Effect of behaviour of Holstein-Friesian and Simmental bulls on semen quality

Wpływ behawioru buhajów rasy holsztyńsko-fryzyjskiej i simentalskiej na jakość ich nasienia

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ABSTRACT

The aim of the study was to determine the relationship between the behaviour of Holstein-Friesian and Simmental bulls and the quality of their semen. A total of 76 breeding bulls of the Holstein-Friesian and dual-purpose Simmental breeds were investigated. Analysis was made of the response of bulls to humans and other bulls, facial hair whorl position and length, scrotal circumference, and semen characteristics (mean ejaculate volume, mean sperm concentration, and sperm wave motion). The age and breed of the bulls had a statistically significant effect on semen quality, scrotal circumference and the animal's response to an unfamiliar human (P<0.05; P<0.01). The coefficients of correlation between the bull's response to a handler and to other bulls averaged 0.73. In general, only weak correlations were found between behavioural traits of the bulls and quality of their semen. It is worth noting a good correlation (r=0.50; P<0.05) between hair whorl position and sperm concentration in Simmental bulls.

Keywords: bulls, behaviour, semen quality

STRESZCZENIE

Celem pracy było zbadanie relacji między behawiorem buhajów rasy holsztyńskofryzyjskiej i simentalskiej a jakością ich nasienia. Badaniami objęto 76 buhajów hodowlanych rasy holsztyńsko-fryzyjskiej i rasy simentalskiej w typie użytkowym mięsno-mlecznym. Analizie poddano reakcje buhajów wobec człowieka oraz innych buhajów, położenie i długość układu włosów na czołach buhajów, obwód moszny oraz cechy ich nasienia (średnią objętość ejakulatu, średnią koncentrację plemników i ruch falowy masy plemników). Stwierdzono między innymi statystycznie istotny wpływ wieku i rasy buhajów na jakość nasienia, obwód moszny oraz reagowanie zwierząt na obcego człowieka (P<0,05; P<0,01). Wartości współczynników korelacji między reakcjami buhajów na osobę z obsługi a reakcjami na inne buhaje wynosiły średnio 0,73. Generalnie wykazano co najwyżej słabe korelacje między cechami behawioru buhajów a jakością ich nasienia. Warto odnotować dobrą korelację (r=0,50; P<0,05) między położeniem układu włosów a koncentracją plemników w nasieniu buhajów simentalskich. Słowa kluczowe: buhaje, behawior, jakość nasienia

INTRODUCTION

Behavioural characteristics of cattle played a key role in the domestication and breeding of animals while being an important indicator of their welfare assessment (Sambraus, 1997; Price, 1999; Stricklin, 2001; Glenske, et al., 2010).

These include the animals' ability to breed under man-made conditions (Jezierski, 2004). Because cattle show a specific response to external stimuli, it was possible to develop reproductive techniques (artificial insemination, embryo transfer) and integrate them into breeding practice; these techniques eliminated the need for direct contact of animals at mating (Nehring and Staehr, 2001; Houpt, 2005).

In the case of stud bulls, their semen has for many years been collected after sexual stimulation and through conditional and unconditional reflexes (Orgeur and Signoret, 1990; Petherick, 2005). Human-animal interactions in the relationships between bulls and handlers are of considerable importance in this respect. This concerns both adult animals and proper handling of young bulls (Perrey, 2000; Phillips, 2002).

Over the last ten years, cattle behaviour has been implicated to be related to fertility (Cooke, 2010). It was suggested, for instance, that the temperament of bulls may be correlated to the facial hair whorl and semen quality. It was also observed that productive type and breed of cattle may have a significant effect on these relationships (Meola, et al., 2002; Evans, et al., 2005; Shirley, et al., 2006).

Therefore, the aim of this study was to determine the relationships between the behaviour of Holstein-Friesian and Simmental bulls and the quality of their semen.

MATERIAL AND METHODS

A total of 76 breeding bulls, including 43 Holstein-Friesian (at the average age 48 months, SD=25,0) and 33 Simmental bulls of the dual-purpose type (at the average age 36 months, SD=27,7) from the Małopolskie Biotechnology Centre company in Krasne, were investigated. Bulls were housed individually under a uniform system of feeding.

The following behaviours were evaluated:

- the bull's response to the approach of an unfamiliar human (Table 1);

- the bull's response to the assessment of its facial hair whorl (Table 2);

- the bull's response to handlers during routine handling in stalls, at feeding and during other routine procedures (Table 3);

- the bull's response to other bulls in areas where they come in contact (e.g. in an adjoining stall or in the outdoor area) (Table 4).

Libido of the bulls was evaluated according to Wierzbowski and Żukowski (2007) (Table 5).

Facial hair whorl position relative to eye level was evaluated according to the method reported by Grandin et al. (1995) (Figure 1).

Hair whorl length on the forehead was also determined (D) (Figure 2). To this end, photographs of bulls' heads were analysed digitally using UTHSCSA Image Tool ver. 3.0.

The following bull semen characteristics were considered as mean values from the last nine ejaculates of semen:

- mean ejaculate volume (cm³);
- mean sperm concentration (thous./mm³ ejaculate);

- sperm wave motion assessed according to the following scale (Kupferschmied, 1999): 0 – no wave motion; 1 – very weak wave motion; 2 – weak wave motion; 3 – moderate wave motion; 4 – strong wave motion; 5 – very strong wave motion. Scrotal circumference of each bull was measured during the analysis.

The following statistical methods were used in the experiment:

- Kolmogorov-Smirnov test with the Lilliefors correction, to test for goodness of fit between individual variables and a normal distribution;
- Kruskal-Wallis test, to determine the effect of breed (Holstein-Friesian, Simmental) and age of the bulls (less than 36 months of age, over 36 months of age);
- Spearman rank correlation coefficients, to evaluate the relationships between the analysed traits.

The calculations were made with the software Statistica ver. 9.0.

Table 1. Behaviour scores of bulls based on their response to an unfamiliar human Tabela 1. Ocena zachowania buhajów na podstawie reakcji buhajów na obcą osobę

Scale	Description of behaviour
1	Very calm. No response to unfamiliar human presence.
2	Calm. Animal shows interest in an unfamiliar human by rising and
	observing; touching the animal generally presents no problems.
3	Nervous. Animal rises quickly and rapidly, shifts its weight from one foot to
	the other; difficult to handle.
4	Very excitable. Animal snorts nervously and hits the floor with fore leg; safe
	approach to animal not possible.

Table 2. Behaviour scores of bulls based on their response during hair whorl measurement

Tabela 2. Ocena zachowania buhajów na podstawie reakcji buhajów podczas oceny układu włosów

Scale	Description of behaviour
1	Very calm. Assessment of hair whorl presents no problems the first time,
	animal submissive to human will.
2	Calm but alert. Assessment of hair whorl presents no problems the first time, but direct contact with the bull requires gradual reduction of the flight distance.
3	Nervous. Assessment of hair whorl possible but not the first time – the bull requires more than 1 attempt at assessment.
4	Very excitable. Assessment of hair whorl not possible due to the bull's constant restless behaviour during attempts at assessment.

Table 3. Behaviour scores of bulls based on their response to handlers*

Tabela 3. Ocena zachowania buhajów na podstawie reakcji buhajów wobec osób z obsługi*

Scale	Description of behaviour
1	No handling problems during all routine procedures.
2	Sporadic problems during routine procedures, but the bull is manageable for the handler. Animal tends to be excitable, but shows no aggressive behaviour.
3	Frequent problems with bull control and handling during routine procedures. Animal is excitable and shows aggressive behaviour.
4	Bull handling is very difficult. Animal is very excitable and aggressive.

* Score accounts for previous behaviour of individual bulls during rearing and use. Assessment was made independently by 3 attendants in daily contact with animals and the score was averaged.

Table 4. Behaviour scores of bulls based on their response to other bulls* Tabela 4. Ocena zachowania buhajów na podstawie reakcji buhajów wobec innych buhajów*

Scale	Description of behaviour
1	Bull always tolerant of other animals.
2	Bull generally tolerant of other animals.
3	Bull sporadically gets excitable in response to the presence of another animal.
4	Bull sporadically shows aggressive behaviour.
5	Bull very aggressive in every contact with another animal.

* For explanations, see Table 3.

Table 5. Assessment of bull libido*

Tabela 5. Ocena libido buhajów*

Scale	Description of behaviour
0	Lack of sex drive. Complete lack of interest. Bull does not sniff or attempt to
	mount; it stands next to the teaser animal or attempts to retreat.
1	Very weak sex drive. During 10-minute waiting, bull only sniffs the teaser
	animal with no attempt to mount.
2	Weak sex drive. Taken to the teaser animal, the bull sniffs it, retreats, sniffs
	again, makes a hesitant attempt at mounting, sniffs again, and makes
	another mount attempt within 10 minutes.
3	Moderate sex drive. Taken to the teaser animal, the bull sniffs it and begins
	mount attempts after 2-3 minutes.
4	Strong sex drive. Taken to the teaser animal, the bull attempts to mount it
	at once but remains calm and is manageable for the handler.
5	Very strong sex drive. Taken to the teaser animal, the bull begins mount
	attempts at once and becomes unmanageable for the handler.

* For explanations, see Table 3.

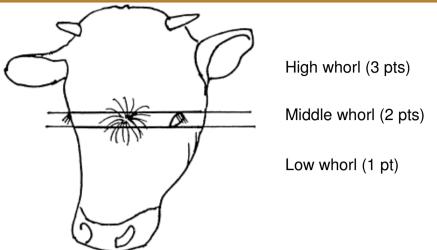


Figure 1. Determination of hair whorl position in bulls (according to Grandin et al. 1995).

Rycina 1. Określanie położenia układu włosów u buhajów (według Grandin et al. 1995).

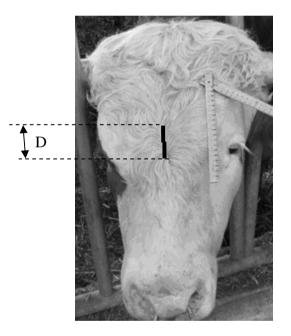


Figure 2. Hair whorl length on the forehead of a bull (photo A. Makowska). Rycina 2. Długość układu włosów na czołach buhajów (fot. A. Makowska).

RESULTS

In most cases, there were no statistically significant differences in behaviour between younger and older bulls (Table 6) except the bull's response to the approach of an unfamiliar human. Compared to younger bulls, older animals responded more quickly and more nervously, shifting their weight from one foot to the other, and presented more handling problems (respectively 2.6 \pm 0.57 and 2.9 \pm 0.60, P<0.05). In addition, bulls differed in hair whorl position and scrotal circumference (P<0.05; P<0.01). Semen parameters were 11-25% better in older compared to younger bulls.

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Several statistically significant differences were found between the analysed traits according to breed (Table 7). Simmental bulls responded more nervously to unfamiliar human presence (2.8 ±0.46) compared to HF bulls (2.6 ±0.66) (P<0.05). Likewise, Simmental bulls were characterized by larger scrotal circumference compared to HF bulls (40.8 ±3.56 vs. 35.9 ±4.10; P<0.01) and higher mean sperm concentration (1343.3 ±391.57 vs. 1102.9 ±388.41 thous./mm³ ejaculate) (P<0.05). The other differences between the breeds were not significant.

For both older and younger bulls, statistically significant (P<0.05; P<0.01) coefficients of correlation were found between (Table 8):

- the bull's response to handlers and the bull's behaviour towards other bulls (r=0.57–0.88);

- the bull's response to the presence of an unfamiliar person and its behaviour towards handlers, towards other bulls, and during hair whorl measurement (r=0.30–0.44, 0.42–0.44 and 0.42–0.66, respectively);

- sperm concentration and sperm mass movement (r=0.62-0.64).

In older bulls it is worth noting good relationships between libido and their behaviour towards handlers and other bulls (r=0.61 and 0.72, respectively), and between scrotal circumference and the animal's response to unfamiliar human presence (r=-0.47). In younger bulls, hair whorl position was positively correlated to the behaviour of bulls towards handlers and other bulls (r=0.42).

There were over twice as many significant correlations between the analysed traits in HF compared to Simmental bulls (Table 9). The coefficients of correlation were similar for both groups between:

- the assessment of bull's libido and its behaviour towards other animals (r=0.43–0.50);

- the bull's response to handlers and its behaviour towards other bulls (r=0.63-0.83);

- the bull's response to unfamiliar human presence and its behaviour during hair whorl measurement (r=0.45–0.60);

- mean sperm concentration, scrotal circumference and sperm mass movement (r=0.38–0.46 and 0.66–0.76, respectively).

In addition, significant relationships were found in HF bulls for their response to humans and other bulls (r=0.34–0.55), and between these and hair whorl position (r=0.61–0.66) (P<0.05; P<0.01). Meanwhile, in Simmental bulls hair whorl position was correlated to scrotal circumference (r=0.67; P<0.01) and sperm concentration (r=0.50; P<0.05).

Discussion

Because of rigorous selection and handler safety, each breeding bull is given special treatment by the breeders, who pay particular attention not only to animal housing conditions but also to positive human-animal interactions (Phillips, 2002). According to Waiblinger et al. (2006), these relationships exist in the sphere of social behaviour. This was confirmed by our study, which showed that depending on breed and age of animals, the coefficients of correlation between the bull's response to a handler and its response to other bulls averaged 0.73.

Until the 1990s, it was believed that bull behaviour is unrelated to semen quality and animal behaviour was assessed subjectively based on the observer's evaluation using a scoring scale (Burrow, 1997). It is also believed that bull libido, which is also assessed subjectively, has no significant effect on semen quality, although it is of crucial importance during natural mating or semen collection (Leamaster and Duponte, 2007; Walker, et al., 2009).

In our study, we used subjective methods and assessed the response of animals to individual handlers and other bulls, also taking account of bull libido. The results obtained confirmed that there were no or only weak correlations between the behaviour of bulls subjected to this evaluation and semen characteristics.

Research findings from the last sixteen years (Grandin, et al., 1995; Randle, 1998; Lanier, et al., 2001; Broucek, et al. 2007) suggested that facial hair whorl position and shape in bulls may be related to their behaviour, in particular their temperament. This is attributed to the fact that during embryonic development, skin, its products and the nervous system arise from the same germ layer, or ectoderm (Fletcher and Weber, 2010). As a result, research was undertaken in bulls to determine the relationship between facial hair whorl position and shape, and traits of reproductive performance. The possible occurrence of these relationships is ascribed to the fact that hair follicles and testes develop during the same period of fetal life in bulls. By way of example, Meola et al. (2002) found that young Aberdeen Angus bulls having facial hair whorl with round epicentre were more likely to have satisfactory spermatozoal morphology scores compared to animals with a non-round epicentre.

On the other hand, Evans et al. (2005) reported that hair whorl and semen quality are expected to be less correlated in Holstein-Friesian compared to Aberdeen Angus bulls, attributing this to the differences in the breeding work with these cattle breeds. A significant effect of genetic factors on the relationships under discussion was also indicated by Shirley et al. (2006). In our study, we did not find statistically significant differences in hair whorl position and length between Holstein-Friesian and Simmental bulls, although the latter showed greater variation in this regard. In addition, for both breeds there were only weak correlations between hair whorl position and length, and semen characteristics. The highest coefficient of correlation (r=0.50) was found in Simmental bulls between hair whorl position and sperm concentration.

CONCLUSION

In summary, Holstein-Friesian and dual-purpose Simmental bulls show similarity in terms of the correlation between their behaviour and semen quality. This may result from the similar breeding work with both breeds, compared to purebred beef cattle. The fact that hair whorl position of Holstein-Friesian bulls is positively correlated to their libido and behaviour towards handlers and other bulls is evidence that these traits are related. Based on the research to date, the present authors do not recommend using facial hair whorl position in Holstein-Friesian and dual-purpose Simmental bulls for semen quality evaluation.

Trait	Parameter	Age	P		
		≤ 36	> 36 months		
		months		_	
	Ν	51	25	-	
	Mean ± SD	3.0 ±0.31	2.9 ±0.49		
Libido	Median	3.0	3.0	0.165	
	Minimum-Maximum	2.0-4.0	2.0-4.0		
Bull's response to	Mean ± SD	2.4 ±0.54	2.7 ±0.84		
handlers	Median	2.3	2.3	0.152	
nanuers	Minimum-Maximum	1.7-4.0	1.3–4.0		
Bull's response to	Mean ± SD	2.6 ±0.59	3.0 ±1.09		
other bulls	Median	2.7	2.7	0.256	
	Minimum-Maximum	2.0-4.7	1.3–5.0		
Bull's response	Mean ± SD	2.2 ±0.50	2.3 ±0.68		
during hair whorl	Median	2.0	2.0	0.511	
measurement	Minimum-Maximum	1.0-3.0	1.0–3.0		
Bull's response to	Mean ± SD	2.6 ±0.57	2.9 ±0.60		
an unfamiliar human	Median	3.0	3.0	0.029	
an unianinal numan	Minimum-Maximum	2.0-4.0	1.0-4.0		
	Mean ± SD	1.6 ±0.63	2.4 ±0.74		
Hair whorl position	Median	2.0	3.0	0.001	
	Minimum-Maximum	1–3	1–3		
Hair whorl length	Mean ± SD	1.2 ±0.85	1.2 ±1.32		
(cm)	Median	1.2	1.2	0.648	
(CIII)	Minimum-Maximum	0.0-3.3	0.0–4.6		
Scrotal	Mean ± SD	35.8 ±3.40	42.9 ±2.44		
circumference (cm)	Median	36.0	43.0	0.000	
	Minimum-Maximum	29.0-42.0	39.0–50.0		
Sperm mass	Mean ± SD	1.6 ±0.61	2.1 ±0.48		
movement (pts)	Median	1.5	2.1	0.002	
movement (pts)	Minimum-Maximum	0.1-3.0	1.1–2.8		
Mean sperm	Mean ± SD	1089.7	1447.1		
concentration		±377.2	±357.44		
(thous./mm ³	Median	1115.6	1428.5	0.001	
ejaculate)	Minimum-Maximum	113.0-	790.7–		
		1900.0	2100.0		
Mean ejaculate	Mean ± SD	5.8 ±1.58	6.5 ±1.47		
volume (cm ³)	Median	5.6	6.9	0.048	
	Minimum-Maximum	3.8-10.0	3.5-8.9		

Adamczyk et al.: Effect Of Behaviour Of Holstein-Friesian And Simmental Bulls On Semen Quality Table 6. Effect of bulls' age on their behaviour and semen characteristics Tabela 6. Wpływ wieku buhajów na ich zachowanie i cechy nasienia

Trait	Parameter	Br	Breed			
		Holstein-	Simmental			
		Friesian				
	Ν	43	33			
	Mean ± SD	3.0 ±0.42	2.9 ±0.32			
Libido	Median	3.0	3.0	0.136		
	Minimum-Maximum	2.0-4.0	2.0-3.7			
Pull'a raananaa ta	Mean ± SD	2.5 ±0.65	2.4 ±0.69			
Bull's response to handlers	Median	2.3	2.3	0.561		
nanuleis	Minimum-Maximum	1.7–4.0	1.3–4.0			
Bull'a raananaa ta	Mean ± SD	2.7 ±0.76	2.7 ±0.87			
Bull's response to other bulls	Median	2.7	2.7	0.814		
	Minimum-Maximum	1.7–4.7	1.3–5.0			
Bull's response	Mean ± SD	2.3 ±0.59	2.2 ±0.53			
during hair whorl	Median	2.0	2.0	0.415		
measurement	Minimum-Maximum	1.0–3.0	1.0–3.0			
Bull's response to	Mean ± SD	2.6 ±0.66	2.8 ±0.46			
an unfamiliar	Median	3.0	3.0	0.036		
human	Minimum-Maximum	2.0-4.0	1.0-3.0			
	Mean ± SD	1.7 ±0.71	2.0 ±0.79			
Hair whorl position *	Median	2.0	2.0	0.18		
	Minimum-Maximum	1–3	1–3			
Hair whark langth	Mean ± SD	1.3 ±0.94	1.1 ±1.12			
Hair whorl length	Median	1.2	1.1	0.315		
(cm)	Minimum-Maximum	0.0–3.3	0.0-4.6			
Saratal	Mean ± SD	35.9 ±4.10	40.8 ±3.56			
Scrotal	Median	35.0	40.5	0.000		
circumference (cm)	Minimum-Maximum	29.0–45.0	31.0–50.0			
Shorm mass	Mean ± SD	1.7 ±0.56	1.9 ±0.65			
Sperm mass	Median	1.7	2.0	0.175		
movement (pts)	Minimum-Maximum	0.1–2.8	1.0–3.0			
Maan anarm	Mean ± SD	1102.9	1343.3			
Mean sperm		±388.41	±391.57			
concentration (thous./mm ³	Median	1185.2	1262.0	0.031		
ejaculate)	Minimum-Maximum	113.0-	706.1-			
ejaculale)		1748.6	2100.0			
Mean ejaculate	Mean ± SD	5.8 ±1.37	6.4 ±1.76			
volume (cm ³)	Median	5.8	6.3	0.144		
	Minimum-Maximum	3.8–8.5	3.5–10.0			

Adamczyk et al.: Effect Of Behaviour Of Holstein-Friesian And Simmental Bulls On Semen Quality Table 7. Effect of bulls' breed on their behaviour and semen characteristics Tabela 7. Wpływ rasy buhajów na ich zachowanie i cechy nasienia

* - In 13 Simmental bulls, hair whorls were irregular and their position could not be determined

_	Libido	Bull's	Bull's	Bull's	Bull's	Hair	Hair	Scrotal	Sperm	Sperm	Ejaculate
-		response	response	response	response	whorl	whorl	circumference	mass	concentration	volume
Trait		to	to other	during hair	to an	position	length		movement		
		handlers	bulls	whorl	unfamiliar						
				measurement	human						
Libido	_	0.61**	0.72**	0.36	0.31	0.22	0.13	0.00	-0.14	-0.14	-0.09
Bull's response to handlers	-0.03	_	0.88**	0.39	0.44*	0.28	0.28	-0.03	-0.12	-0.01	-0.34
Bull's response to other bulls	0.24	0.57**	_	0.31	0.42*	0.41	0.18	0.08	0.00	0.04	-0.18
Bull's response during hair whorl	0.11	0.22	0.37**	_	0.66**	-0.15	0.22	-0.30	0.24	0.30	-0.17
measurement Bull's response to an unfamiliar human	0.19	0.30*	0.44**	0.42**	_	-0.04	0.06	-0.47*	0.12	-0.07	-0.08
Hair whorl position	0.19	0.42**	0.42**	0.09	0.29	_	-0.14	0.65*	-0.18	0.13	-0.25
Hair whorl length	-0.09	0.12	0.17	0.20	0.13	0.09	_	-0.24	-0.17	0.03	-0.27
Scrotal circumference	-0.09	0.25	0.16	0.03	0.26	0.24	0.19	_	0.19	0.39	-0.05
Sperm mass movement	-0.18	0.23	0.05	-0.16	-0.22	-0.02	-0.10	0.19	_	0.62**	-0.14
Sperm concentration	-0.34	0.22	0.20	-0.02	0.04	-0.19	0.08	0.30*	0.64**	-	-0.36
Ejaculate volume	-0.25	-0.01	0.02	0.19	0.16	-0.26	0.13	0.16	-0.23	-0.09	_

Note: Coefficients above diagonal are for bulls aged >36 months, those below diagonal are for bulls aged ≤36 months * - significant coefficients at P<0.05; ** - significant coefficients at P<0.01

	Libido	Bull's	Bull's	Bull's	Bull's	Hair	Hair	Scrotal	Sperm	Sperm	Ejaculate
-		response	response	response	response	whorl	whorl	circumference	mass	concentration	volume
Trait		to	to other	during hair	to an	position	length		movement		
		handlers	bulls	whorl	unfamiliar						
				measurement	human						
Libido	_	0.30	0.50**	0.20	0.23	-0.05	0.12	-0.31	-0.18	-0.19	-0.20
Bull's response to handlers	0.18	_	0.83**	0.24	0.31	0.16	0.12	0.04	0.16	0.26	-0.28
Bull's response to other bulls	0.43**	0.63**	-	0.17	0.26	0.24	0.06	0.04	0.17	0.15	-0.20
Bull's response during hair whorl	0.24	0.34*	0.46**	_	0.45**	-0.09	0.10	0.00	0.17	0.25	0.04
measurement Bull's response to an unfamiliar	0.24	0.46**	0.55**	0.60**	_	0.00	0.07	-0.08	0.01	0.00	-0.11
human Hair whorl position	0.34*	0.66**	0.61**	0.18	0.30	_	0.11	0.67**	0.31	0.50*	-0.27
Hair whorl length	-0.08	0.22	0.23	0.28	0.16	0.11	-	-0.38*	-0.11	-0.06	-0.25
Scrotal circumference	-0.05	0.42**	0.31	0.07	0.21	0.34	0.31	_	0.26	0.46**	0.05
Sperm mass movement	-0.15	0.28	0.15	-0.12	-0.12	-0.03	-0.05	0.43**	-	0.76**	-0.29
Sperm concentration	-0.35*	0.17	0.20	0.00	0.05	-0.31	0.20	0.38*	0.66**	_	-0.48**
Ejaculate volume	-0.14	0.09	0.11	0.15	0.21	-0.11	0.23	0.33*	0.02	0.14	_

Note: Coefficients below diagonal are for Holstein-Friesian bulls, those above diagonal are for Simmental bulls * - significant coefficients at P<0.05; ** - significant coefficients at P<0.01

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