

THE ACROSOME REACTION AS A PREDICTOR OF NON-RETURN RATES IN *Bos indicus* AND *Bos taurus* BULLS

Feliciano Silva¹, A.E.D.; Ramalho², M.F.D.; Rodrigues³, L.H.;
Freitas¹, A.R.; Hossepian², V.; Kastelic¹, J.P.

¹ Embrapa-CPPSE, Caixa Postal 339, São Carlos-SP., Brasil

² Faculdade de Ciências Agrárias e Zootecnia-UNESP, Jaboticabal-SP., Brasil

³ Lagoa da Serra Inseminação Artificial, Sertãozinho-SP., Brasil

Introduction

Prior to fertilization, spermatozoa must go through complex changes (capacitation), culminating with the acrosome reaction (AR) on the zona pellucida (Lenz et al., 1988; Yamagimachi, 1988). The AR, a prerequisite for fertilization, is heparin dependent (Uguz et al. 1994). Heparin binding by spermatozoa (Marks and Ax, 1985) and many other *in vitro* methods have been used to predict fertility. Despite exhaustive efforts, a simple and accurate method of predicting fertility remains elusive.

The objective of the present study was to determine the relationship between *in vitro* assessment of the AR and fertility of spermatozoa from *Bos indicus* (*B. indicus*) and *Bos taurus* (*B. taurus*) bulls.

Materials and Methods

Frozen-thawed semen from 6 *B. indicus* (Nelore) and 8 *B. taurus* (Simmental, Red Angus, Limousin, Blonde d'Aquitaine) bulls was used. Spermatozoa were washed once in a modified Tyrode's buffer (TALP) containing BGM-1 and BSA, as previously described (Parrish et al., 1988). After resuspension in the same solution (final concentration, 20×10^6 spermatozoa/ml), heparin (10 ug/ml) was added and the suspension was incubated for 4 h at 38°C. Lysophosphatidylcholine (LPC; 100 ug/ml) was then added and incubation continued for an additional 15 min.

Examinations for sperm motility were conducted immediately after the semen was thawed. In addition, examinations for sperm motility, sperm viability, and AR rate (percentage of spermatozoa which had undergone the AR), were conducted immediately after addition of heparin (0 h), 2 h later, and 15 min after the addition of LPC (designated as 4 h). Sperm progressive motility was estimated by examination at 400X. To estimate sperm viability, cells were stained with Trypan Blue and examined at 400X. To determine AR rate, air-dried smears were stained with naphtol yellow S-erythrosin B and Giemsa-Trypan Blue (modified from Kovacs and Foote, 1992). Under light microscopy (1000X), 200 spermatozoa

appeared much lighter in the acrosomal area. An AR index was calculated as the AR rate at 4 h divided by the AR rate at 0 h. Semen fertility was determined from 60 to 90 day nonreturn rates (NRR) collected by the Lagoa da Serra Insemination Centre. The average number of inseminations per bull was 33.6 (range, 10 to 65).

A Student's t-test was used to determine if there was a difference between *B. indicus* and *B. taurus* bulls for NRR, motility, viability, AR rate and AR index. Pearson correlation coefficients were determined between NRR and the AR rate (0, 2 and 4 h) and the AR index. Linear regression was used to predict the NRR (dependent variable) from the AR index (independent variable) and a 95% confidence interval was determined.

Results and Discussion

There were no significant differences between *B. indicus* and *B. taurus* bulls for NRR or sperm motility (Table 1). However, sperm viability was consistently significantly higher in the *B. taurus* bulls (Table 1). Sperm motility and viability both decreased progressively with time. To some extent, this deterioration may be due to the addition of heparin, which has been shown to decrease pH of the suspension (Parrish et al. 1980). There was no significant difference between *B. indicus* and *B. taurus* bulls for AR rate or for AR index. The AR rate increased slightly from 0 to 2 h (means, 6.2 versus 8.0%) but was profoundly greater at 4 h (mean, 24.6%), following addition of the LPC. At 4 h, the AR rate ranged from 14 to 34% (*B. indicus*) and from 17 to 46% (*B. taurus*). The Pearson correlation coefficients between NRR and AR rate at 0, 2 and 4 h were -0.43, -0.48 and 0.18, respectively. The AR index ranged from 2.0 to 5.7 (*B. indicus*) and from 3.4 to 7.3 (*B. taurus*). The Pearson correlation coefficient between NRR and AR index was 0.68. For the regression model predicting NRR from the AR index, the coefficient of regression was 0.46 ($P < 0.008$) and the equation was: $\text{NRR} = 49.17 (\pm 6.79) + 4.75 (\pm 1.49) \times \text{AR index}$ (Figure 1).

In the present study, the AR index, the relative increase in the AR rate from 0 h to 4 h (after LPC) had the highest correlation with NRR and a regression equation was derived predicting NRR from the AR index. Therefore, the AR rate was associated with *in vivo* fertility, consistent with previous studies (Lenz et al., 1988; Ax and Lenz, 1987; Feliciano Silva, et al., 1996). In a preliminary study, there was a direct association among *in vivo* fertility, *in vitro* fertility and AR rate in *B. indicus* bulls (Watanabe et al., 1996). It has recently been shown that plasma proteins are involved in both capacitation and the AR (Bellin et al., 1996). Those proteins possess a high affinity for heparin, predisposing the spermatozoa to capacitation and the AR.

In summary, assessment of the AR *in vitro* was a good predictor of fertility *in vivo*. In the future, assessment of AR may be a useful adjunct for the breeding soundness evaluation of the bull.

References

- AX, R.L.; LENZ, R.W. Glycosaminoglycans as probes to monitor differences in fertility of bulls. **Journal of Dairy Science**, v.70, p. 1477-81, 1987.
- BELLIN, M.E.; HAWKINS, H.E.; OYARZO, N.J.; VANDERBOOM R.J.; AX, R.L. Monoconal antibody detection of heparin-binding proteins on sperm corresponds to increased fertility of bulls. **Journal of Animal Science**, v. 74, p.173- 82, 1996.
- FELICIANO SILVA, A.E.D.; RAMALHO,M.F.D.; CAMPANELLI, A.C.;WATANABE,Y.F.; RODIGUES, L.H.; FREITAS, A.R.; HOSSEPIAN, V. Taxa de reação acrossômica como indicador de fertilidade de touros. In: **2o. Congresso Brasileiro de Raças Zebuinas**. Uberaba,MG, 27-30 Outubro,1996. Anais... 1996.
- KOVACS, A.; FOOTE, R.H. Viability and acrosome staining of bull, boar and rabbit spermatozoa. **Biotechnic and Histochemistry**. v. 67, p119-24, 1992.
- LENZ, R.W.; MARTIN, J.L.; BELLIN, M.E.; AX, R.L. Predicting fertility of dairy bulls by inducing acrosome reaction in sperm with chondroitin sulfates. **Journal of Dairy Science**. v. 71, p.1073-77, 1988.
- MARKS, J.L.; AX, R.L. Relationship of nonreturn rates of dairy bulls to binding affinity of heparin to sperm. **Journal of Dairy Science**, v.68, p.2078-82, 1985.
- PARISH, J.J.; SUSKO-PARISH, J.L.; WINER, M.A.; FIRST, N.L. Capacitation of bovine sperm by heparin. **Biology of Reproduction**, v. 38, p.1171-80, 1988.
- WATANABE, Y.F.; FRANSCSCHINI, P.H.; FELICIANO SILVA, A.E.D.; LÔBO, R.B. Relação entre a fecundação in vitro e a reação acrossômica em touros Nelore com alto e baixo desempenho a campo. In: **2o. Congresso Brasileiro das Raças Zebuinas**. Uberaba,MG. 27-30 outubro, 1996. Anais... 1996.
- YANAGIMACHI, R. MAMMALIAN FERTILIZATION. IN: KNOBIL, E.; NEIL, J. **The Physiology of Reproduction**. Raven Press. New York, 1988. p.135-85.
- UGUZ, C.; VREDENBURGH, W.L.; PARRISH, J.J. Heparin - induced capacitation but not intracellular alkalinization of bovine sperm is inhibited by Rp-adenosine - 3.5 - cyclic monophosphorothioate.

Table 1. Mean (\pm SD) for nonreturn rates (NRR), sperm motility and viability, and measures of acrosome reaction (AR) in *B. indicus* (n = 6) and *B. taurus* (n = 8) bulls. The probability (Prob.) of a difference between the two types of bulls is shown in the last column.

Measurement	<i>B.indicus</i>	<i>B.taurus</i>	All bulls	Prob.
NRR (%)	65.8 \pm 13.4	72.4 \pm 8.7	69.6 \pm 11.0	.29
Motility (%)				
Post-thaw	70.0 \pm 12.6	78.8 \pm 6.4	75.0 \pm 10.2	.11
0 h	63.3 \pm 22.5	72.5 \pm 7.1	68.6 \pm 15.6	.29
2 h	48.3 \pm 26.4	53.4 \pm 14.1	51.4 \pm 19.6	.63
4 h	28.3 \pm 22.3	36.2 \pm 13.0	32.8 \pm 17.3	.41
Viability (%)				
0 h	31.2 \pm 10.4	44.5 \pm 12.0	38.8 \pm 12.9	.05
2 h	27.1 \pm 9.6	40.4 \pm 7.9	34.7 \pm 10.8	.01
4 h	20.6 \pm 7.9	32.4 \pm 10.1	27.4 \pm 10.8	.04
AR rate (%)				
0 h	6.8 \pm 1.0	5.8 \pm 2.9	6.2 \pm 2.2	.40
2 h	9.5 \pm 2.0	6.9 \pm 3.6	8.0 \pm 3.2	.14
4 h	24.0 \pm 7.1	25.0 \pm 9.8	24.6 \pm 8.4	.83
AR index	3.6 \pm 1.3	4.8 \pm 1.6	4.3 \pm 1.6	.17

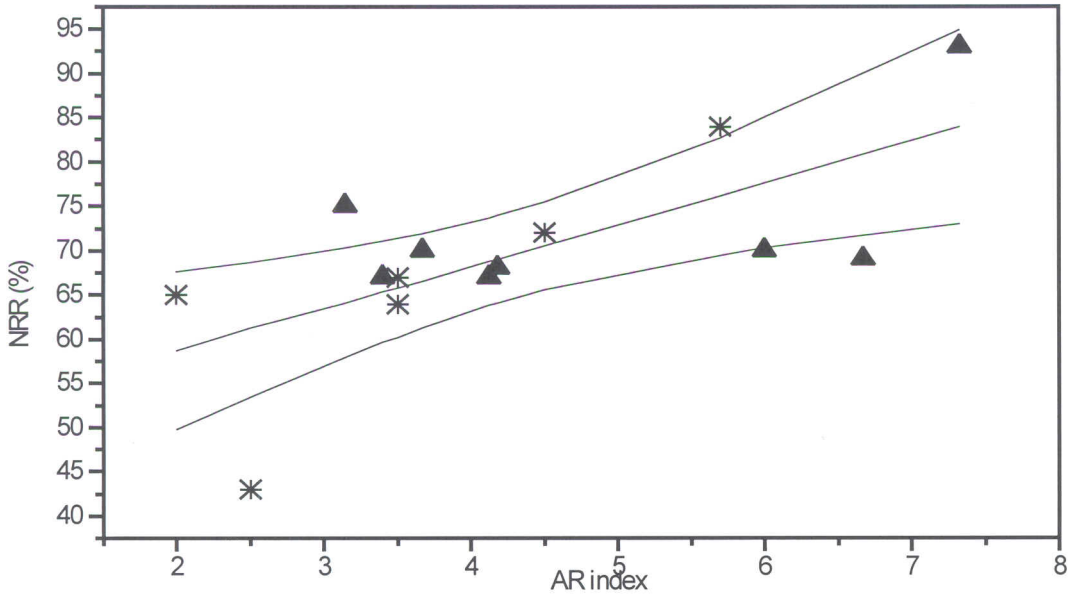


Figure 1. Linear regression line and 95% confidence intervals for nonreturn rate (NRR) as a dependent variable and acrosome index (AR index) as an independent variable for *Bos indicus* (star) and *Bos taurus* (solid triangles) bulls.