Physical characteristics of ejaculates produced by insemination boars depending on the interval between successive ejaculate collections

Cechy fizyczne ejakulatu knurów inseminacyjnych w zależności od upływu czasu między pobraniami kolejnymi ejakulatów

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Abstract

The ejaculate characteristics of Polish Landrace boars showed a significant correlation with the intervals between the successive ejaculate collections. The effect of insemination use intensity was however varied. Rising frequency of ejaculate collection led to a systematic and fairly even fall in ejaculate volume. Ejaculate sperm concentration remained at a relatively high level when ejaculates were collected with a frequency of 7 to 3 days but further shortening of the interval between the successive collections led to a drastic decrease in sperm concentration. An increase in ejaculate collection frequency to every four and fewer days resulted in a significant decrease in the number of spermatozoa present in the produced ejaculates and a concomitant decrease in the number of insemination doses prepared from these ejaculates, with an escalation of such changes.

Keywords: boar, ejaculate quality, ejaculation frequency

Streszczenie

Wykazano, że cechy ejakulatu knurów rasy pbz wykazują istotną zależność od odstępu między kolejnymi pobraniami ejakulatu. Wpływ intensywności inseminacyjnego użytkowania jest jednak zróżnicowany. Zwiększanie częstotliwości pobierania ejakulatów skutkuje systematycznym i w miarę równomiernym zmniejszaniem objętości ejakulatów. Koncentracja plemników w ejakulacie utrzymuje

się na relatywnie wysokim poziomie przy pobieraniu ejakulatów z częstotliwością co 3-7 dni, ale dalsze skracanie czasu między kolejnymi pobraniami skutkuje drastycznym zmniejszaniem koncentracji plemników. Zwiększanie częstotliwości pobierania ejakulatu do co 4 i mniejszej liczby dni skutkuje istotnym zmniejszaniem liczby plemników w wydalanych ejakulatach i adekwatnym zmniejszaniem liczby porcji inseminacyjnych wytwarzanych z tych ejakulatów, przy czym następuje eskalacja tych zmian.

Słowa kluczowe: częstotliwość ejakulacji, jakość ejakulatu, knur

Detailed abstract

Badania przeprowadzono na materiale 126 ejakulatów, pobranych od 6 knurów rasy polskiej białej zwisłouchej (po 21 ejakulatów od każdego knura). Ejakulaty pobierano zachowując stałe i regularne odstępy między kolejnymi pobraniami. Od każdego knura pobrano po 3 ejakulaty w odstępach 7-dniowych oraz po 3 ejakulaty w odstępach 6, 5, 4, 3, 2, i 1-dniowych. Każdy ejakulat poddano ocenie ustalając następujące cechy fizyczne: objętość ejakulatu, koncentrację plemników, odsetek plemników o ruchu postępowym, ogólną liczbę plemników w ejakulacie i liczbę dawek inseminacyjnych uzyskanych z jednego ejakulatu. Zebrane dane pogrupowano według kryterium odstępu między kolejnymi pobraniami ejakulatu.

Wykazano, że cechy fizyczne ejakulatu zależą od odstępu czasu między kolejnymi pobraniami ejakulatu. Wpływ intensywności inseminacyjnego użytkowania jest jednak zróżnicowany. W przypadku objętości ejakulatu zwiększanie częstotliwości pobierania ejakulatów skutkuje systematycznym i w miarę równomiernym zmniejszaniem objętości ejakulatów. Koncentracja plemników w ejakulacie utrzymuje się na relatywnie wysokim poziomie (powyżej 360 x 10^{3}·mm^{-3}) przy pobieraniu ejakulatów z częstotliwością co 3-7 dni, ale dalsze skracanie czasu między kolejnymi pobraniami skutkuje drastycznym zmniejszaniem koncentracji plemników w ejakulacie. Ejakulaty o bardzo dużej liczbie plemników (powyżej 90 mld) pozyskuje się przy częstotliwości ejakulacji mniejszej niż co 4 dni. Zwiększanie częstotliwości pobierania ejakulatu do 4 i mniejszej liczby dni skutkuje istotnym zmniejszaniem liczby plemników w wydalanych ejakulatach i adekwatnym zmniejszaniem liczby porcji inseminacyjnych wytwarzanych z tych ejakulatów, przy czym następuje eskalacja tych zmian.

Uwzględniając wyniki przedstawionych badań w praktyce inseminacyjnego użytkowania knurów jako optymalną częstotliwość pobierania ejakulatów należy zalecać pobieranie ejakulatów co 4-5 dni. Ejakulaty pobierane z częstotliwością co 1-2 dni mają wyraźnie obniżone wskaźniki ilościowe.

Introduction

Insemination plays an increasingly important role in pig reproduction and is associated with a number of advantages. It favours an economical use of sires,

facilitates the organization of reproduction in the herd and reduces the risk of spreading infectious diseases. The chief criterion in the selection of boars at insemination centres is their breeding value which considerably exceeds the mean breeding value of the population of young boars evaluated in this country. The breeding value of boars is of great importance. However, at insemination centres, it is important that the ejaculates obtained from insemination boars should retain high quality throughout the service period (Oberlender et al., 2012; Schulze et al., 2014). That is why efforts are made at insemination centres to use the semen of boars providing high-quality ejaculates with respect to the physical parameters (Robinson and Buhr, 2005). Numerous studies have shown that the quality and quantity of obtained ejaculates are affected by a number of genetic and environmental factors, including among others the breed and the age of the boar (Kondracki et al., 2012; Oh et al., 2006; Rutten et al., 2000; Smital, 2009), the size of testes (Clark et al., 2003; Colenbrander and Kemp, 1990; Colenbrander et al., 1993), the season of the year (Ciereszko et al., 2000; Wysokińska et al., 2009) and the season-related daylight duration (Knecht et al., 2014; Rivera et al., 2005; Sancho et al., 2004), piggery microclimate conditions and the dietary regime (Kemp and den Hartog, 1989; Louis et al., 1994;). The frequency of use of the reproduction boar is a significant factor (Falkenberg et al., 1992; Frangež et al., 2005). An excessive frequency of ejaculate collection can lead to disorders in spermatogenesis and negatively affect the physical parameters of the ejaculates. On the other hand, if sires provide semen at excessively long intervals, sperm quality deteriorates since the spermatozoa remain in epididymal tails for too long.

The present work was aimed at identifying the effect of the interval between successive ejaculate collections on the physical parameters of Landrace ejaculates.

Materials and methods

The material for the study was constituted by 126 ejaculates collected from six Landrace boars. All the ejaculates were collected from boars aged from 26 to 30 months. The ejaculates were manually collected from each boar at seven-, six-, five-, four-, three-, two- and one-day intervals, according to the following schedule: 3 ejaculates collected at seven-day intervals; 3 ejaculates collected at six-day intervals; 3 ejaculates collected at five-day intervals; 3 ejaculates collected at four-day intervals; 3 ejaculates collected at three-day intervals; 3 ejaculates collected at two-day intervals; 3 ejaculates collected at one-day intervals.

In order to eliminate the human factor, the ejaculates were collected from all the boars by a single person. Each ejaculate was evaluated to determine the following physical parameters: ejaculate volume, sperm concentration, percentage of progressively motile spermatozoa, overall number of motile spermatozoa in the ejaculate, number of insemination doses obtained from the ejaculate.

Ejaculate volumes were determined after decanting the gelatinous fraction, on the basis of ejaculate weights measured with electronic scales. Ejaculates sperm concentrations were identified with the photometric method using an Accucell spectrophotometre manufactured by the French company IMV - Technologies. The method consists in measuring the intensity of light passing through a sperm

suspension in a sperm-isotonic sodium chloride solution. The percentages of spermatozoa with progressive motility were determined microscopically. "At a 200-fold zoom, the percentage of correctly motile spermatozoa in the overall number of sperms present in the field of vision of the microscope was determined. On the basis of the obtained results, the WINSUL computer software was used to compute the number of motile spermatozoa in the ejaculates and the number of insemination doses possible to prepare from a single ejaculate.

The results were statistically processed using analysis of variance according to the following mathematical model:

$$Y_{ij} = \mu + a_i + e_{ij}$$

where: Y_{ij} – trait value, μ – population mean, a_i – effect the interval between successive ejaculate collections, e_{ij} – error. Significance of between-group differences was verified by means of Tukey's test at $P \leq 0.05$.

Results and discussion

The data showing the physical parameters of the boar ejaculates in relation to the lapse of time between the successive collections have been presented in Table 1.

Table 1. Physical characteristics of ejaculates produced by insemination boars, depending on the interval between successive semen collections (means±Sd)

Tabela 1. Cechy fizyczne ejakulatu knurów w zależności od odstępu czasu między pobraniami nasienia

Item	The interval between the successive ejaculate collections (days)						
	7	6	5	4	3	2	1
Number of ejaculates	18	18	18	18	18	18	18
Ejaculate volume (ml)	383.89^{a} ±97.56	341.11^{ab} ±10.16	333.89^{ab} ±63.9	291.67^{bc} ±58.03	288.94^{bc} ±82.82	292.22^{bc} ±70.67	233.39^{c} ±86.81
Sperm concentration ($x10^3$ mm^{-3})	348.33^{ab} ±154.24	485.00^{a} ±262.46	394.44^{ab} ±143.04	398.33^{ab} ±109.23	364.44^{ab} ±128.58	287.78^{bc} ±143.96	175.00^{c} ±38.07
Motile spermatozoa (%)	73.89^{ab} ±5.01	72.78^{ab} ±4.61	75.00^{a} ±5.14	71.11^{ab} ±3.23	71.11^{ab} ±3.23	69.17^{ab} ±16.55	67.78^{b} ±6.47
Number of motile spermatozoa in the ejaculate (mld)	92.64^{a} ±28.47	105.60^{a} ±29.83	98.51^{a} ±40.91	80.74^{c} ±20.98	70.61^{cd} ±25.38	54.39^{d} ±18.56	27.59^{b} ±6.07
Number of insemination doses	37.77^{a} ±10.1	38.67^{a} ±9.71	35.89^{a} ±14.31	31.67^{ac} ±8.41	26.56^{cd} ±9.12	19.89^{d} ±6.9	10.78^{b} ±3.15

Different superscripts mean significant differences among means within particular rows; lower-case letters: $P \leq 0.05$

The data reveal a significant correlation between the ejaculate parameters and the interval between the successive ejaculate collections. The diminishing time intervals between the successive ejaculate collections were accompanied with a decrease in ejaculate volumes. The greatest ejaculate volumes were identified in the ejaculates collected at seven-day intervals. The growing frequency of ejaculate collection, from a seven-day to a four-day interval, was accompanied with a gradual fall in the volume. The volumes of ejaculates collected every four, three and two days remained at a similar level and were at least 92ml lower than the volumes of ejaculates collected every seven days ($P \leq 0.05$).

The time elapsing between the successive ejaculate collections also affected sperm concentration in the ejaculates and the percentage of progressively motile spermatozoa, although to a lesser degree than in the case of ejaculate volume. The sperm concentrations in the ejaculates collected at seven-, six-, five-, four- and three-day intervals remained at a relatively high level (348-495 x $10^{3} \cdot mm^{-3}$). It was only the increase in semen collection frequency to every two days that led to a significant lowering of sperm concentration (to 287.8 x $10^{3} \cdot mm^{-3}$), and the lowest sperm concentration was identified in ejaculates collected at one-day intervals. It amounted to 175.0 x $10^{3} \cdot mm^{-3}$ and was almost 173 x $10^{3} \cdot mm^{-3}$ lower than in the ejaculates collected at three-, four-, five- and six-day intervals ($P \leq 0.05$).

The data in Table 1 show that the percentages of progressively motile spermatozoa in the ejaculates collected at seven- and six-day intervals between the successive collections remained at relatively high and similar levels, approximately 72.8-73.9%, and they even slightly rose (to 75.0%) at the five-day interval. Increasing the ejaculate collection frequency to an interval below five days resulted in a decrease in sperm motility in the ejaculates. The percentage of progressively motile spermatozoa in the ejaculates collected at one-day intervals was 67.78%, i.e. 7.22% less than in the ejaculates obtained at five-day intervals. This difference proved significant at $P \leq 0.05$.

The most important characteristic of insemination boar ejaculates is the total count of motile spermatozoa. It determines the number of insemination doses that can be prepared from a single ejaculate (Kondracki et al., 2013b). The data in Table 1 show that with seven-, six- or five-day intervals between successive collections the number of spermatozoa in the ejaculates remained at a very high level (over 90 bn motile spermatozoa). Shortening the intervals between the successive collections to 4, three, two and one day resulted in a decrease in the ejaculate sperm count by at least 10 billion per each day subtracted from the interval between the successive ejaculate collections.

Similar tendencies were observed in the case of the number of insemination doses to be obtained from a single ejaculate. The data in Table 1 show that the ejaculates obtained at seven- and six-day intervals provided the most insemination doses (approximately 38 doses per ejaculate). In turn, shortening the time between the successive ejaculate collections led to a reduction in the number of obtained insemination doses. The ejaculates collected at three-, two- and one-day intervals provided, respectively, 12, 19 and 28 fewer insemination doses than the ejaculates collected at six- or seven-day intervals ($P \leq 0.05$). It should also be noticed that the

dynamics of the fall in the number of obtained insemination doses clearly increased as the time between successive ejaculate collections diminished (Figure1).

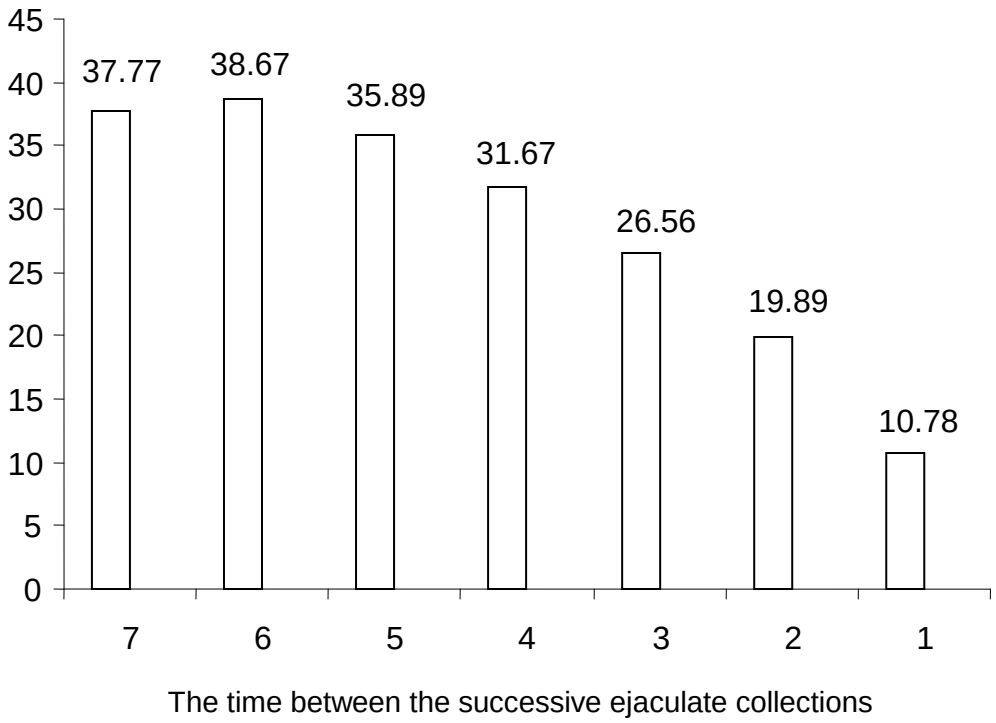

Figure1. The number of insemination doses prepared from an ejaculate, depending on the interval between the successive ejaculate collections

Rysunek 1. Liczba dawek inseminacyjnych możliwych do uzyskania z ejakulatu w zależności od upływu czasu między kolejnymi pobraniami ejakulatu

The presented data justify the conclusion that the interval between the successive collections affects the physical parameters of boar ejaculates. The reduction of the interval between ejaculate collections leads to a systematic decrease in ejaculate volume, sperm concentration and total ejaculate sperm count, as well as, consequently, to a decrease in the number of insemination doses possible to be prepared from an ejaculate. Data indicating the influence of insemination use intensity on ejaculate characteristics have also been obtained in other studies. Magistrini et al. (1987) proved the relationship between ejaculate characteristics and the intensity of insemination use of stallions. Ritar et al. (1992) documented the existence of such an interrelationship in their study of goats. There are also studies available that show the dependence of boar ejaculate parameters on the frequency of insemination use. According to Bonet et al. (1991) and Strzeżek et al. (1995), greater frequency of ejaculate collection from boars reduces the concentration and total sperm count in the ejaculates, as well as lowering sperm motility and increasing the percentage of spermatozoa with morphological defects. A study of Large White boars has revealed that ejaculates collected at four-day intervals are characterized

with larger volumes, and greater numbers and higher motility of spermatozoa than ejaculates obtained at one-day intervals (Umesiobi, 2010).

According to Frangež et al. (2005) boar ejaculates collected once or twice a week have the greatest volumes, whereas those obtained on an everyday basis exhibit considerably reduced volumes. The same as the present work, the above study has revealed that day-to-day ejaculate collection leads to a decrease in sperm concentration in boar ejaculates. The data juxtaposed in Table 1 show that increasing the frequency of ejaculate collection from a seven-day to a four-day or even three-day interval only slightly changes sperm concentration in ejaculates. However, increasing ejaculate collection frequency to a two-day interval results in a fall in sperm concentration in the ejaculates, by approximately 100 x 10^{3}·mm^{-3}. When collected every day, the sperm concentration fell to the level of barely 175 x 10^{3}·mm^{-3} i.e. approximately 200-300 x 10^{3}·mm^{-3} fewer than in the ejaculates collected at an interval of 3-7 days. It seems then that sperm concentration is relatively insusceptible to increasing the intensity of the insemination use of boars. However with a very high frequency of ejaculate collection (every one or two days), there follows a drastic fall in sperm concentration in the produced ejaculates. This is not only significant in the context of insemination dose production. It has been found out that ejaculate sperm concentration is connected with morphological traits of spermatozoa (Kondracki et al., 2011, 2013a; Rijsselaere et al., 2004) and this in turn affects the fertilization potential of the spermatozoa (Chenoweth, 2005; de Paz et al., 2011; Hirai et al., 2001; Ostermeier et al., 2001). Effects of high collection frequency on the physical characteristics of ejaculates have also been observed by Pruneda et al. (2005). Their study has revealed a fall in ejaculate volumes and sperm concentrations and counts in ejaculates of Pietrain boars which ejaculated twice a day in comparison with ejaculates collected at one-day intervals between the successive ejaculations. This frequency also affected sperm motility which amounted to 20% in the ejaculates of the intensively exploited boars, whereas the percentage value exceeded 80% in the case of the other group.

Sperm motility in ejaculates is considered to be one of the most important ejaculate quality parameters (Kommisrud et al., 2002) that affects the fertilization potential of semen. The study has revealed the percentage of motile spermatozoa to range from 75.00% at five-day intervals between the successive ejaculate collections to 67.78% at one-day intervals. The data in Table 1 show that increasing the ejaculate collection frequency reduces the percentage of spermatozoa with progressive motility. Umesiobi (2010) observed ejaculates collected every four days to be characterized with greater sperm motility than ejaculates collected at one-day intervals. Bonet et al. (1991) have found that prolonged and very frequent collection of ejaculates (every two days over the period of 12 months) leads to a decrease in the percentage of progressively motile spermatozoa in the ejaculates. Similar conclusions have been arrived at by Strzeżek et al. (1995) who found that collecting ejaculates from boars on an everyday basis over a period of 10 days led to a reduction in sperm motility to a level of 54-58%. The results of the present work show that frequent ejaculate collection, even over a shorter period of time than in the case of Bonet et al. (1991) and Strzeżek et al. (1995) studies, also leads to a considerable decrease in sperm motility. Different results were obtained by Huang and Johnson (1996) who found that, even over the period of three weeks, day-to-day semen collection from

Landrace and Large White hybrids made it possible to obtain ejaculates with good sperm motility, at the level of 72%.

For the purposes of insemination practice, the most important ejaculate parameter is the number of spermatozoa in ejaculates that exhibit progressive motion, since it determines the number of insemination portions that can be obtained from an ejaculate (Kondracki et al., 2014). Table 1 shows the greatest numbers of spermatozoa in the ejaculates collected at six-day intervals, with relatively numerous spermatozoa also observed in the ejaculates collected at seven- and five- day time intervals. Increasing the ejaculate collection frequency to every four days resulted in a decrease in the total sperm count in the ejaculates. This entails a corresponding decrease in the number of insemination doses obtained from the ejaculates, which, as it results from the data presented in Figure 1, clearly tends to progress along with the shortening interval between successive ejaculate collections. In a study by Miclea et al. (2007) ejaculate collections at seven-day intervals produced 23 insemination doses per ejaculate, whereas ejaculates collected every three days provided barely 18 insemination doses.

The data presented in this work are confirmed by the results of studies conducted on Pietrain boars (Pruneda et al., 2005) which have revealed that increasing the frequency of ejaculate collection leads to a fall in the number of spermatozoa in the ejaculates. According to Frangež et al. (2005), this can result from a depletion of sperm reserves stored in the epididymides and lead to lower numbers of piglets in litters. This hypothesis is supported by the results of Umesiobi (2010), who proved that sows inseminated with sperm collected every 96 hours give birth to 30% more piglets than sows fertilized with semen collected every 24 hours. The deterioration in fertilization results can not only stem from quantitative changes in the produced ejaculates but also from a worse quality of produced spermatozoa, as a result of incorrect sperm maturation processes (Bonet et al., 1992). Disorders in the processes of sperm maturation can be caused by a shorter sojourn of spermatozoa in the epididymal ducts (Pruneda et al., 2005), as a consequence of frequent ejaculation (Bonet et al., 1992).

Conclusions

The physical characteristics of ejaculates depend on the interval between successive ejaculate collections. The effect of insemination use intensity is however varied. In the case of ejaculate volume, rising frequency of ejaculate collection leads to a systematic and fairly even fall in ejaculate volume. Ejaculate sperm concentration remains at a relatively high level (more than 360 x $10^{3} \cdot mm^{-3}$) when ejaculates are collected with a frequency of 3 to 7 days but further shortening of the interval between the successive collections leads to a drastic decrease in sperm concentration in the ejaculates. Ejaculates containing very high numbers of spermatozoa (over 90 billion) are obtained with an ejaculation frequency of not fewer than four days. An increase in ejaculate collection frequency to every four and fewer days results in a significant decrease in the number of spermatozoa present in the produced ejaculates and a concomitant decrease in the number of insemination doses prepared from these ejaculates, with an escalation of such changes. Considering the practical use of the results of the presented analyses in boar

insemination, ejaculate collection every 4 or 5 days should be recommended as the optimal frequency. Ejaculates collected with a frequency of 1 and 2 days have significantly lower quantitative indices.

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