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EFFECT OF COLLECTION FREQUENCY ON PRODUCTION, QUALITY AND STORAGE OF YOUNG BUCKS SEMEN

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ABSTRACT

This work is focused on one of the most interesting factors in artificial insemination: the semen collection frequency. Eighteen bucks from the IRTA sire line were exposed to one of three different semen collection rhythms (6 males/rhythm). We collected two ejaculates per male with an interval of 10-15 minutes between them, one, two or three days per week. We assessed the ejaculates in reference to sperm quality, production of insemination doses and sperm conservation capacity. We found that sperm concentration and insemination doses produced per ejaculate, decreased as collection frequency increased. On the other hand, the number of insemination doses produced per week was higher in the more intensive rhythms than in the extensive one, without any significant effect of rhythm on sperm motility and normal acrosome percentage after 72h of storage.

INTRODUCTION

Numerous authors over the last several years have studied semen collection rhythms (MCNITT, 1981). For example, the number of collections per day can vary between one (BODNÁR et al., 1996; BUNACIU et al., 1996) and four (LÓPEZ et al., 1996; BUNACIU et al., 1996), although beginning with the third ejaculate, semen volume, sperm concentration and number of insemination doses produced decrease (LÓPEZ et al., 1996). Collection days per week can also vary from one (BENCHEIKH, 1995; BUNACIU et al., 1996) to daily collections (BODNÁR et al., 1996). In this case, characteristics such as volume and sperm concentration decrease as collection rhythm increases (BENCHEIKH, 1995; BODNÁR et al., 1996; BUNACIU et al., 1996), whereas number of spermatozoa collected per week is higher in the more intensive rhythms (BENCHEIKH, 1995). Different results can be found in literature about other characteristics such as motility (BENCHEIKH, 1995; BODNÁR et al., 1996).

The previous authors show that semen collection frequency can affect not only sperm quality but also production of insemination doses. That´s why we think it´s an interesting factor in artificial insemination practice.

The present work was carried out to study the effect of three different semen collection rhythms on semen quality, production of insemination doses and storage capacity for 72h, in young bucks.

MATERIAL AND METHODS

Eighteen young bucks (7 months old) from the terminal sire line selected by the IRTA, were exposed for the period May-July 1999 to one of the following three rhythms:

Extensive: 2 ejaculations on Tuesday
Semintensive: 2 ejaculations on Monday and another 2 on Thursday
Intensive: 2 ejaculations on Monday, 2 on Wednesday and 2 on Friday

The ejaculates were collected with an interval of 10-15 minutes between the first and the second one.

Volume and colour of the ejaculate were noted (to detect contaminations with urine), and motility was assessed between 0 and 100% using a light microscope at x100. A semen sample was taken to be diluted 1:100 in 3% NaCl (FINZI et al., 1995) and counted in a Bürker Chamber to calculate sperm concentration.

The ejaculate was stored at 35ºC in a waterbath until dilution 10 minutes after collection. All samples were diluted 1:10, in a commercial saline extender for rabbit semen (KUBUS S.A., Madrid, Spain).

After collection and dilution of the second ejaculate from each male, both ejaculates were mixed. A semen sample was then fixed in a 2% glutaraldehyde solution for assessment of the percentage of sperm abnormalities and normal acrosomes by a bright field contrast microscope at x1000. Afterwards, we took a sample of 1.5 ml to be stored for 72h at 16ºC. At 24h, 48h and 72h after collection time, sperm motility and percentage of normal acrosomes were assessed in this stored sample.

Only ejaculates not contaminated with urine, with a percentage of motile spermatozoa higher than 50% and at least enough sperm concentration for preparing one insemination dose, were considered useful ejaculates. If one of the two daily ejaculates per male wasn´t useful, we didn´t mix them and we worked with the useful ejaculate. The insemination doses were calculated at 15 million of spermatozoa per dose.

From the previous results, we calculated the useful collection rate (useful ejaculates/total collections tried) and the insemination doses produced per ejaculate and per week for the three rhythms (insemination doses = (sperm concentration x semen volume)/15 million).

Statistical analysis was carried out using the LSMLMW program (Mixed Model Least-Squares and Maximun Likelihood Computer Program (HARVEY,1987).

RESULTS AND DISCUSSION

- Useful collection rate

The overall mean rate of useful collections obtained was 62 ± 7.1%. There was no significant difference between the mean percentage of useful collections of the three collection rhythms (Table 1). BENCHEIKH (1995) found a significantly higher useful collection rate in the extensive rhythm.

Table 1: Useful collection rate

Collection frequency	**n**	**Mean ± s.e.**
Extensive rhythm	108	0.62 ± 0.11
Semintensive rhythm	210	0.64 ± 0.10
Intensive rhythm	322	0.60 ± 0.10

We observed no difference because, the decrease of the useful collection rate of the first daily semen collection in the intensive rhythms, was compensated by the increase of the rate of the second daily semen collection in these rhythms (Table 2). In addition, the number of observations in BENCHEIKH´s experiments (1226 collections in the extensive rhythm vs 2856 in the intensive one, and 391 collections in the extensive rhythm vs 701 in the semintensive one) was higher than our number of observations.

Table 2: Useful collection rate. (n=number of observations)

Collection frequency	Fist ejaculate Mean ± s.e. (n)	Second ejaculate Mean ± s.e. (n)
Extensive rhythm	0.59 ± 0.095 (54)	0.64 ± 0.095 (54)
Semintensive rhythm	0.43 ± 0.088 (105)	0.86 ± 0.088 (105)
Intensive rhythm	0.42 ± 0.085 (162)	0.79 ± 0.085 (160)

- Semen quality characteristics

Among the semen characteristics studied - volume per ejaculate, motility and normal acrosomes at collection time, sperm abnormalities and sperm concentration - only the last one showed significant differences between rhythms (Table 3).

Sperm concentration decreased significantly as collection frequency increased. BENCHEIKH (1995), BODNAR et al. (1996) and BUNACIU et al. (1996) also obtained higher concentrations in the extensive rhythms. This is likely associated with a decrease of the epydidimal reserves due to the use of intensive semen collection frequencies (BENCHEIKH, 1995). In contrast to our results, BENCHEIKH (1995) presents significantly higher means for volume/ejaculate and motility at collection time in the extensive rhythm compared to the intensive ones. BODNÁR et al (1996) also found a significantly higher mean volume in the extensive rhythm whereas they didn´t find significant differences in motility means between rhythms.

Volume and concentration were assessed in each of the two daily ejaculates from each male, whereas the rest of the characteristics were assessed after the ejaculates were mixed (to simplify the work), so there are more observations for volume and concentration. The low numbers of observations for normal acrosomes are due to the unavailability of the data for the first four weeks of the experiment.

Although we obtained a high mean percentage of abnormal spermatozoa (29.8%) compared with most literature, which is around 20% (FINZI et al., 1995; MARTÍN, 1992), this mean is in accordance to other authors such as DUCCI et al. (1993), who found a mean percentage of 33.5% of abnormal spermatozoa for the first ejaculate and for the second ejaculate a mean of 28.64% ($p<0.01$). Environmental temperatures higher than 25ºC suffered by the bucks for some days during the experimental period could have induced an increase in the mean percentage of abnormal spermatozoa, as was described by FINZI et al. (1995). In addition, these were young males at the beginning of their breeding life and some of them still showed a high percentage of cytoplasmic droplets.

Table 3: Ejaculate characteristics. Mean ± s.e. (n= number of observations)

	Extensive rhythm	Semintensive rhythm	Intensive rhythm	Overall mean
Concentration (10^6/ml)	317.26 ± 43.41^a (55)	257.72 ± 42.09^b (115)	183.92 ± 39.57^c (173)	----
Volume (ml)	0.99 ± 0.083 (52)	0.76 ± 0.080 (107)	0.76 ± 0.079 (172)	0.84 ± 0.05 (331)
Motility (%)	70.93 ± 4.63 (40)	68.84 ± 4.51 (93)	70.60 ± 4.50 (137)	70.13 ± 5.16 (270)
Normal acrosomes (%)	76.37 ± 4.24 (17)	82.48 ± 4.11 (34)	80.77 ± 3.87 (48)	79.87 ± 4.46 (99)
Sperm abnormalities (%)	27.29 ± 3.77 (40)	35.48 ± 4.26 (84)	26.63 ± 4.38 (128)	29.8 ± 3.36 (252)

Values with different superscript differ significantly. ($p<0.05$)

- Insemination doses/useful ejaculate and per week

Due to the significant differences found among rhythms in the mean concentrations, there were also significant differences among insemination doses produced per ejaculate in the three collection frequencies. Table 4 shows that the number of insemination doses produced per ejaculate decreased as collection frequency increased.

Table 4: Mean number of insemination doses/ejaculate and per week. Calculated for doses of 0.5 ml and 15 million spermatozoa.

Collection frequency	**n**	**Doses/ejaculate Mean± s. e.**	**n**	**Doses/week Mean±s.e.**
Extensive rhythm	51	20.45 ± 1.94^{a}	44	24.84±9.68
Semintensive rhythm	100	13.99 ± 1.82^{b}	38	45.52±9.87
Intensive rhythm	155	10.88 ± 1.71^{b}	43	37.89±9.70

Means in the same column with different superscript differ significantly. ($p<0.05$)

The intensive rhythms yeilded more doses per week than the extensive one. The semintensive rhythm yeilded 20 doses more than the extensive rhythm (the most commonly used rhythm) and the intensive rhythm obtained 13 doses more than the extensive one (Table 4). Despite the large differences found between the means of the intensive rhythms compared to the mean of the extensive one, these differences weren´t statistically significant. The overall mean was 36.08 ± 5.64 insemination doses/week.

The values of insemination doses/week can vary from 0 doses, when no useful ejaculate is obtained from the buck, to more than 80 doses, when all the ejaculates produced by the male under the intensive system are useful. In addition, there is a lower number of observations when we analyze insemination doses produced per week, than when we analyze insemination doses produced per ejaculate. These factors may account for the lack of significance despite the large numerical differences. In relation to this, BENCHEIKH (1995) found that the number of total and living spermatozoa collected per week increased significantly in the intensive rhythms.

- Conservation capacity

Finally, we studied the effect of semen collection frequency on semen conservation capacity. With this aim, we assessed sperm motility and percentage of normal acrosomes at 24, 48 and 72 hours of storage.

Table 5. Sperm motility. Mean ± s.e. (%). (n=number of observations)

Time of conservation	**Extensive rhythm**	**Semintensive rhythm**	**Intensive rhythm**	**Overall mean**
0h	70.93 ± 4.63 (40)	68.84 ± 4.51 (93)	70.60 ± 4.50 (137)	70.13 ± 5.16^{a} (270)
24h	36.18 ± 4.63 (40)	30.76 ± 4.53 (92)	27.55 ± 4.57 (129)	31.50 ± 5.15^{b} (261)
48h	28.93 ± 4.63 (40)	27.60 ± 4.62 (85)	21.53 ± 4.58 (128)	26.02 ± 5.14^{c} (253)
72h	26.18 ± 4.63 (40)	20.78 ± 4.62 (85)	18.29 ± 4.59 (127)	21.75 ± 5.14^{d} (252)

Values with different superscript differ significantly. ($p<0.05$)

No significant differences were found among the motility means of the three rhythms during the storage period and we didn´t find any significant rhythm x time of storage interaction.

On the other hand, the overall sperm motility mean decreased significantly as the storage time inceased (Table 5).

With regard to the percentage of normal acrosomes, no significant differences were found among the means of the three rhythms during the storage period and we didn´t find any significant rhythm x time of storage interaction. The overall mean percentage of normal acrosomes also decreased significantly as storage time increased (Table 6). The decrease in the percentage of normal acrosomes was not as pronounced as for motility. In fact, the mean after 24h of storage was a 12.30% lower than the mean at collection time and, after 72 h of storage was a 24.68% lower than the mean at collection time.

Table 6: Percentage of normal acrosomes. Mean ± s.e. (%). (n= number of observations)

Time of conservation	Extensive rhythm	Semintensive rhythm	Intensive rhythm	Overall mean (n)
0h	76.37 ± 4.24 (17)	82.48 ± 4.11 (34)	80.77 ± 3.87 (48)	79.87 ± 4.46 [a] (99)
24h	58.94 ± 4.24 (17)	76.50 ± 3.92 (41)	76.11 ± 3.77 (54)	70.05 ± 4.48 [b] (112)
48h	57.94 ± 4.24 (17)	73.85 ± 3.92 (41)	68.43 ± 3.78 (53)	67.08 ± 4.48 [c] (111)
72h	53.24 ± 4.24 (17)	67.70± 3.92 (41)	59.70 ± 3.92 (54)	60.15 ± 4.97 [d] (112)

Means in the same column with different superscripts differ significantly. ($p<0.05$)

This experiment was carried out with young bucks and for a period of only two months. To confirm the results another study for a longer period with bucks of several ages should be carried out.

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RESUMEN

Este trabajo se centra en uno de los factores que más interés puede tener en inseminación artificial: el ritmo de recogidas seminales al que es sometido el conejo macho. Se utilizaron 18 machos de la línea de alto crecimiento del IRTA que fueron sometidos a 3 ritmos de recogida diferentes (6 machos/ritmo). En los 3 ritmos a estudio se recogieron 2 eyaculados por macho con un intervalo de 10-15 minutos, siendo la frecuencia de extracciones de 1, 2 o 3 días a la semana. Se evaluaron características referentes a la calidad seminal, a la producción de dosis seminales y a la capacidad de conservación del semen. De este modo, se comprobó que la concentración espermática del eyaculado, así como el número de dosis seminales/eyaculado disminuyen conforme se intensifica el ritmo de recogida. Sin embargo la producción total de dosis/semana es mayor en los ritmos más intensivos, no apreciándose ningún efecto significativo del ritmo sobre la motilidad y el porcentaje de acrosomas normales del semen durante las 72h de conservación.

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