during cryopreservation; n_straw = number of accepted straws.

[3]Analysis did not converge.

CONCLUSIONS

With a large data set, including more than 3,000 bulls, genetic parameters for semen characteristics could be estimated with good precision. Semen characteristic traits are heritable and can be used in genetic evaluation of NR bulls. Andrology traits measured at the test station were highly correlated with corresponding traits measured at the AI center, and a future genetic evaluation could preferably be based on data from both. The most promising traits to consider would be volume, concentration, motility after freezing, and number of accepted straws. The first 2 traits ensure more sperm cells per collection, motility after freezing ensures frozen semen with good quality, and number of straws is the final product of the AI center.

ACKNOWLEDGMENTS

We thank Geno (Hamar, Norway) for access to data and to The Research Council of Norway (Oslo) for funding (project no. NFR-255097/E50). We especially thank Simon Reisvaag, Ragnhild Gjeset, and Sigbjørn Karlsen at the Geno AI center for all of their help and support. The authors have not stated any conflicts of interest.

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5.4 General Discussion

5.4.1 Importance of bull fertility

Good fertility is fundamental to breeding and continued animal production. Semen characteristics play an important role in this context because such traits can be used as indicators for cattle fertility. Poor semen quality can for example cause the inseminated cow to return to oestrus because the sperm cells fail to fertilize the egg. Berry et al. (2014) writes that "practically no genetic variation has been found for non-return rate measured in AI bulls" but explains this to be due to selection (only bulls with sufficient semen quality are considered for use in AI), and also because of the standardization of straws for number of sperm. The standardization ensures that all semen straws that are used contain the same semen volume and concentration of sperm cells, and the quality testing of the collected semen ensures that the motility and percentage of normal sperm cells are at a satisfactory level. Positive genetic correlations have been estimated between semen quality traits and female reproductive performance in cattle (Johnston et al., 2013; Hagiya et al., 2018), which suggests that semen characteristics can serve as a proxy for cow fertility and that selection for semen quality might improve cow fertility, and the other way around. In wild populations, the selection for fertility is strong because individuals, as well as sperm cells, that have an advantage in mating and fertilization are more likely to convey their genes to the next generation. Standardization of straws with respect to semen quality limits natural selection to operate and masks the bull's true genetic potential as a sire, which could lead to deterioration of male fertility over time. Furthermore, use of new technology, such as sex separated semen is gaining popularity, and because this method benefits from bulls with excellent semen quality, the demands for better semen quality may increase in the future. Thus, male reproductive traits should be monitored to ensure sustainability of a breeding program and might also have an additional economical value in a longer time perspective.

Data from many years of quality testing of semen collected at the performance test station and at the AI center of Norwegian Red (Table 3) made it possible to contribute, through papers I-III, to the limited literature on the genetics of bull fertility (Tables 1 and 2), with estimates of heritability, genetic correlations, and genetic change over time, based on large datasets.

5.4.2 Heritability of semen characteristics

Data from the performance test station was well suited for estimating genetic parameters because these bulls were unselected with respect to semen characteristics. The heritability of semen volume and concentration were both estimated to be 0.14, while the heritability of motility in fresh samples and sperm defects were 0.03 and 0.02, respectively (Paper I). Only 4 % of the semen collections were recorded with a defect, and this, combined with the binary nature of the trait and the linear model analysis, resulted in a low heritability. When comparing the heritability estimates for volume, concentration and motility measured at the performance test station with those found by others (Table 1), our estimates were slightly lower. Berry et al. (2014) reported average heritabilities of 0.20 for volume, and 0.17 for concentration, in their meta-analysis, while their heritability estimate for motility in fresh samples was 0.05. The bull's young age and inexperience with the semen collection routine might have contributed to a lower heritability than what would have been found with older, and better trained bulls. As expected, the heritability estimates using data from the AI center (Paper III) for the older and more experiences bulls were higher for semen weight (volume) (0.22), and sperm concentration (0.20), however, the heritability for motility in fresh samples remained low as at the performance test station (0.03). The routine evaluation of motility at the AI center, as at the performance test station, consist of subjective measurements under a microscope, where the percentage of motile sperm

cells are given with 10% or 5% intervals (performance test station and AI center, respectively). This imprecise recording might be a reason for the low heritability. A more precise measurement, for example by the use of CASA, might improve the phenotyping, as well as increase the heritability of the trait.

The estimated heritability of motility change during cryopreservation of 0.05 and for motility after freezing of 0.08 (Paper III) were lower than comparable estimates found by Berry et al. (2019), of 0.21 and 0.13. In the latter study, heritability of motility in fresh samples was as high as 0.37. The large variation in heritability estimates for motility in the literature (Table 1) might be due to variable levels of precision of measurement, trait definitions, number of observations and bulls in the dataset, as well as breed differences.

5.4.3 Genetic correlations between semen characteristics

Another objective was to estimate genetic correlations between semen characteristics, estimates that are largely lacking in the literature (Table 2). This was clearly shown in the meta-analysis performed by Berry et al. (2014) where genetic correlation estimates for many of the trait combinations were missing in the literature, and if found, the pooled mean estimates were based on only 1 to 4 studies. We expected the considerable number of observations and bulls in our study to provide the limited literature available with precise estimates. For traits measured at the performance test station, we found all the genetic correlations to be favorable (Paper I). The genetic correlation (standard error) between motility and volume, and motility and concentration were 0.57 (0.15) and 0.71 (0.16), respectively, implying that selection for more semen and sperm per semen collection would genetically result in bulls with more motile sperm cells. Our estimate of the genetic correlation between volume and sperm concentration was also favorable (0.30), but with a high standard

error (0.24). Unlike the estimates based on data from the performance test station (Paper I), the genetic correlations between semen characteristics measured at the AI center were not all favorable (Paper III). The genetic correlation between concentration and motility, for example, ranged from -0.06 to -0.33, depending on the motility trait. Similarly, the genetic correlation between semen weight and sperm concentration had turned negative, -0.33 (0.09), and the genetic correlation between volume and motility appeared to be close to zero (0.01 – 0.15).

Our results indicate that the genetic correlations between volume and concentration, and concentration and motility, are favorable early in a bull's life when they depend on early maturity, but become negative, or close to zero, later. In the literature, there is a lack of consensus on the size of these genetic correlations as well (Table 2). In the meta-analysis performed by Berry et al. (2014), the genetic correlation between volume and concentration was estimated to -0.16 (0.10), but this pooled mean was obtained on basis of only 4 studies, with individual estimates ranging from -0.72 to 0.06. The pooled-mean estimate for the genetic correlation between concentration and motility was also based on only 4 studies, in which the estimates were ranging from negative to positive, but the overall result was clearly positive with a small standard error (0.61 ± 0.10). For traits recorded at the AI center, we had to consider the possibility of the estimates being affected by selection because the bulls here had been through andrology testing at the performance test station and bulls that did not fulfil the minimum criteria for semen production were excluded at this point. This was an issue when estimating genetic parameters for semen characteristics at the AI center, in general, and could be a reason for the difference in results between the two data sources. With information on which bulls that were selected we estimated the standardized selection intensity for the semen characteristic traits at the performance test station (Paper III) and found it to be small for all traits (*i*, the standardized selection intensity, ranging from 0.036 for volume to 0.10 for concentration). This suggests that the genetic parameters estimated for traits measured at the AI center were not affected by selection of any significant size for these traits and that the differences in

genetic correlations between concentration and motility, and maybe also between volume and concentration more likely depended on the maturity of the bulls.

Genetic correlations between corresponding traits at the performance test station and AI center for semen weight and concentration were 0.83 and 0.78, respectively (Paper III). For motility in fresh samples the genetic correlation was lower (0.49), suggesting that this trait might not be genetically the same throughout a bull's life.

5.4.4 Genetic trends of semen characteristics

Having performance test station data going back to 1994, we could estimate genetic trends for bull fertility in the Norwegian Red population. All genetic trends were unfavorable and significantly ($p < 0.05$) different from zero, but the changes were very small (Paper I). This means that the bull breeding soundness evaluation at the performance test station used to exclude poor semen producers from becoming AI bulls had not been sufficient to improve the genetics of semen characteristics in the population over time; a consequence of the low standardized selection intensity found for the semen characteristics after the performance test. The unfavorable genetic trends could result from correlated selection responses to other traits. The additional traits for which we had data at the performance test were body weight at 150 and 330 days of age, as well as average weight gain in between. Traditionally, because Norwegian Red is a dual-purpose breed, daily weight gain has been considered when selecting bulls from the performance test. This selection could potentially have caused the negative genetic trend observed for the semen characteristics. Therefore, we estimated genetic correlations between body weight traits and the semen characteristics (Paper II). We found that the genetic correlations were positive and favorable, except the unfavorable genetic correlations (although with high standard errors) between daily weight gain (DWG) and

sperm concentration (-0.25 ± 0.15) and DWG and motility (-0.53 ± 0.34). To our knowledge, only one other study have estimated the genetic correlation between semen characteristics and DWG, and similar to us they found a negative genetic correlation between motility and DWG of -0.36 (Smith et al., 1989). These results mean that selection for DWG can be one explanation for the negative genetic trend observed for concentration and motility, but indirect selection for other traits should not be excluded as a cause. For example, as mentioned, a weak unfavorable genetic correlation has been estimated to milk yield (Hagiya et al. (2017) that could affect the semen characteristics negatively. The standardization of straws used for insemination limits natural selection for fertility to take place and may hide the actual differences in fertility between bulls used for AI, contributing to the negative trend for semen characteristics. Inbreeding and accumulation of recessive homozygotes affecting fertility could be another cause.

5.5 Identified knowledge gaps for future study

There is a need to increase the knowledge on the genetics of bull fertility in Norwegian Red and other populations. For our population, estimation of the genetic correlations between semen characteristics and traits in the total merit index should be one priority. Cow fertility, health traits, and milk yield would be the most important traits because they have high relative weight in the total merit index and may therefore have the potential to explain the unfavorable genetic trends for semen characteristic traits. Knowledge about such genetic correlations is important in order to breed for a sustainable population with good fertility. Estimates of such genetic correlations are also largely lacking in the literature.

The close to zero standardized selection differential for semen traits after the performance test (Paper III) shows that the bull breeding soundness evaluation has mainly been used to identify the very poorest semen producers, rather than to select for improved bull fertility. We have shown

that semen characteristics are heritable (Paper I and III), and with ~ 8,000 bull calves being genotyped each year as potential candidates to become an AI bull, genomic breeding values can be predicted for the traits in the routine genetic evaluation of Norwegian Red and be used in selection of bull calves to the performance test station. Currently, data from both stations can be used when estimating breeding values for semen characteristics, increasing the accuracy of the breeding values. At the AI center, semen weight, sperm concentration, and motility are genetically uncorrelated, or negatively correlated. However, the average age of bulls used in AI and semen production in Norway has decreased after the gradual implementation of genomic selection from 2013, onwards. Our results suggest that both the heritability of semen characteristics and genetic correlations between the traits are not the same throughout the bulls' life. With decreasing age and maturity among the semen producers, the results from the performance test station, with favorable genetic correlations between all semen characteristics, might be more relevant for the semen characteristics measured at the AI center in the future. To ensure that the next generations of Norwegian Red bulls provide a good amount of semen, with a high sperm concentration and motility, all these traits need to be considered when selecting bull calves to the performance test. Because of a low percentage of bulls being recorded with sperm defects (4 %) and the analyzed trait being binary (whether a defect was observed or not), the heritability estimated from a linear model was low (0.02). Applying a threshold model would be an alternative and would likely increase the heritability, but this model refers to another scale than the linear model (Gianola, 1981). The estimated heritability of 0.02 found in Paper I is considerably lower than the heritability of the percentage of abnormalities that were estimated in other populations (Kealey et al., 2006; Garmyn et al., 2011; Corbet et al., 2013). Measuring sperm defects on a continuous scale, either subjectively or with CASA, would likely increase the heritability and make the trait better suited for use in selection of bull calves. Despite the frequency being low, likely due to prior selection, sperm defects should be part of the routine genetic evaluation of semen characteristics because the trait was found with an unfavorable genetic trend, and a sufficient

proportion of normal sperm cells are vital for fertilizing the egg. Motility after freezing is also an important trait to monitor and select for. This trait also had the highest heritability among all the motility traits considered (Paper III). Paper I suggest that one should ensure that bulls have reached puberty before phenotyping, with puberty being defined with the minimum requirements for motility and concentration that are required for fertilizing an egg. Further, we suggested that phenotyping at the AI center could be improved by recording each single ejaculate and not by mixing the two ejaculates if less than 20 minutes had passed in between collections (Paper III). This recording might mostly affect semen weight. Number of straws made from a semen collection is an economic important trait because it is the final product of the AI center. Furthermore, the trait has a moderate heritability of 0.18 (Paper III) and was estimated with favorable genetic correlations with all semen characteristics measured at the performance test station. The trait includes both number of sperm cells (semen volume x sperm concentration) and semen quality because only collections with above a minimum threshold for motility and normal sperm cells are filled on straws. In our dataset, this trait was assigned a missing value if minimum requirements were not met. The trait might be a better candidate for genetic study, and potential selection, if a zero was assigned here instead. Then the semen collections (and bulls) that failed to produce straws would be penalized instead of excluded. With regards to the traits analyzed, breeding values should be predicted based on semen weight, sperm concentration, motility, and defects.

Summing up, estimating genomic breeding values for semen characteristics measured at both stations and using those when buying bull calves to the performance test station are advised. This will allow to continuously monitor genetic trends, and to use breeding values for semen characteristics as additional information when selecting bull calves to the performance test station, e.g. to omit bull calves with very low estimates of breeding values for the traits or choosing the best half-sibs on breeding values for semen traits given similar total merit index.

A review article by (Butler et al., 2020) shows that genes important for both total number of spermatozoa, sperm motility, and percentage of living spermatozoa have been identified in several bovine populations. SNPs that affect motility after cryopreservation have also been identified (Dai et al., 2009; Hering et al., 2014). Because all potential Norwegian Red AI bulls are genotyped, it is possible to perform studies in search of alleles/haplotypes important for the semen characteristics in this population as well. A first approach would be to perform a genome-wide association study with the aim to identify Quantitative Trait Loci for the semen characteristic traits. If SNPs that have a large effect on semen characteristics are found, those can be considered when buying bull calves to the performance test station to select for better semen characteristics in Norwegian Red. Moreover, deleterious homozygotes can be found by searching for haplotypes that are common in the population, but never occur in a homozygote state in live animals (Kadri et al., 2014). Furthermore, mildly deleterious recessives or incompletely penetrant lethal genes can be searched by using runs of homozygosity (ROH) mapping as for example done for female fertility in Finnish Ayrshire (Martikainen et al., 2020). ROHs are regions on the genome where both DNA strands are identical by decent, and the length of the runs indicate how recent the inbreeding occurred, with longer regions being more recent. This information can be used to calculate local measures of inbreeding and inbreeding depression along the chromosome, identifying the haplotypes that are deleterious for bull fertility. Such studies could be performed in Norwegian Red to increase our knowledge of the genetics of bull fertility in this population and have more and better tools for increasing semen characteristics in the future and ensure great fertility on the bull side as well.

5.6 Conclusion

We have shown that semen characteristics are heritable in the Norwegian Red population and that breeding values can be estimated and used to select

for better bull fertility. This is important because the bull breeding soundness evaluation, carried out at the performance test station and used to select bulls for semen production, has not genetically improved the semen characteristics over time as shown by the slightly unfavorable genetic trends observed for all semen characteristics measured at the performance test station. The standardized selection intensity after the test was close to zero, but the negative genetic trends could also result from the negative genetic correlation that was estimated to daily weight gain. Including semen characteristic traits in routine genetic evaluations of Norwegian Red would provide breeding values that can be used to improve bull fertility and to monitor genetic trends. Using all available data and considering both semen volume, sperm concentration, motility (after freezing) and defects is advised. The genetic correlations between traits depend on the maturity and experience of the bulls, being positive early in life and becoming negative, or close to zero, for some traits later. The analyzed sperm characteristics measured at the performance test station had high genetic correlations to the same traits at the AI center, but less for motility in fresh samples than for semen amount and sperm concentration. More knowledge and better tools for selecting for fertility in bulls is important because good fertility is fundamental to breeding and continued, sustainable, animal production.

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ISBN: 978-82-575-1836-3
ISSN: 1894-6402

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