ISSN - 0102 - 0803

REVISTA BRASILEIRA DE REPRODUÇÃO ANIMAL

BRAZILIAN JOURNAL OF ANIMAL REPRODUCTION

PUBLICAÇÃO OFICIAL DO COLÉGIO BRASILEIRO DE REPRODUÇÃO ANIMAL - CBRA

Rev. Bras. Reprod. Anim. | Belo Horizonte | v.27 | n.3 | p.313-606 | Jul./Set. 2003

Técnicas de fertilização *in vitro* no bovino Crioulo da Patagonia Argentina

(In vitro fertilization techniques in the Argentine Creole Patagonic bovine)

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RESUMO

Neste trabalho foi avaliado o uso do sistema de produção in vitro de embriões utilizando-se espermatozóides de bovino Crioulo da Patagônia e Holstein. Não foi encontrado nenhum efeito de raça confirmando-se a possibilidade do uso destas técnicas para conservação dos recursos genéticos para produção de alimentos e a agricultura. Entretanto, outros estudos são necessários para melhoria do sistema de produção de embriões in vitro.

PALAVRAS-CHAVE: Fertilização *in vitro*, bovino, raças autóctones.

ABSTRACT

In this paper the use of in vitro embryo production system using Creole Patagonic and Holstein spermatozoa was evaluated. No breed effect was found so we confirm the possibility of use of these techniques for conserving animal genetic resources for food and agriculture. However, more studies are needed to improve the in vitro embryo production system.

KEY WORDS: in vitro fertilization, bovine, autochthon breeds.

INTRODUCTION

The Argentine Creole Patagonic is descendant of the original cattle brought with the Spanish conquest of the New World. These animals have been adapted to the local environments for around 400 years. According to Cunningham (1999) it is necessary the application of improved reproductive technologies for conservation of genetic resources. Detailed studies about gamete and fertilization physiology are a prerequisite for developing effective assisted-breeding programs for autochthon species. At the moment, little information is available about reproductiive characteristics of Creole Patagonic bovine, and nothing is known about the performance of in vitro embryo production and the use of these embryos for transferring to a recipient animal or freezing for later transfer. So, in the way to know more about the reproduction of this autochthon breed the aim of these experiments was to investigate the performance of in vitro fertilization with Creole spermatozoa using Holstein spermatozoa as control.

MATERIALS AND METHODS

Semen preparation

Frozen semen from 2 Holstein and 2 Creole Patagonic bulls with proven fertility were used in this study. Straws were thawed and diluted individually in a capacitation media (CM) consisting of TCM-199 supplemented with 3.88 mg/ml caffeine-sodium benzoate and 0.02 mg/ml heparin sodium salt plus antibiotic-antimicotic solution (100 UI/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml de amphotericin B) and 10% of FCS. Diluted semen was washed by centrifugation twice for 5 min at 500 x g. The supernatants were discarded and the sperm pellet was resuspended to 2 ml in CM. After the second washing step motile sperms were selected by swim-up technique as described by Parrish et al. (1986).

Seminal analysis

The viability of the spermatozoa was assayed and the acrosome status was evaluated by hypo-osmotic swelling test (HOS) and by double stain (Trypan blue-Giemsa) respectively. The hypo-osmotic solution consisted of sodium citrate 7.35 g/l and fructose 13.51 g/l. The final osmolarity was adjusted to 150 mOsm/l, pH 7.2. Aliquots (10µl) of semen were individually mixed with 500 µl of the pre-warmed hypo-osmotic solution and incubated at 35ºC for 25 min. The percentage of sperm integrity membrane was calculated as the number of spermatozoa with swollen tail (Jeyedran et al., 1984). The double stain technique was done according to Kovács et al. (1992). Briefly, the viability testing stain buffer consisted of 0.25% trypan blue in 0.81% NaCl (isotonic solution, pH 7.0). The fixative was composed of 86 ml of 1.0 N HCl plus 14 ml formaldehyde solution (37% w/w) and 0.2 g neutral red. The acrosome stain was 7.5% Giemsa stock solution in distilled water (pH 6.9) prepared fresh before use.

In vitro maturation.

Ovaries were obtained from freshly slaughtered cows at an abattoir and were washed twice with saline solution plus antibiotic at 32 °C. The cumulus-oocyte complexes (COCs) were aspirated from 2 to 6 mm follicles into a 5 ml syringe fitted with an 18-gauge needle containing 1 ml of PBS supplemented with 10% FCS and antibiotic-antimicotic solution. COCs were selected and only those containing 3 or more layers of compact cumulus cells and an evenly homogeneous granulated ooplasm were selected for IVM. Oocyte-cumulus complex were washed twice in TCM-199 with Earle´s salts, L-glutamine, 50 U.I./ml PMSG, 25 mM sodium pyruvate, 10 % de FCS and antibiotic-antimicotic solution. Oocytes were allocated randomly in groups of 10 -15 to 100 µl culture droplets, covered with oil and cultured for 24 h at 39 °C in a humidified atmosphere of 5% CO_2 in air.

IVF and embryo production.

The spermatozoa were suspended in in vitro fertilization media (IVFM) consisting of TCM 199 plus 10% FCS, antibiotic-antimicotic solution without heparin and caffeine-benzoate to get a final concentration of 1.5 x 10^6 cells/ml. Oocyte-cumulus complex were washed twice in IVFM and were allocated randomly in groups of 10-15 to 100 µl fertilization droplets, and cultured for 16 h at 39°C in a humidified atmosphere of 5% CO_2 in air. After that the penetration rate was assayed by fixation and staining of a part of the oocytes. The other oocytes were transferred to a conical tubes containing 2 ml of embryo culture media (ECM: TCM-199 with Earle´s salts, L-glutamine, 10% FCS, plus 100 UI/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml de amphotericin B) of ECM, vortexed for 30 sec. to remove cumulus cells, washed twice in ECM and placed in groups of 15 to 20, to 100 µl culture droplets containing ECM. Then the zygotes were assessed for cleavage at 24 h.

Results and discussion

No differences were found in the spermatozoa characteristics between Creole and Holstein after the swim-up procedure (Table 1, p>0.05). Previously we have studied the pattern of acrosome reaction that suffer the Creole spermatozoa in the IVF media and the effect of presence of cumulus-oocyte-complexes (Matas et al., 2003) and it was similar to the control. In the same way, a similar penetration rate was obtained with both breeds. However, a reduced number of embryos were recovered (Table 2). These data suggest the possibility of use with a relative success in the conservation of genetic resources and it could be a model for other endangered bovine breed worldwide. However more studies are needed to improve the fertilization rate and embryo development for producing high quality embryos in quantity.

Table 1. Percentage of sperm with acrosome intact

Breed	HOS +	Intact	Acrosome Reacted	Membrane Damaged	% Acrosome reacted/ alive
Creole	45.65±4.18	27.21±6.36	19.35±4.21	53.45±4.40	43.79±9.86
Holstein	47.69±3.27	34.31±5.37	20.84±2.01	44.85±4.77	39.50±5.69

Table 2. Number of penetrated oocytes, penetration rate (PEN), and cleavage rate according to the breed of bull used to obtain the semen.

Breed	Penetration rate	Embryos
Creole	60.82 (59/97)	10.44 (7/67)
Holstein	55.10 (54/98)	15.38 (10/65)

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Análise morfológica de embriões bovinos das raças Nelore e Holandês fecundados *in vivo*, antes e após congelação em três protocolos pelo método "one step"

(Morphological analisys of embryos derived by in vivo methods from holstein and nelore cows, before and after three cryopreservation methods using the "one step" procedure)

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RESUMO

Este estudo foi conduzido para se acumular subsídios que justifiquem as diferentes respostas de embriões Bos taurus taurus e Bos taurus indicus frente a procedimentos idênticos de congelação. Para isso confrontou-se morfologicamente 70 embriões bovinos das raças Nelore e Holandesa fecundados in vivo, investigando-se a estrutura de embriões recém coletados (a fresco) e após a congelação-descongelação em três protocolos utilizando-se o método "one step". Foram observadas diferenças no padrão e concentração das inclusões lipídicas e mitocôndrias. As características morfológicas dos embriões levam a crer que as estruturas celulares sejam relativamente melhor preservadas na congelação controlada, em relação à