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USE OF DIFFERENT DILUENTS WITH A LOW NUMBER OF SPERMATOZOA BY INSEMINATION DOSE IN RABBIT.

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ABSTRACT

The aim of this study was to show the efficacy of artificial insemination employing three different diluents (A, B and C). These diluents were : (A) Galap (IMV, France), (B) DLBR (Tris-citric-glucose), (C) MA24 (Laboratorios Ovejero, León, Spain). The inseminations were done in a commercial farm. A total of 8 million of spermatozoa per dose were inseminated. A total of 2125 artificial insemination were done (601 with A, 756 with B, and 768 with C). No differences were found in fertility (66%) and litter size at birth (10.21± 0.10). These results showed that low concentration of spermatozoa can be used with different extenders to obtain normal fertility rate and prolificacy in a commercial farm.

INTRODUCTION

In Spain, artificial insemination (A.I.) in rabbit meat production has become widespread recently. Artificial insemination is valuable tool for rabbit producers because the population of males as well as reproductive management time could be reduced.

Although storage time for refrigerated semen could be 72 hours, the practical application of artificial insemination is performed only with fresh or briefly stored diluted semen. Generally, 16 to 20 million spermatozoa are employed per dose (Theau-Clément and Roustan, 1980; Rebollar *et al.*, 1992 and 1995 ; Alvariño *et al.*, 1996), so the number of inseminations per male is reduced to 10-30. Viudes de Castro and Vicente (1996) showed that it was possible to obtain normal fertility rate and prolificacy employing a lower concentration of spermatozoa per dose (4 million spermatozoa per dose, 74% does gave birth, 9.5 live young rabbits), so an ejaculate of 200 - 400 million spermatozoa may contain sufficient number of motile sperm to inseminate 40-80 does.

One of the most important factors which could affect the results is the diluent (Egea and Roy, 1992).

The aim of this work was to compare the response of three diluents when the A.I. was carried out in a commercial breeding farm using a low concentration of spermatozoa per dose.

MATERIALS AND METHODS.

Animals :

Does from a crossbred AxV line were inseminated with semen of adult males belonging to a growth line (line R). Lines V and A were selected on the basis of litter size at weaning (Estany *et al*., 1988). Line R was selected on the basis of growth rate from weaning

to slaugther (28- 63 days of age, Estany *et al.,* 1992). The experiment was performed from October to January.

Semen collection :

Semen was collected from males kept in a separate place, under a 16 hours/ day photoperiod. Two ejaculates per male were collected each week using an artificial vagina. Each ejaculate was examined under microscope and the percentage of motile sperm was subjectively estimated. After semen evaluation, only ejaculates over 70% progressive motility were pooled and extended in three different diluents : MA24 (Laboratorios Ovejero, León, Spain), Galap (IMV, France) and DLBR (Tris-citric-glucose, Viudes de Castro and Vicente, 1996). Sperm concentration was measured by a Thoma-Zeiss counting cell chamber and after that, the number of spermatozoa per dose was adjusted to 8 million. The semen was diluted at room temperature (20-25ºC).

To measure acrosomal integrity (NAR) of each pool, samples taken from each were fixed with glutaraldehyde 2% in Dubelcco's phosphate buffered saline (Pursel and Johnson, 1974) and the proportion of sperm with normal, intact acrosomes was estimated using interference contrast optics at a magnification of x750.

Insemination :

Two days before insemination does were checked for receptivity. If the vulva colour was pale or white, females were injected subcutaneously with 12 I.U. of PMSG (Gonaser, Hipra). Nonpregnant does at the 12^{th} day post insemination were injected intramuscularly with 0.2 ml of cloprostenol (Planate, Schering-Ploug).

Using a curved glass pipette (0.5 mm diameter) does were inseminated with 0.5 ml of extended semen. The inseminations were made within two hours following semen collection. After insemination does were immediately injected with 0.8 μg buserelin acetate (Hoechst). Reproductive status of doe (nulliparous, primiparous lactating and multiparous lactating or not lactating) was noted. Around 200 A. I. were carried out every week on a commercial farm. Females were assigned randomly to the three experimental groups (Galap, DLBR, MA24).

Statistical analysis :

Chi-squared test with Yate's correction was used to analyze the effects of reproductive status and diluent on fertility rate at birth. The effects of extender, reproductive status and their interaction on prolificacy were evaluated by an analysis of variance. Total kits born was included as a covariate in the analysis of the number of kits born alive.

RESULTS AND DISCUSSION

No differences in acrosomal integrity were observed between diluents (86%). Conception rates, total kits born and kits born alive were not different among groups. The overall averages were 66%, 10.21 ±0.10, and 9.53 ±0.11, respectively (Table 1).

In general, artificial insemination is performed with a relatively high number of spermatozoa per female, (16-30 million) from a heterospermic pool stored a short period of time (2-24 hours), with a doe:buck ratio around 10-15 (Rebollar and Alvariño, 1997). In previous studies, with a simple diluent prepared in our laboratory (DLBR), we have demonstrated that it is possible to work with a heterospermic pool with a low number per dose for 24 hours (6 and 12 million, 0-12 and 24-30 hours respectively), increasing the doe:buck ratio to 30-40 (Viudes de Castro *et al*, 1999).

Table 1 : Effect of diluent on conception rate and litter size at birth of A. I. does.

Diluent	No. Inseminations	Conception rates n (%)	Kits born alive (n) Mean ±SE	Total kits born (n) Mean ±SE
Galap	601	398 (66%)	9.63 ±0.21	10.40 ±0.19
DLBR	756	497 (66%)	9.36 ±0.18	10.16 ±0.17
MA24	768	507 (66%)	9.60 ±0.18	10.08 ±0.17
TOTAL	2125	1402 (66%)	9.53 ±0.11	10.21 ±0.10

Mean ± SE : LS Means ±standard error.

In the present study we demonstrated that it is possible to reduce the number of spermatozoa to 8 million using two commercial diluents (MA24 and Galap) in farm conditions without effects on the conception rate, or numbers of kits born and born alive. Makers of the commercial diluents suggest preparing the insemination doses with 30 million spermatozoa. This work indicates that it is not necessary with auto-insemination. These results are in agreement with those obtained by Maertens and Luzi, (1995), with two different diluents, and by Castellini *et al*, (1999) who reduced the sperm dose per female to 10 million.

The effect of reproductive status is shown in Table 2 Nulliparous does had higher conception rates (84%) than primiparous or multiparous does (lactating or not, 63-67%); while total number of kits born and born alive were higher in primiparous lactating does (10.20 ±0.26). This is in agreement with Perrier *et a.*, (1998) and Viudes de Castro *et al.* (1999).

No interactions were found between reproductive status and diluent for the total number of kits born or the number born alive.

In general the reproductive results obtained are comparable with other experiments in industrial rabbitries (Pizzi *et al.,* 1996 ; Alvariño *et al*., 1996 ; Viudes de Castro *et al*., 1998).

Table 2 : Conception rate and litter size according to the physiological status of does.

Status of doe	No. Inseminations	Conception rates n (%)	Kits born alive (n) Mean ±SE	Total kits born (n) Mean ±SE
Nulliparous	244	204 (84%)^{a}	9.17 ±0.23^{b}	10.24 ±0.22
Primiparous, lactating	240	161 (67%)^{b}	10.20 ±0.26^{a}	10.66 ±0.25
Multiparous, lactating	1336	842 (63%)^{b}	9.50 ±0.11^{b}	10.02 ±0.11
Multiparous, not lactating	305	196 (64%)^{b}	9.24 ±0.11^{b}	9.93 ±0.22
TOTAL	2125	1402 (66%)	9.53 ±0.11	10.21 ±0.10

a,b, : values in the same column with different superscripts differ statistically (P<0.05).

Mean ± SE : L.S. Means ±standard error.

The type of diluents used does not limit the possibilities of insemination with a low number of spermatozoa.

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