INDUCTION OF HYPERACTIVATION IN BULL SPERM IN DIFFERENT INCUBATION CONDITIONS

Poletto M.*[1], Nervo T.[1], Bertero A.[1], Ricci A.[1], Vincenti L.[1]

[1]DPT di Scienze Veterinarie ~ Torino

The sperm cells must undergo two processes which are essential to ensure their ability to penetrate the zona pellucida and fertilize the oocyte: capacitation and hyperactivation that in vivo physiologically occur within the female genital tract.
With capacitation the spermatic movement pattern is subjected to changes which can be evaluated through computer analysis: they become more rapid and linear; furthermore during this phase the acrosome reaction happens. The hyper-activated sperm shows an increase in curvilinear velocity (VCL), a widening of the lateral movements of the head (ALH) and the flagellum and a gradual decrease in speed.
Several techniques were identified and proven to achieve in vitro capacitation of bovine semen, whereas the different proposed procedures for the induction of hyperactivation are still under investigation.
The aim of the study was to induce in vitro sperm capacitation and hyperactivation of four Piedmontese breed bulls whose fertility was proven. Capacitation was induced with the standard methods for in vitro fertilization (IVF) in cattle (1), whereas to achieve hyperactivation the sperm cells were incubated in a medium added with Procaine, a molecule commonly used as a local anesthetic, but able to induce sperm hyperactivation by increasing membrane permeability to calcium ions(2).

We analized the sperm of four Piedmontese breed bulls, for each animal three paillettes were thawed out by placing them in a water bath, set to 37°C, for 1 minute. The sample thus obtained has been divided into 2 parts. One of them was processed using swim up, with an incubation of 45' at 38,5°C in an atmosphere of 5% CO2. The other share has been used as it was.
The latter was shared and incubated at 38,5°C in an atmosphere of 5% of CO2 after dilution with FERT TALP (C1, suspension), FERT TALP-PHE (C2, capacitation), FERT TALP-PROCAINE (C3, hyperactivation), FERT TALP-PHE-PROCAINE (C4, capacitation and hyperactivation). After swim-up, both sample with separated semen and that with sperm which didn't separate (C7 capacitation, C8 hyperactivation), were resuspended in FERT TALP-PHE (C5 capacitation), FERT TALP-PROCAINE (C6 hyperactivation).
All the obtained shares were evaluated through computer-assisted semen analysis (CASA), immediately after dilution (T0) and after 15', 30', 60' of incubation (T1, T2, T3).
The statistic analysis, performed with SPSS 19, proved that semen capacitation (VSL and BCF increased for samples C5 and C7. VSL C1= 93,96 µm/s ±6,48 C5= 96,52 µm/s ±3,38 C7= 95,02 µm/s ±4,25 – P< 0.05 - BCF: C1= 31,27 Hz ±7,09 C5= 38,13 Hz ±3,42 C7: 34,69 Hz ±5,4 – P< 0.05 and progressive) and hyper activation (VCL increased C1= 211,10 µm/s ± 22,78 C3= 235,39 µm/s ± 39,09 C4= 245,32 µm/s ±41,17 C6= 248,03 µm/s ±44,51 C8= 272,52 µm/s ±35,29 – P< 0.05 - and percentage of progressive cells decreased C1= 47,5% ±4,38 C3= 21,5% ±13,32 C4= 20,30% ±11,25 C6= 8,38% ±11,18 C8= 3,63% ±3,68 – P< 0.05) occurred with values statistically greater in samples that undergoing swim up (C5,C6,C7,C8), compared with those used as they were.
Time evolution of analyzed parameters showed a faster decay in samples treated with swim-up, probably because of stress-induced damages correlated with this procedure.
Finally, performing an individual analysis of the bulls, it was possible to observe a wide variability between subjects. Confirmation of this fact based on a larger number of samples and correlation between field fertility and in vitro capacitation and hyper activation response could allow the use of these tests as fertility indicators for bulls.

1. Guerin P, Guyader-Joly C, Mermillod P, Leguienne B, La produzione in vitro e la crioconservazione dell'embrione nella specie bovina. 1997. Summa, Riproduzione dei Ruminanti, anno 14, 9, 83-99.