EFFECT OF SEMEN CONCENTRATION ON RABBIT ARTIFICIAL INSEMINATION WITH FRESH OR 24 HOURS STORED SEMEN

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Abstract - The effect of semen concentration on fertility and prolificacy has been studied, considering a range from 60 to 8 million spermatozoa/dose. A total of 1386 artificial inseminations were carried out using fresh or 24 hours preserved semen. A limit placed between 26 and 20 million spermatozoa/dose was established in order to maintain a good performance of artificial insemination. A dose of 20 million affects negatively fertility and a dose of 14 million also lowers the litter size. Overall the lowest advisable limit for semen concentration should be over 20 million, and 26 million are considered to be enough to allow good artificial insemination results.

INTRODUCTION

One of the factors which restrict the number of males in a rabbit farm or a rabbit semen production centre is the dilution rate of the ejaculates.

The number of spermatozoa needed to guarantee the fertilization in rabbits has been subjected to different proposals, from 9 x 10^4 (CHANG and CASIDA, 1948), 4 x 10^5 (CHANG, 1946), 10^6 (WALES *et al.*, 1965) 10^5 - 10^6 (ROUSTAN, 1982), 8 - 10 x 10^6 (COSTANTINI, 1986), 5 x 10^6 (KHALIFA, 1994) or 10^6 - 20^6 (ADAMS, 1972). WALES *et al.* (1965) found that fertility rate is lowered under 2 x 10^5 spermatozoa, and that no fertilization is obtained under 4 x 10^4 spermatozoa.

Sperm concentration in rabbit ejaculates is around 500 x 10^6 spermatozoa/ml (BATTAGLINI *et al.*, 1992), so a dilution rate of 1:100 should theoretically cover the minimum spermatozoa required to fertilize rabbit oocytes. Nevertheless the usual practice in artificial insemination (A.I.) is associated to a dilution rate between 1:5 and 1:10 and no information is available about the real influence of sperm concentration when A.I. is performed on a large scale basis. The aim of this work is to determine the lower limit of sperm concentration able to maintain a satisfactory fertility and prolificacy when diluted sperm is preserved during 24 hours.

MATERIAL AND METHODS

A total of 1386 inseminations where performed on lactating females on day 4 postpartum. Ovarian activity was stimulated by 20 UI of PMSG (Sincro Gest, Laboratorios Ovejero, León, Spain) injected (i.m.) 48 hours before A.I.

Ejaculates were collected using an artificial vagina and semen quality was assessed under microscope. Only ejaculates with over 60% of mobile sperm were selected. Sperm concentration was determined by a Burker's counting camera (BAGLIACCA *et al.*, 1987; DIAZ *et al.*, 1989) after pooling the selected ejaculates. The original pool was used to reach progressive dilution rates by adding a commercial extender (MA 24, Laboratorios Ovejero, León, Spain). Final sperm concentrations were 64, 32, 26, 20, 14 and 8 million spermatozoa/ ml (Experiment I). In Experiment II and III, does were inseminated with fresh semen, comparing 60 vs 30 (Experiment II), and 60 vs 20 (Experiment III) million spermatozoa/dose. In Experiment I diluted sperm was preserved during 24 hours at 18° C using a programmable fridge.

Ovulation was induced by 20 mg of GnRH (Inducel GnRH, Laboratorios Ovejero, León, Spain). One ml. of diluted sperm was used to inseminate each female.

Statistical analysis of the results was carried out using the non parametric Analysis of Variance (CATMOD procedure) for means comparison of fertility and the ANOVA (GLM procedure) followed by the Duncan test to compare the means of prolificacy (SAS, 1987).

RESULTS

The results obtained in Experiments I, II and III are shown in Tables 1 and 2.

In Experiment I with 24 hours stored semen a limit placed between 26 and 20 million spermatozoa/dose was established in order to maintain a good performance of A.I. either for fertility or for prolificacy ($p<0.001$).

When using fresh semen the decrease of sperm concentration from 60 to 30 did not affect the A.I. performance, but significant reduction in the conception rate was detected for A.I. carried out with 20 million spermatozoa /dose ($p<0,05$).

Litter size was affected by spermatozoa concentration, so that mean litter size was lowered 1.4 and 2.3 pups when 14 or 8 million spermatozoa/doe were employed ($p<0,01$).

Table 1 : Effect of sperm concentration on the fertility and prolificacy of does inseminated with 24 hour stored diluted semen (Experiment I)

Sperm concentration (million /dose)	Conception rate %	Born dead/litter (Mean ± S.E.M)	Litter size/at birth (Mean ± S.E.M)	
64	72,91 A (96)	0,69 ± 0,18	7,14 ± 0,41	a
32	78,70 A (198)	0,37 ± 0,16	7,79 ± 0,31	a
26	74,50 A (51)	0,07 ± 0,04	7.10 ± 0,5	a
20	65,21 B (46)	1,21 ± 0,43	7,67 ± 0,64	a
14	61,38 BC (145)	0,46 ± 0,19	6,26 ± 0,34	b
8	45,80 C (131)	0,51 ± 0,23	5,40 ± 0,46	c

( ) Number of inseminations
A,B,C: Significant difference ($p< 0,001$)
a.b.c: Significant difference ($p< 0,01$)

Table 2 : Effect of sperm concentration reduction from 60 to 30 and 60 to 20 million/dose on fertility and prolificity of does inseminated with fresh semen

	Experiment II		Experiment III	
Sperm concentration million /dose	60	30	60	20
Conception rate %	73,97 (196)	79,65 (231)	66,87 b (157)	54,07 a (135)
Dead born/litter (mean ± SEM)	1.21 ± 0,25	0,98 ± 0,18	0,28 ±0.07	0,74 ± 0,24
Litter size at birth (Mean ± SEM)	8,04 ± 0,28	7,67 ± 0,22	8,25 ± 0,36	8,05 ± 0,35

( ) Number of A.I.
a, b: Significant difference ($p< 0,05$)

DISCUSSION

The limits established in this work seem to be higher than proposed by other researchers, but in fact we are not proposing the minimum sperm concentration to allow fertilization but to obtain good A.I results. If we accept that 26 million spermatozoa per insemination are necessary to reach an optimum performance, that means that about 20 million live spermatozoa are effectively employed per insemination, which differs greatly from other proposals (CHANG, 1946; CHANG and CASIDA, 1948; WALES *et al.*, 1965; HELLEMANN *et al.*, 1979; WEITZE, 1981; ROUSTAN, 1982; BATTAGLINI *et al.*, 1992) but agrees with Adams results (ADAMS, 1972).

The mean litter size at birth was less sensitive to low semen concentrations as only a negative effect was detected at 14 and 8 million spermatozoa/dose. We should remark that in our work structural damage of spermatozoa linked to cooling have not been evaluated, but we assume that the number of intact spermatozoa is well above 0,3 million, reported previously as enough to get satisfactory results in A.I. (WEITZE, 1981).

A similar work studying the effect of dilution rate, although with frozen-thawed semen showed that the conception rate and litter size at birth was maximum for a dilution rate of 1:10, but decreased for a dilution rate of 1:25, 1:50 or 1:100, the litter size being specially affected (THEAU-CLEMENT and ROUSTAN, 1982). In our work the mean sperm concentration was 585 million spermatozoa/ml, so a dilution rate of 1:29 gives 20 million per ml, which is in the range of relatively poor results cited above.

The attempt to determine the lower limit of sperm concentration when A.I. is performed with 24 hours stored semen has led to a clear conclusion: a dose of 20 million negatively affects fertility and a dose of 14 million also negatively affects prolificacy. When fresh semen is employed it seems clear that the reduction from 60 million to a half does not affect either fertility or prolificacy but the reduction to a third, which means 20 million spermatozoa/dose lowers the conception rate by 12%. Overall, experiments I, II and III agree, so we can conclude that the lowest advisable limit should be over 20 million spermatozoa / dose. In practice that means that 26 million spermatozoa per dose is enough to allow good results when A.I. is performed either with fresh or 24 hour preserved diluted semen.

Acknowledgements - This work has been supported by the MINER (Spain) through the CDTI Project 95.0002 and the PATI Project 56/ 95. Authors are grateful to DELGADO F., OCHOA M.J., GUTIERREZ M.F., RAMIRO J.L., DE GRACIA J., SANZ C. and CARRASCO J. for their technical assistance.

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