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ANTIMICROBIAL ACTIVITY OF PORCINE OVARIAN FOLLICULAR FLUID ON *Bacillus cereus* AND COLIFORM BACTERIA *in vitro*

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Abstract: Potential antimicrobial activity of follicular fluid and its selectivity remains open questions in field of human and animal reproduction. In our work we examine antimicrobial activity of porcine follicular fluid on coliform bacteria, as Gram-negative, and *B. cereus*, as Gram-positive, by incubation of different dilatations of bacteria with follicular fluid in cultivation medium and microbiological analysis followed by. According to our results, growth of coliform bacteria is stimulated by porcine follicular fluid and has an inhibitor effect on *B. cereus*, the most probably caused by lysozyme activity. Further study of the antimicrobial effect of follicular fluid against other strains and biochemical analysis in order to define its mechanism, are necessary.

Key words: follicular fluid, antimicrobial effect, coliform bacteria, *Bacillus cereus (B. cereus)*

Introduction

Follicular fluid fills the follicle antrum, surrounds the oocyte and granulose cells, and contains steroids, gluco protein hormones, plasma proteins, muco-polysaccharides and enzymes. It is especially rich in content of hyaluronic acid whose degradation products participate in signal transduction and activation of lymphocytes which leads to pro-inflammatory reaction.

Increasing number of studies confirm the close correlation between immune and reproductive system, and even pre-ovulatory occurrences use mechanisms of inflammation, so the presence of macrophages, T and B lymphocytes, antigen of presenting and NK cells, differently distributed depending on the stage of cyclic changes on follicle during menstrual cycle (*Craig et al, 1996*), is inevitable. In follicular fluid the presence of cytokine, IF-γ, interleukin,

prostaglandin, proteolytic enzymes and other products of immunological agents were detected.

Role of hyaluronic acid, as well as components of immune system, is basically development, and maturation and finally attresion of follicles which includes their participation in processes of initiation of apoptosis of follicular and luteal cells, followed by phagocytes of the cell debris and tissue reparation by macrophages.

In all cyclic changes of the follicle, and composition of follicular fluid, besides this main role and immune endocrine interaction, presence of immunological agents still indicates the presence of antimicrobial trait of follicular fluid, so scientists have come up with the idea and basis for investigation and detection of main effectors, carriers of these traits, as well as potential selectivity in relation to Gram-positive and Gram-negative bacteria.

In our paper we analyze potentially antimicrobial activity of porcine follicular fluid on coliform bacteria and *B. cereus* for the purpose of obtaining additional knowledge in realization of the correlation between immunological and reproductive system.

Material and methods

For the purpose of this research, 30 pre-ovulatory ovaries of Landrace pigs were taken from the slaughterhouse of the Institute for Animal Husbandry, Belgrade-Zemun and transported to the Laboratory in isolation medium for maturation of oocytes *in vitro* (*Torner et al, 2001*) in thermos on 30°C. Isolation medium contained: 0.95 g PBS-Dulbecco with Ca^{2+}/Mg^{2+} (Serva), 100 mg Glucose (Zorka), 3.6 mg Na-pyruvate (Serva), 4 mg Streptomycin (Sigma), 0.5 ml Gentamycine (Sigma), 1 ml Heparin (Galenika) and 30 mg BSA (Sigma).

Using syringe and sterile needle follicular fluid was extracted from individual follicles and filtered using filter with micro-spores size of 50 μm, and in this way the complex with egg and cumulus cells was separated, they remained on the filter, from the follicular fluid which passed through the filter and remained in the dish.

In the first experiment, into each little well 5 ml of cultivation medium was added (*Torner et al, 2001*) which contained: 100 ml of water, 220 mg $NaHCO_3$ (Serva), 0.5 mg Gentamycine (Sigma), 2.2 mg Na-pyruvate (Serva), 1510 mg TCM 199 Hepes Modification (Sigma) and 30 mg BSA (Sigma). Into small well marked as B2, 0.1 ml of follicular fluid was added, and in B4 0.2 ml of follicular fluid in all four small wells, i.e. B1, B2, B3 and B4, 0.5 ml dilution of coliform bacteria was added, in B1 and B2 dilution of 10^{-4}, and in B3 and B4 of 10^{-5}. Controls were made in the following way: in 2 small wells, like in others, 5 ml of cultivation medium, and then in one of them (K5) 0.5 ml bouyon where bacteria where

cultivated, which is control for bacteria medium, and in the other one (K6) 0.2 ml of follicular fluid, which was control for follicular fluid.

In the second experiment, into each of the small wells 4 ml, except into small well 4, where 3ml of cultivation medium was added as well as 1 ml in each well of dilution *B. cereus* of 10^{-5}, except into well 5 where instead of bacteria dilution 1 ml of bouyon was added used for cultivation of bacteria and represents control for bacteria medium. Into small well 2 5 oocytes were added, and in well 4 2 ml of follicular fluid.

Dishes containing small wells were incubated first on 30°C, and then on 37°C. From each of the small wells material was analyzed microbiologically by placing of content on solid medium in Petri dish and counting of bacteria colonies using indirect method 3h and 24h subsequent to incubation.

Results and discussion

In the first experiment, results show that follicular fluid promotes the growth of coliform bacteria. Controls were regular (Figure 1.b). In B2 and B4 (Figure 1.a) containing follicular fluid increased number of coliform bacteria colonies are noticed compared to B1 and B3 where there was no follicular fluid, and this is evident from results presented in table 1 3h after incubation.

Figure 1.a Analysis of antimicrobial activity of porcine follicular fluid on coliform bacteria

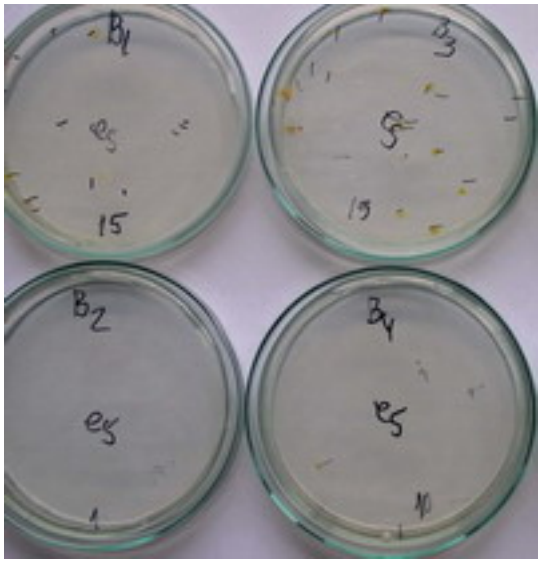

Fig. 1.b Controls for bacterial medium and follicular fluid

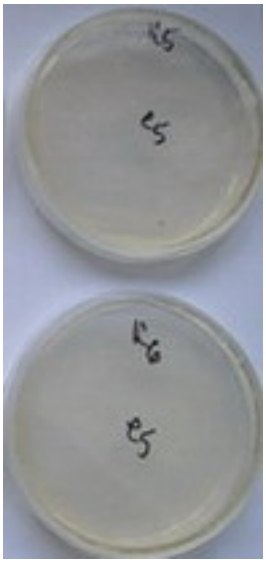

Table 1. Number of colonies of coliform bacteria from culture medium on Petri dish 3 and 24 h after incubation

Petri dish with cultivation sample	B1	B2	B3	B4
Number of colonies 3 h after incubation	300.000	1.200.000	500.000	2.600.000
Number of colonies 24 h after incubation	40.000	1.400.000	100.000	100.000

Results of the second experiment show the antimicrobial activity of follicular fluid on *B. cereus*. In Petri dish 1 and 2, results of the bactericide activity of the oocyte on bacteria of this strain are presented. From Figure 2 and Table 2. it is evident that there is considerable decrease of number of bacteria colonies 24 h after incubation in Petri dishes 3 and 4, and decrease of this number is more expressed in dish 4 containing the follicular fluid, in comparison to Petri dish 3 where there is no follicular fluid. Controls were correct, and marked on figure with number 5 (no bacteria, control for bacteria medium) and 6 (with bacteria, without follicular fluid and oocytes, control for cultivation medium for oocytes and follicular fluid).

Figure 2. Analysis of antimicrobial activity of porcine follicular fluid and oocyte on *B. cereus*

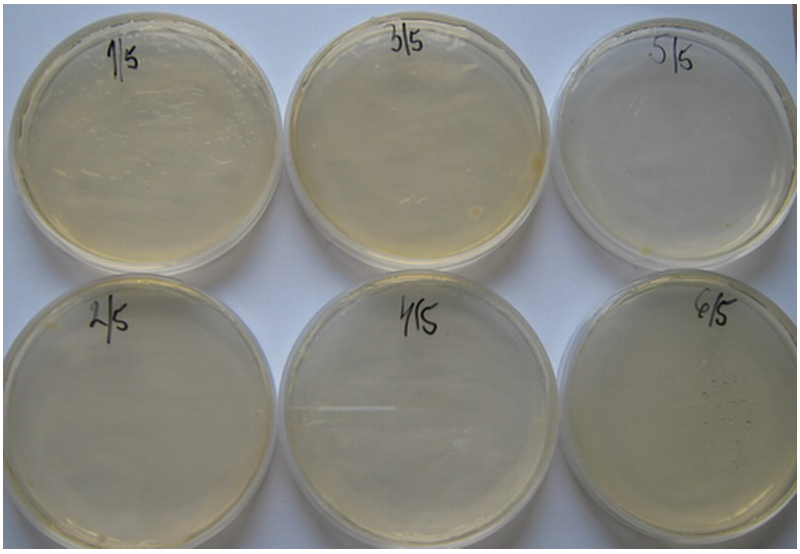

Table 2. Number of colonies of *B. cereus* from culture medium on Petri dish 3 and 24 h after incubation

Petri dish with cultivation sample	1	2	3	4
Number of colonies 3 h after incubation	100.000	200.000	200.000	800.000
Number of colonies 24 h after incubation	100.000	0	0	100.000

In our paper it is clearly confirmed that it is difficult to obtain precise results which unambiguously show that full presence or absence of antimicrobial activity of follicular fluid in regard to given strains, but conclusions were made based on comparison of the increase or decrease in percentages of the number of colonies, with or without presence of follicular fluid. Reason for this is effect of numerous factors of the environment where cultures are cultivated, so in the first trial we had in results obtained 24h after incubation identical number of colonies in B3 and B4, i.e. with and without follicular fluid, since here we also had higher dilution of bacteria, but more expressive presence of antibiotics in cultivation medium after 24 h, which of course induced decrease of number of bacteria colonies.

In the research of the antimicrobial activity of human follicular fluid in regard to strains *E. coli, P. aeruginosa, S. agalactiae, L. monocytogenes, C. albicans,* results showed bacteriostatic activity in regard to all colonies, where *E. coli* and *S. agalactiae* were the most sensitive strains (*Kably Ambe et al, 1995*).

By investigation of the selectivity of antimicrobial activity of the human follicular fluid in regard to Gram-positive and Gram-negative bacteria, as well as *C. albicans*, it was confirmed that follicular fluid had inhibitory effect on Gram-positive bacteria, whereas the growth of Gram-negative and *C. albicans* was stimulated by incubation using follicular fluid (*Gürgan et al, 1993*).

In our work with coliform bacteria, the increase of bacteria colonies in culture with follicular fluid was evident, which proved that it promotes the growth of coliform bacteria which are Gram-negative, whereas in case of *B. cereus* as Gram- positive bacteria, follicular fluid inhibits the growth of this strain.

Mechanism used for achievement of antimicrobial activity of follicular fluid remains unknown, and it is considered that it includes elements of immunological system, based on low content of hyaluronic acid and presence of these elements in the follicle. Previous researches with human follicular fluid have demonstrated that there is no statistically significant change in estradiol, progesterone, transferin, iron and protein concentrations, and most probably lysozyme is main agent of antimicrobial activity of this fluid (*Stepanović et al, 2003*).

Conclusion

Follicular fluid according to our results demonstrates antimicrobial activity selectively, with positive effect on growth of coliform bacteria and negative on growth of *B.cereus* and probably using lysozyme in this activity.

In order to come to final results of the research on this topic, it is necessary to carry out additional researches with other Gram- positive and Gram-negative bacteria, as well as biochemical and molecular-biological analysis of endocrine immunological active components for the purpose of determination of mechanisms and level of activity, as well as potential varying in different stages of menstrual cycle.

Acquired knowledge can be applied in the field of maturation of follicle and oocyte, role of immune system in ovulation and field of protection from bacterial infections of human and animal reproductive system.

Antibakterijsko dejstvo svinjske folikularne tečnosti iz jajnika na koliformne bakterije i *Bacillus cereus in vitro*

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Rezime

Potencijalno antibakterijsko dejstvo folikularne tečnosti i njegova selektivnost su otvorena pitanja u oblasti reprodukcije ljudi i životinja. U našem radu ispitujemo antibakterijsko dejstvo folikularne tečnosti svinje na koliformne bakterije, kao Gram-negativne, i *Bacillus cereus*, kao Gram-pozitivne bakterije, zasejavanjem kultura na kultivacionom medijumu i daljom mikrobiološkom analizom brojanjem kolonija izraslih na medijumu nakon inkubacije medijuma za ćelijsku kulturu jajnih ćelija sa i bez folikularne tečnonsti. Rezultati pokazuju da folikularna tečnost stimuliše rast koliformnih bakterija, a deluje inhibitorno na B.cereus, gde je za ovu aktivnost najverovatnije odgovoran lizozim. Potrebna su dalja ispitivanja antibakterijskog dejstva folikularne tečnosti u odnosu na druge sojeve i analize mehanizma ostvarivanja ove aktivnosti.

References

CRAIG L. BEST, JEFFREY PUDNEY, WILLIAM R. WELCH, NATALIE BURGER, JOSEPH A. HILL (1996)**:** Localization and characterization of white blood cell populations within the human ovary throughout the menstrual cycle and menopause. Hum Reprod, 11(4):790-797.

TORNER H., KUBELKA M., HELEIL B., TOMEK W., ALM H., KUZMINA T., GUIARD V. (2001): Dynamics of meiosis and protein kinase activities in bovine oocytes correlated to prolactin treatment and follicule size. Theriogenology 55 885-899.

KABLY AMBE A., VIELMA VALDEZ A., ARREDONDO JL., BELTRAN ZUNIGA M., ALVARADO DURAN A. (1995): Evidence of antimicrobial activity of the follicular fluid: an evaluation and analytical study of samples obtained in an assisted reproduction program. Ginecol Obstet Mex. 63:152-7

T.GÜRGAN, B. URMAN, K.S. DIKER, L. DELILBASI, H.A. KISNISCI (1993): Human follicular fluid from pre-ovulatory follicles in patients undergoing in-vitro fertilization inhibits the in-vitro growth of Gram-positive micro-organisms. Human Reproduction, Vol 8, No. 4, pp. 508-510

STEPANOVIĆ S., ĐUKIĆ S., VELJKOVIĆ M., ARSIĆ B., GARALEJIĆ E., RANIN L. (2003): Antimicrobial activity of human follicular fluids. Gynecol Obstet Invest, 56(3):173-8.