

BACILLUS CEREUS AND SELENIUM HAVE GOOD EFFECT ON *IN VITRO* MATURATION OF OOCYTES

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It is well known that selenium has positive effect on the reproductive tract either in female or male individuals, but cytology and biochemistry are not well elucidated. In this paper cytologically and microbiologically is shown that bacteria *Bacillus cereus* and selenium have large positive and synergistic influence on maturation of oocytes *in vitro*. This is caused by the bactericide effect on coliform bacteria which are abundant in the digestive tract. Taking account that anus and vagina are often nearby each other and that even sterility can be caused by coliform bacteria in the upper parts of the female reproductive tract, we found microbiologically and cytologically in oocyte in the presence of coliform bacteria, and *Bacillus cereus* and selenium, that number of coliform bacteria decreases, and cytologically oocytes cultured in such medium *in vitro* have more vitality than in culture medium without at least selenium supplement. These investigations should be continued with the aim of optimization of conditions for maturation of oocytes and fertilization *in vitro* for embryo transfer of domestic animals as well as model for *in vivo* reproduction.

Key words: reproduction, oocyte; *in vitro* maturation (IVM); *Bacillus cereus*; selenium; coliform bacteria

BACILLUS CEREUS И СЕЛЕНОТ ИМААТ ДОБРО ВЛИЈАНИЕ НА ЗРЕЕЊЕТО НА ЈАЈЦЕВИТЕ КЛЕТКИ *IN VITRO*

Веќе долго време се знае дека селенот има позитивно влијание на репродуктивниот тракт и кај женските и кај машките единки, меѓутоа на хемиско и биохемиско ниво ова поволно влијание не е расветлено. Во овој труд цитолошки и микробиолошки е покажано дека бактеријата *Bacillus cereus* и селенот имаат посебно поволно синергистичко влијание на зреењето на јајцевите клетки *in vitro*, покрај другото и поради тоа што имаат бактерицидно дејство врз колиформните бактерии кои го населуваат репродуктивниот тракт (јајчниците, јајцеводот, матката кај женските единки и тестисите кај машките единки). Покрај тоа цитолошки, во присуство на селен и *Bacillus cereus* зреењето на јајцевите клетки *in vitro* е поуспешно отколку без присуство барем на селенот како додаток во култивациониот медиум. Истражувањата ќе бидат продолжени со цел да се оптимизираат условите за созревање на јајцевите клетки и за оплодување.

Клучни зборови: репродукција; ооцити; зреење *in vitro* (ИВМ); *Bacillus cereus*; селен; колиформни бактерии

1. INTRODUCTION

The effect of selenium on the reproductive tract *in vivo*, when added in the food for domestic animals is well known, and *in vitro* needs for Se were investigated, and the need for selenium is 5 µg/ml (Jeong Y. W. et al., 2008), especially in

combination with insulin and transferrin. In last few years it is also elucidated that selenium is the most abundant in the nucleus of *Xenopus laevis* oocytes (Popescu B. F. et al., 2007). In male individuals if Se and vitamin E were added to the food for animals, sperm better penetrated Zona pelucida and greater effect seemed to be of Se (Guzman M.

et al., 1997); Nickel A. et al. (2009) investigated selenoamino acids, and concluded that those forms of organic selenium in diet *in vivo*, which contained selenoamino acids, but not seleno derivatives selenobetaine and selenocystamine, are effectively transported by various intestinal and renal amino acid transporters and are thus available for selenium metabolism and therapeutic approaches.

Bacillus cereus was isolated from cabbages and soil. It is widespread in nature, Gram-positive, or Gram-variable bacteria, viable, with optimal temperature for growth at 37–48 °C (Radojčević M., Mihajlović B., Trbić B., 1970). According to Wikipedia, in large amount it can cause abortions.

Coliform bacteria are abundant in the digestive tract. According to the fact that anus and vagina are often nearby each other in mammals, the aim of our cytological and microbiological investigation was to elucidate, effects of these bacteria and selenium on *in vitro* maturation of porcine oocytes, as a model system for *in vivo* meiosis in mammals.

2. MATERIAL AND METHODS

Porcine (Landras) ovaries (30) were obtained from the slaughterhouse in the Institute for Animal Husbandry. Ovaries were transported to the laboratory in isolation medium in a thermos container at 30°C within 2h of slaughter, and they were washed in fresh isolation medium immediately after arrival. Isolation medium for *in vitro* culture of oocytes contained: 0.95 g PBS-Dulbecco with Ca²⁺/Mg²⁺ (Serva), 100 mg Glucose (Zorka), 3.6 mg Na-pyruvate (Serva), 4 mg Streptomycin (Sigma), 0.5 mg Gentamycin (Sigma), 1 ml Heparin (Galenika), 30 mg BSA (Sigma) and 100 ml distilled water.

In every slot 5 ml cultivation medium (Torner H. et al., 2001) was added which contained: 100 ml distilled water, 220 mg NaHCO₃ (Serva), 0.5 mg Gentamycin (Sigma), 2.2 mg Na-pyruvate, 1510 mg TCM 199 Hepes Modification (Sigma) and 30 mg BSA (Sigma).

In the first line was cultivation medium with coliform bacteria, and Se with concentration of 4g/l (in inorganic form), and in the second one, in cultivation medium oocytes (3 per slot) and coliform bacteria were added in the same concentrations (both 10⁵ and 10⁴/ml) with Se as in the first line, and *Bacillus cereus* in the same concentrations. Controls are without oocytes in the last row.

Blocks of slots were put for incubation, first 1 h at 30°C, and then at 37°C. From every slot material is given to microbiological analyses by introducing content from blots on solid medium and counting bacterial colonies by the indirect method after 3 h and 24 h of incubation of slots at 37°C. Cytological observation was done, at the beginning, 24 h and 38 h of cultivation on the stereo microscope (40×) (EUinstruments).

The experiment was repeated 3 times, and the statistical method F test was performed.

3. RESULTS

After 3 h of incubation of blocks with slots with culture media, bacteria, selenium and oocytes, no visible changes were observed in the microbiological, or cytological level. That means that our work satisfied sterility conditions. After 24 h in slots where oocytes were not added, coliform colonies were visible (yellow spots) (Fig.1), and no coliform bacteria or *Bacillus cereus* colonies were observed in slots where oocytes and selenium were implemented. The microscopic view shows after 24 and 38 h of incubation very vital oocytes where *B. cereus* and selenium were added (Fig. 2), too.

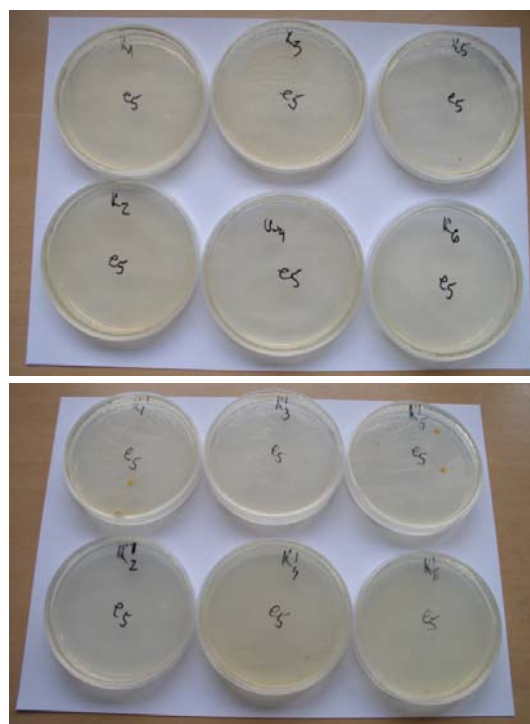


Fig 1. Negative influence of oocytes, Se and *Bacillus cereus* on coliform bacteria

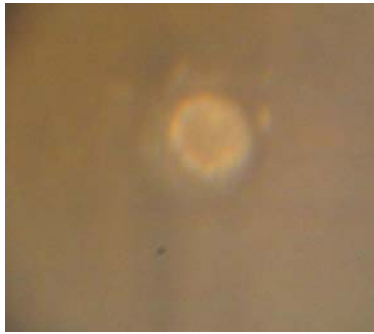


Fig. 2. Microscopic view (40×) of maturated porcine oocyte (38 h *in vitro*)
(Photo: Dr Vesna Krnjaja, work: Dr nat.sci.Tatjana Smiljaković and Marija Kojić)

The upper picture represents the first day of cultivation, down the second day. Oocytes and Se, and *B. cereus* are in the down line of both pictures. Coliform bacteria colonies are yellow spots. Oocytes on the cytological level *in vitro* survived in all combinations with *B. cereus* and selenium, and good vitality of oocytes after 38 h was observed (Photo: Dipl. Arch. Stanislav Marinkov, work: Dr Tatjana Smiljaković and Dipl. spec. vet. Ljiljana Stojanović).

On Fig. 3 colonies of coliform and *B.cereus* bacteria are presented.

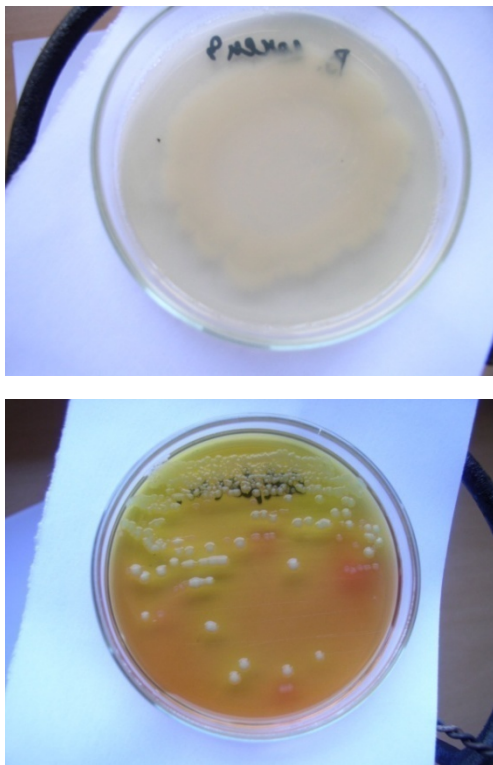


Fig. 3. *Bacillus cereus* on the first Petri plate and coliform bacteria on the second plate
(Photo: Dipl. Arch. Stanislav Marinkov, work: Dipl. vet. spec. Ljiljana Stojanović)

4. DISCUSSION

According to Paszkowski T. et al, 1995, patients with unexplained infertility had significant decreased follicular selenium levels as compared with those with tubal infertility or male factor. This indicated that Se is very important for reproduction and many experiments have been done on experimental animals to explain the role and needs for good fertility. According to Choi IS et al, 1994. Se is important in oocyte proteins, and he successfully added Se to proteins by selenocisteinil-tRNA *in vitro*. First trials were by adding selenium into food to animals in the form of inorganic (in our experiments, too) sodium selenite (Thorlacius-Ussing O. et al., 1986), but nowadays it is clear that in feed organic form of selenium must be included, and there are many investigations to find which forms, by which pathways and in which amounts should be done for different animals in different reproduction phases or on vitro conditions. By Raghu H. M. et al., 2002 was found that supplementation with FSH and EGF significantly increased the maturation of buffalo oocytes and the yeald of blastocyst was higher in media containing EGF and insulin-transferrin and Se. According to Popescu B. F. et al. selenium is more abundant in nucleus than in cytoplasm.

Transport of the transporter modifier RS1 is important for transport of middle large molecules (Se-transporters) into oocyte (in *Xenopus*) (Valentin M. et al., 2000) but pathways are still unclear, how it travels and gives signals into the oocyte from plasma membrane to nucleus. Therefore investigations in this field should be done.

On the other hand, the effect of selenium on coliform bacteria was very rarely investigated, which is the problem in reproduction of mammals. There are some trials to stop infections with inulin into feed for domestic animals, and investigations of immune capabilities of the reproductive system, but this is the first preliminary paper in which we show that Se has a very good effect for decreasing the amount of coliform bacteria, especially with addition of *Bacillus cereus*, into the *in vitro* culture of oocytes. Therefore, our investigations will be continuous with organic selenium and bacterial cultures, for *in vitro* maturation of oocytes and possibly fertilization because it was shown that for oocytes, sperm and fertilization Se has a wonderful effect. The fact that those lines of *Bacillus* are widespread in the nature and not

pathogenic, but must be abrogated for pregnancy time, because it was shown that *B. cereus* for example is dangerous during pregnancy (can cause abortions).

5. CONCLUSION

In this research paper we conclude that selenium and *Bacillus cereus* have a very efficient anticolidiform effect on in vitro conditions, by simulation of in vivo conditions of reproductions. Vagina and penis are often nearby anus where coliform bacteria are usually abundant. Therefore we recommend disinfection of anus before coitus in vivo and supplementation of at least selenium in fertilization in vitro. Effect of *Bacillus cereus* on coliform bacteria is also approved. Following investigations will be done with the aim to elucidate these effects.

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