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VARIABILITY OF THE MAIN CHARACTERISTICS OF RABBIT SEMEN (*).

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Abstract

The quanti-qualitative characteristics of rabbit semen were evaluated and correlated and their repeatability was estimated. During the period June-December 1991, 270 semen samples were collected from 19 N.Z.W. bucks reared in an experimental rabbitry. The traits examined were: ejaculate volume, spermatozoa/ml and spermatozoa/ejaculate, pH and motility. The variability factors studied were: buck, temperature (month) and collection order. Very high variability between bucks was observed for all the semen traits except pH. The volume ranged from 0.31 to 1.15 ml (mean = 0.76 ml), density/ml, 280 to 1049 million (mean = 561), total spermatozoa, 196 to 634 (mean = 420), percent motility, 55.3 to 90.2 (mean = 76) and pH, 6.71 to 8.42 (mean = 7.3). Temperature and the collection order also affected semen characteristics: with high temperatures (summer months) performance was worse and the second ejaculate had less volume but a greater number of spermatozoa/ml. The correlations were rather high for volume vs spermatozoa/ejaculate (0.43), pH vs sperm./ml and sperm./ejac. (-0.43 and -0.62 respectively), motility vs spermatozoa/ml (0.47) and spermatozoa/ejaculate (0.30) and motility vs pH (-0.50). The repeatability estimations fluctuated between 0.12 (motility) and 0.32 (spermatozoa/ml).

Introduction

Interest in artificial insemination is increasing in all the countries where intensive rabbit raising is practiced. The main factors that insure the success of this reproduction technique must be studied. The quanti-qualitative traits of semen must be known to select bucks which produce large quantities of good quality semen whith good fertilizing ability. To date few studies have been done on this subject and the results of those available cannot be easily compared and generalized. Some works (1, 4, 10, 12, 15) provide general information while others give more specific details on the main factors affecting semen characteristics, such as breed (2, 6, 7, 9), body weight (7),

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collection order (5, 10) and season (3, 5, 6, 8). Some researchers (5, 10) also evaluated the correlation coefficients (r) among some of these traits.
The present research was conducted to evaluate the variability of the main semen traits and to ascertain their repeatability.

Materials and Methods

For the trials 19 mature N.Z.W. bucks (aged 7 months) were used. They were reared in an experimental rabbitry with natural ventilation and heated during the winter; the cages were flat-deck. The photo-period was 16 hours/day and the animals were fed a commercial feed (17% crude protein, 16% crude fiber, 2550 DE kcal/kg) ad libitum. The semen was collected weekly from June to December 1991 and during this period daily temperatures were recorded. When possible, a second ejaculate was collected after 15 minutes. Immediately after the collection the following variables were analyzed: ejaculate volume, pH, spermatozoa/ml and motility. The motility was estimated subjectively placing a drop of semen on a microscope slide at 37° C and determining the progressive motile sperm with a video camera and monitor connected to the microscope. The sperm concentration was determined on semen diluted 1:100 with an IMV photometer (520 nm).
Statistical analysis was carried out according to the following linear model:

$$Y_{ijkl} = m + a_i + b_j + c_k + e_{ijkl}$$

Y_{ijkl} = experimental items;
m = overall mean;
a_i = fixed effect of buck (1..19);
b_j = fixed effect of month (1..7);
c_k = fixed effect of collection order (1..2);
e_{ijkl} = residual effect.

To evaluate the repeatability buck effect was considered random. Experimental data were processed by the PROC CORR, GLM and VARCOMP (SAS, 14).

Results and Discussion

The statistical significance of the studied effects is reported in Table 1 where is showed that buck and month effects were always significant while the collection order never reach the significance probably because second ejaculates were little numerous.
The least square means (LSM) for all the considered traits show very high

individual variabilities except for pH (Table 2).
Volume ranged from 0.31 to 1.15 ml (mean = 0.76 ml); the quantity was very low in only two males (0.31 and 0.36) and very high in three (0.99-1.01-1.15). The values fluctuated from 0.57 to 0.94 ml for the remaining 14 bucks. Regarding spermatozoa/ml and spermatozoa/ejaculate the differences between bucks were even greater, fluctuating from 279.5 to 1048.9 (mean = 561) and 195.6 to 634.1 (mean = 420), respectively. The pH values varied little (6.71 to 8.42, mean = 7.3) while there were marked differences in the percent motility (55.3 to 90.2, mean = 76).
The variability of semen traits was also very high for individual bucks as shown by the low repeatability (Table 3), particularly for spermatozoa/ejaculate (0.20) and motility (0.12).
The effect of the collection order (Table 4) was observed for volume, spermatozoa/ml and motility. The second ejaculate had less volume, but a higher concentration of spermatozoa/ml as previously observed (13) and the motility was also higher. The differences were not significative.
The collection period (Table 5) had a considerable influence on the semen traits; the number of spermatozoa/ml was significantly lower during the warmer months. Since spermatogenesis requires 38-45 d (4) influence of the high temperatures does not appear immediately. In fact the concentration/ml decreased gradually after June reaching a minimum in September, which reflects the negative effect of the high temperatures of August. The total number of spermatozoa showed a similar trend, while the observed variations in volume, in our opinion, cannot be ascribed to the collection month. The pH values were rather similar. The highest motility occurred in October, the lowest in August and September; a similar trend was oberved for concentration.
The correlation coefficients (Table 6) showed that increased volume decreased spermatozoa/ml (r=-0.23) but total number of spermatozoa increased (0.43); furthermore pH was negatively correlated with density (-0.43) and total spermatozoa (-0.62) and with motility (-0.50). Finally the more concentrated semen (per ml and ejaculate) was characterized by a higher spermatozoa motility (r= 0.47 and 0.30).
The results obtained in this investigation are in agreement with those of other researchers regarding the average values for the different traits and their high variability, that can be deduced from the large standard deviations. The spermatozoa/ml and ejaculate are higher than those previously reported (13) probably because of the different collection frequency that in the present study was once a week only.
No other author gives detailed data concerning different males; regarding the same buck, only Neira and Bustamante (11) reported that the repeatability of ejaculate volume was 0.71 (in our research it was 0.24).
The data regarding the effect of season on the semen characters cannot be compared because the results differ greatly from work to work and environmental temperatures were never indicated.

The correlation values obtained were similar to those reported by other Authors, except for the correlation between volume and pH which was -0.07 compared to 0.22 reported by Carvajal (5); for volume vs motility our value (-0.09) differed from that (-0.34) of Jarpa Mendez (8).
Based on the data presented it is possible to conclude the following: quanti-qualitative semen characteristics vary greatly between bucks and also in the same buck. Therefore it is necessary to carry out an accurate selection on many males, evaluating at least 20 semen samples/animal.
Also, controlling of the environmental conditions may help to eliminate the negative effects of high temperatures.

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Table 1 - Significance of the effects.

Effect	Volume ml	Spermatozoa/ ml No. x 10^6	Spermatozoa/ ejaculate No. x 10^6	pH	Motility %
Buck	**	**	**	**	**
Collection order	n.s.	n.s.	n.s.	n.s.	n.s.
Month	**	**	**	*	*

** : $P \leq 0.01$; * : $P \leq 0.05$;
n.s. : not significant.

Table 2 - Semen characteristics in different bucks (LSMeans -DSE).

Buck No.	Volume ml	Spermatozoa/ ml No. x 10^6	Spermatozoa/ ejaculate No. x 10^6	pH	Motility %
1	0.85	641.7	545.4	7.02	83.0
2	0.80	385.3	308.9	7.15	76.8
3	0.36	1048.9	377.4	7.11	84.1
4	0.82	740.0	607.2	7.06	87.1
5	0.65	518.5	336.5	7.15	75.1
6	0.57	480.9	273.9	7.26	75.0
7	0.70	494.2	346.7	7.23	82.4
8	0.94	407.7	383.8	7.36	75.0
9	0.68	371.9	252.4	8.42	55.3
10	0.80	549.8	439.4	7.92	64.2
11	1.15	483.2	556.4	6.83	65.3
12	0.99	488.2	483.7	7.29	71.7
13	0.69	727.7	502.7	6.71	90.2
14	0.91	279.5	254.5	7.27	65.2
15	0.69	882.9	608.0	6.95	85.7
16	1.01	628.2	634.1	7.09	84.4
17	0.81	535.2	433.5	7.45	77.3
18	0.31	704.1	218.9	7.41	82.4
19	0.63	310.4	195.6	7.99	58.5
DSE	0.32	250.4	230.3	0.49	11.5
No.	270	270	270	130	117

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Table 3 - Repeatability of semen characteristics.

Volume	0.24
Spermatozoa/ml	0.32
Spermatozoa/ejaculate	0.20
pH	0.24
Motility	0.12

Table 4 - Semen characteristics in two successive ejaculates (LSMeans).

		I	II
Volume	ml	0.80	0.71
Spermatozoa/ml	No. x 10^6	561.9	594.1
Spermatozoa/ejaculate	No. x 10^6	423.9	421.8
pH		7.35	7.25
Motility	%	74,2	78,8

Table 5 - Semen characteristics in different months (LSMeans).

Month	Volume ml	Spermatozoa/ml No. x 10^6	Spermatozoa/ejaculate No. x 10^6	pH	Motility %	Temperature mean±s.d. ° C
June	0.99A	536.9	531.7 C	7.29	76.3	24.1 ± 3.9
July	0.78a	515.6	401.9	7.19A	76.6	25.9 ± 5.5
August	0.71	478.5	339.1	7.40B	72.2A	26.5 ± 3.7
September	0.76	428.1 a	325.3 A	7.34	75.5	23.6 ± 7.8
October	0.73	718.5 A	524.8 C	7.08A	81.2B	17.8 ± 2.8
November	0.64b	689.8 A	440.1 BC	7.31	77.2	16.0 ± 1.4
December	0.67	562.9 b	377.9	7.54B	74.9	16.3 ± 1.2

A..C: $P \leq 0.01$;
a..b : $P \leq 0.05$.

Table 6 - Correlation coefficients (r) of semen characteristics.

Items	Spermatozoa/ ml	Spermatozoa/ ejaculate	pH	Motility
Volume	-0.23 **	0.43 **	-0.07	-0.09
Spermatozoa/ml	-	0.67 **	-0.43**	0.47**
Spermatozoa/ejaculate		-	-0.62**	0.30**
pH				-0.50**
Motility				-

**: $P \leq 0.01$.