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# **How body condition, current nutrition and Finn gene status influence litter size in Norwegian White Sheep.**

- A study in flushing and antifleushing.**

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## Abstract

The effect of body condition, current nutrition and Finn gene status on litter size was studied using 111 ewes of Norwegian White Breed, in the age from 1.5 to 6.5 years. The last 11 weeks before mating in mid-November the ewes were divided into three groups, where one group was fed to increase their body condition (BC), one was fed to keep a constant BC, and the last was fed to decrease BC. All groups were offered forage ad libitum as the sole feed, where forages differed considerably in energy concentration. Forty-eight of the ewes were stalled individually, whereas 63 were stalled in group pens. In addition to using the well-known body condition score (BCS) from 1-5 to describe the fatness of each ewe, also Body Mass Index (BMI) was calculated for each individual. Of the 111 ewes there were 41, 59 and 11 ewes with Finn gene status 0, 1 and 2, respectively. The litter size was registered with ultrasound measurement in January. Results showed a significant effect of BMI at mating ( $P < 0.0001$ ), giving an increase of 0.20 lambs per 10-point increase in BMI, or an increase of 0.26 lambs per increase in BCS ( $P < 0.001$ ). Finn gene status was significant in the BCS model ( $P = 0.03$ ) and tended to be significant in the BMI model ( $P = 0.05$ ), giving an extra of 0.30 and 0.27 lambs, respectively, for each increase in Finn gene status. Current nutrition, measured as daily changes in BCS or BMI before mating did not influence litter size significantly. The presence of Finn genes seemed to be more powerful on ewes in high BC than in low. There were significant differences in litter size between ewes in the Increasing diet as opposed to both the Constant ( $P < 0.05$ ) and Decreasing ( $P < 0.01$ ) diets. The High conditioned ewes (BCS  $> 3+$ ) got significantly ( $P < 0.01$ ) more lambs (2.88 vs 2.13) than the Low conditioned ewes (BCS  $< 3-$ ). On average, the 1.5-year-old ewes got 0.41 lambs less than the adult ewes.

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# 1. Introduction.

Flushing has been a way of increasing litter size in ewes for decades. Even so, it is not fully known whether it's the positive energy balance and thereby increasing body condition (BC) at the time of mating, or the BC at mating, as a consequence of previous good feeding, that gives the bigger ovulation rates. With this study we wanted to find out which of these two factors are most important for determining litter size in ewes.

Flushing is primarily regarded as an improved nutritional status the last few weeks before mating. Nottle et al. (1997) suggested that the preovulatory flushing effect can exceed the possible negative effect from poor nutrition 6 months before ovulation. As an example feed-restricted Merino ewes that lost one-seventh of their body weight between 6 and 4 months, but recovered this loss over the next 3 months, responded to a 10-day preovulatory lupin-grain supplement with an average 0.57 extra ovulations per ewe (1.63 vs 1.06) compared with their previously restricted but "unflushed" contemporaries. Ewes that were experiencing an undernutrition about 2 months before mating, did not get the same flushing effect as the ewes having this experience 6 months before mating, probably because their reduced body condition and weight couldn't be increased enough on only a 10-day flushing regime, to express their genetic potential of ovulation rate (McDonald et al., 2011).

Gunn et al. (1969) described the importance of having the right body condition at the time of mating. Both across breeds and management systems, the highest ovulation rate is at a BC score of 3-3.5 on a 5-point scale. Ewes that were already gaining weight and were in a high body condition did not show a response to flushing (Nottle et al., 1997).

The Norwegian White Sheep (NKS) is known to be a fertile sheep breed, with an average of 2.31 total born lambs per mated adult ewe (2 years or more at lambing) (Langaker and Lystad, 2018). Of the 2.31 born lambs, 2.21 were liveborn and 4,4% stillborn. Apart from year 2012 (2.28 born lambs), the total number of lambs born per mated adult ewe has varied between 2.34 and 2.38 (2016), from 2011 to 2017. The high fertility is partly due to the presence of a fertility gene origin from the Finnish Landrace breed, which in single presence is known to increase the number of fetuses with 0.3 and in double presence with 0.6-0.7 (Boman, 2013).

Big litters of 4, 5 and 6 lambs are a challenge for the farmer and an ethical dilemma. The risk of birth difficulties is increasing for each extra lamb above 2 (Gløersen, 2018). Big litters also increase the risk of stillbirth- and pregnancy-related health issues like abdominal hernia, which is the protrusion of the abdominal contents through a defect in the abdominal wall, caused by the extra pressure of fetuses and placentas (LiveCorp, 2019). There is an economic gain related to letting ewes raise triplets, especially if three finished lambs are successfully produced directly from summer pasture, but also if some extra feeding is required. However, triplets lead to a higher risk of mastitis on the ewe (Waage and Vatn, 2008), and give higher demands to pasture quality and high milk production from the ewe. This might also require higher amounts of concentrates if the quality of roughage isn't good enough.

## 2. Materials and Methods.

### 2.1 Experimental design.

The data from this study was collected between late august 2017, when the ewes came back from summer pasture for lamb weaning, and until ultrasound measurement of the fetuses in mid-January 2018 on pregnancy day 35-50.

In total, there were 119 ewes included in the study, between the age of 1.5 to 6.5 years at mating. At weaning, each ewe were assigned a body condition score (BCS) according to (Russel, 1984), which varied between 2.5 and 4.5. Body condition score is a better estimate of the fat- and muscle development in the ewe, rather than body weight alone, since the skeletal size of the ewes will vary among individuals. The scoring system goes from 1 to 5, where 1 is almost complete lack of body fat and 5 is a thick layer of body fat outside the skeleton. All ewes were sheared during the first week of September.

The ewes were randomly allocated to three feed levels designed to increase, maintain or decrease BC. The intention was that at mating, ewes on a high energy diet would have increased its BC with 1.0 score, and ewes on a low energy diet would have decreased its BC with 0.5 score. For the NKS breed, one point of BC equals about 13% of BW, or 11-12 kg. To reduce their BCS from 3 to 2,5 ewes need to lose 5,5 kg in 11 weeks, which means about 70 g/day. From mating and until ultrasound measurement, the ewes were fed solely with grass silage of medium harvesting time for ad libitum intake. This should make the normal conditioned ewes to eat for maintenance and allow poor conditioned ewes to display some compensatory growth, due to their slightly higher intake capacity (INRA, 1989). Protein, mineral and vitamin were covered according to NRC (2007) for all groups.

All ewes in the study were tested for a gene of origin from the Finnish Landrace, referred to as “Finn gene status”, where carrying-ewes are expected to have higher fertility than non-carrying ewes (Boman, 2013). The ewes were either free for the Finn gene (Finn gene status 0), had the gene in single presence (Finn gene status 1), or had the gene in double presence (Finn gene status 2). Despite random allocation of the ewes to each feeding group it was assured that the groups were fairly balanced for the Finn gene statuses, so there were representatives with Finn gene status 0, 1 and 2 in all three groups (Table 1). Of the 111 animals there were 41, 59 and 11 ewes with Finn gene status 0, 1 and 2, respectively.



Table 1. Distribution of ewes in the study.

Feeding Group	Total number of Ewes	No of ewes in trial group		No of ewes with Finn gene status		
		Individual	Group pens	0	1	2
<b>Increasing</b>	35	15	20	15	18	2
<b>Constant</b>	39	14	25	11	22	6
<b>Decreasing</b>	37	15	22	15	19	3

## 2.2 Feeding regime.

A group of 48 ewes (trial group 1), with a wide variation in BCS, were placed in individual stalls with registration of feed intake four days a week. They were distributed to three groups, and fed diets differing in energy concentration. The remaining 72 sheep (trial group 2) were stalled in three large group pens with ad libitum intake of the three diets. The goal was to let the groups either increase (Increasing group), maintain (Constant group) or decrease (Decreasing group) BC the following 11 weeks until mating in mid-November. To achieve this the three groups were fed grass silage of different maturity stages (early, medium and late harvesting time (HT)) (Table 2). The feeding was adjusted during the 11 weeks to obtain the planned change of BC (Table 3). The last six weeks before mating the Decreasing group was fed late harvested grass silage mixed with straw (Late+straw30; 70% silage and 30% straw on dry matter basis). The silage and energy intakes of the individually stalled ewes during the study is shown in Table 4.

Table 2. Chemical composition of grass silage and concentrate.

	Harvesting time				Concentrate
	Early	Medium	Late <sup>1</sup>	Late+straw30 <sup>1</sup>	
Dry matter, g/kg	213	241	252	331	958
g/kg DM					
Organic matter	933	923	933		927
Crude protein	179	125	116	101	162
Starch					40.6
NDF	482	565	647	741	192
iNDF	81	121	183		
Fat	36.1	30.9	29.8	22.1	34.5
Watersoluble carbohydrates	36.5	25.5	15.3	11.6	4.80
Lactic acid	70.7	68.2	66.3		
Formic acid	9.6	9.0	1.1		
Acetic acid	13.4	11.9	8.6		
Propionic acid	3.6	2.7	1.0		
Butyric acid	0	0	8.5		
Ethanol	8.6	6.8	9.5		
Ammonia N (g/kg N)	90	102	109		
pH	4.17	4.46	4.27		
NEL, MJ/kg DM <sup>2</sup>	6.7	5.8	5.1	4.8	6.8
AAT, g/kg DM <sup>3</sup>	71.8	68.4	65.3	63.7	104
PBV, g/kg DM <sup>4</sup>	54.5	7.3	3.5	-8.6	-26

<sup>1</sup>Late+straw30= harvesting time 3 mixed with barley straw accounting for 30% of the mixture on dry matter basis. Treated with 9.4 g urea per kg DM in straw, and 0.2 g Na<sub>2</sub>SO<sub>4</sub> per g urea to increase the content of Nitrogen and Sulphur of the straw.

<sup>2</sup>Net energy lactation.

<sup>3</sup>Amino acids absorbed in small intestine.

<sup>4</sup>Protein balance in rumen.

Table 3. Feeding regime from 11 weeks before and during mating.

Weeks before mating	Increasing	Constant	Decreasing
11-7	Early HT	Medium HT	Late HT
6-0	Early HT	Late HT	Late HT-straw30

Table 4. Silage and energy intakes of the ewes<sup>1</sup> in trial group 1 throughout the experimental feeding.

	Increasing	Constant	Decreasing	SEM	P-value
Silage intake, kg DM/d					
Week 11-7	2.28 <sup>a</sup>	1.63 <sup>b</sup>	1.51 <sup>b</sup>	0.06	<0.001
Week 6-0	2.02 <sup>a</sup>	1.32 <sup>b</sup>	1.44 <sup>b</sup>	0.06	<0.001
ME intake, MJ/d					
Week 11-7	25.5	16.5	13.4	0.59	<0.001
Week 6-0	23.1	11.9	12.1	0.59	<0.001
NEL intake, MJ/d					
Week 11-7	15.1 <sup>a</sup>	9.6 <sup>b</sup>	7.6 <sup>c</sup>	0.33	<0.001
Week 6-0	13.8 <sup>a</sup>	6.7 <sup>b</sup>	6.7 <sup>b</sup>	0.33	<0.001
CP intake, g/d					
Week 11-7	402	206	170	8.65	<0.001
Week 6-0	380	154	157	8.65	<0.001
AAT intake, g/d					
Week 11-7	163	112	98	3.90	<0.001
Week 6-0	146	86	92	3.90	<0.001
PBV intake, g/d					
Week 11-7	119	12,5	0,66	2,33	<0.001
Week 6-0	126	4,89	-0,33	2,33	<0.001

<sup>1</sup>48 ewes with individual registration of feed intake.

The ewes in the Constant and Decreasing groups gained more weight than wanted during week 11-7 before mating. The feeding level for those two groups were therefore modified from week 6: The Constant group changed to the late harvested silage and the Decreasing group got 30% of their dry matter ration as straw.

### 2.3 Management around mating.

In Norway, it is most common to let the ewes have their first lambs at the age of one, so the 1.5-year-old ewes in this study was having their second pregnancy. At ultrasound measurement, 111 ewes were confirmed pregnant. Seven ewes were confirmed without any fetuses, and one ewe was taken out of the study in November due to mastitis. The seven non-pregnant ewes came from all three feeding groups and both individual and group stalled. All calculations and tables in this article are based on the 111 ewes that were confirmed pregnant in January. Of these 111 ewes there were 58 from 2.5 to 6.5 years, referred to as “adult” ewes, and 53 1.5-year-old ewes.

### 2.4 Measurements of body weight, BCS and BMI.

The ewes in the group pens were weighed 7 times, and the ewes in individual stalls were weighed 8 times during the feeding period (Table 5).

*Table 5. Weighing dates of ewes during feeding period.*

<b>Weighings in trial group 1 (individual stalls):</b>	<b>Weighings in trial group 2 (group pen):</b>
28 <sup>th</sup> of August	
1 <sup>st</sup> of September	1 <sup>st</sup> of September
20 <sup>th</sup> of September	11 <sup>th</sup> of September
2 <sup>nd</sup> of October	25 <sup>th</sup> of September
9 <sup>th</sup> of October	9 <sup>th</sup> of October
30 <sup>th</sup> of October	30 <sup>th</sup> of October
20 <sup>th</sup> of November	20 <sup>th</sup> of November
4 <sup>th</sup> of December	4 <sup>th</sup> of December

All the ewes were given a BCS 3 times: 30<sup>th</sup> of August, 18<sup>th</sup> of October and 21<sup>st</sup> of November. They were also assigned a BCS at 15<sup>th</sup> of December, but this measure was only used in the calculation for those few ewes that were mated after 15<sup>th</sup> of December. Most of the ewes were mated close to the BC measurement of 21<sup>st</sup> of November. All three times, BC scoring was done independently by the same two trained assessors, and their average score for each ewe was used. In addition to the scores 1-5 there were used + and -, which gave an extra or less of 0.25 BCS. A BCS of 3+ gives 3,25 points. Lowest score is 1 and highest is 5. This gave many possible values between two scores.

The body height and length of all ewes were measured in order to calculate body mass index (BMI) (Figure 1). This gave an objective measure of fatness, as opposed to BCS which is a subjective measure of fatness. The ewes' BMI was expected to be between 110 and 200. The ewes' BMI was calculated two times in the experimental period, first time on 1<sup>st</sup> of September, and last time on 20<sup>th</sup> of November, as:

$$\text{BMI} = \text{weight (kg)} / (\text{withers height (m)} \times \text{length (m)})$$

BMI is presumably a good measure of body reserves in non-pregnant animals and in early pregnancy, but is not usable for an animal in late pregnancy (Chavarría-Aguilar et al., 2016).

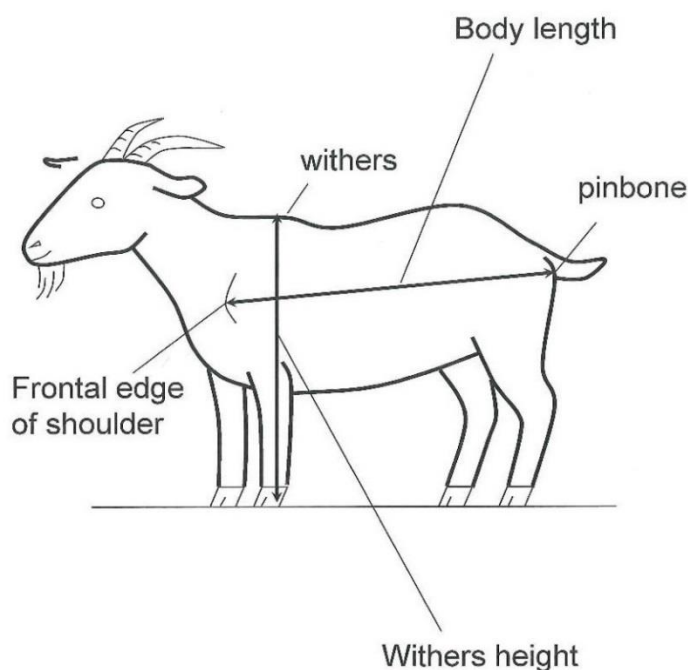


Figure 1. Showing how height and length of sheep were measured.

## 2.5 Statistical model.

A statistical regression model where the ewes' actual BMI at mating and daily BMI-change until mating are included in the model as continuous variables was used. Each ewe's observed daily BMI-change from weaning and until mating was used as a continuous measure of current nutrition in the model, irrespective of the feeding group (Increasing, Constant, or Decreasing) each ewe was assigned to. The model describes the number of fetuses registered by ultrasound as a linear effect of BMI at mating, plus the daily BMI-change until mating, an effect of the interaction between these two, and the Finn gene status.

$$y = \text{BMI}_{\text{mating}} \times b_1 + \text{BMI}_{\text{change}} \times b_2 + (\text{BMI}_{\text{mating}} \times \text{BMI}_{\text{change}}) \times b_3 + \text{Finn gene status} \times b_4 + \varepsilon$$

There was also used a model to calculate the effect of litter size using BCS instead of BMI. In this model we included Finn gene status and age of ewe. Age of Ewe being either 1.5-years-old or adult.

$$y = \text{BCS}_{\text{mating}} \times b_1 + \text{Finn gene status} \times b_2 + \text{Age of Ewe} \times b_4 + \varepsilon$$

Body Condition score at weaning (BCS start) was tested in a model with the variables “BCSstart”, “BCSmating”, “Interaction BCSstart x BCSmating”, and “Finn gene status”, but “BCSstart” had no effect (P=0.76). Therefore, it was not included in the final model.

Ordinary linear models were chosen even if observations of litter size (y) is not normally distributed. For practical purposes this may still give reasonable results (T. Ådnøy, personal comm.). All statistic calculations were done using the statistical program RStudio.

To be able to study the difference between ewes in low, medium and high BC at mating, we created three groups of BCS and BMI in addition to the regression model presented above. We tried to make logical intervals relative to the measuring scale and to have even number of ewes in each group. The testing of the groups was done using an ANOVA model and running a “Contrast-test” between the 3 groups with 95% confidence level.

Body Condition Score groups:

Low (L) = Below 3- (< 2.75)

Medium (M) = 3- to 3+ (2.75 – 3.25)

High (H) = Above 3+ (> 3.25)

Body Mass Index groups:

Low (L) = < 140

Medium (M) = 140 - 159

High (H) = ≥ 160

Correlations between variables were calculated using Pearson correlation method in RStudio.

Simple linear regression was calculated to show the relationship of the BMI and BCS variables.

## 3. Results

### 3.1 Overall effects of feeding groups, age of ewes and litter size.

The goal was that ewes in Increasing group would increase their BC with 1 BCS and Decreasing group would lose 0.5 BCS. From weaning until mating, the variation in BCS increased considerably from being concentrated around 3, to being scattered throughout the whole scale, from 1 to 5.

In Increasing group, the change in BCS varied from -0.38 to 1.75. Only one ewe lost BCS in Increasing group, and most of them gained at least 0.5 BCS from weaning to mating. The average BCS change for all ewes in the Increasing group was 1.02 BCS. Constant group ended up losing 0.57 BCS in average. In Decreasing group, the loss in BCS varied from 0 to 2 BCS. Only one adult ewe in Decreasing group kept a constant BCS of 3 from weaning to mating, and the rest lost from 0.25 to 2 BCS. The total average BCS lost in Decreasing group was 0.87.

Table 6 show that the biggest difference in litter size between 1.5-year-old and adult ewes appeared within the Constant group. The adult ewes got 0.66 more lambs in average than the 1.5-year-olds even though the percentage presence of Finn gene status 1 and 2 were much bigger in the 1.5-year-olds than in the adult ewes (0.47% vs 0.14%). The difference between adult and 1.5-year-old ewes within Increasing group were only 0.1 lamb in average. This was mainly due to the ewe with 6 lambs. Without her, the difference in average litter size between adult and 1.5-year-old would have been 0.33 lambs.

Table 6. Average values of BMI, BCS, Body Weight and Litter Size according to feeding group and age of ewes.

Feeding Group	Number of ewes		Total BMI change	BMI at mating <sup>1</sup>	Total BCS change	BCS at mating	Weight change g/day	Weight at mating kg	Litter Size <sup>2</sup>
Increasing	Adult	20	27	175	1.15	4.42	183	101	2.90
	1.5 years	15	22	144	0.84	3.82	145	77	2.80 <sup>3</sup>
Constant	Adult	17	13	160	-0.49	2.88	62	89	2.71
	1.5 years	22	12	133	-0.62	2.45	35	67	2.05
Decreasing	Adult	21	8	151	-0.77	2.52	5	83	2.33
	1.5 years	16	5	129	-1.00	2.09	-1	67	1.94
All Groups	Adult	58	16	162	-0.026	3.28	58	91	2.64
	1.5 years	53	13	130	-0.318	2.73	55	70	2.23
Total average		111	15	149	-0.17	3.02	70	81	2.44

<sup>1</sup>BMI varied between 107 and 204.

<sup>2</sup>Litter size determined by ultrasound measurement and later confirmed at parturition.

<sup>3</sup>Without the ewe with 6 lambs, this average would have been 2.57 lambs.

The litter size in the Increasing feeding group was significantly higher than in the Constant and Decreasing group (Table 7). There were not significant differences between Constant and Decreasing group.

Table 7. Contrasts between feeding groups in estimated litter size.

Feeding Groups contrast	Estimated difference in litter size	P-value
Increasing-Constant	0.52	0.04
Increasing-Decreasing	0.70	0.004
Constant-Decreasing	0.17	0.69

Of the ten 1.5-year-old ewes that got triplets only one had Finn gene status 0 (Table 8). Half of them was in H-BCS group. Feeding Group and BCS Group had a high correlation ( $r=0.74$ ), which means that feeding level, tells a lot about what body condition they were in at mating.



For adult ewes: In both Increasing and Constant group 70% of the adult ewes got 3 lambs or more. Increasing group had 35% with 4 or 5(one ewe) lambs, while Constant group had 12% with quadruplets. Both “extreme” litters of 5 and 6 lambs were in Increasing group.

Decreasing group had 43% with more than two lambs, and only one of these ewes had quadruplets (this ewe had Finn gene status 1). Of single lambs, there were 20% in the Increasing, and respectively 12% and 14% in the Constant and Decreasing group.

For 1.5-year-old ewes: In Increasing group 60% of the 1.5-year-olds got 3 lambs or more. In those 60% there were two ewes with quadruplets and one ewe with 6 lambs in addition to triplet litters. In Constant group there was 18% triplets and no ewes with more than 3 lambs. The amount of single lambs was 13% for both Increasing and Constant group. In Decreasing group there were no ewes with more than 2 lambs and only one ewe with a single lamb (6.25%).

Table 8. Distribution of ewes relative to litter size.

Litter Size	Number of ewes	Age of ewe		Finn Gene status			Feeding Group			BCS Group <sup>4</sup>		
		1.5 years	Adult	0	1	2	In	Co	De	H	M	L
1	15	6	9	3	10	2	6	5	4	6	6	3
2	48	34	14 <sup>1</sup>	22	22	4	6	18	24	4	14	30
3	35	10	25	14	19	2	13	14	8	13	16	6
4	11	2	9	2	7	2	8	2 <sup>2</sup>	1 <sup>3</sup>	7	3	1
5	1		1		1		1			1		
6	1	1				1	1			1		

<sup>1</sup>Nine out of the 14 adult ewes were in the Decreasing group.

<sup>2</sup>Both ewes had Finn gene status 1, and BCS 3 and 3-.

<sup>3</sup>This ewe had Finn gene status 1 and BCS 2.

<sup>4</sup>H=high (Above 3+), M=medium (3- to 3+), L=low (Below 3-).

### 3.2 Statistical effect of BMI and BCS using Linear Models.

Table 9 shows the effect of BMI on litter size. BMI mating and BMI change are presented as a deviation from the mean. BMI at mating had a significant effect on litter size and gave an increase of 0.2 lambs per 10 points increase in BMI. Finn gene status tended to have a significant effect on litter size and gave an increase of 0.27 lambs per increase in Finn gene status. Daily BMI change until mating and the interaction between BMI at mating and BMI change were insignificant for the litter size.

Table 9. Linear model showing effect of BMI on litter size.

<b>Variables</b> <b>y = litter size</b>	<b>Estimated effect</b> <b>on litter size (b)</b>	<b>Std. Error</b>	<b>P-value</b>
<b>BMI mating (per 10 points)</b>	0.20	0.052	<0.001
<b>BMI change (per 10 points)</b>	-0.0057	0.076	0.93
<b>Interaction BMI mating and BMI change</b>	0.000024	0.0003	0.93
<b>Finn gene status</b>	0.27	0.133	0.05

Table 10 shows the effect of BCS on litter size. The model did not include change in BC before mating because it was highly insignificant and didn't bring any new information to the model. The model shows an increase of 0.26 lambs per increase in BCS, an extra 0.30 lamb for each increase in Finn gene status, and an increase of 0.37 lamb from 1.5-year-old to adult ewes.

Table 10. Linear model showing effect of BCS on litter size.

<b>Variables</b> <b>y = litter size</b>	<b>Estimated effect on</b> <b>litter size (b)</b>	<b>Std. Error</b>	<b>P-value</b>
<b>BCS at mating</b>	0.26	0.09	<0.01
<b>Finn gene status</b>	0.30	0.14	<0.05
<b>Age of ewe</b>	0.37	0.18	<0.05

### 3.3 Effect of low, medium and high BMI and BCS on litter size.

Body condition score and BMI gave similar results for litter size (Table 11). The difference in litter size between the ewes in L and H group was 0.75 lambs for BC and 0.70 lambs for BMI (Table 12). The high correlation ( $r=0.63$ ) between BMI and BCS is presented in Figure 2, for 1.5-year-old and adult ewes. The figure shows that thin ewes have bigger difference between BCS and BMI than fat ewes.

There was significant difference in litter size between the Low and the High group for both BCS and BMI. There were no ewes from Increasing group with BC below 3, and no ewes from Decreasing group above 3+ at mating. All ewes with BC of 4 and higher was in the Increasing feeding group. Of the 17 ewes with BC 4+ and better, there were only two 1.5-year-olds. Of the 10 ewes with BC 2- and less, there were eight 1.5-year-olds. All three Finn gene status variations were expressed in all three BCS and BMI groups.

Table 11. Obtained litter size according to BCS at mating (11a), and BMI at mating (11b) (ewe age and Finn-gene excluded).

11.a

11.b

Average litter size based on BCS at mating.				Average litter size based on BMI at mating.			
BCS groups <sup>1</sup>	Number of ewes	Litter size	P-value	BMI groups <sup>2</sup>	Number of ewes	Litter size	P-value
L	40	2.13	<0.001	L	42	2.07	<0.001
M	39	2.41		M	34	2.56	
H	32 <sup>3</sup>	2.88		H	34	2.77	

<sup>1</sup>BCS groups: L=low (Below 3-), M=medium (3- to 3+), H=high (Above 3+).

<sup>2</sup>BMI groups: L=low (<140), M=medium (140-159), H=high ( $\geq 160$ ).

<sup>3</sup>All ewes from Increasing feeding group was in the H BCS group, except from one ewe in M.

Table 12. Contrasts between low, medium and high conditioned ewes, based on BCS (12.a) or BMI (12.b) groups, alone.

12.a

BCS group contrast <sup>1</sup>	Estimate <sup>2</sup>	Std. Error	P-value
M – L	0.28	0.20	0.339
H – L	0.75	0.21	0.002
H – M	0.47	0.21	0.082

12.b

BMI group contrast <sup>3</sup>	Estimate <sup>2</sup>	Std. Error	P-value
M – L	0.49	0.21	0.05
H – L	0.70	0.21	0.003
H – M	0.21	0.22	0.60

<sup>1</sup>BCS groups: L=low (Below 3-), M=medium (3- to 3+), H=high (Above 3+).

<sup>2</sup>Estimated difference in litter size based on BCS and BMI group.

<sup>3</sup>BMI groups: L=low (<140), M=medium (140-159), H=high (≥160).

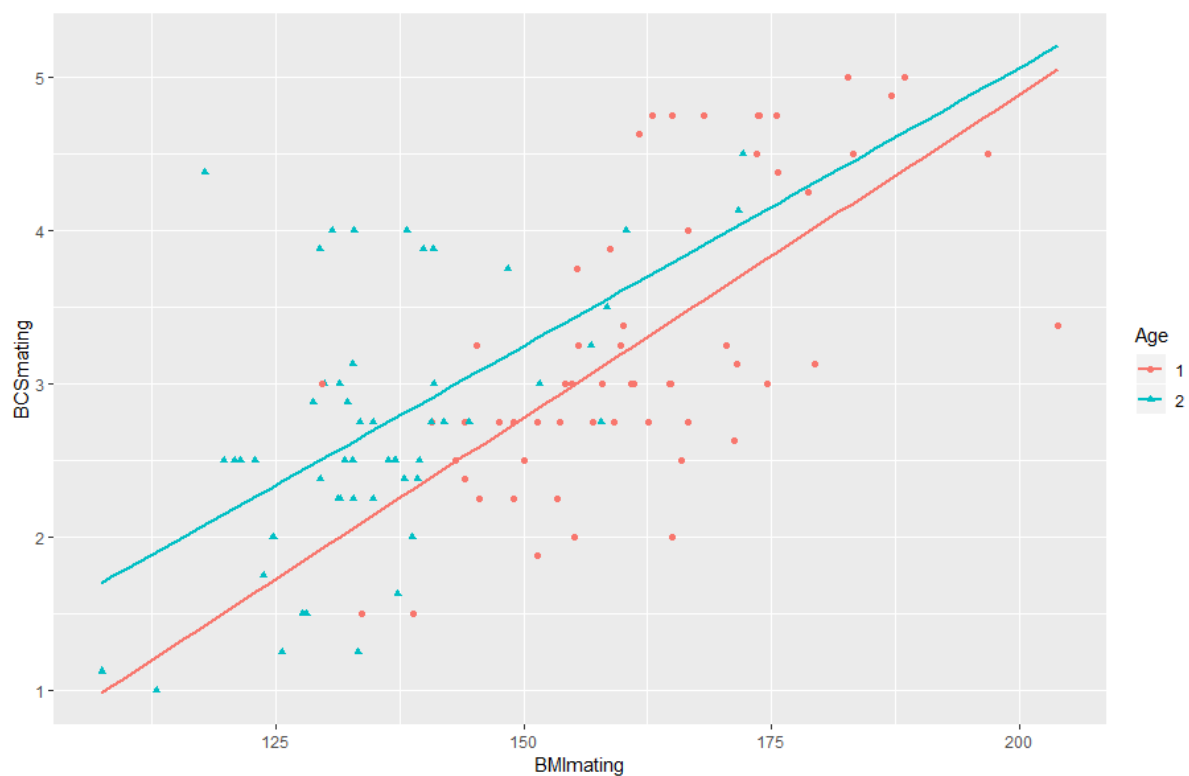


Figure 2. Plot showing correlation between BCS and BMI measurements, with regression lines for each group showing what BMI tells about BCS. Red (Age 1) are the adult ewes. Green (Age 2) are the 1.5-year-old ewes.

### 3.4 Effect of Finn gene status.

There was greater difference in litter size between the lower and upper BCS-group within ewes with Finn gene status 1, than within ewes with Finn gene status 0 (0.72 vs 0.44 lambs). Both statuses had the same average for L BCS ewes. The ewes with Finn gene status 2 were too few to be drawing any conclusions based on their litter sizes.

Table 13. Finn gene status' effect on litter size based on BCS at mating.

<b>Average litter size, based on Finn gene and BCS at mating.</b>				
<b>Finn gene status</b>	<b>BCS at mating<sup>2</sup></b>	<b>Litter size</b>	<b>Number of ewes</b>	<b>Litter size</b>
<b>0</b>	L	2.17	12	2.37
	M	2.31	16	
	H	2.61	13	
<b>1</b>	L	2.17	23	2.44
	M	2.29	17	
	H	2.89	19	
<b>2<sup>1</sup></b>	L	1.83	6	2.72 <sup>3</sup>
	M	3.35	4	
	H	6.00	1	

<sup>1</sup>Eight out 11 ewes with Finn gene status 2 was 1.5 years old.

<sup>2</sup> BCS groups: L=low (Below 3-), M=medium (3- to 3+), H=high (Above 3+).

<sup>3</sup>Among the ewes with Finn gene status 2 there was one ewe with 6 fetuses. This ewe increased average litter size considerably, and without this ewe, the average in this group would have been 2.4 which is the same as the average as the ewes with Finn gene status 1 have.

In the same way as with the BCS groups in Table 13, Table 14 show a greater difference in average litter size within ewes from Increasing group relative to within ewes from Decreasing group. Increasing group had 0.35 more lambs for ewes with Finn gene status 1, than for ewes with status 0. In Constant group there was close to no difference in average litter size between the Finn gene statuses. The difference was small also in Decreasing group.

*Table 14. Finn gene status' effect on litter size based on Feeding Group.*

<b>Average litter size, based on Finn gene and Feeding Group.</b>			
<b>Finn gene status</b>	<b>Feeding Group</b>	<b>Number of ewes</b>	<b>Litter Size</b>
<b>0</b>	Increasing	16	2.53
	Constant	10	2.36
	Decreasing	15	2.20
<b>1</b>	Increasing	18	2.88
	Constant	21	2.32
	Decreasing	20	2.16
<b>2</b>	Increasing	2	5.00
	Constant	6	2.33
	Decreasing	3	2.00

Figure 3 and 4 show plots of the BMI and BCS at mating according to litter size. For BCS there are several observations on the same spot, but it gives an idea of the variation.

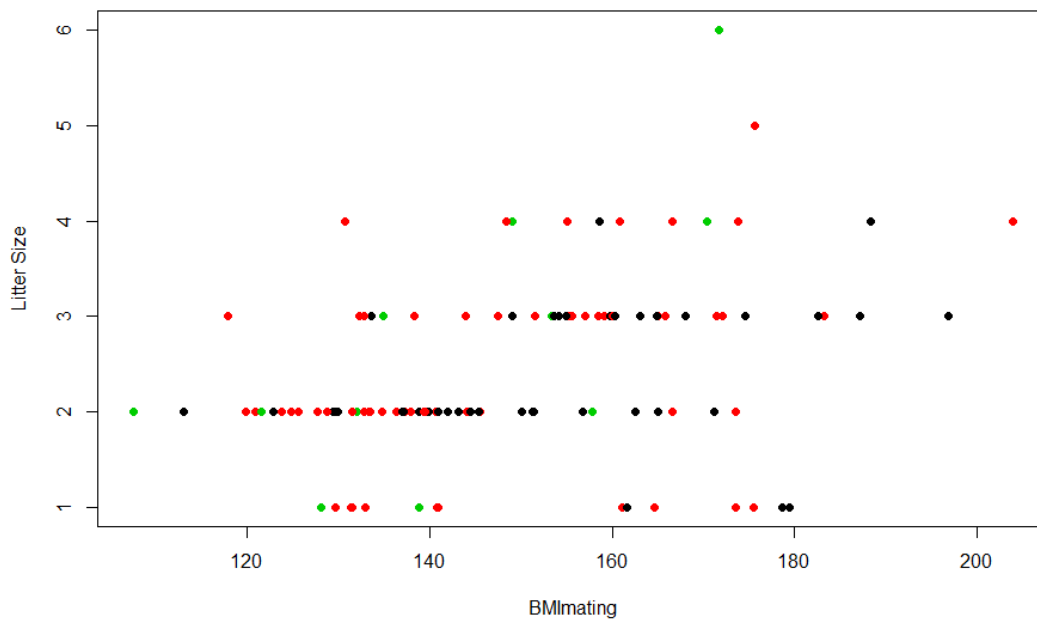


Figure 3. Plot showing distribution of BMI scores at mating according to litter size. Black dots are Finn gene status 0, red are Finn gene status 1, and green are Finn gene status 2.

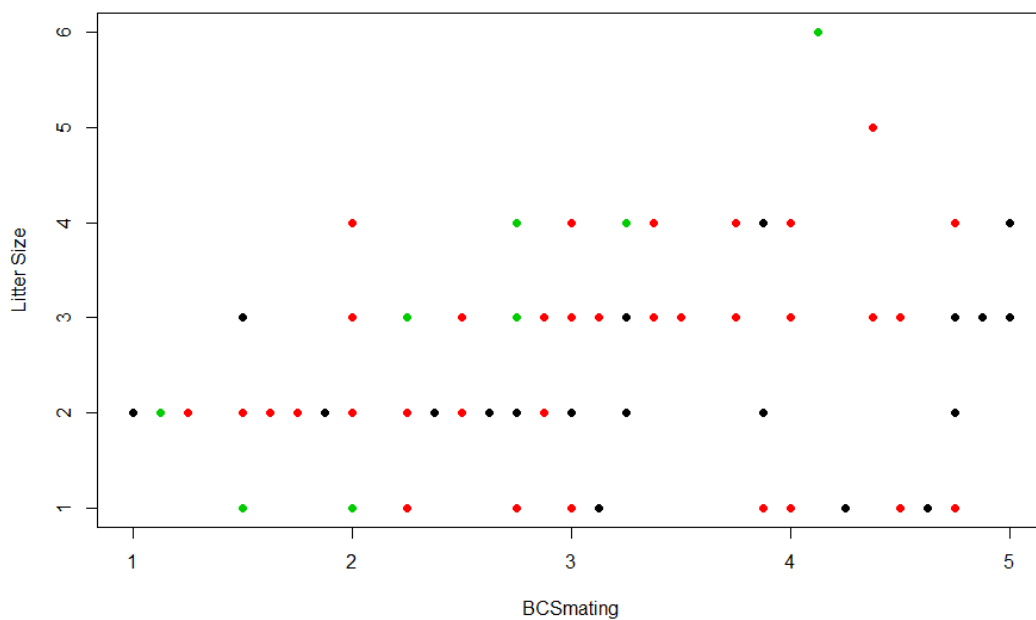


Figure 4. Plot showing distribution of BCS at mating according to litter size. Black dots are Finn gene status 0, red are Finn gene status 1, and green are Finn gene status 2.

## 4. Discussion

The two measuring methods BMI and BCS had a high correlation ( $r$ ) of 0.63. The scale of BMI is a lot longer than for BCS. This may argue that BMI is a more exact measure method, but it's also less practical for the farmers to use and understand. Body Mass Index is supposed to correct for the size of the ewe, using body weight of each individual. Beside errors in the equipment used, it is not so many sources of error using this method. Body condition score, on the other hand, is a subjective measuring method and how each person interprets the scale can vary. Nevertheless, it is an easy method for the farmers to use.

The two plots (Figure 3 and 4), with BCS and BMI show similar effects on litter size for the two measures. Both plots show an interesting distribution around BCS 3 and BMI 150, where most twin litters are below and most litters of three and more lambs are above these values. Single lambs seemed to be randomly distributed, and there was not found any pattern in what ewes that got single lambs, from either of the variables in the study.

The ewes in present study were stalled in a barn for controlled feeding from late august. The majority of ewes in Norway would be on pasture until the end of October, or longer. This makes it more difficult to adjust BC the same way as in the present study. It is often necessary to utilize the pasture for as long as possible due to limited access of harvested roughage. This could cause the ewes to lose some BC the last weeks before housing. If the ewes are out on pasture until the nutritive value of the grass is getting very low, the transition from this to a good access of roughage gets bigger, as opposed to if they are taken in from pasture when the nutritive value of the grass is still decent. However, our study suggests that there is a flexibility of when the ewes are fed for gaining fat deposition (flushed). A short, but more intense feeding period before mating would probably also result in bigger litters, but the effect will vary based on the starting BC. The high-energy feeding can also be restricted to the thin ewes, if the farmer would avoid increasing litters on the ewes that are already in a medium or high BC.



About 6 months before ovulation, the ovarian follicles leave the primordial pool. Poor nutrition at this time can reduce ovulation rate (Robinson et al., 2002). Although it is well known that the nutrition at this time affects ovulation rate, it is not done much research on this topic. This is mainly because the ewes around this time is in lactation with high-growing offspring and therefor ewes and lambs are offered high-quality pasture or have been led to outland pasture where they have no access to supplemental feeding. How the ewe's amount of body fat changes during this season is therefore difficult to influence and is mostly driven by the quality of the pasture, which would vary from year to year dependent on climate and weather.

Nottle et al. (1997) found that a preovulatory lupin-grain supplement (500 g per head a day) the last 10 days before mating gave an average extra 0.57 ovulations per ewe, even though ewes had lost one-seventh of their body weight between 6 and 4 months before ovulation. However, reduced ovulation caused by undernutrition about 2 months before mating, was not as easily compensated for by short time flushing. This was likely due to the reduced body condition at mating, which they were not able to regain in time for mating. Therefor their genetical potential for ovulation rate could not be fulfilled.

In the present study, the ewes in Constant group initially gained weight on their original diet. Therefore, the diets for Constant and Decreasing group were modified during the trial. In the end the ewes in Constant group anyway ended up with positive weight gain, but lost more BC than planned, especially the 1.5-year-old ewes. There was still a difference from the Decreasing group, but the ovulation rates may have been influenced in a different way than they would with completely constant BC during trial period. An unexpected result was that ewes in the Constant and Decreasing group gained some BMI and body weight during trial period, despite losing between 0.5 and 1 BCS (Table 6). The ewes in the Constant group, and the adult ewes in Decreasing, also gained live weight regardless of getting thinner. Thus, there is a clear difference between BMI and BCS as a result of feeding, even though the results for litter size is similar.

The P-values in Table 12 suggests that BMI is a better predictor of litter size when the ewes are thin, while BCS is better for fat ewes. It may be easier to assess the amount of fat, when there is a certain amount of subcutaneous fat present on the animal. Body Mass Index, on the other hand, will include any weight gain whatsoever, if it's muscle growth, skeletal growth, visceral fat, etc.

Table 9 and 10 show that the ewes' fatness at mating is the most important factor for determining litter size in the following pregnancy. High levels of both BMI and BCS at mating gave significant increases in litter size. However, we did not detect any effect on litter size caused by current nutrition, measured as the changes in body condition during the last 11 weeks before mating. This suggests that feeding level at mating did not influence litter size significantly. Despite of Table 7 showing effect of feeding level on litter size, Table 9 and 10 substantiates the claim that this was not due to feeding level itself (current nutrition), but rather an effect of the higher BC obtained by the ewes following 11 weeks on the high-energy diet. Therefore, when knowing the ewes' Finn gene status, age and their current BC, we should be able to tell a lot about the expected litter size.

Table 11 give the same expression as Table 9 and 10, that the BCS or BMI at mating can explain a lot of the variation in litter size. The results match well with the study of Gunn and Doney (1975) on 156 Scottish Blackface ewes, who found that the ewes with highest BC at mating showed the greatest ovulation rates. Since BMI calculations and BCS measurement had a high correlation in this study, and the effects on litter size were similar, it seems reasonable to transfer the effects of BMI at mating over to the more practically feasible BCS. Gunn and Doney (1975) also found that poor BC, irrespective of feeding level, was associated with a delay or suppression of oestrus, and with a high return-to-service rate. In our study 12 ewes had return-to-service and got pregnant on the second oestrus after starting the mating season. This does not include the ewes that got mated the first or second day after starting mating season, and got return-to-service, because they may have been mated too late in oestrus relative to ovulation. Of these 12 ewes there were 7 with BCS between 2 and 2.5. Three ewes had BCS between 4 and 5. Of the 7 ewes that never got pregnant in the present study, 5 had return-to-service at least once during mating period.

From several international studies on fertility in sheep, it has been normal to include body weight (BW) as a variable in the model. In this study, we only used body weight to calculate BMI. There are two main reasons why it was not appropriate to include BW as a variable in our model. The first reason is that half of the ewes was only 1.5-year-old, and therefore still in growth. It differed 45 kg between the lightest and the heaviest 1.5-year-old ewe at mating. Ewes in growth are gaining weight not only from fat deposition, but also from carcass growth. Due to growth they also require more energy if they are going to increase the same amount of fat deposition as adults. This may explain why the 1.5-year-old ewes in the Increasing group did not gain as much BC as the adults, and why the 1.5-year-olds in the Decreasing group lost

more BC than the adults. The extra need of energy for ewes in growth explain why most of the thinnest ewes were 1.5-year-olds, and why few of them were among those with highest BC. Based on this it's fair to assume that young ewes will have a more negative response to malnutrition, than outgrown ewes. The other reason why we did not include BW in the model is because NKS is a synthetic breed bred for best possible production and do not have a breed standard in the same way as many other breeds. It is a mix of several breeds with different body types so the variation in size, width and length is quite big, and gives a heterozygous breed when it comes to adult BW. Adult ewes can be 85 kg or 115 kg when outgrown, without having any known difference in fertility. Therefore, BMI may be a better measure than BW.

Body condition at mating will affect the recommended feeding during pregnancy. Younger ewes, and ewes in low BC, should be fed to gain some weight. This is because ewes with a BC of 2 or less, would get a negative effect on the development of fetal membranes and placenta if kept on a low feed level (Berge, 2016). Norwegian Agriculture advisory recommend keeping the ewes around a BC of 3.5 during the whole pregnancy, and agree with Munoz et al. (2008) that a mild undernutrition during mid-pregnancy is positive for the development of the fetal membranes and placenta (McDonald et al., 2011) for adult ewes in medium and high BC.

Ewes' productivity are expected to be on top at the age of 3-4, and to ensure a healthy long-lasting ewe with high production over many years, it is reasonable to strive for twins on the younger ewes. Berg Olsen (2016) found that 2-year old ewes in their first parity with triplet lambs at autumn weaning had significantly higher risk to be culled because of mastitis, than the same ewes with twins. Using only information about litter size it seems clear that low BC will give less big litters. Nevertheless, it is important to avoid too poor BC because of the recommendations given for feeding level throughout gestation. Vatankhah et al. (2012) found increasing total litter birth weight and total litter weaning weight, with increasing ewe BCS at mating, with maximum values at BCS of 3.5. Khan (1993) showed that higher BCS pre-lambing gave higher total litter weaning weight. He also indicated that although supplementation increased ewe productivity, ewes that were previously in poorer BC did not perform as well as ewes that maintained a good BC throughout gestation. To ensure that the ewes are in good enough condition at parturition to maximize their productivity we need to avoid that the young ewes lose too much BC before mating. It can be achieved by separating the 1.5-year-old ewes from the adults and sorting them based on BC.

More studies are needed to know how young ewes in poor condition (<2.5) are fed optimally during gestation, but based on the results showed in Table 6, the 1.5-year-olds in Constant group got a suitable number of lambs (2.05), and an average BC of 2.45. Presumably, some of the ewes in this group were thinner than they should be, if they are going to get up to a BCS of 3 before parturition. However, it may be a suitable compromise between litter size and body condition. Using breeding values for litter size, or if Finn gene status are known, it may be an option to split the ewes with high genetic fertility from the ones with low genetic fertility. In this way, the ewes with low genetic values for fertility can be fed to a bit higher BC at mating, hopefully without risking any unwanted big litters.

In the Increasing group the 1.5-year-olds got almost as many lambs as the adult ewes (0.1 lamb difference), while the difference was distinctly larger in the Constant and Decreasing groups. The average for the 1.5-year-olds was of course increased a lot by the ewe with 6 lambs. Every one of the 1.5-year-old ewes in the Increasing group that got 3 or more lambs was in a BC between 3+ and 4+. Only one of these had Finn gene status 0 (BC 4), and all four ewes that got twins in this group had Finn gene status 0 despite having BC between 3 and 4-. According to present study, feeding 1.5-year-old NKS ewes to a high BC at mating, especially when Finn genes are present, bring a high risk of litters of 3 or more lambs.

The fact that the high BC ewes with Finn gene status 1 had 0.28 lambs (Table 13) more than the high BC ewes with Finn gene status 0, and the difference for medium and low BC ewes was close to 0, suggests that the Finn gene has a more powerful effect on high conditioned ewes, than poor conditioned ewes. Because the Finn genes give a genetical potential for higher litter size, the ewes in good condition, or optimal environment, will have a higher litter size with, than without these genes. This means, that breeding away from the Finn genes, will decrease the amount of extremely large litters, and the effect will get higher the less ewes there are left with present Finn genes in the population. Table 14 shows the same tendency in Increasing feeding group as for high BCS, with 0.35 more lambs for ewes with Finn gene status 1 compared to ewes with Finn gene status 0. The ewe with 6 lambs was 1.5-year-old with BCS 4.125 and BMI 172 at mating. She had Finn gene status 2 and was in Increasing feeding group. Due to few ewes with Finn gene status 2, we cannot draw any conclusion on the effect of this Finn gene status compared to Finn gene status 0 and 1.

Landau et al. (1995) found Booroola x Assaf crossbred ewes with a high-fertility gene called “the Booroola allele” to have significantly higher ovulation rates, and prolificacy, than ewes without this gene. Also, the ewes carrying this gene had a different response to two types of feeding, based on different amount of ruminally undegradable starch (RUS). The ewes fed the diet with the highest amount of RUS got significantly higher ovulation rates and prolificacy, compared to the ewes on the low-RUS diet. The non-carrier ewes, on the other hand, did not show a significant different response to the two diets.

Sormunen-Cristian and Jauhiainen (2002) tested the flushing effect on purebred Finnish Landrace ewes’ productivity. The study had two different feeding levels of daily concentrate supplement (150 g and 300g barley) and one control group without any supplement. The experimental diets were started about 14 days before mating started. They found increased prolificacy (4 vs 3 lambs) in mature ewes (4-5 years) on the diet with 150g concentrate supplement, but no effect on yearlings and older ewes (7-8 years). They concluded that little was to be gained in lamb production by flushing ewes of the Finnish Landrace. This study had a short period of flushing prior to mating, and the supplement of barley was not particularly high. There were also very few ewes in each group. Thus, it is fair to assume that the ewes would not manage to increase their BC significantly during the short time of flushing and therefore did not have an increase in litter size.

In the present study, more than half of the ewes with Finn gene status 0 had twin litters. They also had a lower frequency of single lambs than the ewes with Finn gene status 1 or 2. The percentage of triplet litters was just above 30% for both Finn gene status 0 and 1. Of these triplet-ewes, 79% and 63% were above BCS 3 at mating, respectively. Of all litters of 4 or more lambs, almost 70% of them came from ewes with Finn gene status 1 or 2 and with BCS  $\geq 3$  at mating.

The effect of Finn gene found in this study matches well with the research of Boman (2013) stating that a single presence of Finn gene gives approximately 0.3 extra lambs, while a double presence of Finn gene gives 0.6-0.7 extra lambs. All registered NKS sheep in the Norwegian sheep-hold register “Sauekontrollen” have a breeding value index showing their expected genetic fertility in form of a litter size index. Every NKS ram that has been progeny tested in Norway, and later used for artificial insemination (AI) has been tested for the Finn gene. In total, all AI rams born from 2000 and later with double presence of Finn gene have produced almost 13 000 producing daughters and had an average litter size index of 128.

The AI rams with a single presence of Finn gene have produced nearly 50 000 producing daughters and had an average litter size index of 109. The AI rams that were free for the Finn gene has produced 55 000 producing daughters and had an average litter size index of 96. About a third of the progeny tested rams born in 2018 had Finn gene status 1, so even though the amount is assumed to be decreasing due to the end of selling AI rams with Finn gene status 2 in 2013 (NSG), it will take a long time before Finn genes are an insignificant factor when discussing litter size in the NKS breed.

The litter size index makes it easier for farmers to control genetic fertility of the next generations and to be able to use rams that gives genetically less fertile offspring if they have unwanted big litters in the herd. When using progeny tested rams, the farmers can also choose if they want rams with or without the presence of Finn gene. Of course, environmental factors will also have a big influence on fertility. It can also be used the other way around, by selecting for more genetically fertile animals.

Based on the results of this study it seems possible to reduce the amount of big litters if the ewes carrying Finn genes are kept in a  $BC < 3$  at mating. This requires testing of the ewes in each herd for their Finn gene status. This is not common today but could be easier to keep track of when genomic selection makes its entrance the next years. If gene testing is not available, the litter size breeding index can be used for the same purpose. Also, every ram that is being progeny tested today, is getting tested for their Finn gene status, so when using rams of this kind, the Finn gene status can be controlled.

Since we did not explore the effect of short time flushing in this study, it would be interesting to repeat the study and add groups that will get the feeding regime of this study the last two weeks before mating, only. Then we could compare the effect of short time flushing or antifleushing with the long-time effects that we see in the present study.

The overall goal from the Breeding Council of Norwegian sheep breeds is to reduce the variation in litter size in NKS. As this study suggests, a significant amount of the variation can be controlled with good feeding management, and condition-scoring before mating. Ewes in high BC at mating will have a higher chance of getting undesirably large litters. On this breed it is currently not realistic, nor wanted, to avoid a certain number of triplets on adult ewes. But more important, from the view of this study, it seems possible to decrease the number of quadruplets or more, to a minimum. Also, if breeding is done consistently on ewes free for Finn genes, the number of ewes with triplets will presumably decrease considerably. With a good overview and management of the BC in the herd from weaning until mating, it should be possible to affect the litter size in the desired direction.

When deciding the target BC at mating one must as well consider what BC is desired for the following pregnancy and lactation, and how this fit into the recommended feeding levels during pregnancy. There is a balance between optimal litter size for each farmer and optimal BC during pregnancy and after parturition. Therefore, a long-term plan for the winter season is needed to achieve optimal results.

## 5. Conclusions

High BCS or BMI at mating significantly increased litter size for both adult and 1.5-year-old ewes in the present study. Current nutrition, measured as daily changes in BCS or BMI until mating did not affect litter size.

The presence of Finn genes seemed to give a stronger effect on litter size for ewes with high BCS at mating than for ewes in low and medium BCS. Finn gene status was significant in the BCS model and tended to be significant in the BMI model when predicting litter size.

The 1.5-year-old ewes lost more BC than the adults when fed low-energy diets and had in average lower BCS and BMI than adult ewes. They also got less lambs in average within each diet.

## Acknowledgements

This assignment marks the end of my masters degree in Breeding and Genetics at Norwegian University of Life Science. Through five busy years I have learned a lot, both theoretically and practically. As long as I can remember, I have been passionate about sheep. Therefore, it was natural for me to choose a subject about sheep for this masters assignment. Management around litter size on ewes is a very relevant issue for sheep farmers nowadays, which made it a very exciting and educational subject to study closer. I want to thank my main supervisor Ingjerd Dønnem and my assistant supervisor Åshild T. Randby for all your help with creating an assignment of this character, regarding both the academic- and the linguistic challenges. I also really need to thank my statistics supervisor, Tormod Ådnøy, for all the hours in your office helping me understand RStudio and with all the calculations that needed to be done. I am truly grateful for the help from all three of you. Finally, I want to thank my roommates and classmates during these five years in Ås. It has truly been an adventure.



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