

## EFFECT OF COLLECTION FREQUENCY ON RABBIT SEMEN PRODUCTION

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**ABSTRACT:** The effect of collection frequency on the volume, concentration, motility and live sperm of rabbit semen was studied. Ten crossbreed Hyla bucks were used in two groups (five per group) during 17 weeks. In the extensive rhythm, two ejaculates per male were collected 10-15 minutes apart on one day each week. In the semi-intensive group two ejaculates per day were collected on two consecutive days each week. The proportion of useful ejaculates obtained decreased as collection frequency increased (0.75 and 0.58 for the extensive and semi-intensive rhythms, respectively). Among the ejaculate characteristics studied – volume/ejaculate, sperm concentration, motility, live sperm – only

volume/ejaculate showed significant differences between rhythms (0.68±0.06 and 0.57±0.01 ml for the extensive and semi-intensive rhythms, respectively). The number of doses (20 million spermatozoa) produced per ejaculate decreased as collection frequency increased, but the number of doses produced per week was higher in the semi-intensive than in the extensive rhythms (28.8±1.5 and 23.3±2.0 doses/week, respectively). These results suggest that semi-intensive rhythms should be used, at least at times of the year that are less favourable for male libido, in order to obtain a larger number of doses per week.

**RÉSUMÉ:** Les conséquences de 2 rythmes de prélèvement de la semence ont été étudiées sur le volume, la concentration, la motilité et le taux de spermatozoïdes vivants, en utilisant 10 lapins adultes de génotype Hyla. Pendant 17 semaines, 5 sujets ont été prélevés deux fois de suite, un jour par semaine (rythme extensif) et les 5 autres lapins ont été prélevés deux fois de suite, deux jours consécutifs (rythme semi-intensif). La proportion d'éjaculats utilisables pour l'insémination artificielle est plus faible en rythme semi-intensif qu'en rythme extensif (0.58 vs 0.75). Parmi les

critères étudiés sur les éjaculats – volume, concentration en spermatozoïdes, taux de motilité, proportion de spermatozoïdes vivants – seul le volume est significativement affecté par le rythme le plus intensif (0.57±0.01 vs 0.68±0.06 ml). Le nombre de doses utilisables pour l'insémination par éjaculat décroît avec le rythme le plus intensif, mais le nombre de doses utilisables produites par semaine est significativement plus élevé avec le rythme semi-intensif (28.8±1.5 vs 23.3±2.0 doses/semaine).

### INTRODUCTION

Artificial insemination, introduced over 20 years ago in rabbit farming (PAUFLER *et al.*, 1979; BATTAGLINI *et al.*, 1986; THEAU-CLÉMENT and ROUSTAN, 1992), has proved a beneficial technique in terms of management, profitability, genetics and health. One of the most immediate benefits has been the reduction in the number of males. As a result, males to be used must be more intensively selected. Breeding males must have good genetic characteristics and provide a good semen yield both in terms of quality and quantity (PANELLA and CASTELLINI, 1990; BATTAGLINI, 1992; CASTELLINI and DAL BOSCO, 1998). The last is affected by genetic factors, by the individual, diet, environmental conditions, and the state of health. In addition, a key factor is the extent to which the male

is used. Several authors have studied various semen collection rhythms that vary from one to four ejaculates per day (BODNÁR *et al.*, 1996; BUNACIU *et al.*, 1996; LOPEZ *et al.*, 1996), on daily or weekly schedules varying from once weekly (BENCHEIKH, 1995; BUNACIU *et al.*, 1996) to daily collection (BODNÁR *et al.*, 1996). Intensification of collection rates increased the quantity of semen per week but inevitably had an adverse effect on quality.

Semen collection rhythms are considered intensive when they entail two successive ejaculates (within 15-30 min) three times per week, extensive with only two successive ejaculates once a week and semi-intensive with two successive ejaculates two times per week (ARROITA *et al.*, 2000). In practice, due to the adoption of the weekly production cycle and the difficulty of freezing rabbit semen, two ejaculates are collected once a week on the day of doe insemination.

Semen stores well for 24 hours, so it seemed appropriate to study the effect upon semen quality of a semi-intensive regime in which two ejaculates per day were collected on two consecutive days.

## MATERIAL AND METHODS

Semen was collected for 17 weeks (July-November) from ten crossbred, 10-12 month old Hyla bucks according to one two rhythms:

Extensive: Two successive ejaculates once a week.

Semi-intensive: Two successive ejaculates on Thursday, another two on Friday.

Males were randomly assigned to the groups and were housed in individual cages with a light cycle of 16 hours light and 8 dark in traditional buildings under non-controlled environmental conditions. Maximum ambient temperatures registered throughout the experiment varied from 18 to 30°C. Rabbits were fed *ad libitum* and had free access to water from nipple drinkers. Semen was collected by artificial vagina with an interval of 10-15 minutes between the first and the second ejaculates. After collection of the second ejaculate from each male, macro and microscopic analyses of semen were performed. Sexual activity (*libido*) was estimated by the time between introduction of the female into the male's cage and ejaculation.

Semen volume, colour, motility (estimated according to ZEMJANIS, 1970), spermatozoa

concentration (using a Burker chamber) and percentages of live spermatozoa were determined for each sample. Ejaculates with a high urine percentage were discarded. The number of useful doses for artificial insemination was determined by dividing the total number of spermatozoa produced in each semen collection (volume x concentration) by 20 million.

Statistical analysis of the results was carried out using  $\chi^2$  for mean comparison of ejaculates obtained and useful ejaculates (ejaculates obtained minus ejaculates with urine) and Student's "t" test to compare the means of volume, concentration, motility, live sperm and *libido*.

## RESULTS AND DISCUSSION

The number of ejaculates obtained from the group subjected to a semi-intensive rhythm was 221 out of 340 (17 weeks x 4 ejaculates x 5 bucks), or 65%, while the extensive group supplied 142 out of 170 (17 weeks x 2 ejaculates x 5 bucks), or 84% (Table 1). From the 363 total ejaculates, 327 useful ejaculates were obtained with only 10% containing urine. No differences were observed between the semi-intensive and extensive rhythms in terms of the percentage of useful ejaculates collected (90%).

Based on the number of ejaculations tried, there was a lower percentage of useful ejaculates from the semi-intensive group. This was caused by the greater failure of bucks to ejaculate. BENCHEIKH (1995) reported a decrease in useful ejaculates in the

**Table 1:** Number of ejaculates tried (ET), ejaculates obtained (OE), useful ejaculates (UE), OE/ET, UE/ET and UE/OE ratios, and *libido*.

	ET	OE	UE	OE/ET	UE/ET	UE/OE	<i>Libido</i> (s)
Semi-intensive group	340	221	199	0.65 <sup>b</sup>	0.59 <sup>b</sup>	0.900	26.4 <sup>a</sup>
Extensive group	170	142	128	0.84 <sup>a</sup>	0.75 <sup>a</sup>	0.901	23.5 <sup>b</sup>

Means in the same column with differing superscripts differ ( $P < 0.01$ ).

**Table 2:** Ejaculate characteristics. Means  $\pm$  standard error (n= number of observations).

	Semi-intensive group (n = 199)	Extensive group (n = 128)
Volume (ml)	0.570 $\pm$ 0.014 <sup>a</sup>	0.680 $\pm$ 0.057 <sup>b</sup>
Spermatozoa (x 10 <sup>6</sup> /ml)	397.7 $\pm$ 11.4	409.0 $\pm$ 14.6
Motility (%)	69.5 $\pm$ 0.92	69.8 $\pm$ 1.2
Live sperm (%)	79.9 $\pm$ 0.70	79.5 $\pm$ 0.8

Means in the same row with differing superscripts differ (P < 0.01).

transition from an extensive rhythm to semi-intensive or intensive rhythms. By contrast, ARROITA *et al.*, (2000) found no significant differences in useful ejaculates among collection frequencies (62%, 64% and 60% for extensive, semi-intensive and intensive, respectively). The percentage of useful ejaculates of the semi-intensive group in the present study was 59%, which did not differ greatly from the value reported by ARROITA *et al.*, (2000). It was much lower than the values of 70 and 69%, reported for intensive and semi-intensive rhythms, respectively by BENCHEIKH (1995). The value in the present study may have been affected by the season in which the trial was conducted (July - November); a period which is not favourable for male *libido*.

The intervals between introduction of the female into the male's cage and ejaculation (*libido*) were significantly shorter in the extensive than the semi-intensive group (23.5 vs 26.4 seconds).

The ejaculate volumes were significantly larger in the extensive group than in the semi-intensive group (Table 2) in agreement with the findings of BODNAR *et al.*, (1996) and BENCHEIKH (1995) who, in shifting from an extensive to a semi-intensive rhythm, noted a decrease in volume. ARROITA *et al.*, (2000), observed a numerical decrease in volume between extensive and semi-intensive rhythms but found no

significant differences between the means.

No significant differences were observed between the two groups for concentration, motility or live spermatozoa.

After observing higher concentrations in the extensive rhythms, BENCHEIKH (1995), BODNAR *et al.*, (1996) and BUNACIU *et al.*, (1996) concluded that sperm concentrations usually decrease significantly due to the effect of frequent ejaculations. In the present study, lack of significant differences in sperm concentrations could be the consequence of the small number of ejaculates obtained per buck and per week with the semi-intensive rhythm.

Table 3 shows the number of insemination doses produced per ejaculate and per week. The extensive rhythm yielded more doses per ejaculate (13.73 $\pm$ 0.56 vs 11.20 $\pm$ 0.39; P<0.01), but fewer doses per week (23.3 $\pm$ 2.0 vs 28.8 $\pm$ 1.5; P<0.05). The number of doses/ejaculate agrees with the values of 9.2 and 10.3 reported by LOPEZ *et al.*, (1996) who used 25 million

**Table 3:** Mean number  $\pm$  standard error of insemination doses/ejaculate and doses/week. Calculated for doses of 0.5 ml containing 20 million spermatozoa.

	Doses/ejaculate	Doses/week
Semi-intensive group	11.20 $\pm$ 0.39 <sup>B</sup>	28.8 $\pm$ 1.5 <sup>a</sup>
Extensive group	13.73 $\pm$ 0.56 <sup>A</sup>	23.3 $\pm$ 2.0 <sup>b</sup>

Means in the same column with differing superscripts differ (A, B: P < 0.01; a, b : P<0.05).

spermatozoa/dose. The number of doses/ejaculate reported by ARROITA *et al.*, (2000), if adjusted to 20 million spermatozoa/dose were 15.3 and 10.5 doses for extensive and semi-intensive rhythms, respectively. These are quite similar to the present study. However, the insemination doses/week reported by the above authors differ greatly from the present findings. Moreover, with semi-intensive rhythms they obtained almost twice as many doses as with extensive rhythms (45.5 vs 24.8). These results may be explained by the useful collection rate. In the present study, there was a clear difference in the useful collection rate between extensive and semi-intensive rhythms (75% and 59%, respectively) whereas ARROITA *et al.*, (2000) found the values to be similar for extensive and semi-intensive rhythms, (62% and 64% respectively). Thus the number of doses/week was only affected by the collection frequency.

The results of the present work indicate that a semi-intensive rhythm with two ejaculates per day collected on two consecutive days, provides less volume but does not worsen other ejaculate characteristics compared with an extensive rhythm. Such results, probably affected by the low number of ejaculates obtained, suggest that semi-intensive rhythms should be used, at least at times of the year that are less favourable for male *libido*, in order to obtain a larger number of doses per week.

## REFERENCES

ARROITA Z., FALCETO M.V., MARTIN RILLO S., DE ALBA C., MORENO C., CIUDAD M.J., RAFEL O., 2000. Effect of collection frequency on production, quality and storage of young bucks semen. *7<sup>th</sup> World Rabbit Congress, Valencia, vol. A, 81-87.*

- BATTAGLINI M., 1992. Fecondazione artificiale, attenti al maschio. *Riv. Coniglic.*, **5**, 47-51
- BATTAGLINI M., COSTANTINI F., BOITI C., CANALI C., 1986. Ormoni steroidei, performance riproduttive dei maschi e fecondazione artificiale nel coniglio. *Riv. Coniglic.*, **3**, 56-59.
- BENCHEIKH N., 1995. Effet de la fréquence de collecte de la semence sur les caractéristiques du sperme. *Ann. Zoot.*, **44**, 263-279.
- BODNÁR K., TÖRÖK I., HEJEL P., BODNÁR E., 1996. Preliminary study on the effect of ejaculation frequency on some characteristics of rabbit semen. *6<sup>th</sup> World Rabbit Congress, Toulouse, vol. II, 41-44.*
- BUNACIU P., CIMPEAU I., BUNACIU M., 1996. Mating frequency effect on spermatogenesis and performance of breeding rabbits. *6<sup>th</sup> World Rabbit Congress, Toulouse, vol. II, 51-54.*
- CASTELLINI C., DAL BOSCO A., 1998. Inseminazione artificiale nel coniglio: fisiologia del maschio, produzione e conservazione del seme. *Large Animals Review*, **4** (3), 87-94.
- LOPEZ J., ALVARIÑO J.M.R., DEL ARCO J.A., BUENO A., SANZ C., 1996. Effect of male rabbit management on semen production. *6<sup>th</sup> World Rabbit Congress, Toulouse, vol. II, 83-86.*
- PANELLA F., CASTELLINI C., 1990. Fattori ambientali e genetici che influiscono sulle caratteristiche del seme di coniglio. *Riv. Coniglic.*, **8**, 39-41.
- PAUFLER S., SCHLOLAUT W., LANGE K., 1979. Post-partale Insemination beim Kaninchen mit Ovulationsauslösung durch synth LH-Release hormone. *Zuchthygiene*, **14** (1), 37-42.
- THEAU-CLÉMENT M., ROUSTAN A., 1992. A study on relationships between receptivity and lactation in the doe and their influence on reproductive performances. *J. Appl. Rabbit Res.*, **15**, 412-421.
- ZEMJANIS R., 1970. Diagnostic and therapeutic techniques in animal reproduction. *2<sup>n</sup> Ed. Balliere Tindall and Cox Ltd, London.*