Scientific Annals of Polish Society of Animal Production - Vol. 14 (2018), No 2, 21-34

Dependence of physical characteristics of ejaculate of Polish Landrace boars on the time interval between successive ejaculate collections

Stanisław Kondracki#, Maria Iwanina, Dariusz Kowalewski, Magdalena Bajena, Anna Wysokińska

Siedlce University of Natural Sciences and Humanities,
Faculty of Natural Sciences, Department of Animal Reproduction and Hygiene,
ul. B. Prusa 14, 08-110 Siedlce; #e-mail: sk@uph.edu.pl

The aim of the research was to determine the dependence of physical properties of boar ejaculates on the time interval between successive ejaculate collections. The material for the study comprised 1954 ejaculates collected manually from boars used for artificial insemination. For all ejaculates, the ejaculate volume, sperm concentration, percentage of motile spermatozoa, and number of spermatozoa in the ejaculate were determined, and the number of insemination doses per ejaculate was calculated. The boars' suitability for artificial insemination was evaluated taking into account the effect of the time passed between ejaculate collections. The ejaculate volume and the concentration and number of sperm in the ejaculate were shown to increase with the time elapsed since the previous ejaculate. The number of insemination doses obtained per ejaculate increased as well. Ejaculates with a high sperm count were obtained when the ejaculation frequency was no more than every 4 days. The percentage of spermatozoa with progressive motility increased with the time elapsed since the collection of the previous ejaculate, but only up to 4 days. Further decreases in ejaculation frequency no longer resulted in increased sperm motility.

KEY WORDS: boar / ejaculate / physical characteristics of ejaculate / time passed between collections

A boar's suitability for artificial insemination is determined by its individual predispositions [15]. Genetic and environmental factors also affect the reproductive performance of the boar [5, 8, 12, 27, 28]. Organizational measures can be taken to improve insemination efficiency in the male. Such measures include changing the frequency of ejaculate collection [2]. Ejaculate collection three times every two weeks, or every 4-5 days, ensures optimal performance in the breeder. However, this cannot be applied to all boars. The individual ejaculation performance of each boar should be taken into account, as impairment

of the spermatogenic and secretory capacity of both the testes and the accessory glands may cause serious functional changes in the tissues of these organs and thus adversely affect the physical characteristics of the ejaculates. Too infrequent use of breeders may also result in deterioration in the quality of sperm, which remain for too long in the tail of the epididymis [7]. In practice, the frequency of semen collection is not always optimal and is subject to large fluctuations [1, 18]. The main reason for the variability in the intensity of use of boars for artificial insemination is changes in the demand for semen. An increase in the demand for boar semen of a particular breed or crossbreed results in an increase in the use of these boars, while a decrease in demand leads to a reduction in the frequency of semen collection. By increasing the frequency of ejaculation, we obtain more ejaculates per unit of time. However, this may affect the quantitative characteristics of the ejaculate, as well as the quality and the fertilization capacity of the sperm.

The aim of this study was to determine the dependence of the physical properties of boar ejaculates on the time between successive ejaculate collections.

Material and methods

The study was carried out at an artificial insemination station belonging to the company Mazowieckie Centrum Hodowli i Rozrodu Zwierząt Sp. z o.o. in Łowicz, on 50 Polish Landrace boars used for artificial insemination. The assessment included all ejaculates collected from each boar for 12 consecutive months. All boars were housed on litter in individual pens with an area of 6.3 m^2, on a concrete floor with heat and moisture insulation. Mechanical, electronically controlled ventilation was used in the pig house. Boars were fed an individually pelleted complete feed, standardized according to boar feeding standards. The feed was given twice a day, at 6:30 a.m. and at 1:00 p.m. The boars had constant access to drinking water from nipple drinkers.

The material for analysis comprised 1954 ejaculates collected manually from the boars [11]. A standard evaluation was performed on all ejaculates to determine the following physical characteristics:

– ejaculate volume
– sperm concentration
– percentage of sperm with progressive motility
– total sperm count in ejaculate
– number of insemination doses per ejaculate

The ejaculate volume was determined after filtering out the gelatinous fraction. Sperm concentration in the ejaculate was determined by the photometric method using a SPERMA CUE electronic photometer. The method involves measuring the intensity of light passing through a microcuvette containing an undiluted ejaculate sample. The length of the light path was 0.7 mm. Sperm motility was determined by microscopic examination. A drop of fresh semen was applied to a heated microscope slide and then covered with a coverslip, also heated to about 37°C. The percentage of spermatozoa displaying normal

motility in the total number of sperm in the field of view of the microscope was determined at 200x magnification. The total sperm count in the ejaculate, the number of insemination doses obtained per ejaculate, and the number of sperm in the insemination dose were calculated using SYSTEM SUL software version 6.1.

The suitability of the ejaculates for insemination was evaluated taking into account the effect of the time passed between successive ejaculations. Ejaculates were divided into seven subgroups depending on the interval since the previous ejaculate collection:

- group I – ejaculates collected 1 day after the previous collection
- group II – ejaculates collected 2 days after the previous collection
- group III – ejaculates collected 3 days after the previous collection
- group IV – ejaculates collected 4 days after the previous collection
- group V – ejaculates collected 5 days after the previous collection
- group VI – ejaculates collected 6 days after the previous collection
- group VII – ejaculates collected 7 or more days after the previous collection

Statistical analysis of the results was performed according to the following mathematical model:

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

where:

Y_{ij} – value of physical trait of ejaculate

μ – mean for population

a_i – effect of time passed since previous ejaculate collection

e_{ij} – error

Significance of differences between groups was determined by the Tukey test.

Results and discussion

Data characterizing the physical characteristics of the ejaculate in relation to the time elapsed since the previous ejaculate was collected are presented in the table. The data show that the ejaculate volume depends on the time passed since the previous ejaculate collection. The volume of the ejaculates increases with the time interval between successive ejaculates. The smallest ejaculate volume (213.95 ml) was found in the case of a one-day interval between successive collections. The ejaculate volume obtained one day after collection of the previous ejaculate was 19.97 ml lower than the volume obtained three days after collection of the previous ejaculate ($P \leq 0.05$) and at least 25.40 ml lower than the volume obtained two days or four days and more after collection of the previous ejaculate. These differences proved to be significant ($P \leq 0.05$).

The sperm concentration in ejaculates collected one and two days after collection of the previous ejaculate was lower than in ejaculates obtained three days or more after collection of the previous ejaculate ($P \leq 0.05$). As the time between successive ejaculate collections

increased, there was a gradual, systematic increase in sperm concentration. The highest sperm concentration was found in ejaculates obtained at intervals of seven days or more (421,830/mm^3).

The data in the table show that the percentage of spermatozoa showing progressive motility was lowest in ejaculates obtained at a one-day interval (70.71%). The highest percentage of sperm with progressive motility was found in ejaculates obtained four, five and six days after the previous ejaculate was collected ($P \leq 0.05$). A decrease in the frequency of ejaculate collection from one to four days was accompanied by a gradual increase in sperm motility. A further increase in the time between successive ejaculations (up to five days or more) did not result in a further increase in sperm motility.

As the time between successive ejaculations increased, a gradual, marked increase was noted in the total sperm count in the ejaculate. In ejaculates collected one day after collecting the previous ejaculate, the total sperm count was 45.55 billion and was at least 6.50 billion lower than in ejaculates obtained two days or more after the previous ejaculate ($P \leq 0.05$). The influence of the time since the previous ejaculate collection on the sperm count in the ejaculate was very pronounced. Each one-day increase in the interval between successive collections resulted in a significant increase in the total sperm count in the ejaculate ($P \leq 0.05$). Ejaculates obtained at seven-day and longer intervals contained the most sperm (73.83 billion). The sperm content in these ejaculates was at least 5.12 billion higher than in ejaculates obtained four or five days after the previous ejaculate was collected ($P \leq 0.05$).

The number of insemination doses that can be obtained from a single ejaculate depends to a large degree on the time passed since the previous ejaculate was collected (Table). Only 17.54 insemination doses were obtained from ejaculates collected one day after the previous ejaculate was collected, whereas 9.69 more insemination doses (27.23 doses; $P \leq 0.05$) were obtained from ejaculates collected seven days or more after the previous ejaculate was collected. As the time between successive ejaculates increased, there was a systematic and statistically confirmed increase in the number of insemination doses obtained per ejaculate.

The quality of ejaculates is determined by numerous genetic and environmental factors, including the frequency with which the boar is used [7]. The present study demonstrated that reducing the time since the previous ejaculate collection resulted in a decrease in ejaculate volume and in the sperm concentration and total sperm count in the ejaculate, and thus also in the number of insemination doses obtained from the ejaculate. Bajena et al. [2] showed that the largest ejaculate volume is obtained when a seven-day interval between ejaculate collections is maintained. Increasing the frequency from seven to four days results in a gradual reduction in volume. The data obtained in the present study show that the smallest ejaculate volume (213.95 ml) is obtained one day after the previous collection, and the volume increases with the length of the interval between successive ejaculates. Similar conclusions are suggested by results reported by Janicki et al. [10] in a study on boars

Table
Dependence of physical characteristics of ejaculate on the time elapsed between successive collections

Trait		Time elapsed since previous ejaculate collection						
		1 day	2 days	3 days	4 days	5 days	6 days	≥7 days
Number of ejaculates		113	120	551	608	142	180	240
Ejaculate volume (ml)	$\bar{x}$	213.95^{a}	239.35^{bc}	233.92^{c}	241.19^{bc}	240.37^{bc}	244.52^{bc}	254.64^{b}
	Sd	72.35	73.54	79.85	81.35	82.70	80.77	93.83
Sperm concentration (1000/mm^3)	$\bar{x}$	315.93^{a}	318.42^{a}	366.28^{b}	386.35^{bc}	404.93^{cd}	414.78^{d}	421.83^{d}
	Sd	80.35	74.95	89.10	88.60	84.28	94.40	105.40
% of sperm with progressive motility	$\bar{x}$	70.71^{a}	70.75^{a}	71.52^{ab}	71.91^{b}	71.69^{cb}	71.89^{cb}	71.46^{ab}
	Sd	2.58	2.64	3.60	3.93	3.76	3.93	3.54
Sperm count in ejaculate (billions)	$\bar{x}$	45.55^{a}	52.05^{b}	57.96^{c}	63.94^{d}	68.71^{e}	70.26^{ef}	73.83^{f}
	Sd	14.76	15.31	16.06	17.87	20.57	19.93	25.29
Number of insemination doses per ejaculate	$\bar{x}$	17.54^{a}	21.18^{b}	22.91^{c}	24.77^{d}	25.81^{de}	27.01^{e}	27.23^{e}
	Sd	6.35	6.52	6.66	7.11	8.16	7.85	9.09

Means in rows designated with different letters differ significantly at P≤0.05

and by Yotov et al. [26] in research on rams. These studies found that as the frequency of ejaculate collection increased, the ejaculate volume and sperm concentration decreased. However, it is worth noting the dynamics of the changes in ejaculate volume as the time between successive ejaculate collections increases. The data from the present study clearly show that the increase in ejaculate volume is greatest when the time interval is increased from one to two days (Fig. 1).

Data obtained by Pokrywka et al. [18] on boars of the Polish Landrace and Polish Large White breeds show that ejaculates obtained one day after the previous collection have the smallest volume, while ejaculates have the greatest volume when obtained at three-day intervals in the case of the Polish Large White breed and at four- and five-day intervals in the case of Polish Landrace. Swierstra and Dyck [23] reported that boar ejaculates obtained at 24-hour intervals have lower volume, sperm concentration and sperm count than ejaculates obtained at 72-hour intervals. According to Frangez et al. [7], boar ejaculates collected once or twice a week have the largest volume, while daily ejaculate collection reduces their volume. Pruneda et al. [19] showed that increasing the frequency of ejaculate collection to twice a day reduces the ejaculate volume, sperm concentration and sperm count in the ejaculate compared to ejaculates collected at one-day intervals.

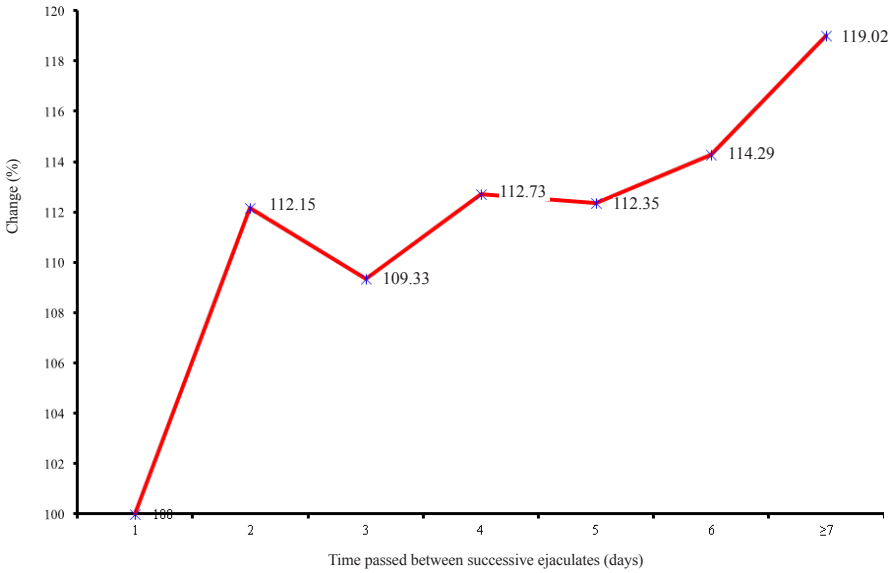

Fig. 1. Change in ejaculate volume depending on the time passed between successive ejaculates (volume of ejaculates collected after 1 day = 100)

The data obtained in this study show that the sperm concentration in the ejaculate increases with the time between successive collections. In the case of a one-day interval, the sperm concentration in the ejaculate was 315,930/mm^3. As the time between collections increased, there was a systematic increase in the sperm concentration in the ejaculate. At intervals of seven days and longer, the sperm concentration was 421,830/mm^3 and was significantly higher than in ejaculates collected at intervals of four days or less. The changes in sperm concentration depending on the interval between successive ejaculate collections are shown in Figure 2.

Frangez et al. [7] showed that daily ejaculate collection leads to a reduction in the sperm concentration in boar ejaculates. According to Strzeżek et al. [21] and Bonet et al. [3], increased frequency of ejaculate collection leads to a reduction in the sperm concentration and total sperm count in boar ejaculates, reduces sperm motility, and increases the percentage of sperm with morphological defects. Somewhat different relationships between the physical characteristics of ejaculates and frequency of ejaculation were reported by Tereszkiewicz et al. [24], who obtained ejaculates from Polish Landrace boars with a frequency of three times to over seven times a month. The data indicate that increased intensity of reproductive use leads to a significant reduction in sperm concentration in the ejaculate

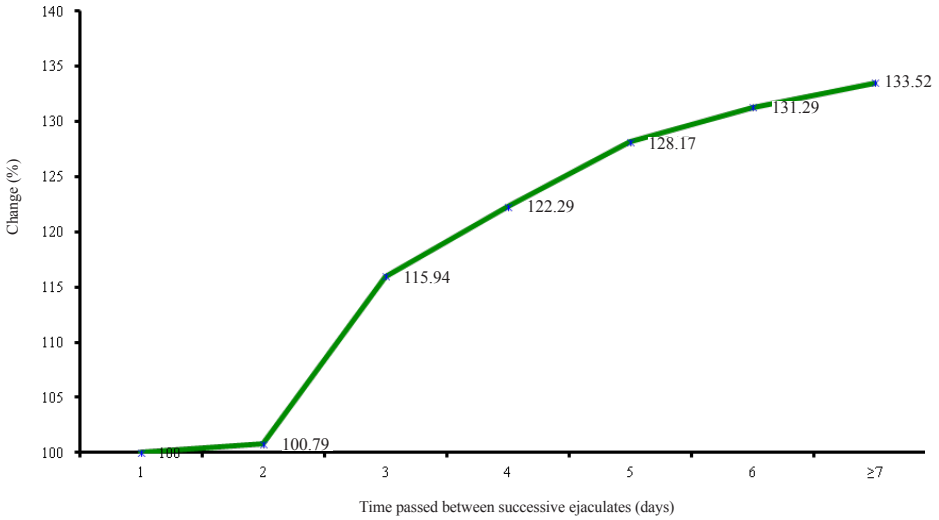

Fig. 2. Change in sperm concentration depending on the time passed between successive ejaculates (sperm concentration in ejaculates collected after 1 day = 100)

accompanied by a minor increase in ejaculate volume and the percentage of spermatozoa with progressive motility. According to Cameron [6], both the ejaculate volume and sperm concentration in the ejaculate are susceptible to changes associated with ejaculation frequency.

The motility of spermatozoa is one of the most important features of ejaculate quality [13], because it influences the fertilization capacity of the semen [5]. Data from our study show that ejaculates collected at one-day intervals had the lowest percentage of spermatozoa with progressive motility (70.71%). The most sperm with progressive motility were observed in the ejaculates obtained at four-, five- and six-day intervals, but increasing the interval to more than four days did not result in any further increase in sperm motility. The changes in the percentage of spermatozoa showing progressive movement depending on the interval between ejaculates are shown in Figure 3.

Milewska [17] showed the highest percentage of motile sperm in ejaculates collected at four-day intervals (71.16%), and the lowest in those collected at 11-day intervals (70.33%). According to Umesiobi [25], the percentage of motile sperm in ejaculates collected every four days is higher than in those collected at one-day intervals. Bonet et al. [3] found that long-term ejaculate collection at a high frequency (every other day for 12 months) reduces the percentage of spermatozoa with progressive motility. Even

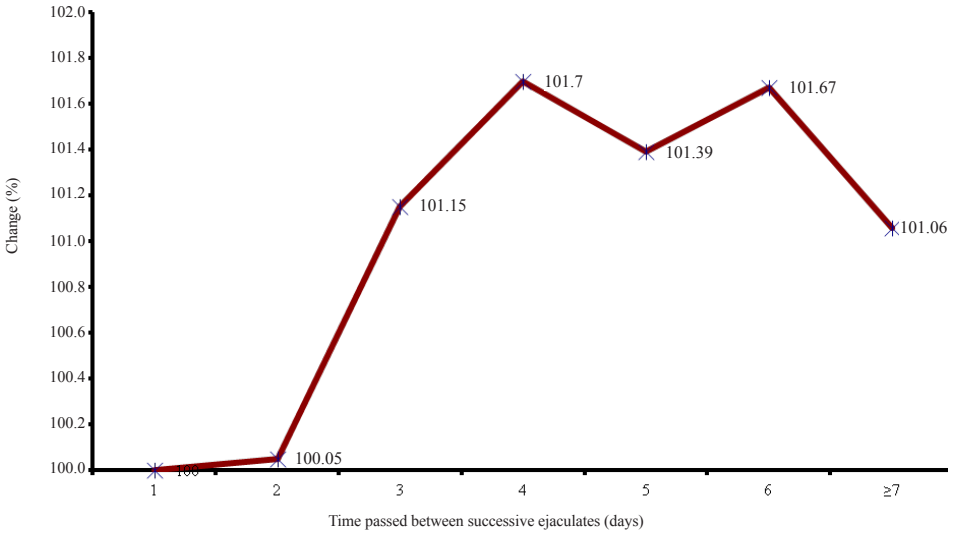

Fig. 3. Change in percentage of spermatozoa with progressive motility depending on the time passed between successive ejaculates (percentage of spermatozoa with progressive motility in ejaculates collected after 1 day = 100)

short-term (10-day) daily collection causes a decrease in the motility of boar sperm to 54-58% [21]. The results of our study indicate that frequent ejaculate collection, even for a shorter time than in the research by Bonet et al. [3] and Strzeżka et al. [21], results in decreased sperm motility.

Huang and Johnson [9] present a different opinion. They believe that ejaculates with sperm showing good motility, at a level of 72%, can be obtained by collecting boar semen daily, even for a period of three weeks. Pokrywka et al. [18] found the smallest percentage of motile sperm in ejaculates collected at intervals of more than seven days, and the highest in those collected at one-day intervals. Pruneda et al. [19], who examined ejaculates collected from Pietrain boars once or twice a day showed a marked decrease in sperm motility (to 20%) when ejaculates were collected twice a day, while in the case of collection once a day, sperm motility remained high (over 80%). The data show that the effect of the frequency of ejaculate collection on sperm motility is not straightforward. Long breaks in acquiring ejaculates (over seven days) seem to have a negative effect on sperm motility [17, 18], but long-term ejaculate collection at short intervals also reduces sperm motility [3, 19, 21]. The data obtained in our study indicate that lengthening the interval between

ejaculate collections slightly improves sperm motility, but only up to a four-day interval. Further increasing the intervals between successive ejaculations no longer results in increased sperm motility.

The number of spermatozoa in the ejaculate is of great utilitarian importance. The number of insemination portions made from one ejaculate depends on the sperm count [14]. The data obtained in this study show that the sperm count in the ejaculate increases with the time passed since the previous ejaculate collection. Ejaculates collected one day after the previous collection contained an average of 45.55 billion sperm, while those collected after an interval of seven days or more contained an average of 73.83 billion sperm. Thus, extending the time interval between successive ejaculates by one day increases the number of sperm in the ejaculate by about 4.7 billion. It is worth noting that the increase in the sperm count in the ejaculate is relatively constant over the range of intervals between successive ejaculations (from one to seven days) (Figure 4).

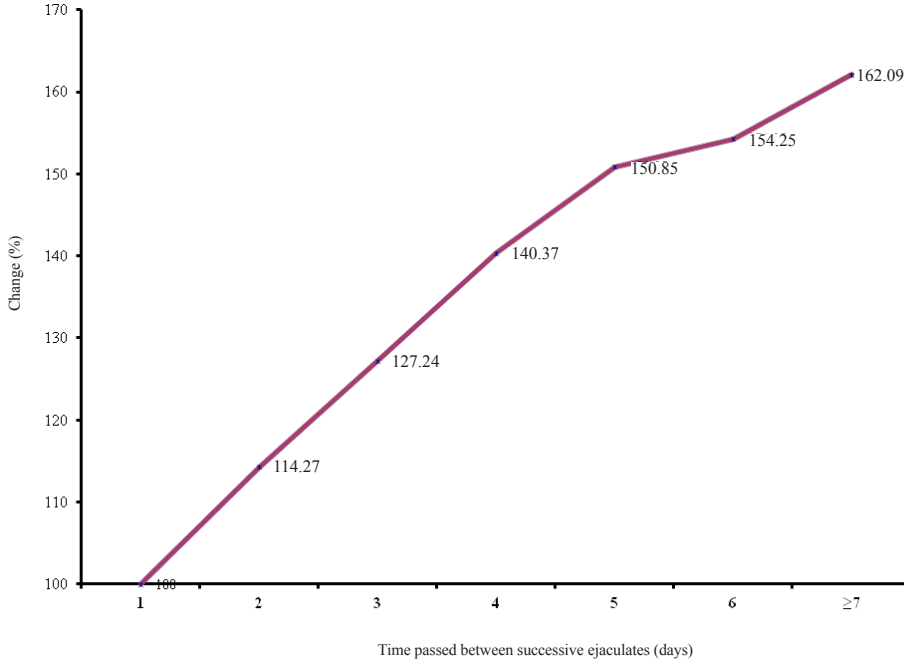

Fig. 4. Change in total sperm count in the ejaculate depending on the time passed between successive ejaculates (sperm count in in ejaculates collected after 1 day = 100)

The data presented show that the time passed since the previous ejaculate collection affects the sperm count in the ejaculate significantly and in a clear manner. Data reported by Suriyasomboon et al. [22] also indicate the influence of the time interval between ejaculates on the ejaculate sperm count. According to Rutten et al. [20], acquiring ejaculates at a frequency of three times a week increases the sperm count in the ejaculate as compared to ejaculates collected more frequently. The observations made in the present study are supported by research on Pietrain boars [19], which found that increasing the frequency of ejaculate collection leads to a decrease in the ejaculate sperm count. According to Frangez et al. [7], this may be due to depletion of the sperm stored in the epididymis, which may result in a reduced number of piglets in the litter. Umesiobi [25] showed that sows inseminated with semen collected every 96 hours give birth to 30% more piglets than sows fertilized with semen collected every 24 hours. Deterioration of fertility rates is associated with quantitative and qualitative changes in ejaculates resulting from abnormalities during sperm maturation [4]. These abnormalities may be due to a reduction in the time sperm spend time in the epididymal duct [19] due to frequent ejaculation [4]. When ejaculates are collected seven times a week, there is a significant decrease in total sperm count in the ejaculate, as well as in the boar's libido [7]. The ejaculate volume, sperm concentration and total sperm count in the ejaculate are lower as well. The percentage of spermatozoa with progressive motility is also lower, and the frequency of sperm with morphological defects increases [21]. A study on Polish Landrace and Polish Large White boars showed the highest sperm count in ejaculates collected 6-7 days after the previous collection [18]. According to Huang and Johnson [9], ejaculates obtained once a week contain the most sperm (16.6 billion), ejaculates collected three times a week contain 10 billion fewer (6.1 billion), and those collected daily contain the fewest (4.3 billion).

The present study showed that as the interval between ejaculate collections increased, the ejaculate volume, sperm concentration, sperm motility, and total sperm count in the ejaculate increased. The number of insemination doses per ejaculate was also found to significantly depend on the time since the previous ejaculate collection. With a one-day interval between consecutive collections, only 17.54 insemination doses were obtained per ejaculate, as compared to 27.23 insemination doses in the case of a seven-day interval or longer. The changes in the number of insemination doses obtained per ejaculate in relation to the time passed since the previous ejaculate collection are shown in Figure 5.

The present study showed the highest sperm counts in ejaculates collected at seven-day intervals. Increasing the frequency of ejaculate collection to every four days or fewer resulted in a reduction in the total sperm count in the ejaculate. This entails a corresponding reduction in the number of insemination doses obtained from the ejaculate, which – as seen in Figure 5 – shows a clear progression as the interval between successive ejaculates

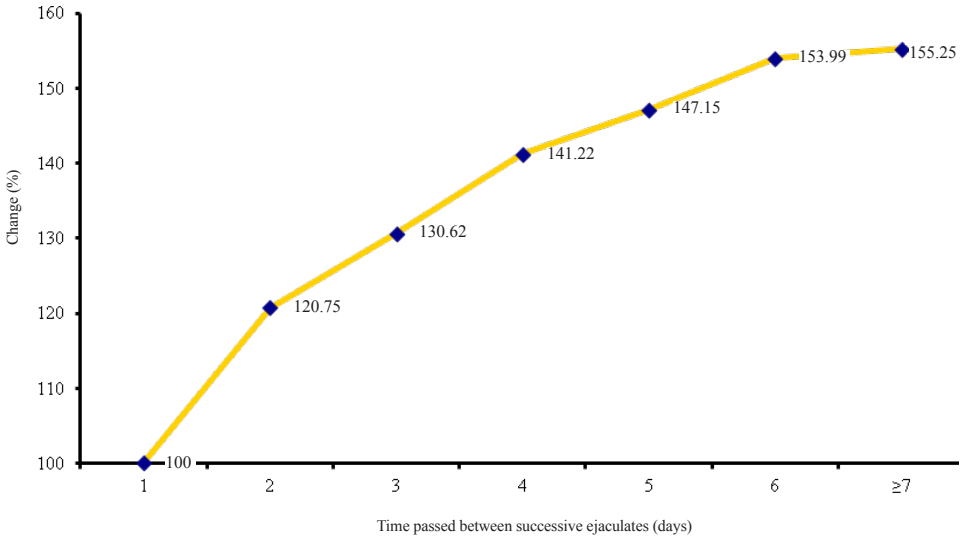

Fig. 5. Change in number of insemination doses per ejaculate depending on the time passed between successive ejaculates (number of insemination doses from ejaculates collected after 1 day = 100)

is reduced. According to Adamiak et al. [1], the increase in the ejaculate sperm count and in the number of insemination doses obtained per ejaculate is greatest when the collection interval is increased to four, five, six, seven, and eight or more days. Similar results were obtained by Miclea et al. [16], who showed that at a seven-day interval between ejaculate collections, 23 insemination doses were obtained per ejaculate, as compared to only 18 insemination doses from ejaculates collected every three days.

According to Milewska [17], the physical characteristics of boar ejaculates show a correlation with the frequency of their collection. This factor determines differences in semen quality, but its impact is not always the same. As the intervals between collections are reduced, most of the ejaculate characteristics show fluctuations, which makes it difficult to establish correlations. Nevertheless, the author states that the ejaculate volume and the total sperm count may be lower in ejaculates obtained more often than every five days, or less frequently than every 10 days.

In conclusion, ejaculate characteristics depend on the frequency of ejaculate collection. As the time passed since the previous ejaculate increases, the ejaculate volume, sperm concentration and total sperm count in the ejaculate increase. The number of insemination

doses obtained from the ejaculate increases as well. Ejaculates with a high sperm count are obtained when ejaculation is no more frequent than every four days. The frequency of ejaculate collection has a much smaller influence on sperm motility. As the time between ejaculate collection is increased, the percentage of sperm with progressive motility increases, but only up to four days; further reduction of ejaculation frequency does not further increase sperm motility. For artificial insemination practice, ejaculates can optimally be collected at four-day intervals.

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