Frequency of Boar Ejaculate Collection and its Influence on Semen Quality, Pregnancy Rate and Litter Size

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Received January 14, 2005
Accepted June 6, 2004

Abstract

Frangež R., T. Gider, M. Kosec: Frequency of Boar Ejaculate Collection: its Influence on Semen Quality, Pregnancy Rate and Litter Size. Acta Vet. Brno 2005, 74: 265-273.

The aim of this study was to determine the best collection frequency for boar ejaculate, based on semen quality, pregnancy rate and litter size. Over a period of 7 months, 480 ejaculates were obtained, at successive collection frequencies of one, two, three and seven times per week, from 12 mature boars of line 54. For each ejaculate, the volume, progressive motility, spermatozoa concentration, sperm morphology and total number of spermatozoa were determined. Two commercial semen extenders, Beltsville Thawing Solution and Merck, were used for semen dilution and preservation. Smaller ejaculate volumes ($P < 0.05$), lower sperm concentrations ($P < 0.05$), and lower total sperm counts per ejaculate ($P < 0.05$) were obtained at collection frequencies of 7 and 3 than at 2 and 1 times per week. Significantly lower progressive sperm motilities at 7 than at 3, 2 and 1 times per week were observed. 1586 sows were inseminated with semen obtained at the four collection frequencies. The conception in sows was highest when inseminated with semen collected once per week and was significantly ($P < 0.05$) lower when collected 7 times per week than at the other three collection frequencies. The number of delivered piglets was also highest in sows inseminated with semen collected once per week and significantly($P < 0.05$) higher than at collection frequencies of 2 and 3 per week. No significant differences in the semen quality parameters measured or in pregnancy rate were found when using either of the two commercial preservation extenders.

These results indicate that the sperm quality decreases with increasing collection frequency and is most pronounced at the collection frequency 7 × per week. Taking into account the fertility rate and litter size, together with the number of semen doses obtained per week, the most suitable ejaculate collection frequency in mature boars of line 54 is two or three times per week.

Boar, semen, frequency, sow, artificial insemination, pregnancy rate

Efficient production of high quality semen of high genetic value is the most important aim of artificial insemination (AI) organizations. The improvement of quality and economics is limited by the number of insemination doses that can be obtained from one ejaculate and the number of appropriate quality ejaculates that can be obtained from a boar per week. Sperm production and its quality are influenced by many environmental factors such as the season (Love et al. 1993; Ciereszko et al. 2000; Corcuera et al. 2002), breed (Borg et al. 1993), age (Swierstra 1973), photoperiod (Sancho et al. 2004; Andersson et al. 1998), ambient temperature (Corcuera et al. 2002), food intake (Kemp et al. 1988), breed and testis size (Colenbrander et al. 1990; Colenbrander et al. 1993). One of the most important factors is the collection frequency, which determines both quantity and quality of sperm and, presumably, the number of spermatozoa in the ejaculate (Swierstra and Dyck 1976). Ejaculate volume, sperm concentration and total sperm per ejaculate were reported to be significantly lower when ejaculates were collected at 24 h than 72 h intervals while the pregnancy rate was significantly higher in sows inseminated with semen collected at 24 h intervals than in sows inseminated with semen obtained at 72 h collection intervals

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(Swierstra and Dyck 1976). The volumes of semen, sperm concentration and total number of sperms in the ejaculate were reported to be significantly larger at a collection frequency of 3 times per week than at 48 h intervals (Cameron 1985a; Cameron 1985b). Sperm output was also found to be higher on a 3-day than on a 1-day collection interval by Swierstra (1973). Other authors reported that the semen collected at high collection frequencies had similar progressive motility and led to higher pregnancy rates than semen obtained at low frequencies (Conrad et al. 1981). Sperm production was significantly higher at semen collection frequency of five times than at three times per two weeks (Kemp et al. 1991). Kemp et al. (1991) reported that higher collection frequencies may result in increased sperm output per day with no effect on semen quality parameters (Kemp et al. 1991).

Further, it was found that high semen collection frequencies affect sperm motility, concentration and total sperm count, and result in increased proportions of abnormal sperm morphology (Strzezek et al. 1995). On the molecular level, no significant changes were found in the phospholipid composition of boar sperm between 24 h and 72 h collection frequencies (Johnson et al. 1969). However, some other important biochemical parameters were reported to be altered by high collection frequencies (Strzezek et al. 1995, 1996).

The experimental design and results on effects of collection frequency on semen quality differ markedly from author to author. Using a high collection frequency can provide a large number of spermatozoa per unit time but, on the other hand, it can lead to compromised libido, semen quality and subsequently fertility. In most studies, no data on fertility rate and delivery of piglets are available. We have therefore sought to determine the effects of four ejaculate collection frequencies and the use of two semen extenders on semen quality parameters in boar line 12.

Even though high collection frequencies have been found to impair many sperm quality parameters *in vitro*, relatively little attention has been paid to *in vivo* fertility. Sweirstra and Dyck (1976) reported that sows inseminated with equal amounts of motile sperm (2.5×10^9) from boars on 24 h and 72 h collection schedules had significantly different pregnancy rates (83% versus 70%, respectively). We have therefore included an assessment of pregnancy rate and the number of live piglets delivered in our study.

Materials and Methods

Animals

Semen was collected between December 2000 and March 2001. Twelve 20-24-month-old boars of line 54 (Piétrain × German Landrace), which are used in a regular AI program, were the subjects of the study. The boars were kept at the Boar Pen Stall under the same controlled environmental conditions and subject to the same weekly collection timetable.

Over the same period, a total of 1586 sows of line 12 (Large White × Swedish Landrace), two to five years of age, were artificially inseminated with the semen collected in accordance with the selection breeding program. The data about parturition rate and number of piglets were collected from April to June 2001.

Ejaculate collection, libido estimation and semen processing

The twelve mature boars were exposed to four ejaculate collection schedules consecutively as follows: single ejaculate collected at a collection frequency of once per week (1 ×), followed by twice per week (2 ×), three times per week (3 ×) and seven times per week (7 ×). Ten ejaculates were collected from each boar at each of the four collection frequencies and a total of forty ejaculates from each boar.

Libido was estimated and scored according to behaviour immediately before semen collection. The semen rich fraction of ejaculate was collected manually by an experienced worker, using the gloved-hand procedure, through a filter (Minitube, Germany) into a glass cup, which enables the gel fraction of ejaculate to be removed. The semen was then transferred immediately to the seminal assessment laboratory.

Semen evaluation: volume, progressive sperm motility, sperm concentration and spermatozoa morphology

Sperm progressive motility (%) was subjectively estimated, always by the same experienced person, under a light phase contrast microscope at 400 × magnification and at 35 °C. The spermatozoa concentration was determined using a Meckler counting chamber. Total sperm number was calculated from the ejaculate volume and sperm concentration. The number of insemination doses for each ejaculate was calculated from the sperm

progressive motility, given that the lower limit per inseminating dose is 3×10^9 spermatozoa. Sperm morphology was assessed on Giemsa stained semen smears under a light microscope at 1000 × magnification. The semen samples obtained from the 8th ejaculate obtained at all four collection frequencies were evaluated. One hundred spermatozoa were analyzed for changes in spermatozoa acrosome, head, neck and tail morphology, and the results expressed as per cent altered morphology.

Semen dilution and semen dose preparation

Two commercial preservation medium were used: one of the most widely used extenders, Beltsville Thawing Solution (BTS), and Merck. Media were prepared and used according to the manufacturer's instructions. The semen was divided into two equal parts and processed as shown in Fig 1.

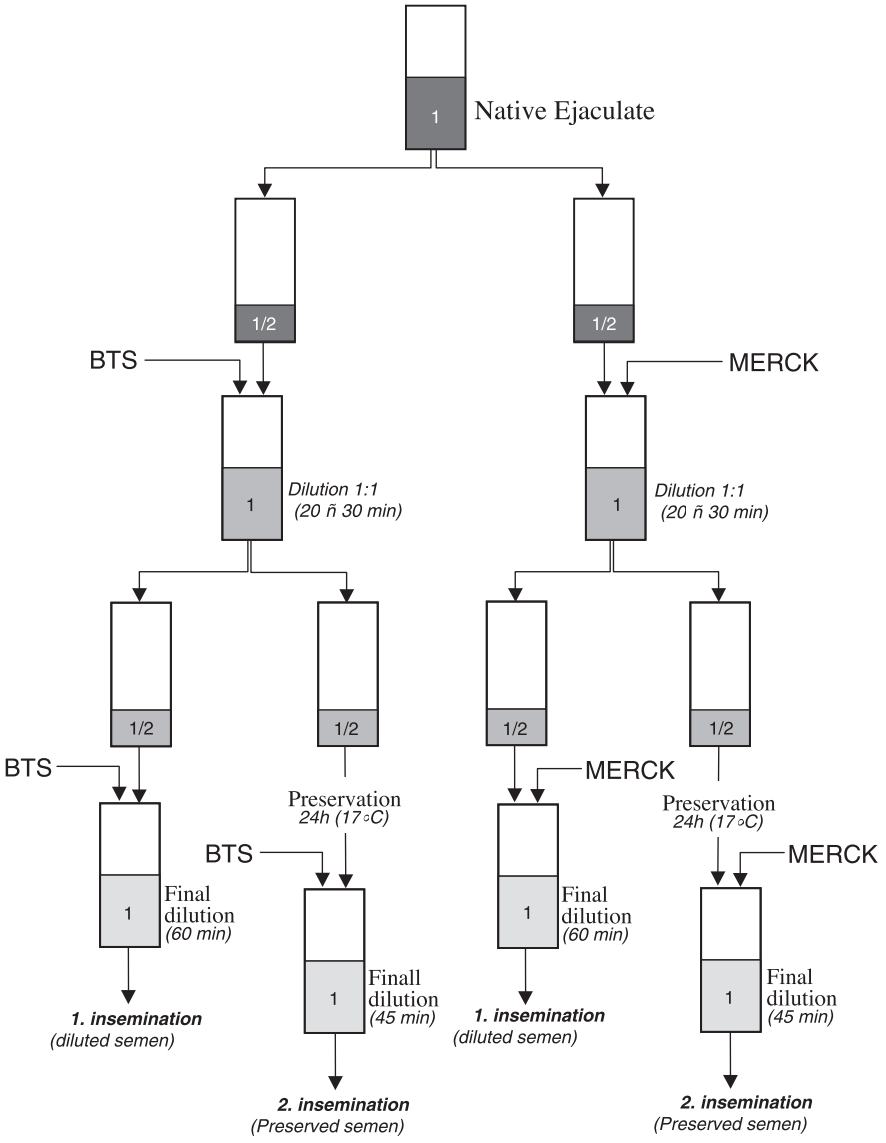

Fig. 1. Dilution and preparation of semen doses. BTS, Beltsville Thawing Soution.

Semen was preserved in BTS and Merck extenders to a final dilution of 3×10^9 spermatozoa in 100 ml of seminal plasma and semen extender. Before AI, the preserved semen doses were analyzed. Progressive motility was redetermined. The sperm doses were prepared only from ejaculates whose progressive motility was 70% or higher.

Artificial insemination of sows with semen from the four different collection frequencies

AI was performed twice, using diluted and preserved semen. The timing of AI was oestrus detection dependent and based on a positive response to back pressure by an experienced technician in the presence of a boar. Detection of oestrus was conducted twice a day (08.00 and 16.00 h). Each sow was inseminated twice; at the time of immobility reflex at 12.00 h with fresh diluted semen, and again next day at 08.00 h with preserved semen from the same boar, stored for 24 h at 17 °C, in order to cover optimally the sow fertility period. One group of sows was inseminated twice with 3×10^9 motile sperm using commercial semen extenders BTS and another group using Merck.

Statistical analysis

The data were expressed in terms of arithmetic means and standard error. The data were subjected to two-way analysis of variance (ANOVA). Tukey's test was used for *post hoc* evaluation. All calculations were performed using the statistical software SPSS version 11 (SPSS Inc., USA). A P-value of ≤ 0.05 was considered statistically significant.

Results

Libido at different collection frequencies

Libido was highest at the collection frequency 2 × and was significantly lower at the collection frequencies 7 × than at frequencies of 1×, 2 × and 3 × ($P < 0.05$). Libido was also lower at collection frequency 3 × than at 2 × ($P < 0.05$).

Effects of four collection frequencies on semen volume, progressive sperm motility, sperm concentration and spermatozoa morphology

A significant effect was observed at 3 × and 7 × collection frequencies on the boar semen quality parameters. A total of 480 ejaculates were obtained and analyzed. Semen quality was found to be dependent on collection frequency.

The ejaculate volume was greatest at collection frequency 2 × and significant lower at 3 × and 7 × than at 1 × and 2 ×collection frequencies. It was also significantly lower at collection frequency 7 × than at 3 × (Table 1). The spermatozoa concentrations were highest at the collection frequency 1 ×. At collection frequency 3 × it was statistically lower than at 1 and 2 ×, and at collection frequency 7 × significantly lower than at the other three collection frequencies. The same is true for the total number of spermatozoa obtained at the same collection frequencies. Progressive sperm motility was highest at a collection frequency 1 × and was significantly lower at a collection frequency of 7 × than at other intervals. The percentage of morphologically altered spermatozoa was estimated in eight ejaculates at each collection frequency. No significant differences in the number of morphologically altered spermatozoa were found between experimental groups (Table 1).

Progressive motility of spermatozoa in native, diluted and preserved boar semen using two commercial extenders

Two commercial semen extenders (BTS, Merck) were used as described in Materials and Methods and shown in Fig. 1.

As described above, the progressive motility was significantly lower at the highest collection frequency in native semen. The same is true and more pronounced for diluted and preserved semen using BTS and Merck extenders. Additionally, progressive motility was significantly lower in semen samples collected at 24 h intervals than in those from 56 h interval collections. No significant differences in progressive sperm motility were found between two semen extenders used at all four collection frequencies.

Number of inseminating doses prepared from ejaculates obtained at four collection frequencies

Only semen samples which had 70% or higher progressive sperm motility were processed and used in AI. In this respect one diluted semen sample at collection frequency 1 ×, five at

Table 1. The semen quality of the boars exposed to the four different ejaculate collection frequencies

Frequency (times/week)	Variable	Mean	SE	Probability
1	Ejaculate volume (ml)	256	9.13	
	Sperm concentration (spermatozoa × 10^6/ml)	289	54.33	
	Total number of spermatozoa (spermatozoa × 10^9)	70	19.13	
	Total number of spermatozoa per boar per week (×10^9)	70	19.13	$P < 0.05$ (2, 3, 7)
	Progressive motility (%)	78	3.63	$P < 0.05$
	*% morphological altered spermatozoa	13.92	5.73	
2	Ejaculate volume (ml) *change as for 1*	258	9.11	
	Sperm concentration (spermatozoa ×10^6/ml)	276	53.20	
	The total number of spermatozoa (×10^9)	67	17.13	
	The total number of spermatozoa obtained from boar per week (×10^9)	133	34.25	
	Progressive motility (%)	77.58	3.63	
	*% of morphological altered spermatozoa	13.92	6.13	
3	Ejaculate volume (ml) *change as for 1*	220	7.68	$P < 0.05$ (1, 2)
	Sperm concentrations (spermatozoa ×10^6/ml)	240	59.27	$P < 0.05$ (1, 2)
	The total number of spermatozoa (×10^9)	51	19.84	$P < 0.05$ (1, 2)
	The total number of spermatozoa obtained from boar per week (× 10^9)	154	59.51	
	Progressive motility (%)	75.64	8.56	
	*% of morphological altered spermatozoa	12.55	3.27	
7	Ejaculate volume (ml) *change as for 1*	198	6.97	$P < 0.05$ (1, 2)
	Sperm concentrations (spermatozoa ×10^6/ml)	153	59.24	$P < 0.05$ (1, 2, 3)
	The total number of spermatozoa (×10^9)	29	14.50	$P < 0.05$ (1, 2, 3)
	The total number of spermatozoa obtained from boar per week (×10^9)	206	101.53	
	Progressive motility (%)	70.17	11.70	$P < 0.05$ (1, 2, 3)
	*% of morphological altered spermatozoa	14.75	5.642	$P < 0.05$ (1, 2, 3)

Results are expressed as the mean ± S.E.M. (n = 120). The values at each collection frequency are significantly different from those at frequencies denoted in parentheses in the column headed 'probability'.
*Results are statistically significantly different ($P \leq 0.05$).

Table 2. Progressive sperm motility in native, diluted and preserved boar semen

Frequency (times per week)	**Native**	**Diluted**		**Preserved**	
		BTS	**Merck**	**BTS**	**Merck**
1×	78.04 ± 3.63	75.87 ± 3.76	75.38 ± 3.73	71.33 ± 7.69	70.67 ± 7.83
2×	77.58 ± 3.63	74.96 ± 5.04	74.64 ± 5.11	68.99 ± 13.16	68.79 ± 13.65
3×	75.64 ± 8.56	72.54 ± 12.47	72.46 ± 12.45	62.54 ± 23.11	62.16 ± 23.47
7×	70.17 ± 11.7	61.30 ± 22.35	61.18 ± 21.50	35.34 ± 31.59*	35.29 ± 31.54*

Results are expressed as the mean ± S.E.M. (n = 120).
*Results are significantly lower than for native and diluted semen at the same collection frequency ($P \leq 0.05$).

2 ×, seven at 3 ×, 36 at 7 ×, together with seven preserved semen samples, using both semen extenders, at collection frequency 1 ×, seven at 2 ×, 22 at 3 ×, 64 at 7 × interval did not meet this criterion and were not used for AI. For AI it is important to know the effects of collection frequency on the number of spermatozoa produced per collection. The number of inseminating doses obtained at four different collection frequencies is given in Table 3.

Table 3. Average number of semen doses for AI obtained per week at different semen collection frequencies

Collection frequency (per week)	Number of semen doses per collection		
	Diluted semen	Preserved semen	Combined
1 ×	8.71	8.20	16.91
2 ×	15.98	15.04	31.02
3 ×	17.26	14.64	31.90
7 ×	14.69	6.85	21.54

Data are presented as the average number of applicable semen doses obtained from twelve boars at four collection frequencies. The numbers of semen doses were the same for each of the two extenders used, and therefore data are presented together.

The highest number of semen doses was obtained at the collection frequency 3 × (Table 3). The sperm quality after 24 h storage of semen samples at 17 °C decreased at collection frequency 3 × and was most pronounced at frequency 7 ×.

The effects of collection frequency on fertility rate and the number of newborn piglets

The semen doses obtained at four different collection intervals were used to inseminate sows. Only semen samples with 70% or with higher progressive motility were used. A total of 1586 sows, of line 12, were artificially inseminated.

Table 4. Percentage of newborn delivered from artificially inseminated sows using two semen extenders

Collection frequency (per week)	% of inseminated swine delivering newborn	
	BTS	Merck
1 ×	87.02 (n = 362)	87.33 (n = 288)
2 ×	84.84 (n = 297)	84.58 (n = 263)
3 ×	78.08 (n = 117)	80.73 (n = 161)
7 ×	74.07 (n = 54)	75.00 (n = 44)

n = number of inseminated sows

No significant differences were found between the two extenders but the fertility rate was significantly lower at 7× ejaculate collection frequency than at the other three collection frequencies.

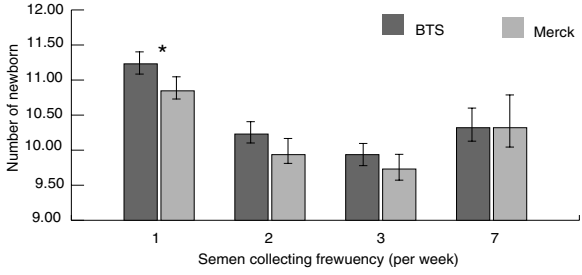

Fig. 2. Average number of newborns per litter delivered after insemination with semen obtained at four different collection frequencies. * Number is significantly different when compared to 2 × and 3 × semen collecting frequency.

The average number of piglets in sows artificially inseminated with semen using BTS extender at collection frequencies 1 ×, 2 × and 3 × was slightly lower but not significantly different ($P > 0.05$).

The average number of piglets was significantly higher ($P < 0.05$) from sows inseminated with semen obtained at collection frequency 1 × than from collection frequencies 2 × and 3 × (Fig. 2).

Discussion

From the results of this study it is clear that higher ejaculate collection frequencies affect certain quality indices of boar semen – volume, concentration, total sperm obtained per unit time, progressive motility and fertility rate. At higher collection frequency the production of sperm per unit time was increased but the number of spermatozoa per ejaculate was decreased (Table 1). In our study a drastic drop in the number of spermatozoa per ejaculate at collection frequency 7 × was observed, probably due to epididymal store spermatozoa depletion. The ejaculate volume is less affected by highest collection frequency. Similar effects concerning spermatozoa concentration and ejaculate volume were described for 13.5-month-old Yorkshire boars (Swierstra and Dyck 1976) and (Cameron 1985a). The collection of frequency three times a week resulted in a greater sperm output, and the boars appeared to sustain a higher level of libido. While Swierstra (1973) found that the sperm output per unit time was greater on a 72 h than on a 24 h collection schedule. In our study we obtained nearly the same average values for total spermatozoa per ejaculate. Small variations may be due to the influence of factors such as breed, age, collection interval, geographic location of the AI centre, and experimental design. High semen collection frequencies increase the number of spermatozoa obtained per unit time. The highest collection frequency (7 ×) was shown here to reduced significantly the number of spermatozoa in the ejaculate, resulting in lower numbers of inseminating doses, especially of preserved semen. Additionally, we found the libido to be significantly lower at a collection frequency of 7 ×, which may constitute a limiting factor for sperm quality and quantity at higher collection frequencies, especially with longer, high collection frequency periods.

The most valuable indices in sperm quality assessment are the number of pregnancies and the number of piglets born per litter. It has been confirmed by many authors that high collection frequencies may have a negative impact on many sperm quality parameters *in vitro*. Our study on *in vivo* indices showed significant differences in pregnancy rates (83% versus 70%), when sows were inseminated with 2.5×10^9 motile sperm obtained from boars on 24 h and 72 h collection intervals, respectively. These results are contradictory to those of Swierstra and Dyck (1976) who found that sows inseminated by semen collected at high (24 h) collection intervals showed a higher pregnancy rate (83%) than sows inseminated with semen collected at 72 h intervals (70%). On the other hand, they found that semen fertility differed with the collection frequency employed, and that some were more fertile at 72 h than 24 h collection frequency. They also found that the litter size was not significantly different between the two collection frequencies. In contrast, we found that, for both extenders used, the number of piglets delivered is inversely proportional to semen collection frequency. One explanation may be that the survival of embryos when using semen obtained at higher frequencies is lower in the later pregnancy period than when semen obtained at lower frequencies is used. Although we used semen with 70% or higher progressive motility, and 3×10^9 of spermatozoa per inseminating dose, a significantly lower percentage of sows inseminated with semen obtained at collection frequency 7 × delivered piglets (see Table 4), although no altered sperm morphology was observed in semen collected at all four frequencies employed.

These results indicate that the quality of spermatozoa is altered at the highest collection frequencies employed. In our study the decrease in progressive motility and subsequent fertility may be a consequence of incomplete maturation due to a faster passage of spermatozoa through the epididymis. Random insemination was performed and therefore it is not expected that the sows are an important factor in fertility rate and numbers of piglets born. High collection frequencies have also been observed by Strzezek et al. (1995) to result in decreased sperm motility, concentration, total sperm and increased percent abnormal sperm and sperm with damaged membranes. Osmotic resistance of spermatozoa was also decreased indicating impaired integrity of spermatozoa membranes. The levels of some biochemical markers measured are also altered by high collection frequency. It is expected that they contribute to the lower pregnancy rate and to the number of live piglets delivered (Strzezek et al. 1995, 1996). Other parameters, such as respiratory activity of sperm (Fülöp 1996) at increased ejaculate collection frequency can affect respiratory activity of the sperm and lead to lower semen viability.

In conclusion, the ejaculate collection frequency affects semen quality and consequently the number of inseminating doses obtained per week. The most suitable ejaculate collection frequencies, taking all factors into consideration, appears to be 2 - 3 collections per week. Our study shows additional data on the effects of semen collection frequency on semen quality indices and on preserved semen, pregnancy rate and litter size. It is evident that the decrease in the semen quality at the highest collection frequency is far more serious than that expressed by sperm morphology and quality parameters alone, and resulted in significantly lower fertility rate and litter size.

Vliv frekvence odběru na kvalitu ejakulátu kance, procento zabřeznutí a velikost vrhu u prasnic

Cílem studie bylo určit optimální frekvenci odběru ejakulátu kance, založenou na kvalitě ejakulátu, procentu zabřeznutí a počtu narozených selat. Během 7 měsíční periody bylo získáno 480 ejakulátů od 12 dospělých kanců linie 54 po úspěšném odběru s frekvencí jednou, dvakrát, třikrát a sedmkrát týdně. U každého ejakulátu byl stanoven objem, progresivní motilita spermií, jejich koncentrace, morfologie a celkový počet spermií. Na ředění a konzervaci ejakulátu byly použity dva komerční extendory Beltsville Thawing Solution a Merck. Odběrem ejakulátu s frekvencí sedmkrát a třikrát týdně byl získán ejakulát s menším objemem ($P < 0,05$), nižší koncentrací spermií ($P < 0,05$) a nižším celkovým počtem spermií ($P < 0,05$) než při odběru s frekvencí dvakrát a jednou týdně. Při odběru s frekvencí sedmkrát týdně byla pozorována signifikantně ($P < 0,05$) nižší progresivní motilita spermií než při odběru s frekvencí třikrát, dvakrát a jedenkrát týdně. 1586 prasnic bylo inseminováno ejakulátem, který byl získán odběrem se čtyřmi různými frekvencemi. Koncepce u prasnic byla vyšší po inseminaci ejakulátem odebíraným jednou týdně a byla signifikantně ($P < 0,05$) nižší při odběru sedmkrát týdně než při zbylých třech odběrových frekvencí. Počet narozených selat byl nejvyšší u prasnic, které byly inseminovány ejakulátem odebíraným jednou týdně a byl signifikantně ($P < 0,05$) vyšší než u ejakulátu odebíraného dvakrát a třikrát týdně. Mezi měřenými ukazateli kvality ejakulátu nebo procentu zabřeznutí prasnic nebyly nalezeny signifikantní rozdíly při použití dvou konzervačních extendorů.

Tyto výsledky ukazují, že kvalita ejakulátu se snižuje se zvyšující se frekvencí odběru, což se nejvíce projevilo při sedmi odběrech týdně. Hodnotíme-li procento zabřeznutí, velikost vrhu a počet dávek ejakulátu získaných za týden, nejoptimálnější frekvencí odběru ejakulátu dospělých kanců linie 54 je dvakrát až třikrát týdně.

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