In Vitro Induction of the Acrosome Reaction in Frozen Thawed Spermatozoa from Different Cattle Breeds

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Abstract

The standard acrosome reaction (AR) in cryopreserved semen doses of dairy and beef cattle breed was analyzed in this study. 18 frozen semen doses from 6 bulls (3 semen samples per bull) of 3 different cattle breeds (2 Charolais, 2 Holstein, 2 Fleckvieh) were *in vitro* incubated in three different test media (Heparin, BSA, or a Heparin-BSA system), using TALP medium, to induce *in vitro* acrosome reaction (AR). The percentage of acrosome reacted spermatozoa was evaluated using the Spermac dichromatic stain, at four different moment (0, 30, 60 or 120 min.). At all time-points, the samples incubated with Heparin-BSA system showed a significant ($P<0.05$) higher percentage of acrosome-reacted spermatozoa as compared to the samples supplemented with Heparin or BSA alone, while the control media (without supplementation) showed the lowest results. A significant influence ($P<0.05$) of cattle breed on the dynamics of the *in vitro* induced AR was also observed. In our study, the response pattern of *in vitro* induced AR depended on the culture media, incubation time and nonetheless cattle breed. In consequence, these factors must be taken into consideration when assessing the sperm acrosome reaction in bulls.

Keywords: *acrosome reaction, bull spermatozoa, Spermac stain*

INTRODUCTION

In order to assess the fertilizing capacity of semen samples, the routine analysis of classical semen parameters might not be enough. Previous studies have suggested the use of *in vitro* acrosome reaction (AR) test might be a useful tool in predicting both field fertility and *in vitro* fertilizing capacity of bull semen (Lessard *et al.,* 2011; Oettle, 1986).

AIMS AND OBJECTIVES

The aim of this study was to evaluate the induced acrosome reaction (AR) in cryopreserved semen doses of dairy and beef cattle breeds, taken into consideration the effect of breed, culture medium supplementation and incubation time on AR test results.

MATERIALS AND METHODS

18 frozen-thawed semen doses from 6 bulls (3 semen samples per bull) of 3 different cattle breeds (2 Charolais, 2 Holstein, 2 Fleckvieh) were used in this study. Aliquotes of 400 µL of semen samples were isolated by swim-up preparation and incubated *in vitro* in three different test media (Heparin, BSA, or a Heparin-BSA system). To achieve capacitation, samples were incubated for 3 hours in TALP medium, at 39°C and 5% CO_2. The acrosome reaction was induced by adding 5mM calcium ionophore A23 187 (Sigma, Europe MO) and incubating the samples for 0, 30, 60 or 120 min as described by Demyda-Peyras *et al*. (2012). The control samples did not contain ionophore. Spermac Stain was used according to the manufacture protocol to assess the acrosome

Tab. 1. The percentage of frozen-thaw bull sperm from dairy and beef bulls that presented an acrosome reaction in Response to Calcium Ionophore Influx as assessed by Spermac Stain

Bull breeds	0 min				30 min				60 min				120			
	C	H	B	H+B	C	H	B	H+B	C	H	B	H+B	C	H	B	H+B
Charolais	34	42	42	49	39	51	50	55	42	56	57	63	45	63	64	73
Holstein	34	43	40	50	37	50	50	56	43	57	56	65	44	63	64	77
Fleckvieh	32	46	44	52	39	52	50	56	43	57	58	65	45	65	66	78

Note: min=minutes; C= control; H= heparin; B= BSA; H+B= heparin+BSA system

reaction. Each sample was examined after staining, under oil immersion and 1000× magnification using a bright field microscope. From each smear, a total of 100 spermatozoa were evaluated for acrosome integrity. The sperm cells were assessed as having normal or altered acrosomes (Paulenz *et al.,* 1995). The normal (acrosomal intact) sperm presented green acrosomal and pink postacrosomal regions, a thick green band with semi-circle form being observed at the tip of the sperm head. The acrosome-reacted/abnormal acrosome presented a pink acrosomal region, or green but the semi-circle band was either discontinuous or missing.

RESULTS AND DISCUSSION

At all time-points, the samples incubated with Heparin-BSA system showed a significant ($P<0.05$) higher percentage of acrosome-reacted spermatozoa as compared to the samples supplemented with Heparin or BSA alone, while the control media (without supplementation) showed the lowest results. A significant ($P<0.05$) influence of cattle breed on the dynamics of the *in vitro* induced AR was also observed (*Tab. 1*).

CONCLUSION

Acrosme reaction **(**AR) induction is a prospective technique in the prediction of both field fertility and IVF results for bull. The main advantages of this technique are the low costs and facility with which it can be executed, thus AR assay may be a useful tool in the increment of the animal improvement Birck *et al.* (2010). The method could also be used as screening test, to identify sub-fertile or infertile bulls, as well as superior fertility bulls Lessard *et al.* (2011). In our study, the response pattern of *in vitro* induced AR depended on the culture media, incubation time and nonetheless cattle breed, our results being accordance with the discoveries made by Demyda-Peyras *et al.* (2012). In consequence, these factors must be taken into consideration when assessing the sperm acrosome reaction in bulls.

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