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EFFECT OF REPRODUCTIVE RHYTHM ON SEMINAL PARAMETERS FROM A RABBIT LINE WITH HIGH GROWTH RATE

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ABSTRACT

The aim of this study was to compare three extensive reproductive rhythms to see if they influenced in the quality of the semen in males belonging to a growth line (line R). The results showed that there were not important differences in production (368 million spermatozoa per buck and week) and quality of ejaculates (motility rate: 79.85 %; normal acrosome rate: 89.03 %; cytoplasmatic drops rate: 10.73 %; abnormalities rate: 7.90 %) between rhythms, so any of the three management systems can be used for this line of males.

INTRODUCTION

Many factors influence in the production and quality of semen such as the male (Castellini, 1996), the genetic origin (growth lines have worse seminal qualities than maternal lines, Egea *et al*., 1992), the season (poor quality in autumn, Panella *et al*., 1990), the photoperiod (16L: 8D, Theau-Clément *et al*., 1995) and the collection frequency (Bencheikh, 1993).

There are few works that compare different reproductive rhythms to study their effect in seminal characteristics. Bencheikh (1993) analysed the effect of three collection rhythms on semen traits; the rhythms employed were: intensive (two successive ejaculates, within 15 minutes, three times per week), intermediate (two successive ejaculates twice a week) and extensive frequency (two successive ejaculates once a week). Bencheikh observed that the best seminal qualities and the highest productions were obtained using the extensive rhythm.

Mocé *et al*. (1999) studied two different extensive rhythms: two ejaculates were collected per buck and week, but in one of the groups the interval between ejaculates was 30 minutes and in the other group ejaculates were collected on different days of the week; the quality of ejaculates and the production obtained per male and ejaculate (211 million spermatozoa) were not different between groups.

The aim of this study is to compare three rhythms to establish the best collection frequency for males belonging to the line R that allow to obtain the best seminal qualities and production for these bucks.

MATERIAL AND METHODS

The work was carried out at the experimental farm of the Department of Animal Science, Universidad Politécnica de Valencia. Twenty-one adult males belonging to a growth line (line R) were used in this experiment. Bucks were accustomed to work with artificial vagina.

The experience was carried out in 1998, during May an June. Males were housed in individual cages and fed (with a commercial diet) and watered *ad libitum*. The photoperiod employed was 16 hours light/day.

Ejaculates were collected early in the morning. Three groups of males (7 animals/group) were established depending on the collection frequency employed: in the first group, three ejaculates were collected per week (1 ejaculate/day, three times a week), in the second and third group, 2 ejaculates were collected per buck and week, but the interval between

ejaculates was 30 minutes for the second group and two days for the third group.
After an adaptation period of 2 months, semen was collected and analysed weekly; 350 individual ejaculates were evaluated, and the following parameters were registered: volume (ml.), motility, concentration (x10^6 spermatozoa/ml.), production (volume x concentration), normal acrosome rate (%), cytoplasmatic drops rate (%) and abnormal spermatozoa rate (%).
Once ejaculates had been collected, the volume and the colour were determined (volume was measured using a graduated tube); ejaculates with abnormal colours (if there were blood or urine, etc.) were thrown away; if ejaculates had gel, it was removed. After that, they were diluted (dilution 1:5) with an extender which contained TRIS-Citric Acid-Glucose (300 mOsm, pH 6.8). Later, an aliquot of the diluted ejaculate was diluted again 1:9 to evaluate the motility (final dilution 1:45); concentration, acrosomal damages and abnormal spermatozoa were evaluated using an aliquot previously fixed employing a solution with 2% of glutaraldehyde (final dilution 1:50).
Kinetic parameters of 262 individual ejaculates were estimated by CASA (Sperm Class Analyzer, Microptic S.A.). Motility was measured at 37ºC under microscope with phase's contrast optics; two drops of ten microlitres from each sample diluted 1:45 were laid over a microscope slide and covered with a micro cover glass (20 x 20 mm.). Two images were taken from each sample (one field from each drop) and were analyzed later by CASA using set-up parameters included on Table 1. Images were analysed by the automatic option (filter Auto-M), after that each case was revised and, if there were some error (confused trajectories, etc.), re-analysed manually until the analysis was correct. The following parameters were taken: track speed (VCL, μm/ second), progressive speed (VSL, μm/ second), linearity (LIN= [VSL/VCL] x 100) and amplitude of lateral head displacement (ALH, μm).

Table 1. Parameter setting.

Frame at frame rate		16 to 25/second
Minimum contrast		100
Minimum data point		7
Low VAP	μm s^{-1}	15
Medium VAP	μm s^{-1}	30
Threshold straightness	%	80

Sperm concentration was measured by a Thoma-Zeiss counting cell chamber and acrosomal damages, cytoplasmatic drops and abnormalities were evaluated under microscope with Nomarski's interference contrast optics at a magnification of x400 and contrast optics at a magnification of x750.
The statistical analysis employed was a General Linear Model of SAS (Statistical Analysis System Institute, 1996) with fixed effects for group, week, ejaculate order within group and donor male within group. Group x week interaction was analysed for all variables.

RESULTS AND DISCUSSION

The effect dues to donor male within the group was significant for all semen related variables, and effects due to week and group x week interaction were significant for all variables except for production and abnormalities rate. Interaction group x week was not significant for cytoplasmatic drop rate either.

Results are shown in Table 2. Group 1 had significantly higher values of volume, motility rate and percentage of abnormal spermatozoa. Groups 2 and 3 had higher values of concentration. There were not differences between groups for production (because ejaculates belonging to groups 2 and 3 had higher values for concentration than group 1, but their volume was lower than ejaculates from group 1), and normal acrosome rate.

Table 2. Seminal characteristics.

GROUP	VOLUME (ml)	MOTILITY (%)	CONCENT.(10^6 spz/ml)	PRODUCT.(10^6 spz/week)	N.A.R. (%)	C.D.R. (%)	A.R. (%)
1	1.93± 0.13^b	83± 1.5^b	197±21^a	325± 26	90± 0.8	9± 0.8	11± 0.7^b
2	1.44± 0.10^a	79± 1.5^a	279± 25^b	375± 35	89± 0.9	11± 1.00	7± 0.4^a
3	1.64± 0.07^a	78± 1.5^a	239± 13^b	403± 27	89± 0.8	12± 1.1	6 ± 0.4^a
TOTAL	1.66± 0.04	80± 0.76	239± 8	368± 14	89± 0.4	11± 0.4	8± 0.2

CONCENT.: Concentration; PRODUCT.: Production (volume x concentration); N.A.R.: Normal acrosome rate; C.D.R.: Cytoplasmatic drop rate; A.R.: Abnormalities rate. $^{a, b}$ Values in the same column with different superscripts differ statistically ($P< 0.05$).

Results for parameters of motility are shown in Table 3. The ejaculate order (first, second and third for the group 1, and first and second for groups 2 and 3) was not significative for any of the parameters studied.

Group was significant for all the kinetic parameters analyzed; group 2 had higher values of VCL, VSL and ALH. Groups 1 and 3 had higher values of LIN than group 2 (64.7 ± 0.98 and 64.6 ± 0.72 vs 58.6 ± 1.44 for groups 1, 3 and 2 respectively). Nevertheless, the importance of this effect should be studied; differences observed on these parameters by Castellini *et al*., 1999 did not affect the fertility rate of does inseminated, especially if a non conserved semen with high number of spermatozoa per dose is employed for artificial insemination.

The effect dues to donor male within the group was significant for all parameters of motility and interaction group x week was not significative for any of the variables studied.

Results obtained in the present work are different from those found by Castellini *et al*., 1999. These authors found higher values of concentration (395-410 million spermatozoa/ml. vs 239 x 10^6 in the present work), VCL (91.25-103 μm/ second vs 32.7 μm/ second) VSL (38.9-41.7 μm/ second vs 19.1 μm/ second obtained in this work) and ALH (3.33-3.91 μm vs 0.96 μm for this study). In the present work higher values were found for percentage of motility (80% vs 68.51-70.23%found by Castellini *et al*., 1999) and linearity (63.5 % vs 40.26-44% found by Castellini *et al*., 1999). These differences could be due to the genetic origin of males employed for the studies or to the protocol of work employed with CASA.

Table 3. Parameters of motility.

GROUP	VCL	VSL	LIN	ALH
1	32.9 ± 0.99^{b}	19.5 ± 0.59^{ab}	64.7 ± 0.98^{b}	0.96 ± 0.04^{ab}
2	38.5 ± 1.13^{c}	21.1 ± 0.78^{b}	58.6 ± 1.44^{a}	1.07 ± 0.04^{b}
3	30.5 ± 0.72^{a}	18.1 ± 0.49^{a}	64.6 ± 0.72^{b}	0.92 ± 0.03^{a}
TOTAL	32.7 ± 0.46	19.1 ± 0.33	63.5 ± 0.5	0.96 ± 0.02

VCL: track speed (μm/ second); VSL: progressive speed (μm/ second); LIN: linearity (LIN= [VSL/VCL] x 100); ALH: amplitude of lateral head displacement (μm). $^{a, b, C}$ Values in the same column with different superscripts differ statistically ($P< 0.05$).

CONCLUSIONS

Any of the rhythms studied in this work give good results for males belonging to the line R; nevertheless, the rhythm which was employed for bucks of the group 1, should not be recommended because, working more (it is collected one ejaculate more/male/week), results obtained using it aren't better than results for groups 2 and 3.

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