

Impact of colostrum and plasma immunoglobulin intake on hippocampus structure during early postnatal development in pigs

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ABSTRACT

The first milk, colostrum, is an important source of nutrients and an exclusive source of immunoglobulins (Ig), essential for the growth and protection from infection of newborn pigs. Colostrum intake has also been shown to affect the vitality and behaviour of neonatal pigs. The objective of this study was to evaluate the effects of feeding colostrum and plasma immunoglobulin on brain development in neonatal pigs.

Positive correlations were found between growth, levels of total protein and IgG in blood plasma and hippocampus development in sow-reared piglets during the first 3 postnatal days. In piglets fed an elemental diet (ED) for 24 h, a reduced body weight, a lower plasma protein level and a decreased level of astrocyte specific protein in the hippocampus was observed, as compared to those that were sow-reared. The latter was coincident with a reduced microgliogenesis and an essentially diminished number of neurons in the CA1 area of the hippocampus after 72 h. Supplementation of the ED with purified plasma Ig, improved the gliogenesis and supported the trophic and immune status of the hippocampus.

The data obtained indicate that the development of the hippocampus structure is improved by colostrum or an Ig-supplemented elemental diet in order to stimulate brain protein synthesis and its development during the early postnatal period.

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1. Introduction

It is well documented that intake of the first milk, colostrum, in addition to providing essential nutrients, also provides passive immunity (immunoglobulins) to prevent infections as well as growth factors, thereby playing an essential role in the growth and survival of newly born ungulates (Tlaskalova-Hogenova et al., 1994; Alonso-Spilsbury et al., 2005). Hence, during the first few days of life, all necessary immunoglobulins and immune factors

must be transferred over the 'open' gut mucosa to the blood circulation of the neonate (Markowska-Daniel et al., 2010; Foisnet et al., 2010). The highest concentration of immunoglobulins is in the first colostrum. Pigs withheld from colostrum ingestion during the first few hours of their life, often die due to the development of diarrhoea – which is supposedly caused by pathogens. Generally, it is accepted that the cause of death is bacterial diarrhoea and general infection due to impaired passive immunity transfer from the mother to the neonate (Gomez et al., 1998). In ungulates this transfer possibly only occurs through the gut mucosa, from ingested colostrum to the neonate. Colostrum is rich in hormones and other biologically active peptides "designed" to be transferred directly to the blood via the "open" gut during the first 24 h of life (Milon et al., 1983; Foisnet et al., 2010). Thus, the colostrum not only provides the neonate with nutrients, it also ensures proper gut development (the process of gut closure after 24 h of birth) (Jensen et al., 2001; Siggers et al., 2011). Past research has shown that full development of the gut functions (e.g., gut motility, absorption and

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secretion) occurs mainly in the early postnatal period and that the colostrum is an important factor in this process (Xu, 1996; Mubiru and Xu, 1997; Marion et al., 2002; Woliński et al., 2003; Korczynski et al., 2006).

It is a common observation that piglets receiving more colostrum are stronger, more alert and have an enhanced suckling behaviour, compared to their weaker, colostrum-deprived, counterparts (Weström et al., 1987; Koldovský, 1995; Zabielski et al., 1998; Rauprich et al., 2000). Moreover, the behavioural parameters directly indicate that the brain status and function can be affected by the colostrum. Protection of the brain from inflammatory insult is the top priority in the first few weeks of life (Choi et al., 2010). So, there is an increasing interest in the potential effects of the colostral compounds on brain development. Only a few studies have evaluated such effects to reveal that the colostrum-borne macromolecules absorbed into the systemic circulation, e.g., lactoferrin, transferrin, IgG, and epidermal growth factor-like proteins are also transported into the CSF (cerebrospinal fluid), in a time-dependent manner through the blood-CSF or blood-brain barrier (Mubiru and Xu, 1997; Harada et al., 2002). CSF flows through the ventricular system, passing over all the regions of germinal activity and contains growth factors and other neurotropic factors, which are important for neuron survival and proliferation, as well as brain development. Even though, the above mentioned information all points towards the colostral components being able to directly affect the fast developing, growing brain, we did not find any data describing the type of association between colostrum rearing and the development of cognitive function (behaviour) in newborn pigs.

In the present study, we chose to study the hippocampus, the brain structure thought to be responsible for memory and learning, more specifically the CA1 area. We focused on the possible immunochemical and morphological relations between colostrum deprivation and the development of hippocampal structures. The main aim of our study was to evaluate the role of colostrum and its most pronounced component—Ig, on the development of the hippocampus in newborn pigs.

2. Materials and methods

2.1. Animals

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 the Local Ethics Review Committee on Animal Experiments in the Malmö/Lund region, Sweden.

The experiment was conducted on cross-bred ((Yorkshire × Swedish Landrace) × Hampshire) pigs (*Sus scrofa domesticus*) obtained from the closed herd belonging to the Swedish University of Agricultural Sciences (Odarslöv research farm, Department of Agricultural Biosystems and Technology, Alnarp, Sweden), where complete management, production and health records are maintained. A total of 63 piglets from 6 litters, born on time and with no complications, were used in the study. Immediately after birth and before the first suckling, the piglets were removed from the sow and placed in a clean, straw-bedded area, under a heating lamp. The piglets were then weighed and divided into 5 experimental groups with randomly chosen pairs of males and females from different litters: newborn, un-suckled controls (NB, $n=7$); suckled pigs which stayed with their sow for 24 or 72 h (Sow, $n=14$); or experimentally fed piglets given either milked colostrum (Col, $n=14$), an elemental diet (ED, $n=14$), or the elemental diet supplemented with purified plasma immunoglobulins (ED + Ig, $n=14$). The pigs were gavage-fed these experimental diets, 10 ml/kg body weight, via a stomach tube every 2 h for up to 24 h (12 feedings). After this, half of the piglets, 7 from each feeding group, were euthanized (at 24 h) while the other half of the piglets, independently of group, were further fed with the ED every 2 h, for up to 72 h. During the feeding experiments, the piglets were housed together in a clean stable area, with an ambient temperature of between 28 and 29 °C, with a dry towel for bedding.

Piglets from the NB group were euthanized just after birth. At 24 or 72 h after the start of the experiment, piglets from the fed groups were euthanized, i.e., 4 from each group for fresh brain sampling (left and right hippocampus, $n=8$) and 3 pigs for the brain morphology after *in situ* fixation (left and right hippocampus, $n=6$).

2.2. Diets

Aliquots of about 1000 ml of the first milk, colostrum (Col), were hand-milked from 5 sows during farrowing, pooled and stored fresh at 4 °C during the feeding experiment. Samples of the colostrum pool were centrifuged at 20,000 × g for 60 min at 4 °C, the lipid layer at the top and the bottom pellet were removed, and the remaining colostrum supernatant was then stored at –20 °C until biochemical analysis.

The elemental diet (ED), contained glucose, free amino acids (Kabiven®), lipids in the form of emulsified soybean oil (Vitalipid adult) and vitamins (Soluvit®, all Fresenius Kabi AB, Uppsala, Sweden), as used in hospitals for total parental nutrition. To make the ED iso-energetic with the sow milk, the pigs received 120 ml ED/kg/day, which corresponds to an energy intake of 0.451 MJ/kg/day.

The ED was supplemented with purified porcine plasma immunoglobulins, 33.5 mg/ml (ED + Ig) in one experimental group. Plasma Ig was purified by ammonium sulphate precipitation (Grodzki and Berenstein, 2010). A pool of citrated blood (500 ml), obtained from about 10 slaughtered pigs at a local slaughterhouse (Scan AB, Kristianstad, Sweden) was centrifuged at 3000 × g for 15 minutes, using a refrigerated centrifuge. The blood plasma was separated and solid (NH₄)₂SO₄ was added to a final concentration of 38%. After an overnight incubation, the precipitate was obtained by centrifugation, washed in 38% (NH₄)₂SO₄ solution, dissolved in distilled water and finally dialyzed against distilled water. The plasma Ig preparation was stored frozen until used.

2.3. Colostrum and blood analyses

At euthanasia of the piglets, blood from the left or right subclavicular vein was collected (2 ml) into commercially available EDTA-treated tubes (Becton & Dickinson) with the addition of 500 KIE/ml of the protease inhibitor aprotinin (Trasylo™, Bayer Health Care, Germany). After centrifugation at 3000 × g for 10 minutes, using a refrigerated centrifuge, plasma was harvested and frozen at –20 °C for later analysis.

Total protein concentration (mg/ml) in the plasma samples and colostral supernatant was determined according to Lowry et al. (1951), using BSA (fraction V, Sigma Chemicals) as the standard.

The level of immunoglobulin G (mg/ml) in plasma samples and colostral supernatant was analyzed by single radial immunodiffusion (Fahey and McKelvey, 1965), using specific antibodies to porcine IgG produced in rabbits (Carlsson et al., 1980) and purified porcine IgG as the standard (Sigma).

2.4. Brain sampling for morphology

The pigs were anesthetized using 0.5–1.5% Fluothane (Zeneca, Gothenburg, Sweden) in a mixture of air and O₂, at approximately 0.5–1 L/min. The brain was *in situ* fixed by transcardial perfusion with 4% formaldehyde in 0.1 M phosphate buffer, pH 7.4. The brain was then isolated and the hippocampus dissected and post fixed in the same fixative solution overnight at +4 °C. The next day the hippocampus was cut into 50-μm-thick frontal slices using a vibratome Vibroslice 752 M (Campden Instruments Ltd, Great Britain). Hippocampal slices were rinsed out with 0.1 M phosphate buffer, pH 7.4 and treated with blocking solution (1% normal goat serum and 0.3% Triton X-100). Double and triple immunofluorescence staining of hippocampal slices was performed. Neurons were identified by monoclonal antibodies, specific to neuronal nuclear protein (NeuN). Polyclonal antibodies against glial fibrillary acid protein (GFAP – specific astrocyte marker) were used for astrocyte detection. Iba1 (ionized calcium binding adaptor molecule 1) was used as the marker for microglial cells. Slices were incubated with primary mouse anti-NeuN antibodies (Merck Millipore, USA), diluted in PBS, pH 7.4 (1:1000), chicken anti-GFAP antibodies (diluted 1:1500) (Abcam, USA) and rabbit anti-Iba1 polyclonal antibodies (diluted 1:1500) (WAKO, Japan) for 16 h at +4 °C. After rinsing, slices were then incubated with secondary antibodies; anti-mouse conjugated with Alexa Fluor 488 (1:1000), anti-chicken conjugated with Alexa Fluor 647 (1:1000) and anti-rabbit conjugated with Alexa Fluor 555 (1:1000) (Invitrogen, USA) for 1.5 h at room temperature. The slices were then rinsed, placed on histological slides and mounted with Fluorescence Mounting Media (Dako, Denmark). Images of hippocampal tissue were analyzed with a confocal FV1000-BX61WI microscope (Olympus, Japan). Estimation of the amount of neurons and microglial cells on images obtained was carried out using UTHSCSA Image Tool software (version 3, University of Texas, San Antonio, TX, USA), by counting the number of NeuN- and Iba-1-positive cells respectively, in equal squares of the different areas within the CA1 hippocampal area, which were limited by the counting frame (650 × 650 px test area). The number of cells per unit area was then calculated.

2.5. Brain sampling for biochemical analyses

After anaesthesia by an overdose of mebumal (Sigma, USA), the pigs' brains were quickly dissected out and the hippocampi were isolated and immediately frozen. The tissue was then homogenized in 10-volumes of 25 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA, 2 mM dithiothreitol, 0.2 mM PMSF and 0.01% merthiolate, at +4 °C. The homogenates were centrifuged at 100,000 × g for 60 minutes at +4 °C. The supernatant containing the water-soluble protein fraction was used to analyze the cytosolic forms of the astrocyte specific proteins, glial fibrillary acid protein (GFAP)

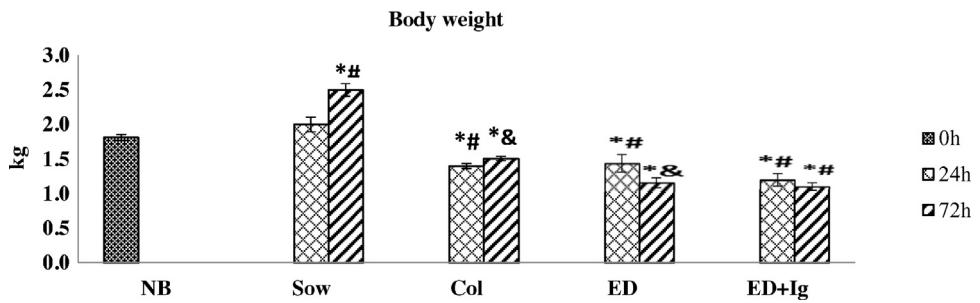


Fig. 1. Body weight (mean \pm SEM) of piglets (males:females – 50:50) at 0 h, 24 h, 72 h after birth. Newborn un-suckled (NB), sow reared (Sow), or piglets experimentally fed with colostrum (Col), or an elementary diet (ED), or an elementary diet with immunoglobulins (ED + Ig); ** p < 0.05; * p < 0.005 compared to NB, # p < 0.001 compared to Sow 24 h, & p < 0.05 compared 24 h to 72 h; n = 5–7.

and calcium-binding protein (S100B). The pellet was subsequently re-suspended in the initial buffer containing additional 2% Triton X-100 (to obtain the membrane protein fraction containing neural cell adhesion molecules, NCAM) and 4 M urea (to extract the filamentous GFAP) and centrifuged at 100,000 $\times g$ for 1 h, respectively. The levels of astrocyte and neuron specific proteins in the resulting fractions were measured with an ELISA, using monospecific, polyclonal antiserum against GFAP and S100B (Sigma, USA) and NCAM (produced in our laboratory, as previously described (Ushakova et al., 1995)). Highly purified GFAP, S100B (Sigma, USA) and NCAM (Protein Lab, Denmark) were used as standards. Optical density was measured using an Anthos-2010 absorbance reader (Anthos Labtec Instruments, Austria).

2.6. Statistical analysis

All data are expressed as mean \pm SEM (standard error of the mean). The two-tailed nonparametric Kolmogorov-Smirnov test and parametric Student's *t*-test were used to assess the differences between samples. All analyses were carried out using Statistica, version 7 (StatSoft, USA). In all statistical analyses p < 0.05 was considered significant.

3. Results

3.1. Body weight and behaviour

All newborn piglets used in the experiment weighed 1.8 ± 0.04 kg. A normal body weight gain of 0.7 kg during the

first three days of life was observed in all piglets suckling from the sows (Sow), up to 2.5 ± 0.09 kg (Fig. 1). However, all piglets in the experimental feeding groups had a decreased body weight (by between 1.2 and 1.4 kg), 24 h after birth. Colostrum-gavaged pigs (Col) displayed an increased body weight (by 10%) 72 h after the onset of feeding. The piglets fed with ED had a decreased body weight 3 days after birth (from 1.43 ± 0.13 to 1.16 ± 0.07 kg). Moreover, the piglets from that group were observed to be weak and lethargic and sometimes vomited and in a few cases developed terminal diarrhoea. The piglets fed with ED + Ig had no such symptoms, however, their body weight was also decreased (1.2 ± 0.09 kg) compared to the Col group, but was not statistically reduced during the first 3 days of postnatal development (1.11 ± 0.06 kg at 72 h) compared to ED group.

3.2. Protein concentration in blood plasma

In the newborn pigs the total concentration of proteins in the blood plasma was 24.8 ± 1.8 mg/ml (Fig. 2). The highest concentration of total protein at 24 h after birth in the blood plasma of pigs was observed in the group that was reared by the sow, under natural conditions (53.2 ± 5.6 mg/ml). During the first day after

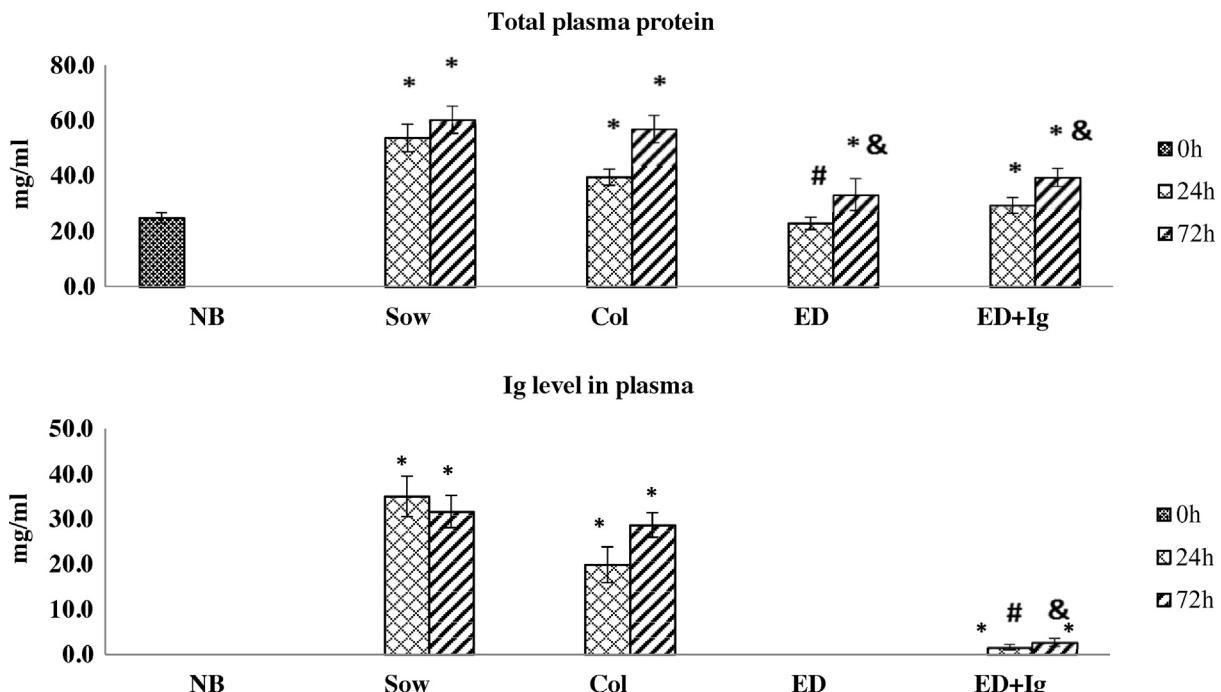


Fig. 2. Total protein and Ig concentration in the pig blood plasma at 0 h, 24 h, 72 h after birth. Newborn un-suckled (NB), sow reared (Sow), or piglets experimentally fed with colostrum (Col), or an elementary diet (ED), or an elementary diet with immunoglobulins (ED + Ig); * p < 0.005 compared to NB; # p < 0.005 compared to Col-24 h; & p < 0.05 compared to Col-72 h; n = 5–7.

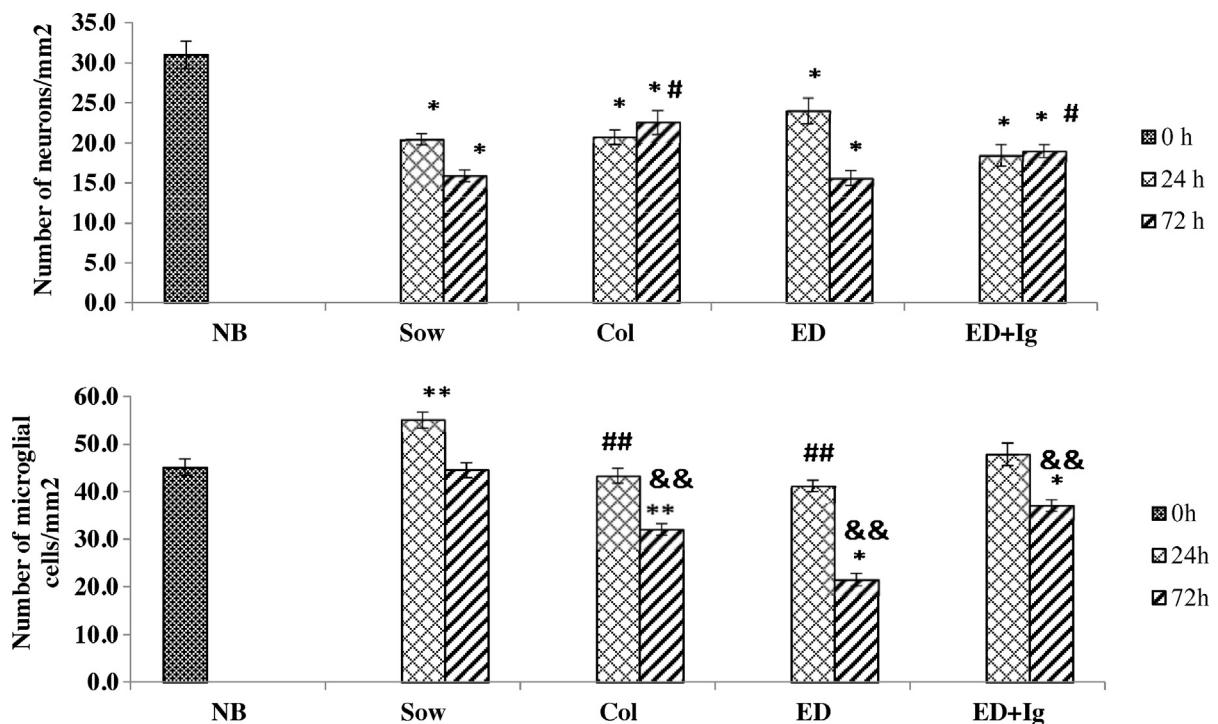


Fig. 3. The number of neurons and microglial cells in the pigs' CA1 hippocampal area (per mm²) at 0 h, 24 h and 72 h after birth. Newborn un-suckled (NB), sow reared (Sow), or piglets experimentally fed with colostrum (Col), or an elementary diet (ED), or an elementary diet with immunoglobulins (ED+Ig); *p < 0.005 or **p < 0.01 compared to NB; #p < 0.005 compared to Sow-24 h; ##p < 0.05 compared to Sow-72 h; &&p < 0.05 compared to Sow-72 h.

birth, total plasma protein concentration was elevated to up to 39.6 ± 6.9 mg/ml in the piglets that were gavaged with colostrum. Elevated plasma protein levels were not observed in the piglets receiving ED, during the first 24 h after birth, with the levels of total protein still similar to those observed in the newborn pigs (22.9 ± 2.2 mg/ml). Dietary supplementation with plasma porcine Ig to the ED, resulted in an increase in plasma total protein of up to 29.4 ± 2.9 mg/ml in piglets at 24 h. During the next 2 days after birth, the level of total protein in all groups studied increased to between 1.3 and 1.4 times that of the initial measurement (56.8 ± 11.6 mg/ml – Col; 33.3 ± 5.7 mg/ml – ED; 39.4 ± 3.2 mg/ml – ED+Ig).

The level of immunoglobulin in the pooled sample of colostrum used for feeding was 84.5 mg/ml. The measurement of IgG concentration in the blood plasma indicated the highest levels of immunoglobulin in sow-reared piglets (35.1 ± 4.5 mg/ml at 72 h, about 50% of total protein) and in those gavaged with colostrum at 72 h after birth (25.3 ± 2.7 mg/ml, 45.3% of TP) (Fig. 2). In the

newborn piglets and those fed with ED, total Ig deficiency was observed. Feeding with ED + Ig resulted in a measurable concentration of immunoglobulin in the blood: 1.6 ± 0.3 mg/ml (4.8% of TP) at 24 h and 2.7 ± 0.4 mg/ml (6.9% of TP) at 72 h after birth.

3.3. The amount of neurons and glial cells in pigs' hippocampus

The maximal number of neurons in the CA1 hippocampal area was observed in the newborn piglets (NB) (Figs. 3 and 4). The number of neurons in this area was diminished at 24 h after birth by 34.2% in the sow-reared group, 33.2% in the Col group and by 40% in the ED + Ig group, compared to the newborn piglets. In the hippocampus of piglets fed with ED, this process was slower and the number of neurons was decreased by only 22.6%. The further decrease in neuron number in CA1 region of the hippocampus was ameliorated in the piglets fed with colostrum and ED + Ig, even though the quantity of neurons 72 h after birth differed significantly

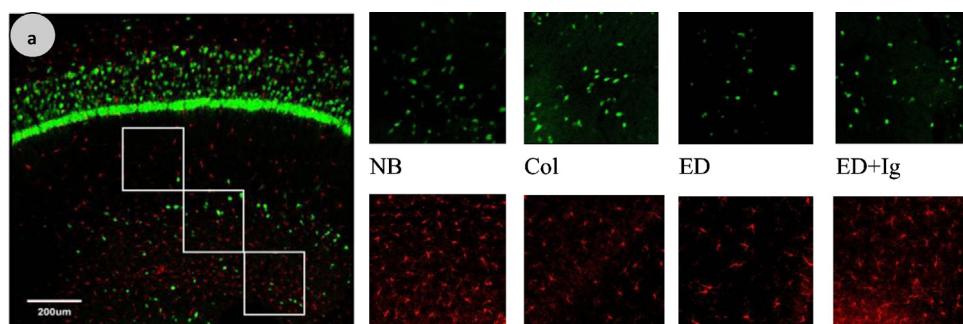


Fig. 4. Double immunocytochemistry identifying neurons (NeuN, green) and microglial cells (Iba-1, red) in the hippocampal CA1 area of pigs (a). The counts of neurons and microglial cells were provided in equal squares of the stratum radiatum at 72 h after birth (b): newborn un-suckled (NB), or piglets experimentally fed with colostrum (Col), or an elementary diet (ED), or an elementary diet with immunoglobulins (ED+Ig). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

compared to that observed at 24 h after birth. In the hippocampus of piglets fed with ED, the decrease in neuron number in the CA1 area continued to up to 50% at 72 h after birth.

After the first to 24 h of postnatal development, the amount of microglial cells in the hippocampus of control piglets (Sow-reared) significantly increased by 21.6%, $p < 0.05$ (Fig. 3). By 72 h of postnatal development it decreased to the newborn level (probably due to migration of the microglia to other areas of brain). No differences in the number of microglial cells in the hippocampus soon after birth were observed between the colostrum (Col) and sow-reared groups (Sow). A significant ($p \leq 0.05$) decrease in the amount of microglial cells 72 h after birth was also observed in this group. In the hippocampus of ED group, 24 h after birth, the number of microglial cells was slightly decreased (about 5%), but by 72 h after birth the amount of microglia were dramatically reduced (to 52%). In the hippocampus of the ED + Ig group, the number of microglial cells were no different with the addition of Ig, 24 h after birth, but by 72 h after birth, the number of microglial cells had decreased to 12%. This data distinguishes the pigs in this group from those in the Col and ED groups.

3.4. The level of neurospecific proteins in the pigs' hippocampus and colostrum

In parallel with the morphological study, the level of astrocyte and neuron specific proteins in the pigs' hippocampus was investigated. Astrocyte specific protein of intermediate filaments glial fibrillary acid protein (GFAP) has soluble and filamentous forms and is responsible for astrocyte cytoskeleton formation. Levels of the soluble form of astrocyte specific GFAP increased from $3.5 \pm 0.9 \mu\text{g}/\text{mg}$ total protein (TP) in the hippocampus of the newborn piglets, to $5.8 \pm 0.7 \mu\text{g}/\text{mg}$ TP in the hippocampus of the colostrum-fed piglets, after 3 days of postnatal development (Fig. 5). The level of filamentous GFAP was also increased from $10.3 \pm 2.7 \mu\text{g}/\text{mg}$ TP in the hippocampus of the newborn piglets, to $19.1 \pm 2.6 \mu\text{g}/\text{mg}$ TP in the hippocampus of the colostrum-fed piglets, during the first 3 days after birth. The increase in GFAP coincided with increasing levels of the other astrocyte specific, calcium-binding protein S100B from $2.03 \pm 0.3 \mu\text{g}/\text{mg}$ TP in the newborn hippocampus, to $4.8 \pm 0.6 \mu\text{g}/\text{mg}$ TP in the hippocampus of the colostrum-fed piglets, after 72 h of development. However, during the first 24 h after birth, the levels of cytosolic astrocyte specific proteins (sGFAP and S100B) were lower in the hippocampus of the piglets that were deprived of colostrum and supplementation of Ig to the ED did not alter the situation. Decreased stimulation of the biosynthesis of astrocyte specific proteins during the first 24 h after birth, lead to overexpression of these proteins in the hippocampus of the piglets fed with ED.

The levels of neuron cell adhesion molecules (NCAM) which engage in multiple neuronal interactions and influence cell migration, axonal and dendritic projection, and synaptic targeting, in the hippocampus of colostrum-fed piglets were not significantly altered within the first three days of postnatal development. The data obtained showed no significant differences between the levels of proteins studied in the hippocampus of control piglets (Sow) and those gavaged during the first 24 h after birth with colostrum (Col) or ED (the level of NCAM was $35–46 \mu\text{g}/\text{mg}$ TP) with tendency to increasing at 72 h. However, ED feeding for 72 h lead to a significant decrease in NCAM level to $28.5 \pm 3.6 \mu\text{g}/\text{mg}$ TP in the hippocampus. Feeding the ED + Ig prevented this decrease in NCAM after 72 h of development ($46.7 \pm 3.4 \mu\text{g}/\text{mg}$ TP).

Interestingly, we observed small concentrations of the mobile soluble forms of the proteins studied in the pooled colostrum: S100B – 7 ng/ml, sGFAP – 18 ng/ml and sNCAM – 35 ng/ml. To compare: in the pasteurized adult cow milk it was not detectable.

4. Discussion

In ungulates, immunoglobulins from the pregnant sow, are not able to cross the placenta and pass into the circulation of the foetus (Salmon et al., 2009). Neonatal pigs are therefore "agammaglobulinemic" at birth and have no form of immune protection at systemic and mucosal sites (Lecce et al., 1991). Their survival depends directly on the acquisition of maternal immunity from the colostrum. The ability of the neonatal intestinal cells to absorb whole macromolecules and transport them intact across the epithelium into circulation is unique to ungulates, e.g., a unique characteristic of the pig intestine during its development (Svendsen et al., 2005). During the short time after birth, the porcine gut is completely "open" to macromolecule absorption, while intestinal "closure" begins approximately 6–12 h after beginning colostrum ingestion and is complete by between 24 and 36 h. Such transfer of macromolecules ensures the uptake of immunoglobulins and other growth factors necessary for proper neonatal development. Data from the present study indicates that colostrum-fed piglets obtain the necessary levels of Ig in the blood plasma after the first 3 days after birth (17–30 mg/ml) and there is no Ig detectable in the ED fed group. However, the data also indicates that the dogma about the "open" intestine in newborn ungulates is not necessarily true. It seems as if colostrum ingestion does not 'shut' the neonatal intestine, but rather 'opens' it, enabling the absorption of intact proteins, e.g., immunoglobulin's. The blood level of immunoglobulin's in colostrum deprives pigs but fed with elementary diet supplement with immunoglobulin's was much more lower than in piglets fed colostrum. Previous data has shown the ability of porcine colostrum to stimulate gastrointestinal DNA and protein synthesis in the neonate (Burin et al., 1992). Our data illustrates that the level of proteins in the blood of ED piglets was not increased during the first 24 h of postnatal development. The immunodeficiency in these pigs leads to weakness, apathy, diarrhoea and body weight reduction during the first 3 days after birth, which may cause a delay in brain development.

Moreover, natural stress during the first few days of life can affect brain development, especially its cognitive function. A pig's brain reaches about 50% of its maximum volume at the age of 4 weeks and grows to about 90% of its maximum volume by the age of 21–23 weeks (similar to that which occurs in humans) (Conrad et al., 2012). The pig brain requires a wide range of colostrum compounds to achieve a maximum rate of protein synthesis and development.

In addition, Ig in the serum of newborn piglets is not detected before suckling and increases steeply after colostrum feeding. It has been shown that absorbed Ig is quickly transferred into the CSF of pigs during natural colostrum suckling, but not after feeding with colostrum of bovine origin (Harada et al., 2002). Thus, the transport of macromolecules into the CSF and brain is selectively specific in neonatal pigs, however the mechanism of transportation is unknown.

The data obtained in our study shows a positive tendency towards an increase in body weight and an increase in the level of total protein and IgG in the blood plasma of sow-reared piglets, during the first 3 days of postnatal development. The colostrum deprived pigs fed ED exclusively for 24 h, not only have a decreased body weight and level of blood plasma proteins, but also display an absolute immunodeficiency which could lead to brain retardation during the first stage of postnatal development. We noted the reduced levels of neurospecific proteins in the hippocampus of piglets fed with ED. It is obvious that a deficiency of immunoglobulins and other colostrum-borne bioactive compounds leads to a reduction in postnatal hippocampal development. It is not excluded that these processes are vital for the development of digestive function. Data from Woliński et al. (2012), obtained from a similarly

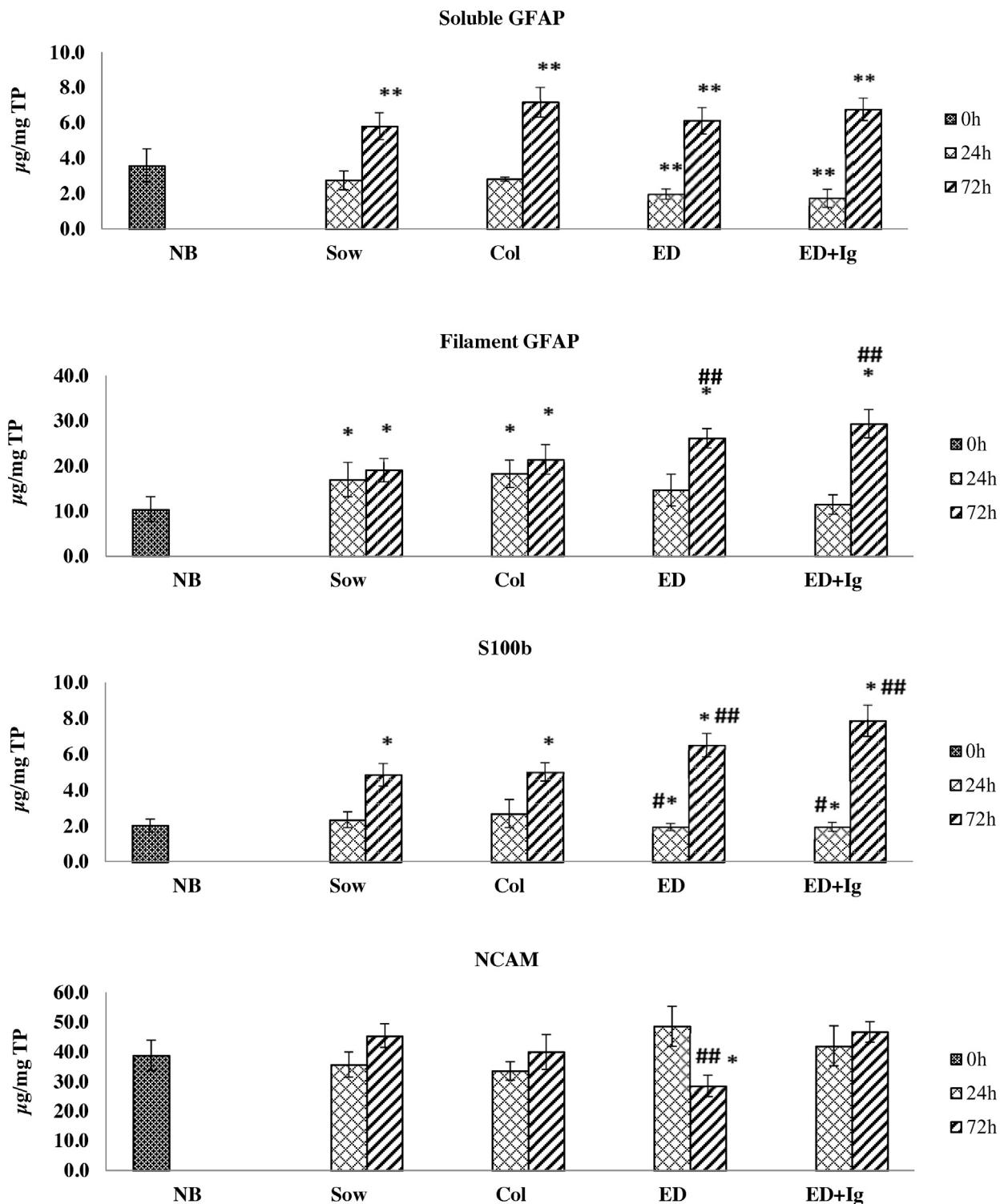


Fig. 5. The level of astrocyte and neuron specific proteins in the hippocampus of piglets during the first three days of postnatal development, at 0 h, 24 h, 72 h after birth. Newborn un-suckled (NB), sow reared (Sow), or piglets experimentally fed with colostrum (Col), or an elementary diet (ED), or an elementary diet with immunoglobulins (ED + Ig); GFAP – glial fibrillary acid protein, S100B – calcium-binding protein; NCAM – neural cell adhesion molecules; TP – total protein; * $p < 0.005$ or ** $p < 0.01$ compared to NB; # $p < 0.01$ compared to Sow-24 h; ## $p < 0.05$ compared to Sow-72 h (as to ED-72 h); n = 5.

designed experiment, shows that immunoglobulins added to the elementary diet affect the morphology of the GI wall towards that observed in colostrum-fed piglets. Moreover, immunohistochemical evaluation of NOS (nitric oxide synthetase) expression in the same study indicated that ED feeding may influence the jejunal myenteric, but not submucosal neurons of newborn pigs.

Moreover, the “newborn” brain probably requires regulation of its ‘development’ and ‘switching on’ pathways for its integration with other organs and tissues. The gut is one of the crucial organs requested such integration because in contrary to other organs during uterine life gastrointestinal tract was not at all necessary for other organ function. Thus, directly after birth in order to obtain the necessary energy/nutrients and develop all functions,

the gut should be quickly integrated with all other organs and tissues, but firstly the functions of the GIT must be regulated. Thus the integration of the GIT with the nervous system is indispensable. We postulate that the majority of the rapid brain developmental changes are required in order to ensure efficient regulation and development of the GIT.

Feeding with ED, instead of colostrum, did not ensure physiological postnatal brain development. The changes observed could provide evidence for the time of migration and maturation of neurons during the early stages of postnatal development, which is strongly dependent on early nutrition with colostrum. Diminishing of neurons in CA1 area of the hippocampus of piglets fed exclusively with elementary diet was slower during first day of postnatal development compare to the Sow and Col groups, however, to 72 h the number of neurons in this area was 50% to newly-generated one at the birth time like in Sow group. Diminishing of neurons in CA1 area of the hippocampus of piglets fed with ED + Ig was not slower during 24 h. But, in the hippocampus of colostrum-fed piglets this process was faster, shortened up to 24 h after birth, whereas 32–34% of neurons were decreased in CA1 area, and the number of neurons was not statistically differ at 72 h. Obtained data allow to suggest the existence of some short-time living (or activated) factors together with Ig in colostrum that can influence to time of neuron migration and differentiation, and may be changed in the artificially saved colostrum during 24 h.

Recent imaging studies show that the formation of neural connections in the central nervous system in early neonatal life is a highly dynamic process (Buffelli et al., 2002; Hays et al., 2012; Feliciano and Bordey, 2013). It is worth noting that hippocampal neurons are arranged in a special, spatial manner, which allows for the precise estimation of their size and location. Changes within the hippocampus during the first 24 h of postnatal development of piglets fed with the elementary diet were coincident with (or induced by) the inhibition of gliogenesis, compared to colostrum-fed pigs. Microglia act as a first line of defence and participate in transforming the innate immunity into an adaptive immune response by recruiting systemic immune cells. Moreover, according to recent reports, microglia have the capacity to direct the migration of neural precursor cells, as well as affect their differentiation (Aarum et al., 2003; Ziv et al., 2006; Beutner et al., 2010; Hays et al., 2012).

Astrocytes, once relegated to a mere supportive role in the central nervous system, are now recognized as a heterogeneous class of cells with many important and diverse functions (Ben Achour and Pascual, 2012; Losi et al., 2012; Molofsky et al., 2012). Major astrocyte functions can be grouped into three categories: guidance and support of neuronal migration during development, maintenance of the neural microenvironment, and modulation of immune reactions by serving as antigen-presenting cells. The concept of astrocyte heterogeneity is critical to understanding the functions and reactions of these cells (Chaboub and Deneen, 2012). Unfortunately, we failed to estimate the number of astrocytes stained by immunohistochemistry because of their tight arrangement during the first few days of postnatal development. But, biochemical data has shown that colostrum deprivation during the first 24 h after birth, lead to the inhibition of astrocyte development during the first few days after birth and faster astrogliosis under decreased microgliogenesis after 72 h of development.

In our experiment the supplementation of Ig to the ED improved the health of the piglets as well as the hippocampus development. Even the low level of immunoglobulins present in the piglets fed ED + Ig, ensured the occurrence of the various developmental changes (as described below) within the hippocampus, similar to those observed in the pigs fed colostrum. These changes

were not observed in the piglets exclusively fed the ED. The data obtained confirms the importance of natural feeding, especially during the first few days of life, with respect to the immune status of the brain. However, the data also indicates that not only is it the immunoglobulins from colostrum which actively participate in brain development, other factors in the colostrum also affect brain development during the early postnatal period, hence the differences observed between the colostrum fed and ED + Ig fed piglets.

We detected a small concentration of neurospecific proteins (S100B – 7 ng/ml, sGFAP – 18 ng/ml and sNCAM – 35 ng/ml) in the pooled store of pig colostrum that could play a crucial role as neurotrophic factors during the first stages of brain development. S100B as a neurotrophic protein was previously detected in biological fluids and in human milk (Gordon, 1997; Amin et al., 2000; Gazzolo et al., 2004; Galvano et al., 2009). According to Galvano et al. (2009), S100B concentration in human milk ($10.41 \pm 4.2 \mu\text{g/L}$) is higher than in other mammalian milks: in cow milk ($3.13 \pm 0.56 \mu\text{g/L}$), donkey ($1.17 \pm 0.26 \mu\text{g/L}$), sheep ($0.25 \pm 0.11 \mu\text{g/L}$) and goat ($0.26 \pm 0.11 \mu\text{g/L}$). This data indicated the presence of a few cell types that express S100B, including mammary epithelial cells and lymphocytes, which may be the source of S100B in the milk. Amin et al. (2000) proposed that the presence of S100B at very high concentrations in human breast milk may be related to its putative neurotrophic role, given that breast-feeding is believed to exert a stimulatory effect on brain maturation.

The study supports the importance of dietary immunoglobulins for hippocampus development during the first few days of life of the piglet. Our data extent the present imagination about such processes as neuro- and microgliogenesis from Sanai et al. (2011) who demonstrated that the infant human subventricular zone (SVZ) of the hippocampus contain an extensive corridor of migrating immature neurons before 18 months of age, but as for the dentate gyrus (DG) and hippocampus, no such observations were made. Data from Sanai et al. (2011), as well as findings from Bland et al. (2010) report that glial cells, being per se immune cells, may play some role in the maintenance and neuronal functions of the hippocampal CA1 pyramidal neurons and granule cells of the dentate gyrus, during postnatal development. Here we propose that the development of the hippocampus is related to dietary immunoglobulins and could serve as possible evidence of altered migration and maturation of neurons during the early stages of postnatal development. Therefore, the modulation of these processes could lead to long-term effects on the modulation of synaptic transmission in adult life. On the other hand, the colostral Ig may play a preventive role against diarrhoea and weight loss, thus in turn preventing brain retardation.

More detailed research is needed in order to corroborate the molecular mechanism of neurogenesis under colostrum deprivation and we hypothesize that Ig and neurotrophic factors (S100B, soluble forms of GFAP and NCAM) from colostrum or milk may participate in the intestinal development and have a trophic effect on the enteric and central nervous systems.

5. Conclusion

1. The study suggests that colostrum ingestion as well as the ingestion of Ig, before gut closure, affects microgliogenesis and neuron migration in the CA1 hippocampal area.
2. The level of neural cell adhesion molecules in the hippocampus is strongly related to colostrum feeding or Ig rearing.
3. Behavioural changes (apathy development) in piglets deprived of colostrum/Ig can be related to retarded brain development but not necessarily to infection.

Conflict of interest

There are no known conflicts of the interest associated with this publication.

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