УДК 612.822 : 612.3 + 591.4 + 636.4

THE IMPACT OF COLOSTRUM ON BRAIN DEVELOPMENT OF THE NEWBORN PIGS

K.O. Goncharova[1], I.O. Osadchenko[1], T.M. Kovalenko[1], G.O. Ushakova[2], S.G. Pierzynowski[3], G.G.Skibo[1]

[1] Department of Cytology, Bogomoletz Institute of Physiology, (chief – prof. G.G. Skibo), Kiev; [2] Department of Biophysics and Biochemistry, Dnepropetrovsk National University named by Oles'Gonchar, Dnepropetrovsk, Ukraine; [3] Department of the Biology, Lund University, Solvegatan, Sweden.

THE IMPACT OF COLOSTRUM ON BRAIN DEVELOPMENT OF THE NEWBORN PIGS
K.O. Goncharova, I.O. Osadchenko, T.M. Kovalenko, G.O. Ushakova, S.G. Pierzynowski, G.G.Skibo

SUMMARY

Colostrum is a source of many bioactive components including antibodies (IgG) as suggested protecting the newborns against infection. In this study we aimed to evaluate the role of colostrum for the brain development in newborn pigs. The hippocampal neurons and microglial cells were identified by specific antibodies and analyzed with confocal microscope and the morphometric assay was provided. The piglets deprived of colostrum revealed a reduction in postnatal hippocampal neuro and microgliogenesis. Feeding with elementary diet instead of colostrum didn't help physiological postnatal brain development. Addition of IgG to elementary diet improved the neurogenesis and supported immune status of the brain supporting the importance of natural feeding in the first weeks of life.

ВПЛИВ МОЛОЗИВА НА РОЗВИТОК МОЗКУ НОВОНАРОДЖЕНИХ ПОРОСЯТ
К.О. Гончарова, І.О. Осадченко, Т.М. Коваленко, Г.О. Ушакова, С.Г. Пієржиновський, Г.Г. Скибо

РЕЗЮМЕ

Молозиво є джерелом багатьох біологічно активних речовин, в тому числі, імуноглобулінів (а саме IgG), які, як вважається, захищають новонароджених від інфекцій. Метою даного дослідження була оцінка впливу споживання молозива після народження на розвиток головного мозку поросят. Методом конфокальної мікроскопії з допомогою специфічних антитіл були ідентифіковані нейрони СА1 зони гіпокампа та мікрогліальні клітини, також був проведений морфометричний аналіз зрізів. У позбавлених молозива тварин було виявлене зменшення постнатального нейро- та мікрогліогенезу в гіпокампі. Годування поросят основною дієтою замість молозива не відтворювало фізіологічний постнатальний розвиток головного мозку. Додавання IgG до основної дієти покращувало нейрогенез та підтримувало імунний статус головного мозку, що підтверджує думку про важливість натурального харчування в перші тижні життя.

Key worlds: colostrum, brain, hippocampus.

Colostrum from several points of view is one of the most mysterious tissues in several mammals' species. Among others ungulate pigs and ruminants are fed without transformed immunity from their dam during intrauterine life [1]. Colostrum contains a lot of biologically active peptides and proteins, which are not produced by infants host endocrine system and must be absorbed from the gut. These components are synthesised in mammary glands. To them belongs: growth factors, hormones e.g. insulin concentration in colostrum is 100 times higher than in blood similar is for prolactine, IGF, leptin, ghrelin, etc, the other group of the active substance are appear after digestion of colostrum/milk proteins [2]. The main non-nutritional components of the colostrum are immunoglobulin's – the super biologically active proteins and absorbable from the gut to the blood during first 24 hours of the life. Thus, the main aim of the studies was to evaluate role of the immunoglobulin of colostrum origin on brain development.

MATERIALS AND METHODS

Animals and diet

The experiment was conducted on 48 healthy piglets (Swedish Landrace X Yorkshire x Hampshire) born on time and with no complication obtained from SLU University Farm, where complete management and health records were maintained. Just after birth pigs were divided into 5 groups by simple randomization**:**: control newborn group – newborn, unsuckled pigs (n=8) (A), native control group – suckled pigs, staying with sow during 24 and 72 hours (n=16) (B), and fed: colostrum group - (C), (n=8), ED group - reared with elementary diet (The elementary diet (Kabiven, Fresenius Kabi AB, Uppsala, Sweden) contained glucose, free amino acids and emulsified soybean oil and was supplemented with vitamins (Soluvit®, Fresenius Kabi AB, Uppsala, Sweden) and (Vitalipid adult, Fresenius Kabi AB, Uppsala, Sweden) (n=8) (ED), and ED-IgG group - reared with ED+Ig (purified serum IgG), (n=8). Pigs were fed up to 24 and 72 hours. The pigs were individually housed and provided with food via a stomach tube with dose 10 ml/kg every 2 hours. The care and use of animals was

conducted in accordance with the principles outlined in the current Guide to the Care and Use of Experimental Animals and was approved by Lund University Ethics Review Committee on Animal Experiments, Sweden.

Brain sampling for morphology

Pigs from group A were euthanized immediately after birth and brain specimens were taken for further analysis. 24 and 72 hours after the experiment start piglets from each group were sacrificed. Animals were anesthetized using 0.5 - 1.5% air mixture of Fluothane (Zeneca, Gothenburg, Sweden) and carrier O_2 at approximately 0.5 l/min and fixed by transcardial perfusion with 4% formaldehyde in 0.1M phosphate buffer. The brains were isolated after perfusion and separated hippocampi were postfixed overnight in the same fixative solution at +4°C. The next day they were cut in 50-мм-thick frontal slices by a vibratome Vibroslice 752M (Campden Instruments Ltd, Great Britain). Hippocampal slices were washed out with 0.1M phosphate buffer and treated in blocking solution containing 1% normal goat serum and 0.3% Triton X-100. Double immunofluorescence staining of hippocampal slices was used. Neuron identification was realized by monoclonal antibodies, specific to neuronal nuclear protein NeuN. Iba1 (ionized calcium binding adaptor molecule 1) was used as the marker for microglial cells. Slices were incubated with primary mouse anti-NeuN antibodies (diluted 1: 1000) and rabbit anti-Iba1 polyclonal antibody (diluted 1:1500) during 16 hours at +4°C. After washing slices were incubated with secondary antibodies: anti-mouse conjugated with Alexa Fluor 488 (1:1000) and anti -rabbit conjugated with Alexa Fluor 555 (1:1000) for 1,5 h at room temperature. Then slices were washed out, placed on histological slides and mounted with Fluorescence Mounting Media (Dako, Denmark). Images of hippocampal tissue were analyzed with confocal FV1000-BX61WI microscope (Olympus, Japan). For morphometric assay the number of neurons and microglial cells in the CA1 hippocampal region was counted for the area unit with using UTHSCSA Image Tool (v.3.0., UTHSCSA, USA).

Statistical analysis

All data were expressed as mean ± SE (standard error), ANOVA (analysis of variance). All analyses were carried out using Statistica, version 7 (StatSoft, USA). In all statistical analyses, $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

The amount of neurons in pig's hippocampus

At birth, the central nervous system has completed most of its early stages of cell division, migration, and specialization. Neuroblasts are continuing to divide only in limited brain regions [3]. Recent imaging studies show that the formation of neural connections in the central nervous system is a highly dynamic process.

The highest amount of neurons in *stratum radiatum* of the CA1 hippocampal area of newborn piglets (group A) was observed. In the first days of life the number of neurons was diminished in C, ED and ED+Ig groups in comparison with group A ($p<0.01$). To the 24th hour after birth the decrease was the 34,2%, 33,2%, 22,6%, 40,6% in the groups B, C, ED and ED+Ig respectively. To the 72th hour the significant increase of neurons number (pd"0.05) in comparison with group B was observed in hippocampi of animals from groups C and ED+Ig (Fig.1). Changes observed could be a possible evidence of changed migration and maturation of neurons on the early stages of postnatal development in dependence of diet.

Neural activity modulates development through biasing the process of formation and elimination, promoting the formation and stabilization of appropriate synaptic connections on the basis of functional activity patterns The iterative formation and elimination of synapses and neuronal branches result in the formation of a much larger number of trial connections than is maintained in the mature brain [4].

The amount of microglial cells in pig's hippocampus

Microglias are cells of the innate immune system, which is the front line of host defense against invading pathogens or pathological cells. The development of microglia is not an isolated process and must take place with the microenvironmental changes associated with neurogenesis, gliogenesis, angiogenesis, and synaptogenesis. Microglial activation during immune activation has been associated with both enhanced and suppressed neurogenesis [5]. The role of microglial responses in these and other circumstances could be construed as either harmful or beneficial, and remains unknown. From the time of birth to the 24th hour of postnatal development the amount of microglial cells in hippocampus of control piglets (group B) significantly increased (21,6%-increase) (Fig.2). To the 72th hour of postnatal development it had decreased to the newborn level probably due to migration of the microglia to other areas of brain. Soon after birth it was revealed no difference between the colostrum (group C) and group B in the number of microglial cells in hippocampus, but it is notably that we did not find increase of microglial cells amount which was revealed in the control piglets to the 24th hour of postnatal development. The significant (pd"0,05) decrease of the microglial cells on the 72th hour was also observed in this group. In hippocampus of ED group to the 24th hour of postnatal development the microglial cells number had slightly decreased (about 5%), but to the 72th hour the amount of microglia dramatically reduced (to 52%) that can indicate the decrease in immune status of the pigs from ED-group. In hippocampus of ED+Ig piglets the number of microglial cells did not show any difference with addition of IgG on the 24th hour. To the 72th hour of postnatal development the number of microglial cells decreased only on 12%. These data distinguish pigs of this group from group C and ED group (Fig.2).

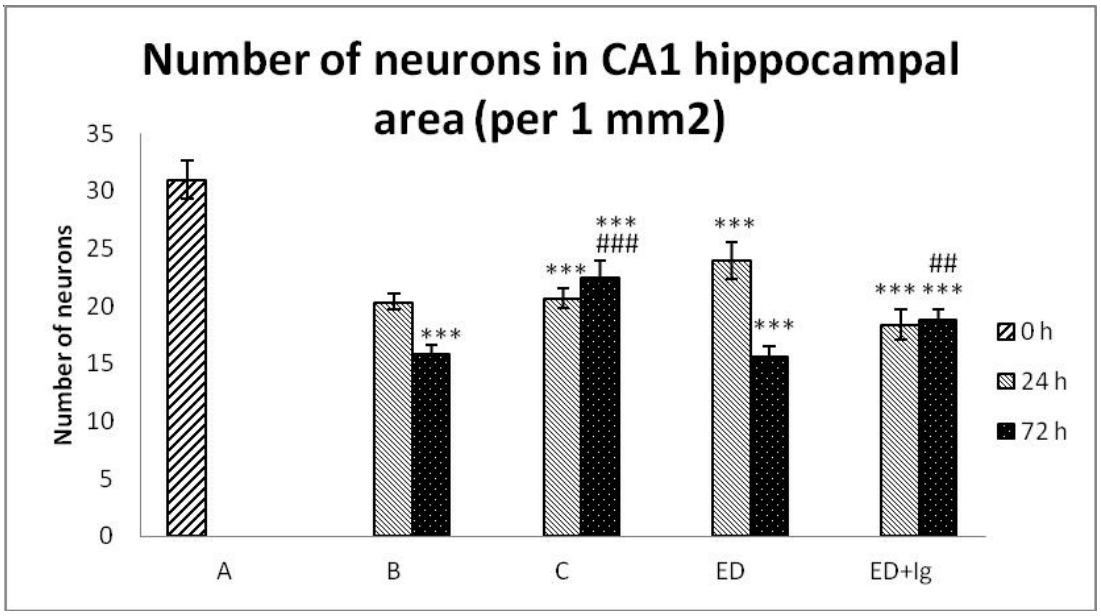

Fig.1. The number of neurons in pig's hippocampus

Group A – unsuckled piglets, group B – suckled piglets, group C – fed colostrum piglets, group ED – fed elementary diet piglets and group ED + Ig – fed elementary diet with addition of immunoglobulin G piglets. *** - the difference is significant ($p \leq 0,01$) in comparison with group A, ## – the difference is significant ($p \leq 0,05$) in comparison with group B.

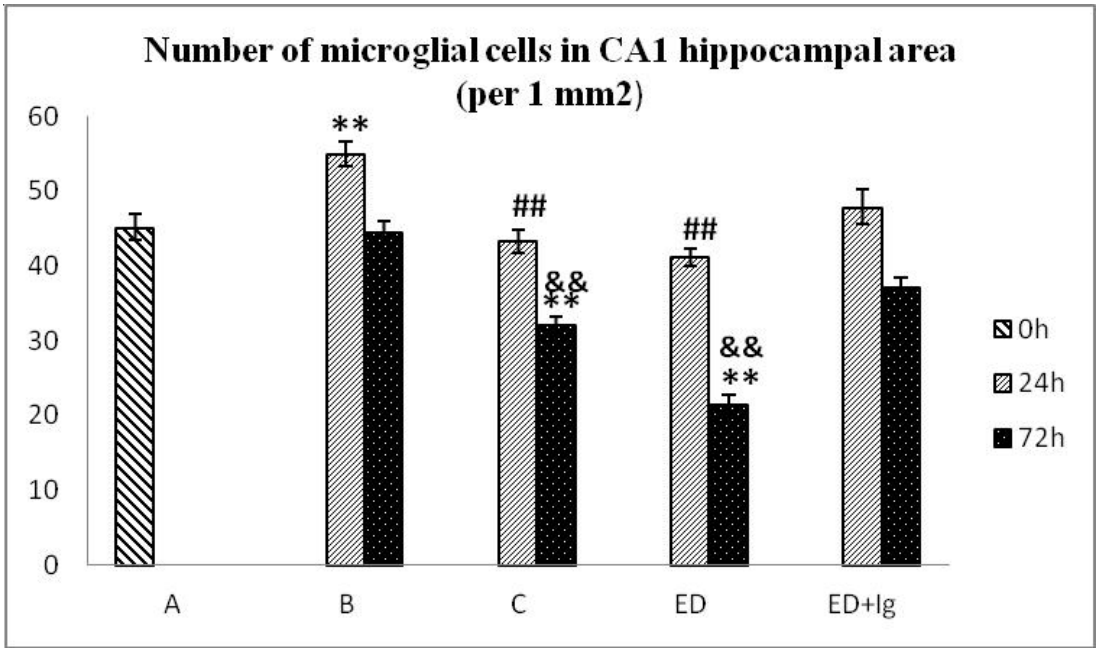

Fig.2. The amount of microglial cells in the pig's hippocampus.

Group A – unsuckled piglets, group B – suckled piglets, group C – fed colostrum piglets, group ED – fed elementary diet piglets and group ED + Ig – fed elementary diet with addition of immunoglobulin G piglets. ** - the difference is significant (pd"0,05) in comparison with group A, ## - the difference is significant (pd"0,05) in comparison with group B on the 24th hour, && - the difference is significant (pd"0,05) in comparison with group B on the 72th hour.

CONCLUSIONS

The piglets deprived of colostrum revealed a reduction in postnatal hippocampal neuro- and microgliogenesis extent the present imagination about these processes in neonatal brain. Feeding with ED, instead of colostrum, didn't ensure help physiological postnatal brain development. Addition of IgG to ED improved the neurogenesis and supported immune

status of the brain supporting the importance of natural feeding in the first weeks of life.

REFERENCES

1. Xu R.J. Development of the newborn GI tract and its relation to colostrum/milk intake: a review // Reprod. Fertil. Dev. – 1996. – V.8, №1. – P. 35–48.

2. Westrцm B.R., Ekman R., Svendsen L., Svendsen J., Karlsson B.W. Levels of immunoreactive insulin, neurotensin, and bombesin in porcine colostrum and milk // *J* Pediatr. Gastroenterol. Nutr. – 1987. – V.6, №3. – P. 460-465.

3. Bishop B. Neural plasticity: Part 2. Postnatal maturation and function-induced plasticity // Phys Ther. – 1982. – V.62, №8. – P. 1132-1143.

4. Hua J.Y., Smith S.J. Neural activity and the dynamics of central nervous system development // Nat. Neurosci. – 2004. – V.7, №4. – P. 327-332.

5. Bland S.T., Beckley J.T., Young S., Tsang V., Watkins L.R., Maier S.F., Bilbo S.D. Enduring consequences of early-life infection on glial and neural cell genesis within cognitive regions of the brain // Brain Behav. Immun. – 2010. – V.24, №3. – P. 329–338.