EFFECT OF COOLING TEMPERATURE ON 24 HOURS STORED SEMEN FOR ARTIFICIAL INSEMINATION OF RABBITS

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Abstract - The effect of cooling temperature during 24 hours on fertility and prolificacy was studied, considering a range from 25 to 6°C. Best results were obtained in the range 17 to 19°C, 18°C being the recommended cooling temperature when the commercial extender MA 24 is employed. In this conditions fertility can overcame 80% and litter size reaches 9 pups/litter. Temperatures over 19 and under 17°C affected negatively the conception rate. Litter size was significantly affected, best results being obtained at 18°C. No correlation has been found between mobility at 24 hours and fertility or litter size.

INTRODUCTION

Artificial Insemination (A.I.) is rapidly approaching the point where it can be employed on a large scale basis in rabbits farms. One of the main problems is the ability to preserve rabbit semen in order to transport it to the farms where AI is performed. Cooling is presently the most practical way to keep rabbit spermatozoa alive for periods of 24-48 hours (SINKOVICS *et al.*, 1983; FACCHIN, 1992; ALVARIÑO, 1993). The most frequently studied range of cooling temperature has been 4 to 6°C (COSTANZO, 1985; BATTAGLINI *et al.*, 1988; BONANNO and FACCHIN *et al.*, 1988; COSTANTINI, 1988; ANSELMINO and TOMATIS 1989; FREYCHAT *et al.*, 1989; PRIGENT, 1989; THEAU-CLEMENT and ROUSTAN, 1991), although recently research has focused on higher preservation temperatures, and better mobility (GOTTARDI, 1993; BERGONZONI and ZAMBELLI, 1994) and acrosomal integrity (BERGONZONI and ZAMBELLI, 1994) has been found at 15°C. Although numerous works have studied the effects of cooling on mobility and spermatozoa alterations, little information is available about the influence on A.I. performances. This work attempts to define the optimum cooling temperature to preserve for 24 hours rabbit semen, according to the fertility and prolificacy obtained through A.I. with a commercial extender. A wide range of cooling temperatures is considered and tested under the same A.I. conditions.

MATERIALS AND METHODS

A total of 1303 inseminations where carried out 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. Mobility was subjectively estimated after dilution on a 0-10 scale, based on the percentage of mobile spermatozoa. The initial appreciation was corrected according to the type of movement (straight, circular or without displacement) and the velocity. The same person assessed mobility in all cases. Only ejaculates with over 60% of mobile spermatozoa were selected. Sperm concentration was determined by a Burker's counting camera (BAGLIACCA *et al.*, 1987; ALVARIÑO, 1993) after pooling the selected ejaculates. The original pools were employed to reach a 30 million spermatozoa/ml concentration by adding a commercial extender (MA 24, Laboratorios Ovejero, León, Spain).

Experimental designs

Experiment I - 880 A.I. were performed to test the effect of cooling temperature. Diluted sperm was preserved during 24 hours at 15 17, 18, 19, 21 and 25° C using a programmable fridge, (Magapor, Zaragoza, Spain), or at 6° and 11° C in a domestic fridge.

Experiment II - 423 females were inseminated with 24 hours preserved semen at 18°C to test the effect of low mobility preserved ejaculates on fertility and prolificacy.
Ovulation was induced by 20 µg of GnRH (Inducel GnRH, Laboratorios Ovejero, León, Spain). One ml. of diluted sperm and a sterilized glass cannula 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 mobility, and the ANOVA (GLM procedure) followed by the Duncan test to compare the means of prolificacy (SAS, 1987).

RESULTS AND DISCUSSION

Results obtained in Experiment I are shown in Table 1. Fertility was significantly affected by cooling temperature, reaching the highest values in a range of 17 to 19°C and decreasing at lower (15, 11 and 6) or higher (21 and 25°C) cooling temperatures ($p < 0.0001$). This could be explained by the effect of temperature on mobility, acrosomal damages and depletion of endogenous energetic reserves. Temperatures under 17°C are not beneficial to rabbit spermatozoa preservation because although metabolic activity slows down, acrosomal damage increases (GOTTARDI, 1993). Temperatures over 19°C would have a negative effect because of a lower acrosome integrity (GOTTARDI, 1993) probably associated to a partial alteration of the ability to employ exogenous energetic resources.

Table 1 : Effect of cooling temperature during 24 hours on A.I. performance of lactating females (day 4 after parturition)

	COOLING TEMPERATURE							
	6°C	11°C	15°C	17°C	18°C	19°C	21°C	25°C
Stimated mobility (0 hours)	8,75	9	8,5	8,3	9	8,75	8,5	9
Stimated mobility (24 hours)	0,5	1,5	3	2,6	2,3	2,1	2,3	3,75
Conception rate (%)	68,31 A (101)	67,36 A (95)	65,21 A (46)	82,89 B (152)	82,85 B (105)	86,60 B (209)	74,50 A (102)	71,42 A (70)
Born dead / litter (Mean I SEM)	1,11±0,30 a	1,07±0,33 a	0,50±0,20 b	0,47±0,11 b	0,41±0,13 b	0,51±0,10 b	0,36±0,16 b	0,46±0,17 b
Litter size (Mean ±SEM)	8,147±0,36 AB	8,872±0,33 A	7,857±0,50 AB	7,299±0,24 B	9,108±0,30 A	8,167±0,24 AB	8,573±0,28 AB	8.000±0,44 AB

( ) Number of A.I.
A,B : significant difference ($p<0,001$)
a,b: significant difference ($p<0,05$)

Our results show that it is possible to preserve rabbit spermatozoa during 24 hours at a wide range of cooling temperatures (6 to 25°C), which is in agreement with previous works (SINKOVICS *et al.*, 1983; BONANNO and COSTANZO, 1985; BATTAGLINI *et al.*, 1988; COSTANTINI, 1988; FACCHIN *et al.*, 1988; ANSELMINO and TOMATIS 1989; FREYCHAT *et al.*, 1989; PRIGENT, 1989; THEAU-CLEMENT and ROUSTAN, 1991; FACCHIN, 1992; ALVARIÑO, 1993). Although recent research showed better mobility and acrosome integrity at 15°C (GOTTARDI, 1993; BERGONZONI and ZAMBELLI, 1994), in our work best A.I. results are obtained at 18 and 19°C, 18°C being the preservation temperature recommended.

Litter size was affected by cooling temperature. The highest value was obtained at 18°C, and the lowest at 17°C ($p<0,001$), although a simple explanation can not be offered to understand this difference. Mortality at birth was increased at 6 and 11°C compared to higher semen preservation temperatures ($p<0,05$). A similar increase in birth mortality was reported in PMSG stimulated does (MAERTENS and LUZI, 1995), which suggest that some mechanisms could exist relating gamete quality and birth mortality. Further research is needed to ascertain if modifications experienced by spermatozoa previous to fertilization are expressed at the end of pregnancy.

Mobility at 24 hours is greatly reduced in relation to the observation made immediately after semen dilution. It seems clear that relatively high cooling temperatures (25°C) show higher mobility than low ones (6 an 11°C), but it has no practical consequences as no correlation has been found between mobility after 24 hours and fertility. Results obtained in Experiment II are shown in table 2. Only pooled ejaculates showing a mobility score under 1 gave rise to a lower conception rate ($p<0,01$), although results were not disastrous and litter size was normal. That means that apparently dead spermatozoa are only in a sleeping state and are reactivated once in the female genital

tract. A similar phenomena can be detected when low mobility spermatozoa are heated (GOTTARDI, 1993) or caffeine is added to the culture medium (EL-GAAFARY, 1994).
Overall our results indicate that best results are obtained with a cooling temperature of 18°C, and that mobility after a 24 hours preservation period does not affect insemination performance if mobility is higher than 0,5. Even when no mobile spermatozoa are observed the conception rate is not disastrous, and a normal litter size is obtained.

Table 2 : Performance of A.I. carried out with low mobility 24 hour preserved semen

Score mobility* (24 hours)	1,5	1	0,5	0
Conception rate %	75,73 A (103)	80,91 A (220)	69,38 B (49)	66,66 B (51)
Born dead/litter (mean±SEM)	0,35±0,16	0,13±0,05	0,33±0,16	0
Litter size (mean±SEM)	8,45±0,32	8,33±0,36	8,40±0,41	8,55±0,56

( )Number of AI
*1,5: Around 15% spermatozoa show straight movement
1,0: Around 10% spermatozoa show straight movement
0,5: Around 5% spermatozoa show straight movement
0: No mobile spermatozoa were observed
A, B: Significant difference ($p<0,01$)

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