Modern Agricultural Science and Technology, ISSN 2375-9402, USA
November 2019, Volume 5, No. 5-6, pp. 43-45
Doi: 10.15341/mast(2375-9402)/03.05.2019/005
Academic Star Publishing Company, 2019
www.academicstar.us

Evaluation in Vitro of Rabbit Spermatozoa with the Aggregate of Gelatin in the Extender for Refrigeration

Marcos Alejandro Puente, I. Covelo, and C. M. Tartaglione

Laboratory of Animal Reproduction, School of Agricultural Sciences, National University of Lomas de Zamora, Argentina

Abstract: In developing countries, rabbit breeding can be a productive option and a response to the problems of malnutrition and rural poverty. The consolidation of this activity requires the availability of techniques that allow the improvement of the reproductive indexes, using selected males that are not within reach of the small farmer. The incorporation of biotechnologies such as artificial insemination can multiply the productive characteristics of a farm. The conservation of semen depends on many factors, one of which is the ability of the extender to provide an optimal environment for spermatozoa. The objective was to evaluate rabbit semen after having been preserved at 15°C for 24 hours using extender with the addition of different doses of gelatin. The data obtained were analyzed using an analysis of variance using the LSD Fisher method. A significant difference ($p < 0.05$) was observed for the treatment with 1% gelatin, concluding that the addition of gelatin to the extender improves the viability and mobility of refrigerated rabbit spermatozoa.

Key words: reproduction, spermatozoa, cryopresrvation, gelatin

1. Introduction

Reproductive techniques have been used for many years in domestic animals. Techniques such as artificial insemination and the conservation of semen through refrigeration or freezing are used routinely in livestock for more than 50 years. As in any livestock species, reproduction is the basis of production. In the rabbit activity, artificial insemination with diluted fresh semen has become a routine procedure. However, a limiting factor for a greater use of this biotechnology is related to the preservation of semen. The current practice of using diluted fresh semen is limited mainly to the rabbits of the farms where the male is located and within a few hours of collecting the semen [1].

The use of refrigerated and stored semen for 2-3 days can facilitate the transport and later use in nearby rabbit farms [1]. To maintain the integrity and motility of the sperm for a longer period, substances have been used to modify the viscosity of the extender. This prevents sedimentation, which helps distribute the gametes more evenly in the extender by acting more efficiently buffer solutions [2]. To increase the viscosity, it is possible to use substances such as methylcellulose, carboxymethylcellulose or gelatin. The objective of this study was to evaluate the rabbit refrigerated semen for 24 hours using extender with the addition of gelatin.

Corresponding author: Marcos Alejandro Puente, Zootechnicist Engineer; research areas/interests: animal reproduction. E-mail: ingmarcospuente@gmail.com.

2. Materials and Methods

2.1 Chemicals

All Chemicals, unless otherwise noted, were purchased at Sigma-Aldrich (St. Louis, MO, USA).

2.2 Animals

In this study, all males and females were sexually mature. We used 10 males Neo Zealander White Breed. The rabbits were kept individually to ambient with

controlled Light (16 L: 8 O). Animals received a commercial diet, and water was provided *ad libítum*.

2.3 Semen Collection and Evaluation

Semen was collected once a week, two ejaculates per male at 30 min intervals. The semen was collected by an artificial vagina at 42°C and in case of presence of mucous plug in the ejaculate it was removed by means of a forceps.

Each ejaculate was evaluated in a macroscopic way observing the color, odor and volume measured in a graduated collector tube. The collected fresh semen was diluted with TCG (0.25M tris hydroxymethyl aminomethane, 88 mM citric acid, 47 mM glucose) and antibiotic. Each ejaculate was divided into four aliquots with the addition of commercial gelatin (Bloom 240 index) in the following concentrations: 0 (control), 0.5%, 1% and 2%. After being stored at 15°C for 24 hours, progressive rectilinear motility, viability by eosin-nigrosin staining and membrane integrity were evaluated by the HOS test.

2.4 Statistical Analyses

For the statistical analysis, the analysis of variance was used with the Fisher LSD method with multiple range contrast for the homogeneity test, considering a level of significance of 5%. The results obtained are expressed as mean values ± standard deviation.

3. Results and Discussion

Table 1 shows the averages and standard deviations of the seminal parameters evaluated for each of the treatments with different percentages of gelatin in the extender. It can be seen that there is a significant difference for motility and viability parameters in the treatment with 1% gelatin. However in the membrane integrity parameter there is no significant difference between the treatments.

Gelatin is a protein that can be of animal or vegetable origin. The stability and gelling power is determined by the Bloom value ranging from 50 to 300. The higher this value is, the higher the gelation intensity. Due to its reliable stability, gelling ability and easy handling, many researchers have incorporated it into seminal extender studies for different animal species such as the rabbit [3, 4], the goat [5] and the pig [6]. The use of gelatin in the extender exerts a beneficial effect avoiding sedimentation of sperm, changes in the composition of the medium and pH.

Table 1 Sperm quality parameters of ejaculates refrigerated for 24 hs. at 15°C with different concentrations of gelatina.

	0%	0.5%	1%	2%
Motility	62.5 ± 2.6^{b}	58.5 ± 4.6^{a}	67.5 ± 2.56^{c}	64.5 ± 3.6^{b}
Viability	67.4 ± 4.7^{a}	65.9 ± 5.6^{a}	70.5 ± 4.7^{b}	64.6 ± 4.3^{a}
Membrane integrity	50.0 ± 6.4^{a}	45.3 ± 8.5^{b}	50.1 ± 7.3^{a}	45.2 ± 7.0^{b}

(a,b,c) Different letter in the same row indicates significant difference ($p < 0.05$).

According to the results of Nagy et al. (2002) [3]the pH differs when the cells settle, the concentration of some toxic metabolites may be greater in that sedimentation zone. Several authors have documented the use of gelatin in the conservation of sperm from different species with disparate results. López-Gatius et al. (2005) [4] observed an improvement in motility. On the other hand, Nagy et al. (2002) [3] observed greater viability and acrosomal integrity in diluted semen conserved during 72 hs. However, Hernández et al. (2004) [2] did not find significant differences in spermatozoids refrigerated with gelatine during 24, 48, and 72 hs. at 12°C, in none of the parameters mentioned above. According to the results of El-Speiy et al. (2014) [7], preserving spermatozoa at 5°C, with the addition of 1.4% of gelatin, an improvement in motility and a decrease in the percentage of dead cells and abnormalities are obtained.

4. Conclusions

Sperm storage in refrigerated form for 24 hours with the addition of 1% gelatin in the extender improves motility and viability parameters. However, in

membrane integrity no differences were observed between sperm with and without gelatin.

References

[1] J. Roca, S. Martinez, J. M. Vazquez, X. Lucas, I. Parrilla and E. A. Martinez, Viability and fertility of Rabbit spermatozoa diluted in Tris buffer extenders and stored at 15°C, *Animal Reproduction Science* 64 (2000) 103-112.

[2] P. Hernández, R. Fernández, R. Avalos, B. Diaz and Olvera V., Effect of the addition of gelatine to rabbit semen stored at 12°C, *Animal Health Magazine* 26 (2004) 197-201.

[3] Nagy Sz., Sinkovics Gy. and Kovács A., Viability and acrosome integrity of Rabbit spermatozoa processed in a gelatina-supplemented extender, *Animal Rrproduction Science* 70 (2002) 283-286.

[4] F. López-Gatius, G. Sances, M. Sancho, J. Yániz, P. Santolaria, R. Gutiérrez, M. Núñez, J. Núñez, and C. Soler, Effect of solid storage at 15°C on the subsequent motility and fertility of Rabbit semen, *Theriogenology* 64 (2005) 252-260.

[5] I. Salvador, J. Yániz, M. P. Viudes de Castro, E. Gómez and M. Silvestre, Effect of solid storage on caprine semen conservation at 5°C, *Theriogenology* 66 (2006) 974-981.

[6] L. Gil, M. Solorzano, C. Malo, D. Pinto, N. Gonzalez, I. De Blas and T. Anso, Effect of the addition of gelling agents in a commercial porcine extender, in: *I Congress of the National Association of Porcine Veterinarians*, Zaragoza-España, 2008.

[7] M. El-Speiy, E. Elkomy and K. Kamel, Effect of adding protein high viscosity (gelatin) in tris extender on semen conservation status, fertility rates, antioxidante status and sex ratio of rabbits, *Global Veterinary* 12 (2014) 840-849.