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ARTIFICIAL INSEMINATION OF RABBITS WITH DILUTED SEMEN STORED UP TO 96 HOURS

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ABSTRACT : The effect of different duration's rabbit semen storage at 18°C (2, 24, 48, 72, and 96 hours) on fertility and prolificacy has been studied in one experiment, where a total of 1447 artificial inseminations have been carried out using a new commercial extender, MA 24. The results indicate a similar fertility (84.14%, 83.56% and 79.73%) when increasing the time of conservation (2, 24 and 48 hours). Difference statistically significant were observed at 72 hours (67.59%) and fertility fell dramatically after 96 hours of conservation (39.23%). The number of total born kits per litter follows the same tendency than fertility, presenting a light descent in the first conservation intervals (8.92, 8.48 and 8.02) and an important reduction in the last ones (7.04 and 5.58). The conclusions of this work indicate that the extender MA 24 reaches a good performance for periods of conservation of the semen up to 48 hours in lactating females inseminated on day 4 postpartum, which can be considered good enough to develop a large scale utilisation of preserved rabbit semen.

RESUME : Insémination artificielle des lapines avec une semence diluée conservée jusqu'à 96 heures.
Cette expérimentation a pour but d'étudier l'effet sur la fertilité et la prolificité du temps de conservation à 18°C de la semence (2, 24, 48, 72 et 96 heures) ; 1447 inséminations artificielles ont été pratiquées, utilisant un nouveau dilueur MA 24. Les résultats montrent un taux de fertilité similaires (84,14%, 83,56% et 79,73%) pour les temps de conservation de 2, 24 et 48 heures, statistiquement différent pour 72 heures (67.59%) et considérablement plus bas (39,23%) pour 96 heures de conservation. Le nombre total de lapereaux nés vivants par portée suit la même tendance que le taux de fertilité, diminuant légèrement avec les premiers temps de conservation (8,92 ; 8,48 et 8,02) et fortement avec les autres (7,04 et 5,58). On peut conclure que le dilueur MA 24 donne de bons résultats pour les périodes de conservation allant de 2 à 48 heures pour les femelles allaitantes inséminées 4 jours post-partum, ce qui peut être considéré comme suffisant pour développer l'utilisation à large échelle chez le lapin de semence conservée.

INTRODUCTION

Artificial Insemination (AI) is being used in Italy, France, Spain and increasingly in the rest of Europe as a tool to improve the efficiency of the rabbit industry on a large scale basis. The advantages of the AI technique include the reduction in the number of males needed in the rabbit farm, the assessment of semen quality, the synchronisation of reproduction in fixed days of the week, and the increase of disease control. The advantages of AI have been admitted by several authors (COSTANTINI, 1986; SANFORD, 1986; HAFEZ, 1987; CHINELLATO *et al.*, 1991; TAWFEEK and EL-GAAFARY, 1991; ALVARIÑO, 1993).

Rabbit semen does not respond to dilution as in other species mainly because of its sensibility to hypertonic solutions (CASTELLINI *et al.*, 1992) and to cryoprotective agents containing hydroxyl groups, such as glycerol (MAURER *et al.*, 1976; HANADA and NAGASE, 1980; ARRIOLA, 1982). Unfortunately an effective cryoprotective agent has not been identified, so freezing is not yet a practical way to preserve valuable semen from selected bucks. Instead numerous attempts have been made to keep diluted semen during short periods of time, usually under 48 hours, by cooling it at 5 to 25°C. Most extenders are based on Tris-citric acid combination associated with egg yolk, which acts as a protective agent (ARRIOLA, 1982; SINKOVICS *et al.*, 1983; MERCIER and RIDEAUD, 1992). Dimethylsulphoxide, ethylene glycol or acetamide showed low toxicity (HANADA and NAGASE, 1980; CHEN *et al.*, 1989) and maintain a good mobility at 20°C (HANADA and NAGASE, 1980). Glycerol has also non toxic effects on diluted semen at least under 5% concentration (CASTELLINI et al., 1992). Ethylene diamino tetra-acetic acid (EDTA) has been employed (ALVARIÑO, 1993) although no studies have been made on a large scale using diluted semen cooled and stored for 96 hours.

Indeed, the possibility to dilate the interval between collection of semen and its application to the female would enlarge the possibilities of performance of the AI, for example in farms without males located far from semen collection centres.

For the development of the AI at industrial level, extenders able to maintain the semen intact during longer periods of time are indispensable on the market. Semen properties must be preserved, in such a way that parameters as reproductive fertility and prolificacy are not altered. A new commercial extender including EDTA, has been tested on a large scale. This work attempts to elucidate the effect of storage of semen up to 96 hours on the fertility and prolificacy of artificially inseminated female rabbits.

MATERIAL AND METHODS

The AI were carried out in "El Señorío de Molina" farm. About 30.000 females of the California×NZW breed, housed in individual cages with controlled light/dark cycles (16h/8h) and fed *ad libitum* a commercial diet, were inseminated on a regular basis, which means that every week around 8.000 AI were performed.

Table 1 : Influences of the semen conservation time on fertility.

Treatment	Nb A. I.	% Fertility	Statistical analysis χ^2	Prob.
2 h.	372	84.14 A		
24 h.	359	83.56 A		
48 h.	370	79.73 A	111.74	0.0001
72 h.	216	67.59 B		
96 h.	103	39.23 C		

The values followed by different letters are statistically different to each other.

The experimental design considered the effect of the storage of diluted semen on the fertility and prolificacy of lactating females. The commercial name of the new extender used to conserve the semen is MA24 (Laboratorios Ovejero, León, Spain).

Semen was collected from bucks kept in individual cages, under a 16L:8D photoperiod. Each ejaculate was examined under microscope and the percentage of mobile sperm was subjectively estimated. Only ejaculates over 60% mobility were pooled and diluted with MA24 to a variable rate in order to reach a final concentration of 30 million spermatozoa per millilitre.

Pooled diluted semen was introduced in a programmable refrigerator and kept at 18°C until AI was carried out. In all cases females were injected 20 IU i.m. of PMSG ("Sincro-Gest", Laboratorios Ovejero, León) 48 hours before AI Ovulation was induced by 20 µg i.m. GnRH injection (Gonadoreline, "Inducel GnRH", Laboratorios Ovejero, León, Spain), immediately after insemination.

1447 lactating females were inseminated on day 4 *postpartum*, using MA 24 as extender at 2, 24, 48, 72 and 96 hours after semen collection.

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

RESULTS

Data of fertility and prolificacy obtained are shown in Tables 1 and 2.

The results reflected in Table 1 indicate a falling of fertility when increasing the time of conservation. Differences were statistically significant at 72 hours and fertility fell dramatically after 96 hours of conservation.

The number of total born kits followed the same tendency than fertility, so that 72 hours of conservation caused a reduction of one kit per litter, this fall being much more accused after 96 hours, up to three kits less. The dead born kits did not present statistically significant differences in relation with the studied treatments.

DISCUSSION

The results provided by this work show three marked periods of performance of the MA 24 extender. In the first period, between 2 and 48 hours, means of fertility are next or higher than to 80%. The second interval, between the 48 and 72 hours of conservation, reflects an important decrease of fertility, with a reduction of 12 points. Periods of conservation longer than 72 hours led to decrease of fertility (around 40%) unacceptable from an economic point of view.

FREYCHAT *et al.* (1989), using as extenders the denominated Tris (STRAZINGER *et al.*, 1971) and that one proposed by BATTAGLINI *et al.* (1982), obtained a fertility of 59.5% and 62.8% respectively, after a period of conservation of 24 hours. The inseminations carried out without conservation (using fresh semen) provided levels of fertility around 77%, while those carried out after a period of conservation of 48 hours were not, in any case, positive (0%). The fertility reached using fresh semen is equivalent to that one obtained in our experience. When studying the period of 24 hours, the results obtained by FREYCHAT *et al.* (1989) are similar to those reflected by FACCHIN *et al.* (1988), located around 64%, whereas in this work a fertility of 80% was attained. The most important differences are appreciated when comparing the periods of conservation, basically due to the null capacity of the above mentioned extenders to preserve the semen during periods of time longer than 24 hours.

BATTAGLINI *et al.* (1988), using the previously mentioned Tris extender adding 20% of egg yolk, with a period of conservation of 48 hours at 5°C, obtained a fertility of 45.6% using does in diverse physiological states, compared to 65.0% achieved with fresh semen.

Table 2 : Influences of the semen conservation time on prolificacy (Mean ± ESM).

Treatment	Nb of litters	Total born/litter	Dead born /litter
2 h.	287	8.92 ± 0.16 A	0.59 ± 0.09
24 h.	274	8.48 ± 0.17 A	0.42 ± 0.07
48 h.	242	8.02 ± 0.18 AB	0.37 ± 0.07
72 h.	125	7.04 ± 0.27 B	0.33 ± 0.08
96 h.	36	5.58 ± 0.44 C	0.30 ± 0.15
F – value		17.86	1.59
Probability		0.0001	0.1749

The values followed by different letters are statistically different to each other

LAZZARONI *et al.* (1992) reported, for periods of conservation of 50, 62 and 72 hours, values of fertility of the 50.98, 60 and 45.45% respectively, using the same extender and identical conservation temperature.

In a work carried out by ANSELMINO and TOMATIS (1989), using an extender of not published composition, acceptable values of fertility are presented for the period between 24 and 72 hours (71.1%) or between 72 and 108 hours (61.1%), although the number of inseminations carried out with each group was relatively low (52 and 18 respectively).

The prolificacy values obtained in our experience approach in good measure those of the works previously mentioned. FREYCHAT *et al.* (1989), obtained 7.74 kits after a period of conservation of 24 hours, while ANSELMINO and TOMATIS (1989), reached 7.5 with a conservation interval between 24 and 72 hours and 6.9 kits after 72-108 hours.

The readiness of an extender able to maintain rabbit semen during periods of time longer than 24 hours would be of great utility for the restructure of the sector, based on the integration of farms with breeding females and fattening rabbits on one hand, and semen production centres for another. With this objective at commercial level, the search of new products has been intensified, with recent launchings of new extenders (MARTÍNEZ, 1996). At the moment, the capable extenders for rabbit semen are located in the phase of conservation between 1 and 3 days, although diverse centres are working to increase the time of conservation. Thus, a new organisation system, based on transportation of rabbit semen from specialised centres to the all female rabbit farms, could be set up.

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