ARTIFICIAL INSEMINATION OF RABBITS WITH DILUTED SEMEN STORED FOR 24 HOURS

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Abstract - The effect of 24 hours rabbit semen storage on fertility and prolificacy has been studied in two experiments, where a total of 1999 artificial inseminations have been carried out using a new commercial extender, MA 24. Globally an excellent result was obtained, without any decrease in fertility or prolificacy, although better performance was detected in experiment II when more experience in the artificial insemination technique had been gained. The fertility rate was over 80% and litter size was higher than 8,5 pups/litter, in lactating females inseminated on day 4 postpartum, which can be considered good enough to develop a large scale utilization of 24 hours preserved rabbit semen.

INTRODUCTION

Artificial Insemination (A.I) 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. The advantages of the A.I. technique include the reduction in the number of males needed in the rabbit farm, the assessment of semen quality, the synchronization of reproduction in fixed days of the week and the increase of disease control. The advantages of A.I. have been admitted by several authors (COSTANTINI, 1986; SANFORD, 1986; HAFEZ, 1987; CHINELLATO *et al.*, 1991; TAWFEEK and EL-GAAFARY, 1991; ALVARIÑO, 1993), in agreement with the fact that A.I. is extending rapidly on a large scale.

Nevertheless rabbit semen conservation is to date one of the main impediments for commercial utilization of the A.I.. Rabbit semen does not respond 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; SINKOVICKS *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 tetraacetic acid has been employed (ALVARIÑO, 1993) although no studies have been made on a large scale using diluted semen cooled and stored for 24 hours. The rabbit production is demanding extenders to preserve diluted semen at least for 12 hours in order to transport it from the specialized enterprises in rabbit semen to the rabbit farms, usually located in an area of one hundred Kms or more. A new extender commercial including EDTA, has been tested on a large scale. This work attempts to elucidate the effect of storage of semen for 24 hours on the fertility and prolificacy of artificially inseminated female rabbits.

MATERIALS AND METHODS

The A.I. was carried out in "Granja Señorío de Molina" farm, where about 30.000 females are being inseminated on a regular basis, which means that every week around 8.000 A.I. are performed.

The experimental design considered the effect of the storage of diluted semen on the fertility and prolificacy of nulliparous and lactating females. The new extender named MA 24 (Laboratorios Ovejero, León, Spain) was tested against the usual extender (skim milk) employed as a routine in the farm to inseminate with fresh semen (no more than 1 hour after semen collection).

Semen was collected from bucks kept in a separated place, under a 16 hours/day 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 to a variable rate in order to reach a final concentration of 30 million spermatozoa per millilitre.

When MA 24 was used as extender pools were introduced in a programmable fridge and kept at 18° C until A.I. was carried out. In all cases females were injected i.m. with 20 IU of PMSG (Sincro-Gest Laboratorios Ovejero, León) 48 hours before A.I. Ovulation was induced by 20 µg of GnRH (Inducel GnRH, Laboratorios Ovejero, León, Spain).

Experimental designs

Experiment I - 1456 lactating females were inseminated, using skim milk (n=294) or MA 24 as extender. In this last group females were inseminated within three hours (n=458) or 24 hours after semen collection (n=704).

Experiment II - 178 nulliparous females and 365 lactating females were inseminated using MA 24 as extender, either 3 hours (n = 244) or 24 hours after semen collection (n= 299). Experiment II was carried out four months later than experiment I.

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

Data of fertility and prolificacy obtained in experiments I and II are shown in tables 1 and 2.

Table 1 : Conception rate and litter size in lactating rabbits after A.I. on day 4 postpartum with fresh (diluted in skim milk or MA 24) or 24 hour stored semen (diluted in MA 24).

Extender	MA 24	MA 24	Skim milk
Storing time	24 hours	3 hours	< 1 hour
Mobility	3,25	8,2	8
Fertility	76,98 a (704)	81,22 ab (458)	83,67 b (294)
Born dead (Mean ± SEM)	0,64 ± 0,07	0,52 ± 0,08	0,81 ± 0,12
Litter size (Mean ± SEM)	8,09 ± 0,12	8,21 ± 0,13	8,03 ± 0,17

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

Table 2 : Conception rate and litter size in nulliparous or lactating rabbits after A.I. with fresh or 24 hour stored semen diluted in MA 24 extender

	NULLIPAROUS		LACTATING	
Storing time	24 hours	3 hours	24 hours	<3 hours
Mobility	2,75	8	3,25	8,2
Fertility	77,77 (90)	73,03 (88)	88,51 (209)	83,97 (156)
Born dead (Mean±SEM)	0,56 ± 0,19	0,57 ± 0,18	0,44 ± 0,11	0,42 ± 0,12
Litter size (Mean±SEM)	7,49 ± 0,34	7,83 ± 0,38	8,79 ± 0,22	8,56 ± 0,25

( ) Number of A.I

In experiment I, A.I. was performed on day 4 postpartum. A significant decrease in the conception rate ($p< 0,05$) was detected when semen was stored 24 hours, compared to A.I. with fresh semen diluted in skim milk. The conception rate was not different when fresh semen diluted in MA 24 was used. No differences were detected in litter size between the different treatments.

In experiment II, nulliparous females showed no significant differences in all studied variables. Conception rate was over 70% and litter size was over 7,5 born per litter, independently of the storing time. In lactating females on day 4 postpartum no significant differences were found, conception rate being over 80% and litter size over 8,5 born per litter.

DISCUSSION

Globally the performance of the A.I. with 24 hours stored diluted semen using the MA 24 extender has been excellent, without any decrease in fertility or prolificity. Only in experiment I a poorer performance was detected compared to fresh semen, but this was not confirmed in experiment II. It must be remarked that experiment II was carried out four months later, and that during this period a higher skill on the artificial insemination technique was gained: semen collection and microscope assessment was quicker and the time from taking semen out of fridge to effective insemination was shorter. This could explain the improvement in the results as it has been observed in previous works (FACCHIN *et al.*, 1988)

Nulliparous females showed lower results in the conception rate than lactating females which does not agree with previous results (ZANIRATO *et al.*, 1988; SZENDRO *et al.*, 1992; REBOLLAR *et al.*, 1994). This could be explained by the fact that the age and weight of females the first time they were inseminated was inadequate: Animals were too young and weight was too low, factors which affect negatively to the A.I. performance (SZENDRO *et al.*, 1992; ALVARIÑO, 1993). According to most of the reviewed experiments, included our own previous work, A.I should be as effective or more so on nulliparous females as on lactating females.

On the contrary the lower prolificacy obtained in nulliparous agrees well with the references which consider that a normal result is one pup under the mean litter size of multiparous females (HULOT *et al.*, 1982)

Overall our results, in agreement with previous statements (BLOCHER and FRANCHET, 1990; DELLA PORTA *et al.*, 1991), should mean great interest in using A.I. in rabbit farms, to overcome the poor results obtained with 24 hour preserved semen in earlier experiments (ANDRIEU, 1974; ROUSTAN, 1982).

New experiments are being carried on in order to stablish how long rabbit semen can be stored, as preliminary results indicate that a 48 hours preservation period or longer could be achieved with satisfactory A.I. performance.

A new organizational system, based on transportation of rabbit semen from specialized centres to the rabbit farms could be set up between all female rabbit farms and semen producers.

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REFERENCES

ALVARIÑO J.M.R., 1993. Control de la reproducción en el conejo. *Ed. MAPA - Mundi Prensa,* 137 pp., Madrid.

ANDRIEU R., 1974. Conservation du sperme de lapin sous forme liquide. *E.N.S.A. Montpellier.*

ARRIOLA J., 1982. Interaction of formaldehyde and of sodium and triethanolamine lauryl sulphate on the motility and fertilizing ability of rabbit and bull spermatozoa frozen in egg-yolk and milk extenders. *Ph. D. Thesis, Cornell University, Itaca New York.*

BLOCHER F., FRANCHET A., 1990. Fertilité, prolificité et productivité au sevrage en insémination artificielle et en saillie naturelle: influence de l'intervalle mise bas - saillie sur le taux de fertilité. *5emes Journees Rech. Cunicole,* **3**, Paris.

CASTELLINI C., BATTAGLANI M., LATTAIOLI P., 1992. Effects of cryoprotectants and freezing on rabbit semen quality. *J. Appl. Rabbit Res.,* **15**, 431 - 438.

CHEN Y., YANG X., FOOTE R. H., 1989. Time breeding of rabbits with fresh and frozen-thawed semen and evidence of acrosome alteration following freezing and thawing. *Anim. Repr. Sci.*, **18**, 35 - 41.

CHINELLATO A., PANDOLFO L., DE BIASI M ., NUMERATTI M., BONIN M. R., POLVORINO DE LAURETO P., FASOLATO L., 1991. Fecondazione artificiale:Standarizzazione del metodo. *Rivista di Coniglicoltura.,* **5**, 49-51.

COSTANTINI F., 1986. "Fecondazione artificiale e ciclizzazione dei parti". *Rivista di Coniglicoltura,* **7**, 13 -20.

DELLA PORTA P., DELLA PORTA D., MACCARIO P., BORNELLI A., 1991. Inseminazione artificiale nei conigli. *Ed. Gruppo Zeta, Italy.*

FACCHIN E., ZANIRATO M.G., GUALTERIO L., VALENTINI A., 1988. A.I. in rabbit breeding. Note 1: A.I. for meat rabbit breeding. *4th WRC,* **1**, 121-129, Budapest.

HAFEZ E.S.E., 1987. "Reproduction in farm animals" *5th Ed., Lea and Febiger, Philadelphia, PA, USA.*

HANADA A., NAGASE H., 1980. Cryoprotective effects of some amides on rabbits spermatozoa. *J. Repr. Fertility,* **60**, 247 - 252.

HULOT F., MARIANA J.C., LEBAS F., 1982. L'établissement de la puberté chez la lapine (Folliculogenése et ovulation). Effet du rationnement alimentaire. *Reprod. Nutr. Dévelop.,* **22**, 439-453.

MAURER R.R., STRAZINGER G.F., PAUFLER S.K., 1976. Embryonic development in rabbits after insemination with spermatozoa stored at 37, 5 or - 196° C for various periods. *J. Repr. Fertility,* **48**, 43-49.

MERCIER P., RIDEAUD P., 1992. Bacteriological study of rabbit sperm and the effects of antibiotic supplements in the conservation medium. *J. Appl. Rabbit Res.,* **15**, 520 - 529.

REBOLLAR P.G., ALVARIÑO J.M.R., UBILLA E., 1994. Grouping of rabbit reproduction management by means of artificial insemination. *World Rabbit Sci.,* **2**, 87-91.

ROUSTAN A., 1982. L' insémination artificelle chez la lapine. *Cuniculture,* **46**, 189 -196.

SANDFORD J.C., 1986. The domestic rabbit. *4 th Ed., Collins press, 8 Grafton St., London, W1,* 127 - 144.

SAS/STAT 1987. Guide for personal computers. Version 6. *Ed. SAS Inst. Inc., Cary NC, USA.*

SINKOVICKS G., MEDGYES I., PALJAK J., 1983. Some results of A.I. in rabbits. *J. Appl. Rabbit Res.,* **6**, 43- 47.

STRAZINGER G.F., MAURER R.R., PAUFLER S.K., 1971 Fertility of frozen rabbit semen. *J. Repr. Fertility,* **24**, 111-117.

SZENDRÓ Z.S., BIRÓ-NEMETH E., RADNAI I., 1992. Considerazioni sui risultati dell'inseminazione artificiale nei conigli. *Rivista di coniglicoltura,* **10**, 39-42.

TAWFEEK M.I., EL-GAAFARY M.N.,1991. Evaluation of A.I. technique as compared to natural mating in association with some productive and reproductive traits in rabbits. Egyptian *J. Rabbit Sci.,* **1**, 13 - 20.

ZANIRATO M.G., GUALTERIO L., FACCHIN E., 1988. A.I. in rabbit breeding. Note 3: Practic al problems in standardization of a new method for A.I. in rabbit breeding *4th WRC,* **1**, 156 - 164. Budapest.