AndroMed® with increased amount of antibiotics

Minitüb GmbH, 2010

1) Introduction

The directive EC 88/407 includes guidelines for the amount of antibiotics in extended bull semen. These guidelines were implemented in the A.I. industry by using semen extenders with a guaranteed amount of antibiotics of proven efficacy per each ml of semen extender . The reference value of a given amount of antibiotics per ml semen extender does not consider different dilution rates applied to bull ejaculates with variable native semen density. Within the wide range of semen density in native bull semen, most of the ejaculates being processed are extended at a dilution rate between 1:8 and 1:20. Especially in the case when low dilution rates are used, the amount of antibiotics per ml extended semen drifts away from the amount of antibiotics supplied with each ml of semen extender.
In consideration of the guidelines stipulated in the directive EC 88/407 with regards to the minimum amount of antibiotics per ml extended semen, the amount of antibiotics per each ml of AndroMed® bull semen extender was increased in order to fullfill the guidelines of directive EC 88/407 with a dilution rate of 1:8 and higher. The revised formula was validated in in vivo- as well as in vitro-examinations.

2) Material and Methods

Four ejaculates of each of six bulls in breeding condition were processed as split samples and used for in vitro-examinations. The aliquots were prepared as follows:

- AndroMed®-new in a high dilution ratio (1:20)
- AndroMed®-new in a low dilution ratio (1:8)
- AndroMed®-standard with 15 million spermatozoa/straw

AndroMed®-new contains the new dosage of antibiotics. The AndroMed®-standard contains the amount of antibiotics used in the past. The semen was processed according to routine lab procedures. An automatic semen freezer was used to freeze the semen.

a) Motility analysis

Aliqots from 2 ejaculates of each of 6 bulls and the CASA system SpermVision were used to analyse the motility of the frozen- thawed semen samples.
The percentage of progressively motile spermatozoa was analysed immediately after thawing as well as after 3 hours of incubation at +37°C.

b) Flow cytometric analysis

The integrity of the plasma membrane (PMAI) and acrosome was analysed in all semen samples with the FITC-PNA/PI-method*, immediately after thawing and again after a 3 hour incubation period at +37°C.
Furthermore the inducibility of the acrosome reaction (IAR) by a Ca-ionophor stimulation during a 3 hour incubation period at +37°C was analysed.

492µl semen suspension of each aliqout were pipetted into each of 3 cups for the determination of the 0-hours- and two 3-hours-values. 3µl PI und 5µl FITC-PNA were added to two samples immediately, incubated for 15 minutes respectively 3 hours at +37°C in 5 % CO2-atmosphere and then analysed.
The third cup received 3.5µl calcium-ionophore and was placed in the incubator. After 2 hours 45 minutes the sample was stained as described above and analysed 15 minutes later

In cup 1 and 2 the amount of spermatozoa with intact plasma membrane and intact acrosome (PMAI) was determined. In cup 3 the inducibility of the acrosome reaction (IAR) was determined.

* FITC = fluorescein isothiocyanate, PNA = peanut agglutinin, PI = propidium iodide

3) Results

a) Motility analysis

The percentage of spermatozoa with progressive motility immediately after thawing and after an incubation period of 3 hours at +38°C was highest in the group AndroMed®-new with a dilution rate of 1:8, followed by AndroMed®- Standard and AndroMed®-new with a dilution rate of 1:20 (Figure 1).

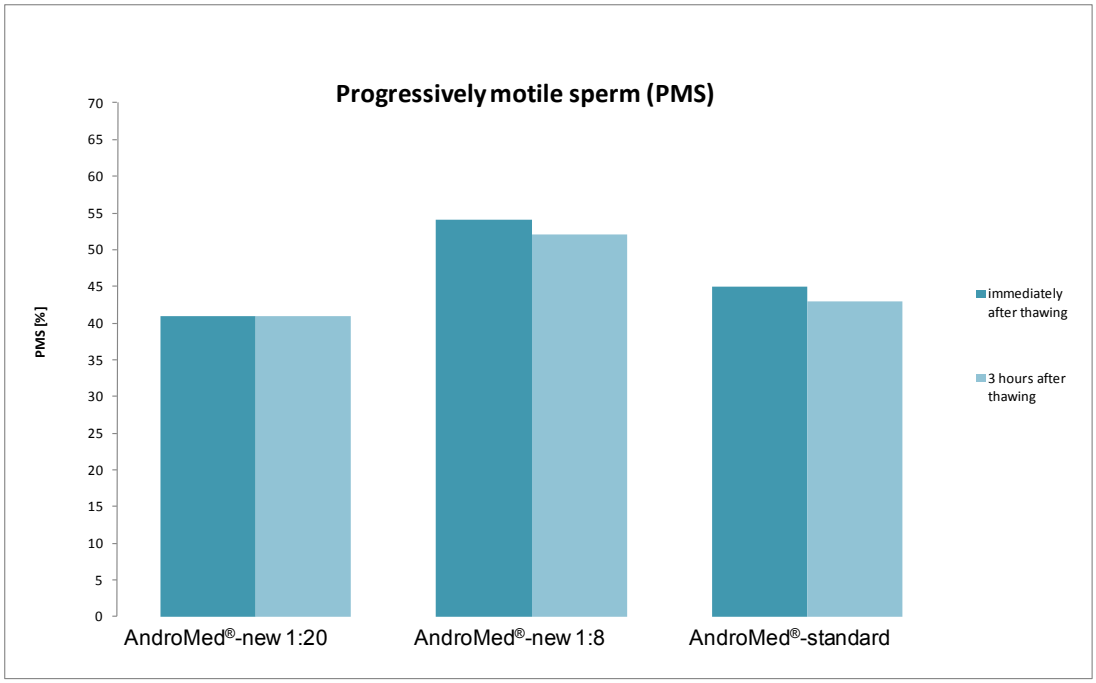

Figure 1: Progressive motile sperm (PMS) immediately after thawing and after 3 hours incubation at +38°C.

b) Flowcytometric analysis

The percentage of PMAI spermatozoa immediately after thawing and after 3 hours of incubation at +38°C was higher using AndroMed®-new in both dilution rates compared to AndroMed®-standard (Figure 2)

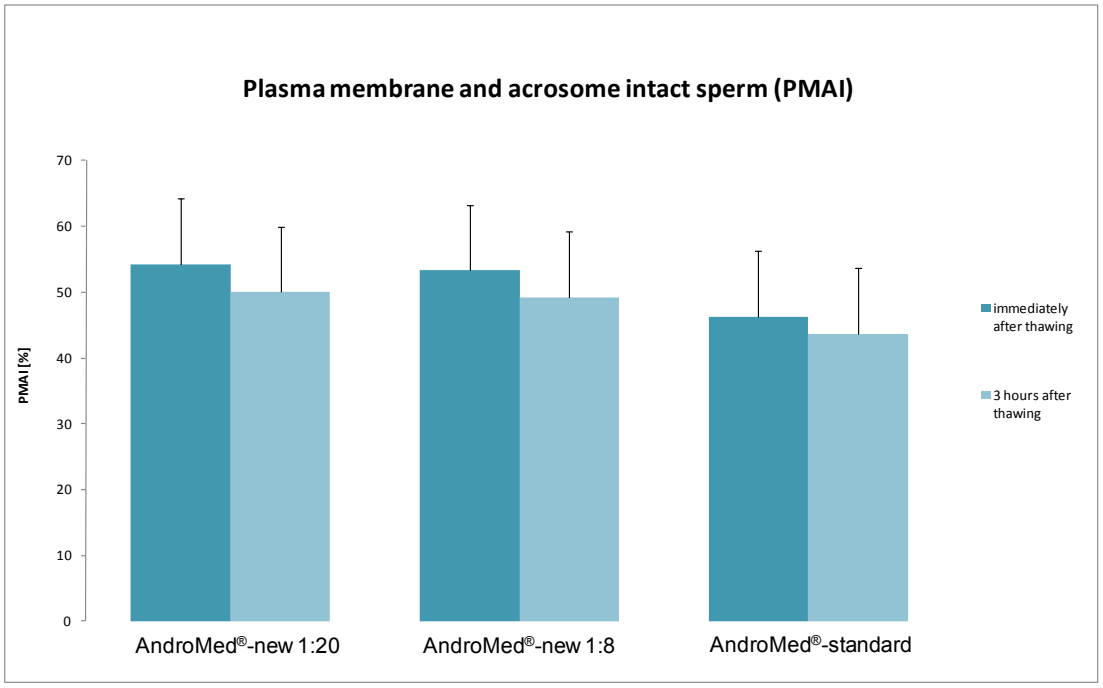

Figure 2: Percentage of Plasma membrane and acrosome intact spermatozoa (PMAI) determined immediately after thawing and after 3 hours of incubation at +38°C

Several studies showed positive correlations between the inducibility of the acrosome reaction and a successful insemination.
In the present study, semen prepared with AndroMed®-new with both dilution rates, 1:8 and 1:20, showed significantly higher values for the inducibility of the acrosome reaction compared to AndroMed®-standard after 3 hours of incubation at +38°C (Figure 3) .

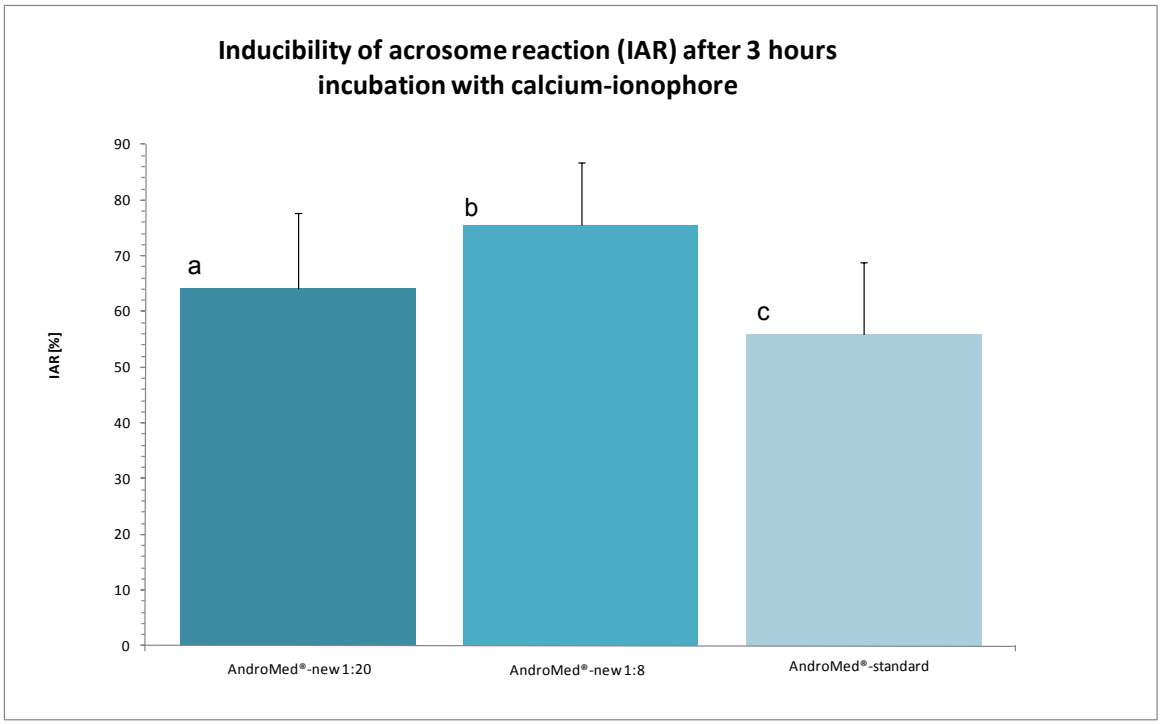

Figure 3: Inducibility of acrosome reaction (IAR) after 3 hours incubation of thawed spermatozoa using calcium-ionophore. Values marked with different letters are different ($p < 0.01$).

4) Field trial

In an additional field trial the first insemination results of split ejaculates using AndroMed®-standard und AndroMed®-new were evaluated. For this purpose split-ejaculates of 7 bulls in breeding condition were prepared with AndroMed®-standard and AndroMed®-new. A total of 882 and 877 first inseminations were done in each group, respectively. The non-return-rate (56 days) increased in the group AndroMed®-new to 68.1 % versus 66.7 % in the group AndroMed®-standard. The differences are not significant.

5) Summary

- The results of the present study show that there are no negative effects on the semen quality due to the increased amount of antibiotics in AndroMed®
- There is no difference in the non-return-rate (56 days) between both test groups
- The non-return-results of the field test correlate with the determined in vitro-results
- The modified AndroMed® composition is able to meet the requirements of the directive EC 88/407 with a dilution rate of 1:8 and higher.

6) References

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