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EFFECT OF COLLECTION RHYTHM ON SPERMATOZOA AND DROPLET CONCENTRATION OF RABBIT SEMEN

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Abstract: The aim of the paper was to analyse the effect of collection rhythms on spermatozoa and droplet concentration of rabbit semen. Thirty adult New Zealand White rabbit bucks were submitted to 3 collection rhythms: every day (D), every week (W), every 2 weeks (2W). The trial lasted 71 days and a total of 790 ejaculates were collected. Volume, concentration of spermatozoa, droplets and their dimensions were evaluated. Ejaculate volume and concentration of spermatozoa were the lowest (0.37± 0.08 mL and 48.9± 43.8x10^6 mL^{-1}, respectively) when the samples were collected daily, whereas the ratio droplets/spermatozoa was the highest (6.4 ± 3.3). The output of spermatozoa per week was the highest with W rhythm (173.4 ± 105.3x10^6), followed by D (134.2± 75.1x10^6) and by 2W (79.5 ± 49.8x10^6) collection whereas the weekly output of droplets was the highest for D (810.2 ± 615.7x10^6). In the following order of collection the spermatozoa concentration was similar in W and 2W while D showed a sharp decline of values after few collections. Semen droplets with respect to spermatozoa showed a more stable trend showing that droplets are able to respond better to high soliciting. Bucks submitted to an intensive rhythm were able to increase about 9-fold (respecting to W) the secretion of droplets by prostate glands. As a result, the ratio droplet/spermatozoa showed the highest values in daily collected semen (6.4 ± 3.3). The repeatability of seminal traits showed that volume and spermatozoa concentration had the highest value followed by droplets, and ratio droplets/spermatozoa. In conclusion, it is possible to affirm that the collection rhythm, besides influencing the concentration of spermatozoa, also affects the rate of droplet production. A collection rhythm every 2 weeks was detrimental to both the spermatozoa and droplet output.

Key words: rabbit, semen, spermatozoa, seminal droplet.

INTRODUCTION

Studies on the effect of collection rhythm on rabbit semen characteristics have been particularly focused on volume, concentration, pH, live and motile cells, and kinetic characteristics of spermatozoa (Carvajal *et al*., 1993; Bencheikh *et al*., 1995; Lopez *et al*., 1996; Theau-Clément *et al*., 1999; Nizza *et al*., 2003).

The effect of collection frequency on other seminal characteristics is lacking. Rabbit semen contains spermatozoa and several other particles produced by different accessory glands that are mainly described as droplets and vesicles. Droplets are very abundant in semen, are comparable in size to spermatozoa and are produced by the prostate gland (Zaniboni *et al*., 2004), whereas vesicles are 70 nm in diameter, are few in number and are unknow (Minelli *et al*., 2003).

The role of these particles is not well known, and very few reports have analysed their composition (Castellini *et al*., 2005) and postulated their many functions. These droplets seem to affect motility rate and spermatozoa capacitation; a capacitative effect has also been hypothesized for vesicles

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(Davis and Davis, 1983). Since droplets are peculiar to rabbit semen, the aim of this study was to analyse the trend of this parameter in rabbit bucks submitted to different rhythms of semen collection.

MATERIAL AND METHODS

Animals and housing

The trial was carried out at the experimental farm of the Department of Biologia Vegetale, Biotecnologie Agroambientali e Zootecniche (University of Perugia). Thirty adult (7 months of age) New Zealand White rabbit bucks were divided into three homogeneous groups and submitted to 3 rhythms of semen collection: every day (D), every week (W) and every 2 weeks (2W). Rabbits were kept under a continuous 16 h light/8 h dark photoperiod (Theau-Clément *et al*., 1995) with an intensity of 40 lux (Besenfelder *et al*., 2005). The ambient temperature ranged from 18 to 27°C. All the bucks had been previously trained for semen collection using an artificial vagina without any pre-stimulation. Before the start of the experiment the bucks had a 10-day abstinence. A total of 790 ejaculates were analysed. To avoid the effect of the stimulus constituted by collecting semen from bucks in the building on the semen characteristics, the order in which the semen was collected from the individuals in the various groups was changed for each collection sequence. The trial lasted 71 days from 1/03/05 to 10/05/05. The refusal to mount was rare, even in daily collected bucks (< 5 %), but when it occurred it was considered as a missing value.

Feeding

The rabbits were fed *ad libitum* (Nizza *et al*., 2000) with a commercial diet containing 175 g/kg protein and 145 g/kg fibre (Luzi *et al.*, 1996) and 10.7 MJ/kg digestible energy. Water was provided *ad libitum*.

Measurements of semen traits

Immediately after semen collection, the volume and concentration of spermatozoa and lipid droplets were evaluated according to international guidelines (IRRG, 2005). Volume was evaluated by weighing the tube before and after collection, according to international guidelines (IRRG, 2005). The concentration of spermatozoa and droplets were estimated using a Thoma-Zeiss cell counter (final dilution 1:200). At the start and end of a trial, the droplet dimensions were measured in all the samples n=10 per group) by SCA®2002 software (Sperm Class Analyzer, Microptic, Barcelona Spain).

Statistical analyses

Semen characteristics were statistically analysed by analysis of variance with the fixed effects being the collection rhythm (CR), the buck within the CR, the collection days (CD) and the interactions CR × buck (CR) × CD (R: Copyright 2002, The R Development Core Team). For spermatozoa and droplet output the CD was omitted from the model.

To study the variability of semen characteristics, a mixed model was used, with the fixed effect of the collection order and the random effect the buck (CR); values were determined within each CR. Repeatability was estimated as $\sigma B^2/(\sigma B^2 + \sigma e^2)$, where σB is the variability of the buck and σe that of error.

RESULTS AND DISCUSSION

The characteristics of the semen samples are reported in Table 1. Ejaculate volume and spermatozoa concentration were lower when the samples were collected daily from the bucks, whereas the droplets/spermatozoa ratio was much higher in the D group. The highest concentration of seminal droplets was recorded for the bucks of the W group. The weekly spermatozoa output was the highest with one semen collection/week, followed by the daily and then 2W collection. In contrast, daily collection

Table 1: Semen characteristics according to the collection rhythm, the buck within the collection rhythm and the collection order.

	Number of ejaculates	Volume (mL)	Spermatozoa ($nx10^6mL^{-1}$)	Doplet ($nx10^6mL^{-1}$)	Ratio droplet/ spermatozoa	Spermatozoa output ($nx10^6wk^{-1}$)	Droplet output ($nx10^6wk^{-1}$)
Mean ± SD	790	0.49±0.12	146±104	373±278	5.6±3.1	142.1±87.2	560±502
R square		0.64	0.68	0.49	0.88	0.50	0.58
Collection rhythm[1]							
D	645	0.37^a±0.08	48.9^a±43.8	314.9^a±226.5	6.4^b±3.3	134.2^b±75.1	810.2^c±615.7
W	95	0.60^b±0.05	260.8^b±127.0	518.7^b±431.4	2.0^a±0.6	173.4^b±105.3	310.5^b±203.4
2W	50	0.62^b±0.06	235.6^b±90.4	348.9^a±84.6	1.5^a±0.4	79.5^a±49.8	88.7^a±41.5
Significance							
Buck (within CR)[2]		*P*<0.01	*P*<0.01	*P*<0.01	*P*<0.01	*P*<001	*P*<0.01
Collection Days		*P*<0.01	*P*<0.01	*P*<0.01	*P*<0.01	-	-
CRxCD[3]		*P*<0.01	*P*<0.01	*P*<0.01	*P*<0.01	-	-

Means in the same column with different superscripts are significant ($P<0.05$). [1] Collection rhythm: every day (D), every week (W) and every 2 weeks (2W). [2]CR: Collection rhythm. [3]CD: Collection days.

increased the weekly output of droplets while the more extensive rhythm showed the lowest value (only 10% of D value).

Throughout the entire collection period (Figure 1) the spermatozoa concentration was similar and quite stable with the W and 2W collection rhythms, while the concentration decreased markedly, about -75%, after a few collections at the daily collection rhythm.

On the other hand, the droplet concentration decreased less with continuous semen collections, and the results of the daily collection rhythm were quite similar to those for the 2W collection rhythm (Figure 2). Semen collected weekly showed the highest droplets concentration throughout the entire experimental period.

As a result, the droplets/spermatozoa ratio (Figure 3) was the highest in daily collected semen; the ratio value was lower but similar in the W and 2W groups.

The repeatability of the seminal traits showed that the volume of ejaculate and spermatozoa concentration had the highest values followed by droplets, and then droplets/spermatozoa ratio (Table 2). The lowest values for enhancing buck variability were recorded with the daily semen collection rhythm. These high repeatability values were in agreement with the results of Theau-Clement *et al.* (2003) that showed that when the main factors of variation are controlled (collection rhythm, environmental conditions, feed and genetic strain) the repeatability of the seminal traits increases about 2-fold (Panella and Castellini, 1990).

The values for mean diameter of droplets at the end of the experimental period were lower to those observed at the beginning in the daily collection rhythm (Table 3); even though the large variability of particles prevented the differences from being significant.

It is widely known (Nizza *et al.*, 2003) that collection frequency affects many seminal traits; intensive rhythms (2-3 collections/week) reduced the spermatozoa number and the seminal volume. Our data confirmed this tendency and also showed that a too extensive rhythm (2W) reduced the spermatozoa output.

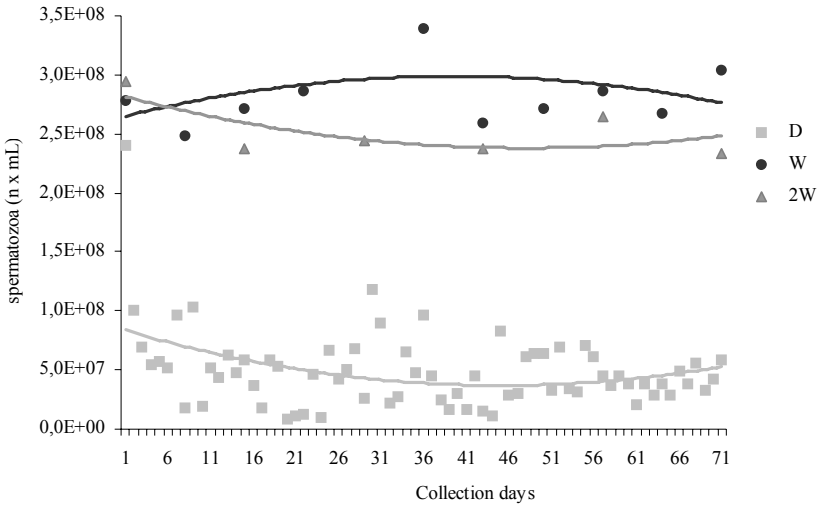

Figure 1. Spermatozoa concentration in bucks submitted to different collection rhythms.

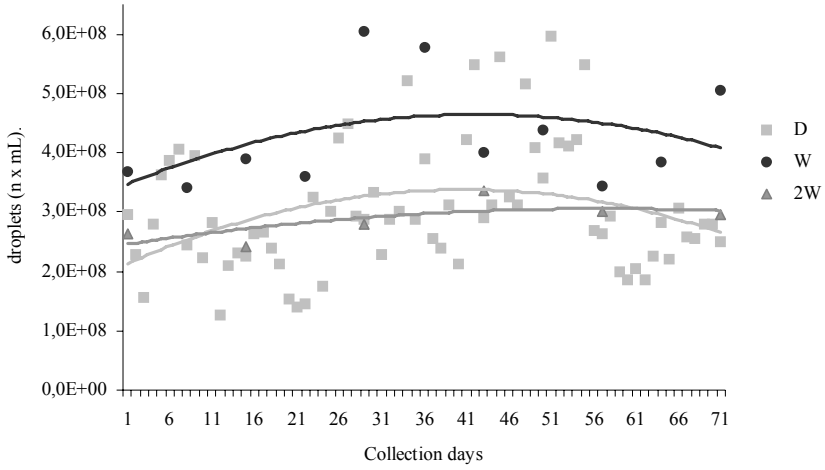

Figure 2. Droplet concentration in bucks submitted to different collection rhythms.

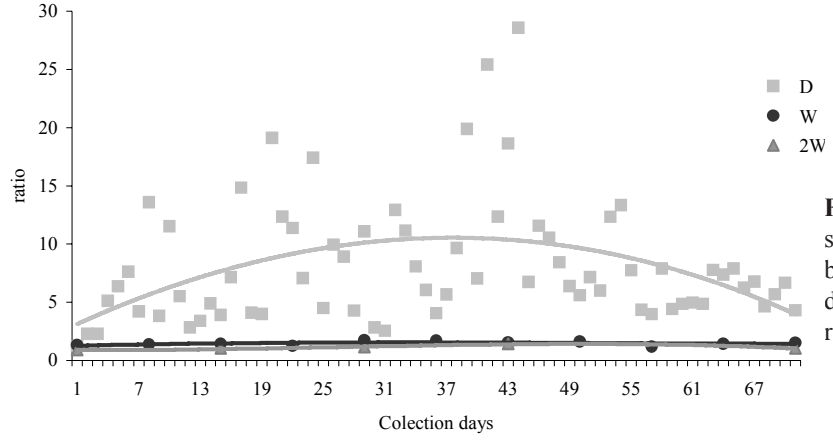

Figure 3. Droplet/ spermatozoa ratio in bucks submitted to different collection rhythms

Table 2: Repeatability (%) of semen characteristics.

Collection[1] rhythm	Volume	Concentration	Doplet	Ratio droplet/spermatozoa	Spermatozoa output	Droplet output
D	36.5	17.0	27.6	10.0	21.8	28.3
W	44.8	47.9	27.1	20.0	37.1	30.2
2W	33.5	56.0	21.6	35.7	38.5	25.3

[1] Collection rhythm: every day (D), every week (W) and every 2 weeks (2W).

Bencheikh (1995) showed that one collection/week (repeated within 15 min) improved many qualitative traits of semen (volume, pH, number of spermatozoa, motility, live sperms); nevertheless, the weekly sperm output was greater with 2 or 3 collections/week, but the increase was slight: e.g. only + 28% of total sperms *vs.* an expected increase of +300%.

When the semen collection rhythm was more frequent than the rate of spermatozoa production, the concentration decreased (Mann and Luttwak-Mann, 1981). The spermatozoa production however is stimulated by semen collection and a too extensive collection probably reduces hormonal release and increases the reabsorption of germ cells along the epydidimus (Boussit, 1989).

The secretion from accessory glands (Mann, 1964) is dependent on the release of testosterone from the testis and is probably affected by such hyper- or hypo-stimulation.

To our knowledge, this is the first report on the effect of collection rhythm on seminal droplets. These droplets, with respect to spermatozoa, seem to maintain a more stable trend and show a better response to high soliciting. The weekly spermatozoa output in the D rhythm decreased by 90% with respect to the theoretical values (concentration obtained in W rhythm × 7) whereas the droplets only decreased of 63%.

An intensive collection rhythm stimulates the prostate gland (Holtz and Foote, 1978; Vasquez and Del Sol, 2003) which can increase its droplet secretion about 9-fold (in comparison with 2W). At the same time, it seems that very intensive collection of semen could result in a decreased droplet size.

The role played by such droplets in *in vivo* spermatozoa behaviour and during transit in the female reproductive tract is uncertain. Some authors (Yamamoto *et al*., 1999; Castellini *et al.,* 2005), hypothesised that the higher cholesterol level of seminal droplets and seminal plasma could render them donors of sterols (Davis, 1980) for protecting spermatozoa against environmental shock and premature acrosome reaction (Davis and Davis, 1983). Thus, when capacitation is induced in the presence of such particles, the spermatozoa are more refractory to undergoing acrosome reaction.

Gojalas *et al.* (2004), comparing human and rabbit spermatozoa, hypothesized that the timing and duration of capacitation is programmed according to the time of egg availability in the oviduct. In

Table 3: Initial and final dimension and shape of droplets (n=10 per group).

Collection rhythm	Initial diameter (µm)	Final diameter (µm)
D	2.12	1.67
W	2.35	2.19
2W	1.98	2.30
SEM	0.20	0.24

Collection rhythm: every day (D), every week (W) and every 2 weeks (2W).

induced ovulators like rabbits, it is probable that the presence of droplets could also modulate the prolonged time (8-16h) needed to reach the highest capacitation percentage. This situation should maximize the possibility that an ovulated egg will meet spermatozoa that are in an appropriate functional state. Besides modulating cholesterol efflux, some authors (Miodrag *et al*., 1995) have suggested that non-sperm particles could have an immuno-modulatory effect by preserving sperm survival in the female reproductive tract.

In conclusion, it can be affirmed that the collection rhythm not only influences the concentration of spermatozoa, but affects droplet production. Too frequent collection rhythms are mainly detrimental to spermatozoa output but one ejaculate every two weeks also has a negative effect on semen production. The repeatability of droplets and spermatozoa production was similar.

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