ACROSOME REACTION PROGRESS IN FROZEN-THAWED AND CAPACITATED BOAR SPERMATOZOA INFLUENCES THE EFFICIENCY OF IN VITRO FERTILIZATION

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Abstract

In this study functional status in boar spermatozoa collected from frozen-thawed semen and capacitated in vitro by caffeine was monitored. The motile spermatozoa of boars (A, B and C) were isolated by Percoll gradient, treated with 1mM or 2mM caffeine and incubated in IVF medium or co-incubated with matured porcine oocytes for 3 hours. Motility, viability, acrosome integrity and fertilizing ability of spermatozoa were evaluated. Motility and viability of spermatozoa lightly decreased during capacitation in all tested boars independently of caffeine treatment (from 50.4±8.5 to 38.8±5.8 and from 56.0±6.9 to 43.9±5.5, respectively). The acrosome reaction was faster for 1mM caffeine-treated compared with 2mM caffeine-treated spermatozoa in all sires but its progress was different in each boar. The mean penetration rate was higher for 2mM caffeine-treated compared with 1mM caffeine-treated spermatozoa. On the other side, monospermy was higher and the mean % ±SD of normal fertilization was significantly higher ($p<0.5$) for 1mM caffeine-treated compared with 2mM caffeine-treated spermatozoa for all tested boars (29.8±6.1 vs. 12.7±5.6). It can be concluded that the progress of acrosome reaction was specific for spermatozoa of each boar and influenced the efficiency of normal IVF process.

Keywords: Boar, frozen spermatozoa, caffeine, acrosome reaction, in vitro fertility

The use of cryopreserved boar spermatozoa is very important because a higher number of insemination doses with the standard fertilizing ability of spermatozoa prepared from the same ejaculate and useful for in vitro fertilization is possible to obtain. Prerequisite for a successful oocyte fertilization and embryo production is a good functional status of frozen-thawed spermatozoa. Many assays for spermatozoa evaluation have been developed for these purposes (Johnson et al., 2007). The acrosome reaction progress in spermatozoa is necessary for fertilization process because only spermatozoa with reacted acrosome can penetrate the oocytes. For that reason the fertilization medium usually contains caffeine, an agent which is known to induce capacitation and acrosome reaction of boar spermatozoa (Funahashi et al., 1999) and increase their penetration into oocytes (Wang et al., 1991). Although more effective techniques for porcine embryo production have been widely developed during the past years, a low incidence of normospermy and high incidence of polyspermy in inseminated oocytes remain a major problem (Funahashi, 2003). It was documented that one of the reasons of polyspermic fertilization could be the quality of spermatozoa (Sirard et al., 1993). Some differences in in vitro fertilizing ability of fresh or frozen spermatozoa among individual boars have been described (Long et al., 1999; Sellés et al., 2003). But only little information is available for prediction of fertilizing ability of frozen-thawed boar spermatozoa (Gadea et al., 2001). Therefore effective methods in the field of boar sperm function evaluation are still needed.

The aim of this study was to monitor the functional status of boar spermatozoa collected from frozen-thawed semen and capacitated in vitro by caffeine. Our attention was focused on the relationship between acrosome integrity changes of spermatozoa and the efficiency of in vitro fertilization.

Material and methods

Semen cryopreservation

The sperm-rich fractions were collected from ejaculates of 2-5 year old boars of Pietrain (A) and Large White (B and C) breeds with proven fertility. The semen with at least 70% motility and 90% acrosome integrity of spermatozoa was frozen according to an adjusted procedure described by Westendorf et al. (1975) and subsequently modified by Cavarajal et al. (2004). After the equilibration in cooling extender (11 % egg yolk (v/v), 0.12 M lactose and 0.11 M trehalose) from 17 to 5°C, semen were resuspended in freezing extender (0,22 M lactose, 22.8% egg yolk (v/v), 1.3% (v/v), Equex STM, Minitübe, Tiefenbach ,Germany and 7.5 % glycerol (v/v) to final concentration of 0.5×10^9 of spermatozoa/ml and frozen in 0.5 mL straws in liquid nitrogen vapour. The semen thawing was carried out by holding straws at room temperature for 15 s and plunging them into water bath at 37°C for 30 s.

Motile spermatozoa separation

After thawing, motile spermatozoa were separated on 40 vs. 60 Percoll-gradient by centrifugation at 520g for 4 minutes. The pellet was removed and washed in BTS medium (Minitübe, Tiefenbach, Germany). The spermatozoa were resuspended (10×10^6 spermatozoa/ml) in mTBM fertilization medium (modified Tris-buffered medium containing 113.1mM NaCl, 3mM KCl, 10 mM, 10 mM $CaCl_2\times H_2O$, 20mM Tris, 11 mM glucose, 5mM sodium pyruvate and 0.4% BSA) supplemented with 1mM or 2mM caffeine. The caffeine treated spermatozoa were either capacitated or used for oocyte insemination.

Spermatozoa capacitation

Both the 1mM caffeine- and 2mM caffeine-treated spermatozoa were capacitated in a humidified atmosphere of 5%CO_2 at 39°C for 3 hours.

Spermatozoa assessment

Thirty minutes after thawing (th), immediately after caffeine treatment (0), and at 1h-intervals during capacitation (1, 2, 3h) the sperm motility, viability and acrosome integrity were assessed. Motility was evaluated subjectively using a phase contrast microscope. For viability, the spermatozoa were stained with bisbenzimide Hoechst 33258 and evaluated immediately by epifluorescence. For acrosome status evaluation, staining according to Farelly was used (Věžník et al., 2004). The percentages of motile and viable spermatozoa, and spermatozoa with intact and non-intact acrosome or without acrosome were determined.

Oocyte maturation and fertilization

Ovaries were collected from cyclic sows at a local abattoir and oocyte-cumulus complexes were isolated by slicing of ovarian cortex. Oocytes were matured in TCM-199 medium with 0.20 mM sodium pyruvate, 0.57 mM cysteamin, 50 $IU\times mL^{-1}$ penicillin, 50 $mg\times mL^{-1}$ streptomycin, 10 % fetal calf serum and gonadotropins (P.G.600 15 $IU\times mL^{-1}$; Intervet, Boxmeer, Holland) in an atmosphere of 5%CO_2 at 39°C for 46-47 h. They were inseminated in mTBM medium with 1mMcaffeine- or 2mM caffeine-treated spermatozoa of each boar at standard gamete ration and co-cultured for 3hours.

Assessment of fertilization

After co-culture, oocytes were incubated in PZM-3 medium (Yoshioka et al., 2002) for 15 hours, fixed, stained with bisbenzimide Hoechst 33258 and examined by epifluorescence. They were considered as penetrated when the oocytes had at least one swollen sperm head and/or a male pronucleus in cytoplasm. Those with more than one swollen sperm head or male pronucleus were considered as polyspermic. Only the oocytes with one male and one female pronucleus and two polar bodies were classified as monospermic. The data were expressed as rates of penetrated oocytes from inseminated, as monospermic or polyspermic oocytes from penetrated and normal fertilized oocytes from inseminated.

Statistical analysis

Data from in vitro fertilization expressed as means ± SD were analyzed by the Student's t-test.

Results

The mean rates±SD of motile and viable spermatozoa after semen thawing were 45.1±8.8 % and 50.4±6.4%, respectively. After separation, the mean percentages ±SD of motile and viable spermatozoa increased lightly to 50.4±8.5 % and 56.0±6.9 % and decreased to 38.8±5.8 and 43.9±5.5 respectively during capacitation.

The acrosome reaction progress was different in each boar and this process was faster for 1mM caffeine-treated spermatozoa compared with 2mM caffeine-treated spermatozoa in all tested boars.

In boar A, the rate of acrosome intact spermatozoa was similar before and after separation and spermatozoa response to caffeine was continual during capacitation. The increase in the rate of acrosome non-intact spermatozoa and decrease in the rate of acrosome intact spermatozoa were observed either between 0h and 2h or between 1h and 3h of capacitation in the 1mM caffeine-treated spermatozoa or 2 mM caffeine-treated spermatozoa (Figure 1).

In boar B, the rate of acrosome intact spermatozoa markedly increased after separation and spermatozoa response started quickly, immediately after caffeine supplementation. The rate of acrosome non-intact spermatozoa was higher in 1mM caffeine-treated spermatozoa compared with 2mM caffeine-treated spermatozoa from 0h to 2 h and was the same for both caffeine treatments in the of end capatitation (Figure 2).

In boar C, the rate of acrosome intact spermatozoa lightly increased after separation and spermatozoa response to caffeine had a one- or two-step character according to caffeine treatment. The first decrease in the rate of acrosome intact spermatozoa occurred between 0h and 1h in both caffeine treatment and the second decrease was observed between 2h and 3h of capacitation but only in 1 mM-caffeine treatment when higher rate of spermatozoa without acrosome was determined compared with 2 mM caffeine-treatment (Figure 3).

After oocyte insemination, the penetration rate was higher for 2 mM caffeine-treated spermatozoa compared with 1 mM caffeine-treated spermatozoa in boars A and B and it was similar for 1 mM and 2 mM caffeine-treated spermatozoa in boar C. The mean ±SD values of penetration and fertilization rates for oocytes inseminated with spermatozoa of all tested boars are showed in the Figure 4. In contrast to penetration, the rate of normally fertilized oocytes was significantly ($p<0.5$) higher after insemination with 1mM caffeine-treated spermatozoa than the rate of those inseminated with 2 mM caffeine treated-spermatozoa.

Figure 1. Acrosome reaction progress in caffeine treated spermatozoa of boar A

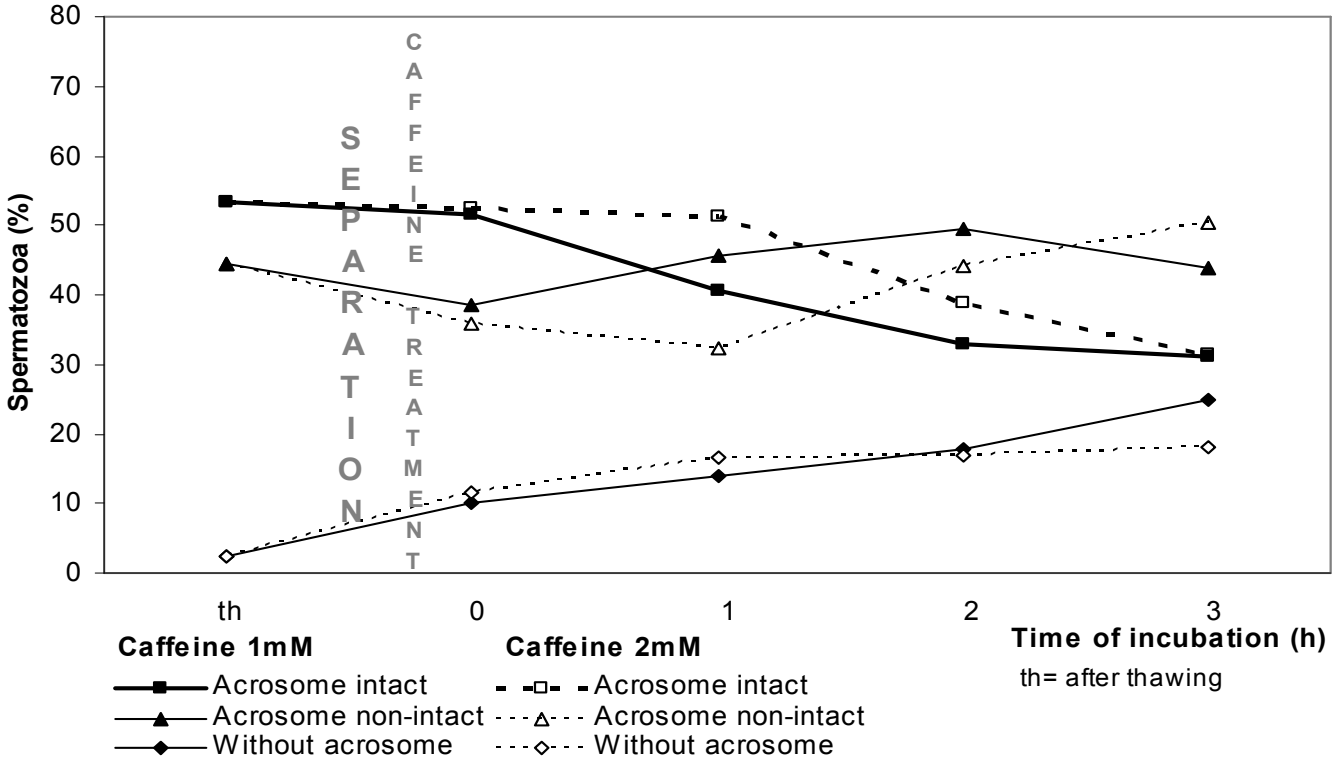


Figure 2. Acrosome reaction progress in caffeine treated spermatozoa of boar B

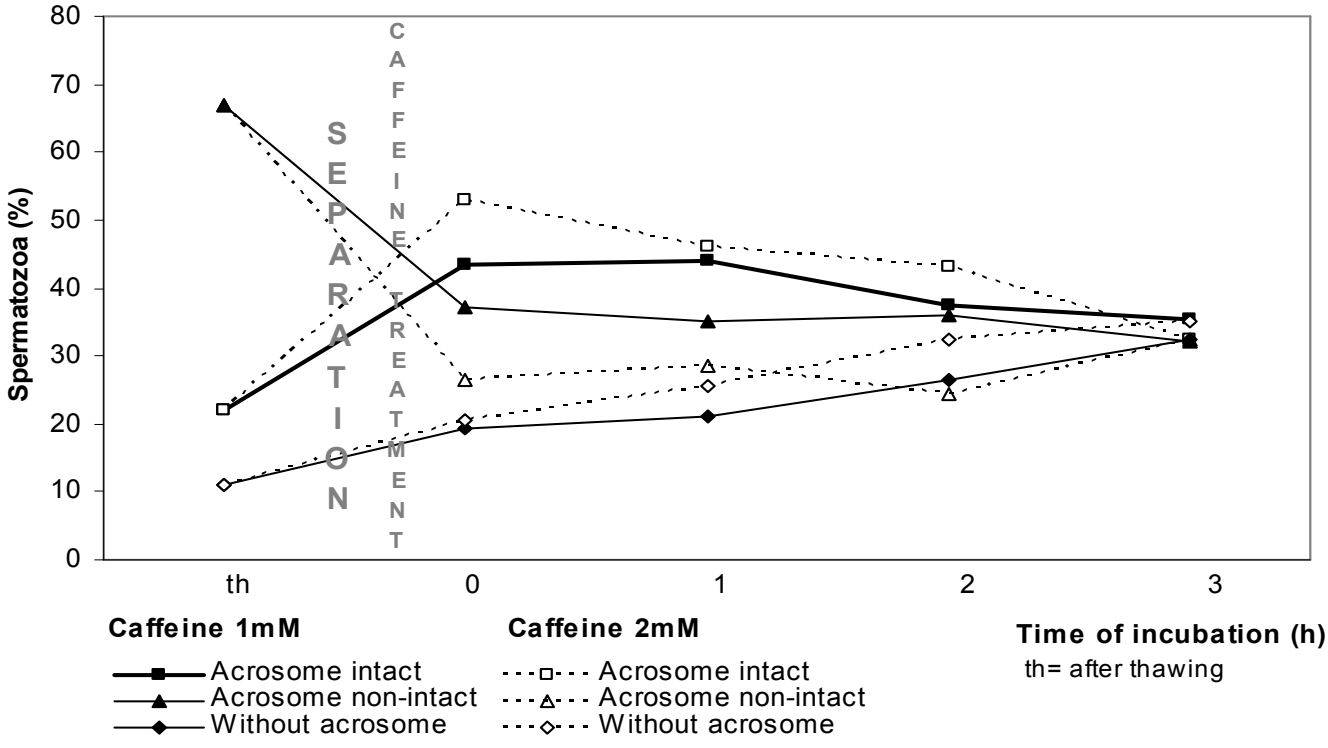


Figure 3. Acrosome reaction progress in caffeine treated spermatozoa of boar C

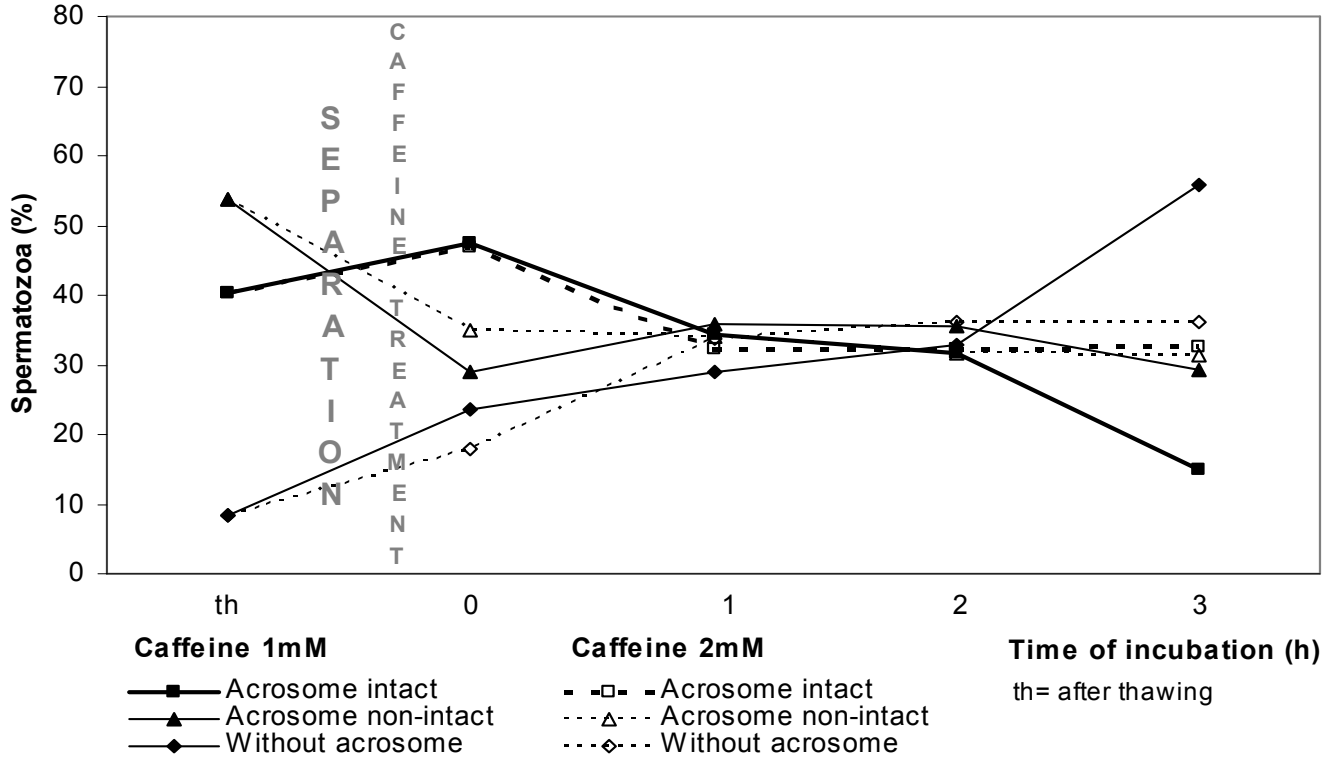
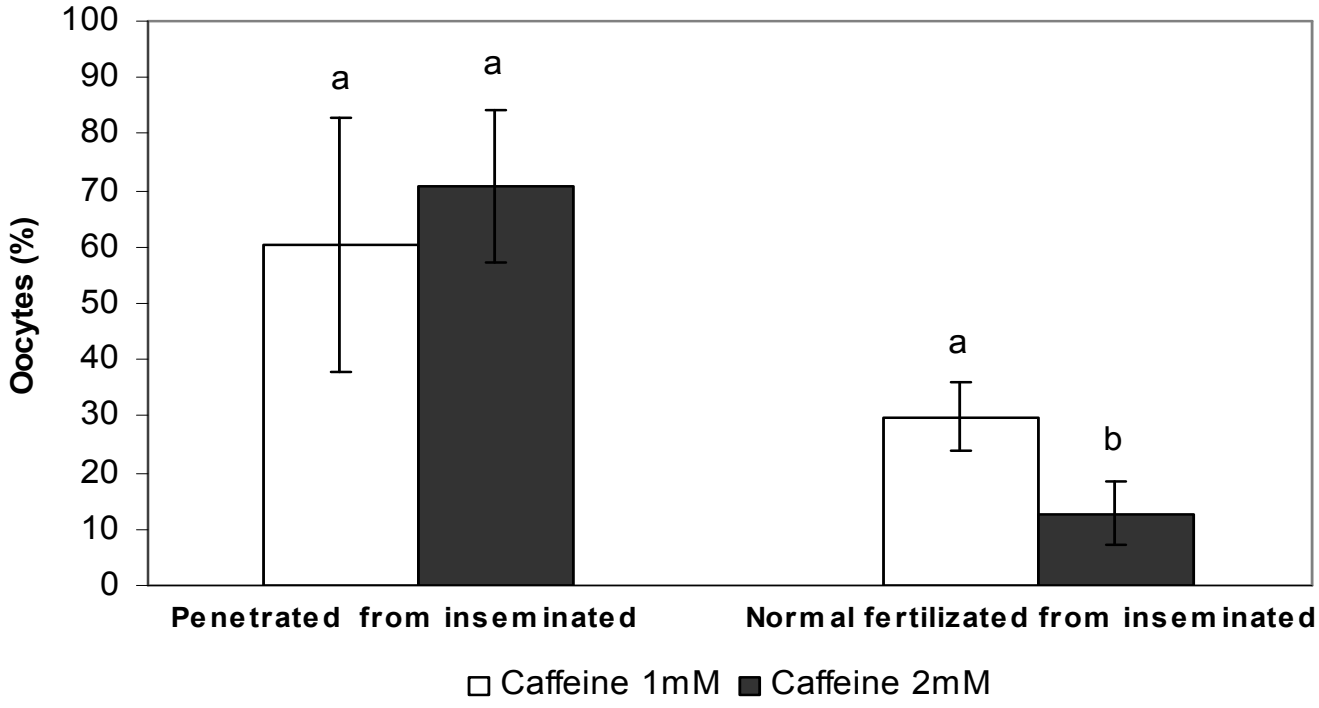


Figure 4. Penetration and normal fertilization efficiency after insemination of oocytes with caffeine treated spermatozoa for all boars (mean ± SD)



Values for the same parameter with different superscripts are significantly different ($p<0.5$).

Discussion

In order to predict the fertilizing ability of cryopreserved boar semen, it is necessary to study functional status of spermatozoa. Therefore we have focused our attention on the changes of basic functional parameters, i.e. motility, viability and acrosome integrity in capacitated spermatozoa to compare them with spermatozoa in vitro fertilizing ability.

Our study proved that the motility, viability and acrosome integrity of frozen-thawed spermatozoa were not negatively influenced by separation or capacitation. We have found that the development of acrosome changes in spermatozoa during capacitation were specific for caffeine treatment because no differences in motility and viability were observed between both caffeine levels.

Although the spermatozoa response to caffeine was different in each of the tested boars, in all sires the acrosome reaction development was faster in some of periods of capacitation for spermatozoa treated with 1mM caffeine than for those treated with 2mM caffeine.

Just as capacitation so fertilization was more effective after insemination of oocytes with 1mM caffeine-treated spermatozoa compared with 2mM caffeine treated-spermatozoa. The higher rates of monospermy and the lower rates of polyspermy were detected in oocytes after their insemination with 1mM caffeine-treated spermatozoa in all tested boars. Funahashi and Nagai (2001) reported that one of the sources of high polyspermy, that is usually observed in IVF system in pig, comes from a high rate of spermatozoa at early stage of spontaneous acrosome reaction. We suppose that in our experiments, the faster development of acrosome reaction induced by optimal caffeine treatment resulted in the decrease of the rate of spermatozoa in early stage of acrosome reaction and consequently in the decrease of polyspermy.

In our study, the faster acrosome reaction progress yielded lesser oocyte penetration but the decrease of penetration was much lower in comparison with that of polyspermy. Therefore the accelerated acrosome reaction positively influenced normal fertilization of oocytes.

Conclusion

It can be concluded that a relationship between the kinetics of acrosome reaction development during capacitation and the efficiency of in vitro fertilization has been found in boar spermatozoa. Normal fertilization was more present at oocytes when faster progress of acrosome reaction was induced in spermatozoa.

Response of spermatozoa to caffeine was specific for each boar; it would be important to modulate caffeine treatment and consequently acrosome reaction progress for individual sires.

Monitoring of acrosome reaction changes in frozen-thawed boar spermatozoa might be used for improvement of in vitro fertilization and embryo production in pig.

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