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EFFECT OF SEMEN COLLECTION FREQUENCY ON THE PROGRESS IN THE MOTILITY OF RABBIT SPERMATOZOA

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ABSTRACT

In the present study the effect of collection regime on rabbit semen development was analyzed. Eight seminal traits, such as overall ejaculate volume (VOL), spermatozoa concentration (CON), motility (MOT), progressive motility (PRO), percentage of progressive motility (% PRO), curvilinear velocity (VCL), beat-cross frequency (BCF) and amplitude of lateral head displacement (ALH) were analyzed. As the number of collections increased, more bucks were able to ejaculate. During collections the semen volume gradually increased from 0.4 ml to 1.0 ml with the average of 0.68 ± 0.34 ml. The average of MOT was 55.55 ± 26.63 % and the average of PRO reached 40.43 ± 27.35 %. The other assessed parameters were CON - 0.547 ± 0.165 x 10^9 ml^{-1}; ALH - 4.18 ± 1.18 µm; BCF - 27.42 ± 7.12 Hz; VCL - 104.04 ± 37.00 $µm.s^{-1}$. A positive correlation between ejaculate volume and the number of collections was found. MOT and PRO significantly correlated with the number of collections. In addition, positive correlation between % PRO and the number of collections was recorded. No correlation between the number of collections and VCL was detected. In the case of ALH, a moderate positive correlation between this parameter and the number of collections was observed. BCF slightly correlated with the number of collections. The results of present study indicate on possibility to increase the values of some seminal parameters, which opens new opportunities for raising the quality of semen directly *in vivo*.

Key words: rabbit; spermatozoa; motility; semen collection frequency; CASA; fertility

INTRODUCTION

In the past ten years, the development of biological sciences has been intensively connected to expansion of technologies, aimed at recognition of principles and control of biological processes of animal reproduction. One of the most important of them is assisted reproduction and artificial insemination. Artificial insemination has probably been the greatest technological advance in animal breeding and the main reasons for its success have been genetic gain, disease control, and the cost-effectiveness of insemination compared to natural mating (Laurinčík *et al.*, 2008). It has also been the most noteworthy example of the successful integration of both research and widespread application (Vishwanath, 2003). Moreover, biotechnological methods include *in vivo* and *in vitro* experiments, as well. In the *in vivo* experiments living animals are used. Conversely, *in vitro* cultivations are explicitly aimed at the work with cells out of organism in such conditions imitating the organism environment (Laurinčík *et al.*, 2008).

The commercial success of artificial insemination of any species depends on the extensive use of genetically superior males to impregnate a large group of females with relatively low doses of spermatozoa per insemination. This requires high semen quality which can be assessed by several parameters (Farrell *et al.*, 1993; Castellini *et al.*, 1996; Castellini *et al.*, 2000; Brun

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et al., 2002; Ducci *et al*., 2002).

In order to predict fertility before insemination, different *in vitro* tests have been developed to determine spermatozoa quality. Computer-assisted semen analysis (CASA) provides a repeatable estimate of many spermatozoa movement criteria and allows determination of specific motion characteristics of spermatozoa. This objective semen assessment technique could be used to evaluate differences between bucks and attempt to predict the *in vivo* performance of semen. Among semen parameters, spermatozoa motility is believed to be the most important characteristics correlated to male fertility (Bostofte *et al*., 1990; Eimers *et al.*, 1994) because of its importance for spermatozoa migration through the female genital tract and for gamete interaction at fertilization (Robayo *et al*., 2008). Many authors observed, that some other CASA motility parameters, or a combination of more ejaculate traits (motility parameters, semen concentration, seminal plasma compounds, spermatozoa morphological parameters) often highly correlated with success of fertilization itself (Ellington *et al*., 1993; Farrell *et al*., 1998; Wood *et al*., 1986; Shibahara *et al*., 2004; Freour *et al.*, 2009; Fréour *et al*., 2010; Hirano *et al*., 2001; Macleod *et al*., 1995; Marshburn *et al*., 1992; Siduhu *et al*., 1997).

The aim of this study was to analyze the progress in spermatozoa motility in male rabbits in relation to the frequency of semen collection.

MATERIAL AND METHODS

As a biological material, the semen (ejaculates) of 34 sexually mature and healthy rabbits (4 - 5 months) of the HYLA breed were used. The males were housed in individual cages, under a photoperiod of 10 - 15 h of daylight. All animals were fed *ad libitum* with a commercial diet and water was provided *ad libitum* with nipple drinkers. The ejaculates were collected 18 times for 11 weeks using artificial vagina (Massányi *et al.*, 2008) from February to May. Each ejaculate was analyzed using the CASA technique with SpermVision 3.0 software using the Makler Countig Chamber (Sefi–Medical Instruments, Germany).

In total, the following sperm characteristics were assessed: motility (MOT), progressive motility (PRO), percentage of progressive motility (% PRO), beat cross frequency (BCF), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH) and spermatozoa concentration (CON). The average ejaculate volume (VOL) for every collection was evaluated, as well. The average value of each of these parameters was computed for every collection individually.

The percentage of PRO of MOT was computed as % PRO = (PRO/MOT) x 100 % for every collection. The correlation between MOT, PRO, % PRO, VOL, CON, VCL, ALH, BCF and the number of collections was assessed by a Spearman correlation coefficient (r_S).

Table 1: Percentage of collected ejaculates in relation to the number of collections

Collection	Number of rabbits	Collected	Percentage
1.	35	18	51 %
2.	35	28	80 %
3.	35	24	69 %
4.	35	32	91 %
5.	35	31	89 %
6.	35	29	83 %
7.	35	27	77 %
8.	35	32	91 %
9.	35	31	89 %
10.	35	32	91 %
11.	35	33	94 %
12.	35	33	94 %
13.	35	33	94 %
14.	35	34	97 %
15.	35	34	97 %
16.	35	34	97 %
17.	35	34	97 %
18.	34	33	97 %

Furthermore, correlation between % PRO, MOT, PRO and VOL was determined through a Pearson correlation coefficient (r_P). All the statistical tests were carried out with the SigmaPlot version 12.0 at the level of significance α = 0.05.

RESULTS

Before every semen analysis, we attempted to collect the ejaculate from all 35 males. In the first collection, only 18 bucks (51 %) ejaculated. By further attempts more bucks were able to ejaculate and in the last collection 34 ejaculates were obtained (97 %). Detailed information is provided in Table 1.

A gradual increase in semen volume during performed collections was recorded. While in the beginning of the process the volume was equal 0.40 ± 0.25 ml, in the last collection the value reached 1.00 ± 0.30 ml (Fig. 1). Overall, the average volume was 0.68 ± 0.64 ml. The correlation between the ejaculate volume and the number of collections (r_S = 0.964) was statistically significant, and the dependence of ejaculate volume on the number of ejaculate collections was confirmed.

By assessing the spermatozoa movement, we detected a moderate increase in motility (MOT) from 74 % to 84 % and also a moderate increase in progressive motility (PRO), which started at 54 % and culminated at 72 %. The average MOT was 55.55 ± 26.63 % and the average PRO reached 40.43 ± 27.35 %. In the case of % PRO, a similar progress in its development was noted. Therefore, a significant positive correlation between MOT (r_S = 0.581), PRO (r_S = 0.651), % PRO and the number of collections was observed (Fig. 2).

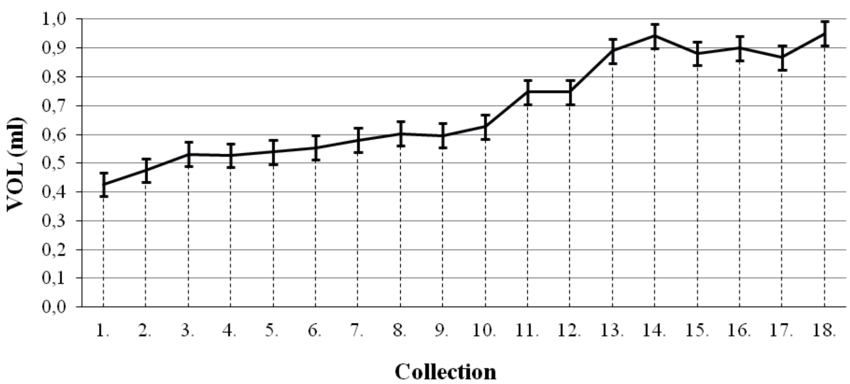

Fig. 1: Ejaculate volume dependence on the number of collections

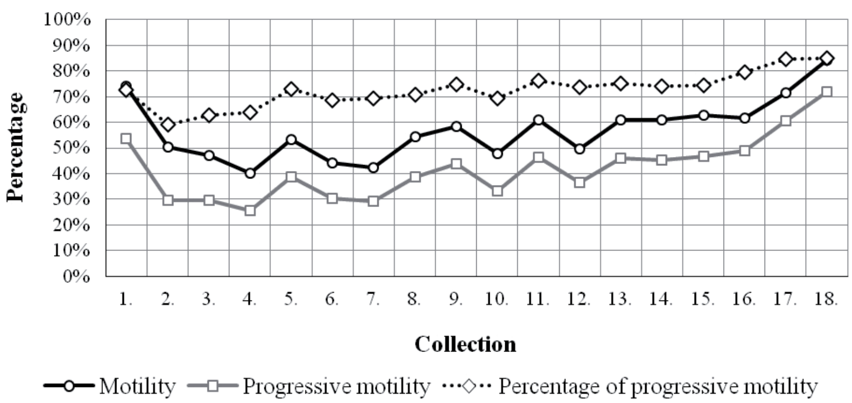

Fig. 2: Development of MOT, PRO and % PRO with regard to the number of collections

Likewise, a positive correlation between the number of collections and the spermatozoa concentration (CON) was observed (r_S = 0.789), whereas the value of spermatozoa concentration varied from 0.37 to 1.05 x 10^6 ml^{-1} (Fig. 3), with 0.55 ± 0.16 x 10^9 ml^{-1} in average.

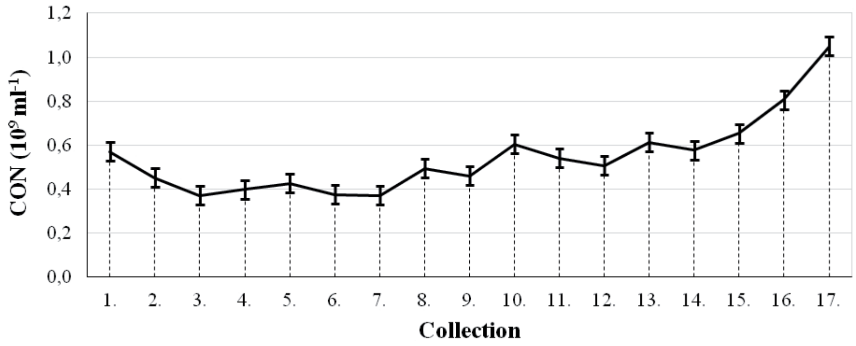

Fig. 3: Spermatozoa concentration development with regard to the number of collections

Motility (MOT) and progressive motility (PRO) correlated to the ejaculate volume (r_P = 0.548 and r_P = 0.640, respectively). In the case of the dependency of the ejaculate volume and spermatozoa motility, positive correlation (r_P = 0.751) between % PRO and the ejaculate volume was observed (Fig. 4).

The relationship between the number of collections and ALH, BCF, VCL was also studied. No correlation between the number of collections and the VCL (r_S = 0.267) value development was detected (Fig. 5). In the case of ALH, a moderate positive correlation (r_S = 0.478) between this parameter and the number of collections was observed (Fig. 6). BCF slightly correlated (r_S = 0.560) to the number of collections (Fig. 7). The average value of VCL was 104.04 ± 37.00 µm.s^{-1}. ALH was represented by 4.17 ± 0.28 µm and the average value of BCF reached 27.50 ± 1.96 Hz.

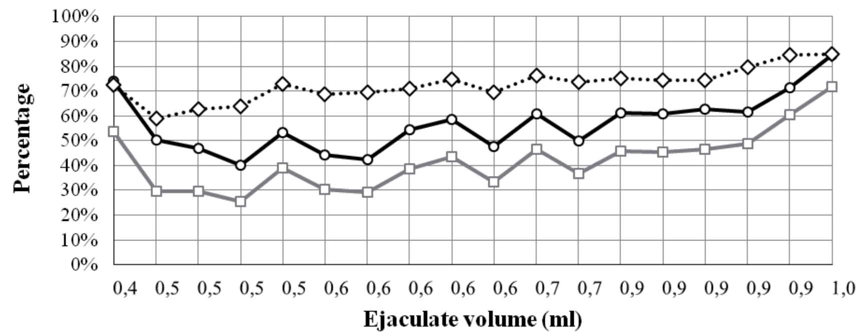

Fig. 4: Spermatozoa motility development with regard to the ejaculate volume

DISCUSSION

For artificial insemination a proper ejaculate with desired seminal parameters is required. Some seminal parameters could have a high relationship to the success of fertilization process. There are several publications, the aim of which lies on analyzing the development of selected seminal traits with the relationship to the regime of semen collection (Castellini *et al*., 2006; Nizza *et al*., 2002). Some of such traits, as shown in present study, may be influenced through regular semen collection. The results of this study indicate on possibility to increase the values of these parameters, which opens new opportunities for raising

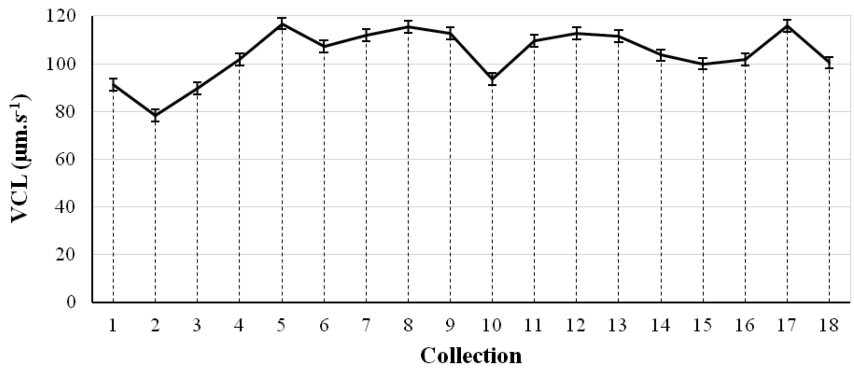

Fig. 5: VCL development with regard to the number of collections

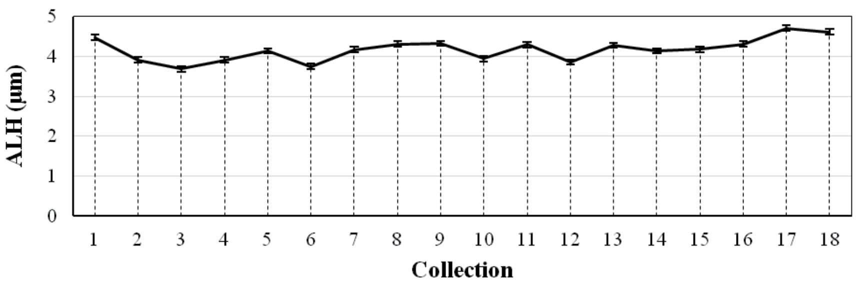

Fig. 6: ALH development with regard to the number of collections

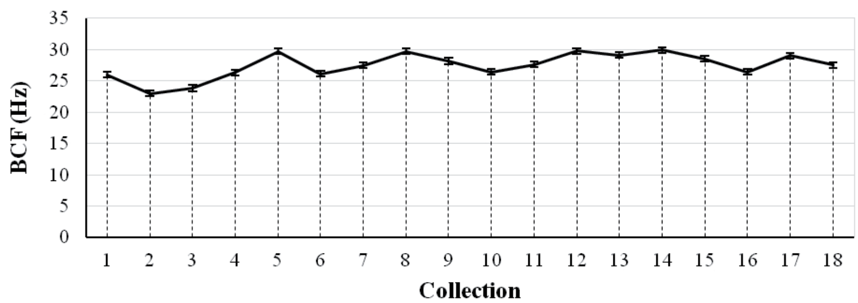

Fig. 7: BCF development with regard to the number of collections

the quality of ejaculates directly *in vivo*.

During continuous semen collections it came to a significant increase in the ejaculate volume. After the first ejaculate collection, its average volume was 0.43 ml and this value increased progressively up to 0.95 ml for the last collection. This increase is caused by the stimulation of the male accessory reproductive organs, which produce more secret as the glands get long-term stimulated. Schneidgenova *et al.* (2011) in their study report an average ejaculate volume 0.68 ± 0.25 ml in 25 rabbits in the spring season, which is similar to the values obtained in our work (0.68 ± 0.34 ml). The similar results (0.60 ± 0.05 ml) were obtained in the work of Castellini *et al.* (2006). Taking into consideration similarity of methods used for collection, it can be concluded that there is probably no significant influence of any other factors than the regime of semen collection on the ejaculate volume development.

Almost three-fold increase in spermatozoa concentration from the beginning (0.37×10^6 ml^{-1}) to the end of collections (1.05×10^6 ml^{-1}) was found. It can be concluded that the rising number of collections generally causes an increase in semen concentration. However, Nizza *et al.* (2002) recorded increase in semen concentration neither after 34 nor 68 semen collections, which were done from July to November. This can be a consequence of season influence on rabbit semen development. Chrenek *et al*. (2011) assessed ejaculates of 10 HYLA males, semen concentration of which reached 0.94×10^9 ml^{-1}. Similarly, in the study of Schneidgenova *et al*. (2011) the average semen concentration was measured as 1.18×10^9 ml^{-1}. Diametrically different results were obtained by Castellini *et al*. (2006), where the CON value was about $0.261 \pm 0.127 \times 10^9$ ml^{-1}. Despite large differences in these values from that of present work ($0.547 \pm 0.165 \times 10^9$ ml^{-1}), it only indicates on individual variability in rabbit semen concentration.

With the number of collections it came to an increase of MOT (74 % - 84 %) and also a moderate raise of PRO (54 % - 72 %) were observed. The average MOT was 55.55 ± 26.63 % and the average PRO reached 40.43 ± 27.35 %. In the study of Schneidgenova *et al*. (2011) the average MOT was 69.94 ± 16.64 %. For the PRO parameter they reported the average value 52.23 ± 20.63 %. These present differences are probably due to individual variations among males. Moreover, it is necessary to take into consideration the occurrence of many factors that influence analyzed seminal traits, such as occurrence of urine in the ejaculate or environmental temperature.

By evaluation of the other motility parameters, we observed no relationship between the number of collections and VCL ($r_S = 0.267$). In contrary, ALH moderately positively correlated ($r_S = 0.478$) with the number of collections. The same applies to the BCF parameter ($r_S = 0.560$). The average value of VCL was 104.04 ± 37.00 μm.s^{-1}, for ALH it was 4.17 ± 0.28 μm and the average value of BCF reached 27.50 ± 1.96 Hz. For VCL Schneidgenova *et al.* (2011) reported an average value of 81.44 ± 24.21 μm.s^{-1}. In the same study the average ALH was 4.03 ± 0.82 μm, the result not very different from those reported in this study. The BCF value is lower as well, when compared to our results (22.81 ± 5.75 Hz). Explanation of this phenomenon lies on the fact that as the frequency of spermatozoa head movement decreases it comes to simultaneous reduction of the linear movement.

Our results suggest that some seminal traits and, thus, the semen quality can be improved by regular semen collection.

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