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Environmental and age effects on the semen semen quality of Austrian Simmental bulls

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Abstract

More than 90% of the breeding stock of Austrian dual purpose Simmental cows are artificially inseminated. Knowledge of factors affecting sperm production and semen quality is of importance with regard to reproductive efficiency and thus genetic improvement as well as for the productivity and profitability of AI centers. Hence semen data from two Austrian AI centres collected in the years 2000 and 2001 were evaluated. In total, 3625 and 3654 ejaculates from 147 and 127 AI bulls, respectively, were analysed regarding various effects on ejaculate volume, sperm concentration, percentage of viable spermatozoa in the ejaculate, total spermatozoa per ejaculate and motility. Effects accounted for were age of bull, collection interval, number of collection on collection day, bull handler, semen collector, day of week, temperature on day of semen collection, in the course of epididymal maturation (average temperature of days 1-11 before collection), and during spermatogenesis (average temperature of days 12-65 before collection), and length of day. Age of bull, collection interval and number of collection were significant for most traits at both AI centres. Temperature, either on day of semen collection or during epididymal maturation or spermatogenesis had important but inconsistent effects on semen production and sperm quality. Results for other effects on semen production were also inconsistent between AI centers.

Keywords: Cattle; Bull; Semen; Artificial insemination; Temperature; Age

1. Introduction

Artificial insemination is one of the most important tools in cattle breeding programs. More than 90% of the breeding stock (about 250,000 herd book cows, Hofinger et al., 2004) of Austrian dual purpose Simmental cows are artificially inseminated. High bull fertility has an important effect on the reproductive efficiency in cattle and thus on the genetic improvement. Appropriate conception rates are dependant on sufficient semen quality and quantity provided by AI centres. Obviously, AI centres are highly interested in collecting semen of maximum quantity and quality for economic reasons. Extensive knowledge of factors affecting sperm production and semen quality is therefore substantial.

As the world's cattle population is located in different climatic zones, environmental effects being related to specific climatic conditions were intensively investigated in different breeds and countries. In many studies season significantly influenced semen production (Mathevon et al., 1998; Stalhåmmar et al., 1989) while other investigations failed to detect an effect of season (Brito et al., 2002b). Seasonal effects are the result of various factors such as temperature, humidity, length of day, feed composition but also management. Consequently significant effects of season on semen production were partly contrary. While e.g. Stalhåmmar et al. (1989) observed the highest sperm concentration and total number of spermatozoa during summer, Mathevon et al. (1998) found higher values during winter and spring. Optimal ambient temperature for semen production was found to be approximately 15-20ºC (Taylor et al., 1985; Parkinson, 1987). Not only temperature on day of collection, but also during epididymal maturation or spermatogenesis up to about 70 days before collection seems to affect semen production (Stephan et al., 1971; Meyerhoeffer et al., 1985; Dorst, 1991;Krämer, 2000). However, notable differences between individual bulls were reported, suggesting differences in particular heat tolerance. Similarly, varying quality of feed may affect semen quality up to several weeks (Peter, 1991).

Bull handler and semen collector are also essential for adequate semen production and quality by being responsible for proper sexual preparation. Mathevon et al. (1998) observed a significant effect of the collection team on ejaculate volume and total number of spermatozoa while concentration and motility did not seem to be affected.

Large effects of collection interval were found by Everett and Bean (1982) and Mathevon et al. (1998). The same was found for frequency of collection. First ejaculates resulted in significantly higher ejaculate volumes, sperm concentration and total number of spermatozoa (Everett and Bean, 1982; Everett et al., 1978) while motility was less affected. Similarly, weekday may influence semen production as observed by Everett and Bean (1982) and Taylor et al. (1985).

Finally, age of bulls was found to be highly significant for ejaculate characteristics in most studies. Generally, semen production and quality are reported to increase with age of bull (Stalhåmmar et al., 1989; Mathevon et al.; 1998; Brito et al., 2002a). However, management and health effects may bias results for age effects. Some older bulls may benefit from preferential treatment while a possible unfavourable state of health may decrease semen quantity and quality of others.

The aim of this study was to investigate various factors influencing semen production of Austrian dual purpose Simmental bulls. Besides for the possible improvement of productivity and profitability of AI centers, results shall be utilized in further studies dealing with genetic evaluation of fertility traits.

2. Materials and methods

2.1. Location, animals and treatment

Data from two Austrian AI centers, Oberösterreichische Besamungsstation GmbH, Hohenzell in Upper Austria (center 1) and NÖ Genetik Rinderbesamung GmbH, Wieselburg in Lower Austria (center 2) were collected in the years 2000 and 2001. The Central Institute of Meteorology and Geodynamics in Vienna provided data on average daily ambient temperature and average humidity. Weather is similar at both locations (distance 130 km), therefore data from the official weather station Ried im Innkreis close to center 1 were used for both AI centers. Average daily ambient temperature during the observation period was 10.4ºC with a minimum of –9.9ºC and a maximum of 23.7ºC. Average humidity was 76% (44-99%) and average length of day 12:10 h (8:19-16:05 h).

In total, 3625 and 3654 ejaculates from 147 and 127 dual-purpose Simmental AI bulls, respectively, were analysed. Average age of bulls was 1292d (center 1) and 958d (center 2). Semen was collected by artificial vagina on three and four days per week in center 1 and 2, respectively. In center 1, approximately 60% of the bulls mounted a dummy and and 40 % a teaser animal. In center 2, most bulls mounted a teaser animal, but a dummy was also available. In center 1, bulls were handled by 7 and semen was collected by 3 different employees. In center 2 no information about bull handlers was available, 5 different people collected the semen. Intervals between collection varied between 0 and 168d with an average of 7.9d (center 1) and 0 and 323d with an average of 7.2d (center 2). In center 1, 60% of ejaculates came from first, 38% from second and 2% from third collection per day. In center 2, 88% and 12% came from first and second collection, respectively (Table 1).

2.2. Semen quality measurements

In the routine evaluation of bull semen the following traits are recorded in the AI centres: ejaculate volume, sperm concentration, percentage of viable spermatozoa in the ejaculate, total spermatozoa per ejaculate, and motility.

Ejaculate volume in ml, a trait describing semen quantity, was recorded gravimetrically in center 1 and volumetrically in center 2. The sperm concentration was determined by a properly calibrated spectrophotometer in both stations. Total number of spermatozoa per ejaculate was calculated as the product of ejaculate volume and concentration. Percentage of viable spermatozoa in the ejaculate was examined under the microscope. The estimation took place before dilution in center 1 and after dilution in center 2. In both AI centers motility is subjectively assessed under microscope using a scale from 1 (worst motility) to 4 in center 2 and 5 in center 1 (best motility). In center 1 mass and forward movement were taken into account whereas mass movement only was considered in center 2 (1 no movement, 2 marginal movement, 3 distinct movement, 4 excellent movement; Weitze and Müller, 1991). In Table 1 means and standard deviations are shown for all traits within number of collection and both AI centers.

2.3. Statistical analyses

All statistical analyses were performed using SAS Version 8.0 (SAS Institute Inc., 1999). Data of both AI centers had to be analysed separately since data recording differed. The procedure MIXED was used to investigate the effects on ejaculate volume, sperm concentration, percentage of viable spermatozoa in the ejaculate and total spermatozoa per ejaculate. For motility, classes 1 and 2 (both centers) and 4 and 5 (center 1) were merged. As the trait was not normally distributed, the procedure GENMOD for the analysis of multinomially distributed data was used. The following statistical model was applied:

$Y_{ijklmnop} = \mu + a_i + age_j + handler_k + collector_l + interval_m + number_n + weekday_o + b_1{*}temp + b_2{*}temp11 + b_3{*}(temp11)^2 + b_4{*}temp65 + b_5{*}(temp65)^2 + b_6{*}dlength + \varepsilon_{ijklmnop}$

where

$Y_{ijklmnop}$ is the individual observation,

μ is the overall mean,

a_i is the random effect of animal i (i = 1 to 147 and 127 for center 1 and 2),

age_j is the fixed effect of age class j (j = 1 to 8; 1 = 16-18 months, 2 = >18-20 months, 3 = >20-22 months, 4 = >22-24 months, 5 = >24-36 months, 6 = >36-48 months, 7 = >48-72 months, 8 = >72 months),

$handler_k$ is the fixed effect of bull handler k (k = 1 to 7, center 1 only),

$collector_l$ is the fixed effect of semen collector l (l = 1 to 3 and 5 for centers 1 and 2),

$interval_m$ is the fixed effect of interval in days since last collection (m = 1 to 4, 1 = 1-3d, 2 = 4-6d, 3 = 7-9d, 4 = > 9d),

$number_n$ is the fixed effect of number of collection on the respective collection day (n = 1 to 3 and 2 for center 1 and 2),

$weekday_o$ is the fixed effect of day of week (o = 1 to 3 and 4 for center 1 and 2),

b_1-b6 are regression coefficients,

temp is the continuous effect of the average ambient temperature on day of collection,

temp11 is the continuous effects of the average ambient temperature of days 1-11 before collection (linear and quadratic),

temp65 is the continuous effect of the average ambient temperature of days 12-65 before collection (linear and quadratic), and

dlength is the continuous effect of length of day.

In addition to the usual data edits, AI bulls with less than 10 collections and collections with missing information were excluded from analysis. As less than 10 records for third collections were available in center 2, only first and second collection were taken into account. Since no information about interval between collections was available for the very first record per bull, it was not considered for analysis. Interactions were tested but not found to be significant and therefore discarded. Humidity was included in preceding calculations but excluded as P>0.10 for all traits and both AI centers. Quadratic continuous effects were included for all traits but excluded when not significant. Additionally, continuous traits were not considered when P>0.10 and other related traits became significant after exclusion of the respective trait.

3. Results

In Table 2 levels of significance for fixed and continuous effects are shown. As data of the AI centers had to be analysed separately due to different data recording, comparisons between centers are not feasible.

Age of bull. Except for motility ($P<0.10$) and concentration ($P<0.01$) in center 2, age class of bull was highly significant for all traits in both AI centers ($P<0.001$, Table 2). In center 1, ejaculate volume continuously enhanced with increasing age of bull (Table 3). Thus, highest ejaculate volumes (Least Squares Mean, LSM, 7.14ml) were achieved by bulls being older than 72 months. In center 2, ejaculate volumes also increased with age, but decreased again for older bulls. Results for total number of spermatozoa per ejaculate followed those for ejaculate volume.

Except for the youngest bulls, sperm concentration decreased with increasing age in center 1 (LSM 1.19×10^9/ml for youngest and 1.01×10^9/ml for oldest bulls), with an optimum for bulls with an age of 18-20 months. Results for center 2 were inconsistent. Highest and lowest concentration were found for bulls in the age classes 48-72 months (LSM 1.07×10^9/ml) and 20-22 months (LSM 0.98×10^9/ml), respectively.

Percentage of viable spermatozoa decreased with age of bull in center 1. However, oldest bulls had medium values. In center 2, differences between age classes were smaller with lowest values for oldest bulls. Results for motility were inconsistent with a slight tendency of decreasing with age.

Bull handler and semen collector. Bull handler was only recorded in center 1 and was found to have a significant effect on ejaculate volume and total number of spermatozoa per ejaculate ($P<0.05$, respectively). Semen collector significantly influenced ejaculate volume in both centers ($P<0.001$) as well as concentration ($P<0.001$) and motility ($P<0.001$) in center 2 (Table 2).

Collection Interval. Increasing collection intervals resulted in higher ejaculate volumes (Table 4). While this result was highly significant for center 1 ($P<0.001$), only a trend ($P<0.10$) could be observed for center 2 (Table 2). Results for total number of spermatozoa followed those for ejaculate volume ($P<0.001$ and <0.01 for center 1 and 2, respectively) with highest values for collection intervals of 6-9 days. Collection interval was also found to be significant for sperm concentration, with optimal values with an interval of 4 to 9 days in center 1 ($P<0.05$) and 4-6 days in center 2 ($P<0.001$; Table 2, Table 4). A collection interval of 4-6 days resulted in the highest values for the percentage of viable spermatozoa in the ejaculate in center 2 (LSM 66,6%, Table 4). In center 1, percentage of viable spermatozoa deceased with increasing collection interval. However, while collection interval was significant for center 2 ($P<0.05$), only a trend could be observed for center 1 ($P<0.10$; Table 2). Motility was not significantly affected by collection interval. Similar to the results for percentage of viable spermatozoa, highest values were observed for collection intervals between 4 and 6 days.

Number of collection. First ejaculates had significantly higher volumes, sperm concentrations and total number of spermatozoa per ejaculate ($P<0.001$; Table 2, Table 4). For percentage of viable spermatozoa a significant effect could be observed for center 1 ($P<0.001$) with higher values in ejaculate 2 and 3 (65.0% in first and 66.2% in second and third ejaculate; Table 2, Table 4). In center 2, percentage of viable spermatozoa did not differ between first and second ejaculate (66.2% and 66.1%, respectively). Motility was not affected by number of collection.

Weekday. Semen was collected on Tuesdays, Wednesdays and Fridays in center 1 and Mondays through Thursdays in center 2. In both centers, ejaculate volume was significantly influenced by weekday ($P<0.01$ and <0.05 for centers 1 and 2; Table 2). In center 1, volume decreased Tuesday through Friday (5.89ml, 5.81ml and 5.58ml, respectively, Table 4) while it was highest on Wednesday in center 2 (6.19ml) All other traits were significantly affected by weekday in center 1 only. Sperm concentration ($P<0.01$) was lowest on Wednesday. The same was found for the percentage of viable ($P<0.001$) and total number of spermatozoa ($P<0.05$). Highest motility values were observed on Tuesday, lowest on Friday ($P<0.001$).

Ambient average temperature on day of collection. Results for ambient average temperature on day of collection were inconsistent for both centers (Table 2, Table 5). In center 2 ejaculate volume and motility decreased (-0.03ml/°C, $P<0.05$, and -0.06/°C, $P<0.001$) with increasing ambient temperature while percentage of viable spermatozoa increased in center 1 (0.05%/°C, $P<0.05$).

Ambient temperature on days 1-65 before collection. Effects for temperature before semen collection were stronger than for temperature on day of collection. However, results were also partly inconsistent for AI centers (Table 2, Table 5).

In center 1, ejaculate volume was significantly affected by average temperature during epididymal maturation ($P<0.05$) but not during spermatogenesis. In center 2 however, solely ambient temperature during spermatogenesis was found to have a significant effect on ejaculate volume ($P<0.01$). Optimum temperature for both stages was approximately 12-16°C.

Additionally a significant negative linear effect ($P<0.001$) of temperature during epididymal maturation and a non-linear effect ($+0.01x10^9$/ml°C $P<0.001$ and $-0.0004x10^9$/ml°C, $P<0.05$, respectively) of temperature during spermatogenesis was found on sperm concentration in center 1. Optimum temperatures for sperm concentration were thus in the range of 14-18°C.

Total number of spermatozoa ($+0.13x10^9$/°C and $-0.005x10^9$/°C; $+0.08x10^9$/°C and $-0.004x10^9$/°C linear and quadratic for center 1 and 2) was also significantly affected in both centers ($P<0.05$ to $P<0.001$). Range of optimum temperature was found to be approximately 10-16°C.

A non-linear significant relationship was observed for temperatures during epididymal maturation and spermatogenesis and percentage of viable spermatozoa in both AI centers ($P<0.05$ to $P<0.001$). However, results for both AI centers were contrary.

Additionally, temperature during epididymal maturation had a non-linear effect on motility in center 1 ($P<0.001$) and a positive, linear effect in center 2 ($P<0.05$) while temperature during spermatogenesis had a significant positive linear effect on motility in center 1 only ($P<0.001$).

Length of day. An increase in length of day resulted in slightly lower ejaculate volumes ($P<0.001$), total number of spermatozoa ($P<0.001$) and motility scores ($P<0.01$) in center 1. In contrast to these results, ejaculate volume increased with length of day in center 2 ($P<0.001$) while sperm concentration and percentage of viable spermatozoa decreased ($P<0.05$ and $P<0.001$, respectively, Table 2, Table 5).

4. Discussion

The increase of ejaculate volume with age of bull is in agreement with previous studies (Everett and Bean, 1982; Taylor et al., 1985; Mathevon et al., 1998; Brito et al., 2002a). Taylor et al., 1985 observed an increase in ejaculate volume until 7 years of age. Afterwards ejaculate volume remained constant until 9 to 10 years of age. This is consistent with the findings of (Brito et al., 2002a). Highest ejaculate volumes were found for bulls older than 9 years, but these did not significantly differ from ejaculate of bulls aged 5-9 years. Other authors (Everett and Bean, 1982; Mathevon et al., 1998) observed an increase up to an age of 4 to 5 years which is consistent with the results in center 2 where ejaculate volumes decreased again for the age class of older bulls. Results for total number of spermatozoa per ejaculate followed those for ejaculate volume, which is in agreement with the studies previously cited (Everett and Bean, 1982; Taylor et al., 1985; Mathevon et al., 1998; Brito et al., 2002a).

Similar to the results for center 1, Taylor et al. (1985) and Mathevon et al. (1998) observed an increase of sperm concentrations up to an age of 20-22 months. In another study (Brito et al., 2002a), age of bull was reported to show no effect on sperm concentration. Results for motility were in contrast to the findings of other studies (Stalhåmmar et al., 1989; Makulska et al., 1993) reporting increasing motility with age of bull. For the effect age of bull it should be noted that results may be influenced by preferential treatment. AI centers are undoubtedly highly interested in producing a maximum number of straws of well demanded proven bulls while at the same time younger test bulls are of less interest. Additionally, less demanded bulls may not reach a higher age leaving a selected group of bulls in higher age classes.

Time of sexual preparation was reported to have significant effects on ejaculate volume, number of doses per ejaculate and post-thaw motility (Komisrud and Andersen Berg, 1996). This may explain why bull handler and semen collector have an essential impact on semen quality and quantity as they are responsible for sexual stimulation and preparation. Mathevon et al. (1998) also observed a significant influence of collection team on ejaculate volume and total number of spermatozoa. However, contrary to the findings in this study, motility and concentration did not seem to be affected by the collection team.

The increase of ejaculate volume with increasing collection interval is in agreement with several studies (Everett et al., 1978; Everett and Bean, 1982; Mathevon et al., 1998). The same authors also observed higher sperm concentrations and a significant increase of total number of spermatozoa per ejaculate with longer intervals between collections, which is in agreement with the findings in this study. For motility, present results are consistent with those of Everett et al. (1978) and Mathevon et al. (1998) who reported optimum intervals of 3 to 5 days for percentage of motile sperm. From the results for collection interval it may follow that AI centers should aim at intervals of 4 to 6 days.

The superiority of first ejaculates with regard to higher volumes, sperm concentrations and total number of spermatozoa per ejaculate is in accordance with many other reports (Everett et al., 1978; Everett and Bean, 1982; Taylor et al., 1985). However, contrary to the present findings, in some of them (Everett et al., 1978; Everett and Bean, 1982) a significantly higher percentage of motile sperm was observed in first ejaculates.

Weekday was included in analyses as it was reported to significantly affect semen production in earlier studies (Everett and Bean, 1982; Taylor et al., 1985). Everett and Bean (1982) included day of the week as a measure of efficiency of the labour force expecting a top sperm production on Mondays after weekend break. However, Tuesday was found to be the best day with regard to semen production. The authors assumed that the employees being more socially interactive after weekends might negatively influence semen production results on Mondays. In another work (Taylor et al., 1985) inconsistent results for the effect of weekday were reported. From the results in this study it is assumed that significant effects of weekdays on semen production are rather caused by partly unknown AI center related routines than by the efficiency of employees. In center 1, only bulls put in quarantine are collected on Wednesdays. This selected group of predominantly young and inexperienced bulls may therefore be one reason for lower semen qualities on Wednesdays.

A testicular temperature that is below body temperature is known to be essential for the production of fertile spermatozoa (Waites, 1970). Higher ambient temperature may result in increased testicular temperatures and thus decrease semen quality. Taylor et al. (1985) observed a significant effect of temperature on day of collection on ejaculate volume and total number of spermatozoa while sperm concentration was unaffected. Highest and lowest temperature classes resulted in an increase of ejaculate volume and total number of spermatozoa indicating a non-linear relationship. In contrast to these findings, only a linear relationship could be detected in the present results. However, the previously mentioned study (Taylor et al., 1985) did not include temperatures before collection which might have biased their results. Under tropical conditions, Brito et al. (2002b) failed to detect an effect of temperature on semen production.

Spermatogenesis and epididymal maturation take about 65 days (Dorst, 1991). Environmental influences at any time during these sensitive stages may thus affect semen quality. Therefore average temperatures between days 1 to 11 (epididymal maturation) and 12 to 65 (spermatogenesis) before collection were investigated. The results for ejaculate volume are in accordance with Meyerhoeffer et al. (1985). In their experiment in which AI bulls were exposed to heat, the authors observed a decrease in ejaculate volume during the first 6 weeks of heat stress. Range of optimum temperature for total number of spermatozoa, which was found to be approximately 10 to 16°C, is consistent with another study (Parkinson, 1987). The author reported a quadratic relationship between daily maximum temperature and total sperm numbers collected one month later with an optimum between 15 and 20°C. Contrary to the present results, in a previously cited report (Meyerhoeffer et al., 1985) a decrease of the percentage of motile sperm 2 weeks after exposure to heat was found. The authors assumed that heat stress might not affect epididymal sperm while an impact during spermatogenesis exists. In the present study, both stages showed an influence on percentage of viable spermatozoa and on motility. Parkinson (1987) also observed a significant quadratic relationship of temperature to percentage abnormal sperm four weeks later. The minimum, and thus, optimum, was achieved with approximately 15-17°C.

Generally, ambient temperatures in the range of 12-18°C were found to be optimal for semen production. Reasons for differences between AI centers and the fact that effects on percentage of viable spermatozoa and on motility score are contrary are unclear. Results may reflect differences in recording of the subjective motility score. Another reason may be that the use of data from the weather station close to center 1 might have been suboptimal for the analysis of center 2. However, average ambient temperatures usually only differ marginally between both locations. Besides, maximum summer temperatures were not remarkably high in the years 2000 and 2001. Little variance in ambient temperature may therefore be another reason for the present results. Other causes for the inconsistencies between AI centers may be found in air-conditioning more or less compensating for minimum and maximum temperatures. Apart from that, no semen is collected for 4-6 weeks during summer in center 1. It should be noted that humidity was not found to have an effect on any of the observed semen characteristics and was consequently excluded from analysis.

Finally, length of day may influence semen production depending on latitude (Salisbury et al., 1978). In intermediate latitudes temperature and photoperiod are reported to have an equally important influence. In the presented work contrary results for AI centers were found. A possible explanation are different lighting systems within AI centers.

To account for the positive correlation between the four covariates (r = 0.30 to 0.89), additional calculations were carried out (data not shown). Results were similar when restricting the models to one covariate only.

From all observed effects, age of bull and number of collection had the highest impact on semen quantity and quality of AI bulls. The age of bull effect is likely to be partly intentionally caused by preferential treatment. The first ejaculate per day is superior to the following. However, as total productivity and profitability increase with a second collection per day it is thus recommended. To increase the results for certain employees, training courses in regular intervals might be helpful. Additionally, specific weekday-related routines and possible improvements should be investigated by the AI centers. The same applies for air-conditioning and lighting system.

Currently, semen production traits are not included in the genetic evaluation for fertility in Austrian and German cattle populations (Fuerst and Egger-Danner, 2002). Results of this work will be utilized in subsequent studies as it is planned to investigate the possible implementation of routine semen evaluation records in the genetic evaluation for fertility.

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Table 1

Means ($\bar{x}$) and standard deviation (sd) for both AI centers, first, second and third collection per day (1 to 3) and traits analysed

	Center 1			Center 2	
	Number of collection			Number of collection	
	1	2	3	1	2
	n=2179	n=1388	n=58	n=3148	n=411
Ejaculate volume (ml)	5.76±2.56	5.13±2.22	5.28±2.34	6.88±2.52	4.82±1.91
Sperm concentration (10^9/ml)	1.26±0.41	1.08±0.36	0.95±0.41	1.10±0.33	0.85±0.33
Total number spermatozoa (10^9)	7.10±3.62	5.37±2.68	4.74±2.78	7.51±3.47	4.05±2.28
Viable spermatozoa (%)	65.8±5.7	67.1±4.9	67.1±5.4	66.5±5.3	65.4±5.4
Motility (1 worst, 4 or 5 best)	2.92±0.40	2.96±0.37	2.95±0.35	3.13±0.68	3.00±0.63

Table 2

Level of significance[1] for fixed and continuous effects and both AI centers (1 = center 1, 2 = center 2); Vol = ejaculate volume; Con = sperm concentration; %viable = percentage of viable spermatozoa; Tot = total number of spermatozoa, and Mot = motility.

	Vol		Con		%viable		Tot		Mot	
	1	2	1	2	1	2	1	2	1	2
Bull's age class	***	***	***	**	***	***	***	***	***	+
Bull handler	*	-	ns	-	ns	-	*	-	ns	-
Semen collector	***	***	ns	***	ns	ns	ns	ns	ns	***
Collection interval	***	+	*	***	+	*	***	**	ns	ns
Number of collection	***	***	***	***	***	ns	***	***	ns	ns
Weekday	**	*	**	ns	***	ns	*	ns	***	ns
Avg. temp. on day of collection	ns	*	ns	ns	*	ns	ns	ns	ns	***
Avg. temp. on days 1-11 before collection - linear	*	ns	***	ns	**	ns	ns	-	***	*
Avg. temp. on days 1-11 before collection – quadr.	*	-	-	-	***	**	-	-	***	-
Avg. temp. on days 12-65 before collection – lin.	ns	**	***	+	*	ns	***	*	***	-
Avg. Temp. on days 12-65 before collection – quadr.	-	**	*	-	-	***	**	**	-	-
Length of day	***	***	-	*	-	***	***	+	**	-

[1] + P<0.10; * P<0.05; ** P<0.01; *** P< 0.001

Table 3

Least Squares Means (Vol = ejaculate volume; Con = sperm concentration; %viable = percentage of viable spermatozoa; Tot = total number of spermatozoa) and arithmetic means (Motility = Mot) for different age classes of bulls and both centers (center 1 = 1, center 2 = 2)

	N		Vol (ml)		Con (10^9/ml)		%viable		Tot (10^9)		Mot[1]	
Age class in months	1	2	1	2	1	2	1	2	1	2	1	2
16-18	640	374	4.66	4.88	1.19	1.06	67.0	66.4	5.43	5.27	2.97	3.24
>18-20	644	803	4.81	5.36	1.20	0.99	67.4	66.5	5.60	5.39	3.00	3.22
>20-22	298	615	5.39	5.89	1.16	0.98	67.1	66.3	6.03	5.85	2.99	3.17
>22-24	150	356	5.56	6.22	1.13	1.01	66.4	66.9	6.16	6.49	3.01	3.16
>24-36	221	693	5.65	6.39	1.12	1.03	65.6	66.8	6.03	6.80	2.88	3.03
>36-48	164	185	6.45	6.62	1.00	1.01	63.9	65.3	6.33	7.07	2.89	2.68
>48-72	897	183	6.42	6.77	1.01	1.07	63.7	66.6	6.51	7.41	2.85	3.09
>72	611	350	7.14	5.83	1.01	1.02	65.2	64.4	7.21	5.87	2.94	2.97
rsd[2]	-	-	1.79	2.17	0.30	0.28	4.0	4.5	2.74	2.94	-	-

[1] The LSMEANS statement is not available for multinomial distribution models for ordinal response data (SAS Institute Inc., 1999). Therefore arithmetic means for the subclasses are presented in this table.

[2] residual standard deviation

Table 4

Least Squares Means (Vol = ejaculate volume; Con = sperm concentration; %viable = percentage of viable spermatozoa; Tot = total number of spermatozoa) for different collection intervals, number of collections per day and both AI centers (center 1 = 1, center 2 = 2)

	N		Vol (ml)		Con (10^9/ml)		%viable		Tot (10^9)	
Collection interval (d)	1	2	1	2	1	2	1	2	1	2
1-3	826	774	5.43	5.93	1.08	0.99	66.1	66.2	5.67	5.95
4-6	861	1686	5.44	5.84	1.12	1.06	65.9	66.6	5.82	6.34
6-9	1369	762	6.05	6.03	1.12	1.03	65.7	66.0	6.64	6.45
>=10	569	337	6.12	6.16	1.08	1.00	65.5	65.9	6.51	6.33
Number of collection										
1	2179	3148	6.00	6.92	1.25	1.15	65.0	66.2	7.27	7.87
2	1388	411	5.60	5.07	1.06	0.89	66.2	66.1	5.76	4.66
3	58	-	5.67	-	0.99	-	66.2	-	5.45	-
Weekday										
Monday	-	1103	-	5.95	-	1.00	-	66.0	-	6.17
Tuesday	1583	492	5.89	5.95	1.13	1.03	66.8	65.9	6.45	6.29
Wednesday	253	859	5.81	6.19	1.04	1.00	64.2	66.3	5.91	6.41
Thursday	-	1105	-	5.89	-	1.04	-	66.5	-	6.22
Friday	1583	-	5.58	-	1.13	-	66.4	-	6.13	-

Table 5

Regression coefficients (b_l = linear, b_q = quadratic) of average ambient temperature on day of collection, on days 1-11 and 12-65 before collection and length of the day on ejaculate volume (Vol), sperm concentration (Con), percentage of viable spermatozoa (%viable), total number of spermatozoa(Tot) and motility (Mot) for both AI centers (center 1 = 1, center 2 = 2)

		Vol (ml)		Con (10^9/ml)		%viable		Tot (10^9)		Mot	
		1	2	1	2	1	2	1	2	1	2
Avg. Temp. on day of coll. (°C)	b_l	-	-0.03	-	-	0.05	-	-	-	-	-0.06
Avg. Temp. on days 1-11 bef. coll. (°C)	b_l	0.05	-	-0.01	-	0.12	-0.03	-	-	-0.17	0.02
	b_q	-0.002	-	-	-	-0.009	0.005	-	-	0.011	-
Avg. Temp. on days 12-65 bef. coll. (°C)	b_l	-	0.08	0.01	-	-0.05	0.11	0.13	0.08	0.15	-
	b_q	-	-0.003	-0.0004	-	-	-0.011	-0.005	-0.004	-	-
Length of day (h)	b_l	-0.13	0.12	-	-0.01	-	-0.31	-0.20	-	-0.15	-