QUALITY AND FERTILITY OF PRESERVED RABBIT SEMEN AT 15° C, IN GELATIN SUPPLEMENTED EXTENDER

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ABSTRACT

The objective of the present study was to evaluate New Zealand rabbit semen viability and fertility diluted and preserved at 15 °C in gelatin supplemented extender. The trial was conducted at the Unidad de investigación Aplicada en Producción Cunícola, Departamento de Preparatoria Agrícola, Universidad Autónoma Chapingo, Mexico, during January-March, 2004. From 10 one year old bucks, semen was collected with artificial vagina 3 days a week, 2 times each day. Collected semen was kept at 35°C in order to be evaluated for volume, color, mass motility viability and normal morphology. Twenty minutes, as maximum, after collection, the evaluated semen was diluted to generate doses with 20 million cells in 0.5 ml volume. Extender had sodium citrate, glucose, and gelatin. Once semen was diluted, temperature was lowered 0.33 °C /min up to 18°C. Before the semen got gel consistency it was packed in 0.5 ml straws. They were seal with polyvinyl alcohol, then the straws were storage in a thermos having glacial acetic acid at 15 °C. Every 24 h after storage semen was evaluated in motility, normal and live spermatozoa. Once the maximum time of viability keeping was known, then the fertilizing ability of preserved semen was evaluated by artificially inseminating lactating does on day 11 post partum after a bio-stimulus consisting on 48h doe-litter separation. Previously to the statistical analysis arcsin transformation was applied to viability and normality and then the general linear model form SAS (V8) was applied to the variables. Fertility was analyzed through proc FREQ and tested by Chi square. Preservation period affected ($P < 0.05$) semen characteristics: motility, viability and normal morphology were reduced as period of preservation increased particularly for motility after 72 h of semen preservation. Preserved semen for 24, 48, and 72 had not significant differences on fertility (84, 73 and 70 %, respectively). Maximum period of semen preservation at 15 °C in gelatin supplemented extender, without affecting seminal characteristics was 72 h. As far as 72 h preservation period, semen fecundity is not affected.

Key words: rabbit, semen preservation, fertility, gelatin, extender.

INTRODUCTION

Artificial insemination is a biotechnological tool used for genetic improvement spread. It is used in all the animal production species with many purpose including production planning and control, which is associated to maximize enterprise profitability. Rabbits are not the exception were artificial insemination is applied with the same objectives.
A limitation factor for rabbit artificial insemination spread in commercial level is related with semen preservation. Fresh diluted semen has been used but its quality can be maintained only for a short period of time (ROCA, *et al*, 2000).

Rabbit semen is more sensible to hypertonic solutions and to cryoprotective agents having hydroxyl groups such as glycerol (CASTELLINI, *et al*., 1992; quoted by LÓPEZ and ALVARIÑO, 1998). Because of that it is necessary to find different substances that allow the semen to keep its quality for longer periods.

Organic extenders have been used for longer periods in both cooling and freezing semen conservation, some of them are Salisbury, Toni Roca, Strazinger, Sinkovics, ETSIA Madrid, and Strazinger mediums (ALVARIÑO, 1993).

A good extender has to have a buffering power to maintain the optimal pH. If additionally a gelatin substance stop the spermatozoa movement, then reducing the temperature, semen conservation for longer periods can be possible without affecting its fertility capacity.

The objective of this study was to evaluate the diluted semen viability when preserved at 15 °C in a gelatin supplemented extender.

MATERIAL AND METHODS

The trial was conducted at the Unidad de Investigación Aplicada en Producción Cunícola, Departamento de Preparatoria Agrícola, Universidad Autónoma Chapingo, Mexico.

One year old (4.5 kg weight) White New Zealand bucks were used. Rabbits were kept in commercial cages (40 x 86 x 32 cm). A food hopper was used to feed the animals. The cages had an automatic watering system with nipple drinkers. Cages were provided with a feet rested. A commercial formula was offered *ad lib*. which had 16% of protein. Clean and cool water was always available.

Bucks were trained for semen collection which was done 3 days a week and 2 times by day using an artificial vagina maintained at 46 °C. Immediately after collection, semen was keep at 35 °C in waterbath in order to be evaluated. Macroscopic and microscopic characteristics were evaluated: volume (ml), color (scale from 1 to 3), gel content (present or absent), estranges corpus (scale from 1 to 3), mass motility (scale from 0 to 3), live spermatozoa (%), and normal sperm morphology (%). Twenty minutes as

maximum, after collection, the evaluated semen was diluted to generate doses with 20 million cells in 0.5 ml volume.

The extender had sodium citrate, glucose, and commercial gelatin, which give to the extended semen a liquid state at 37 °C but gel consistency at 18 °C or lower temperatures. Culling was done at 1 °C by 3 min. Before the semen got gel consistency it was packed in 0.5 ml straws which were sealed with polyvinyl alcohol. Then the straws were stored in a thermos having glacial acetic acid at 15°C.

Every 24 h, stored semen was evaluated in motility, normal sperm morphology and live spermatozoa. Once the maximum time of viability keeping was known through microscopic evaluation then fertilizing ability of semen was evaluated by artificially inseminating lactating does on day 11 post partum after a bio-stimulus consisting on 48h doe-litter separation.

For these records was fitted the following model: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} represents the independent variable, μ the general mean, T_i the preservation period effect, and e_{ij} the experimental error. Previously to the analysis arcsin transformation was applied to viability and normality and then the general linear model form SAS (V8) was applied to the variables. Fertility was analyzed through proc FREQ and tested by Chi square.

RESULTS AND DISCUSSION

Seminal characteristics from fresh and preserved (at 15 °C) semen until 4 days are shown in table 1. The three evaluated characteristics are negatively affected by time conservation, however, still 72 h after collection, semen kept its qualitative characteristics in order to be used in artificial insemination to receptive does.

Table 1. Live and normal cells (%) and mass motility for fresh and preserved semen

Semen Preservation period*	Live cells	Normal cells	Mass motility**
0	94.30^{a}	96.30^{a}	2.70^{a}
24	90.80^{b}	94.50^{ab}	2.20^{b}
48	90.70^{b}	93.44^{bc}	2.00^{bc}
72	88.22^{b}	93.40^{bc}	1.80^{c}
96	84.70^{c}	90.70^{c}	0.50^{d}

* In hours
** In a 0 to 3 scale.
Means with same literal by column are not different ($a \leq 0.05$),

Similar to results found in the present study, NAGY, *et al*. (2001) reported that gel addition had a positive effect in semen quality preserved at 5 °C by 72 h. They found a higher percentage of live cells and undamaged acrosomes, in semen preserved with gel supplemented extender in comparison with no supplemented extender.

As previously stated, semen preservation is a main limitation in rabbits artificial insemination. Semen fecundity capability has to be maintained the necessary time for transportation from the processing centers to the rabbitry where is going to be used. In the first phase of the present study it was determined 72 h as the maximum period of semen preservation without affecting semen characteristics.

In the second phase receptive does were artificially inseminated with preserved semen by 24, 48 and 72 h. As can be seen in table 2 there were not significant differences in fertility when the semen was preserved until 72 h in comparison with 48 and 24.

Semen storage generally deteriorates the spermatozoa quality; the reduction of movement by lowering temperature and supplementing the extender with gelatin was effective in reducing spermatozoa metabolism and movement.
As a consequence of the previous factors, probably there was a reduction of lactic acid generation and it is known that low pH kill the spermatozoa.

Table 2. Fertility of does inseminated with preserved semen in gelatin supplemented extender at three periods of preservation (%)

Semen preservation period*	Inseminated does	Fertility
24	25	84.0[a]
48		72.5[a]
72	20	70.0[a]

* In hours
Means with same literal by column are not different (a≤ 0.05)

Semen preservation has to be done without affecting its quality but at the same time avoiding expensive materials, and having an easy way to manage and transport the semen to the commercial rabbitry.

CONCLUSIONS

Rabbit semen preservation as far as 72 h at 15 °C does not affect its seminal characteristics neither its fertilizing ability when a gelatin supplemented extender is utilized. That allows to reach acceptable fertility levels when preserved semen is utilized to inseminate receptive does.

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