SHIFTS IN IMMUNOGLOBULINS LEVELS IN THE PORCINE MAMMARY SECRETIONS DURING WHOLE LACTATION PERIOD

IWONA MARKOWSKA-DANIEL AND MAŁGORZATA POMORSKA-MÓL

Department of Swine Diseases,
National Veterinary Research Institute, 24-100 Pulawy, Poland
iwonamd@piwet.pulawy.pl

Received for publication March 29, 2010

Abstract

The absolute and relative levels of immunoglobulins (Ig) G, M, and A in porcine mammary secretions throughout lactation period were estimated. Concentrations of each immunoglobulin were determined by ELISA kits. The results of the study confirmed that pigs, like other mammals, have colostral and milk phases of lactation, distinguished by the concentrations of IgG and IgA. The amount of IgG showed a dramatic 27-fold decline from the beginning to day 6 of lactation. The IgM concentration decreased over threefold at the same time. Concentration of IgA at the day 6 of lactation was almost 4-fold lower than that at the beginning of lactation. The relative contribution of IgG and IgA to the total Ig content becomes reversed during lactation. Summarising, in pigs, transformation of colostrum to milk was characterised by dramatic decrease in total Ig content, connected with the change of predominant immunoglobulin. The predominant immunoglobulin in colostrum was IgG, which plays an important role in systemic immunity, while in milk a major Ig was IgA, responsible for the protection at mucosal surface level. As concentrations of Ig in colostrum are very variable, an improved understanding of factors influencing colostrum Ig concentration is now desirable.

Key words: sow, colostrum, milk, immunoglobulins.

Neonatal mortality index is very high in farm animals (about 10%) and disease resistance is greatly influenced by an adequate passive immunisation just after birth (23). Piglets, in contrast to human beings, are dependent on the intestinal transmission of antibodies and other factors present in colostrum, which modulate immune response (23). Beside immunoglobulins (Ig), mammary secretions contain lymphocytes, cytokines, nucleotides, and various growth factors, which may affect the development of the post-natal immune system (4, 18, 24). Since the antigenic specificity of Ig reflects the maternal experience of environmental antigens, immunity acquired through colostrum and milk will protect the piglets against these antigens but not against novel antigens (2).

Just after birth, the newborn is exposed to a massive invasion of potentially harmful pathogens from the environment (23). It should be able to respond immunologically to the pathogens but the innate immune defence in pigs is immature at the time of birth. Therefore, passive immunisation from the sows is required, until the active immune system is fully developed (1, 6, 25).

In piglets, as well as in foals, lambs, and calves, the intestinal absorption of Ig from their mother’s colostrum occurs mainly by a non-specific endocytosis of macromolecules. The details of the absorption process and the mechanism regulating its cessation after 24-36 h of colostrum exposure remain poorly understood (23).

Neonatal vitality often shows a positive correlation with the degree of passive immunisation (6, 26, 27). Circulating Ig, particularly immunoglobulin G (IgG), constitute a key element in the general host defence against environmental antigens. Additionally, immunoglobulin A (IgA) plays an important role in the protection of young piglets being responsible for the protection of animals at mucosal surfaces level.

Because a lot of factors may influence Ig content in mammary secretion (e.g. genotype), there is a need to study Ig concentration in colostrum and milk of sows of different breeds. It is important, not only from veterinary point of view, but also because pigs represent an important experimental model for cost-effective studies in human neonatal nutrition, immunology and developmental immunotoxicology aimed at analysis of the risk of environmental hazards (13, 19, 21).

Here we report on the absolute and relative levels of Ig in colostrum and milk in France hybrids FH 900 multiparous sows, from the start of parturition to the weaning (about 28 d post partum) of their piglets. Data show the amounts of individual Ig classes and their changes throughout lactation.
Material and Methods

Animals. A total of nine multiparous sows, France hybrids FH 900, from local farm were used in this study. The sows were of various lactation numbers (from 3 to 7) and various litter sizes (from 10 to 13 piglets). All sows were maintained in the same environmental conditions and each received the same nutrients. The farm had a closed production cycle and the basic herd consisted of 60 sows. Technological groups of 9-10 sows were formed every 21 d. Ten days before parturition the sows were moved to the individual farrowing pens (2×2.5 m). Farrowing was in all in-all out unit with a thorough cleaning between batches. The prophylactic programme at the time of pregnancy consisted of the vaccination of the sows with inactivated vaccines against atrophic rhinitis and neonatal E. coli diarrhoea, for controlling infectious diseases in the groups of weaning pigs.

All farrowings were attended. The start of parturition was defined as the moment when the first piglet was born.

Local Ethical Commission of the University of Life Sciences in Lublin approved all procedures involved in the study.

Colostrum samples. Colostrum (approximately 10-15 ml) was collected from each sow at 1, 6, 12, and 24 h and milk at 6, 12, 18, and 28 d from parturition beginning, using oxytocin, when necessary. Colostrum and milk samples were centrifuged at 14,000 rpm for 30 min at room temperature and then stored at -20°C.

Measurement of Ig concentrations. Total IgA, IgM, and IgG concentrations in colostrum and milk samples were determined by commercial ELISA kits (ELISA Quantitation Kit, Bethyl Laboratories Inc, USA) accordingly to the manufacturer’s recommendations. The absorbance was recorded at 450 nm using an ELISA plate reader (Multiskan RC, Labsystems, Finland).

The results obtained (in ng/mL) were multiplied by the appropriate dilution factor and expressed as mg/mL.

In each experiment, serial dilutions of standard samples of pig’s reference serum were tested in order to receive calibration curve, which then has been computer adjusted (with the use of FindGraph software programme). From this calibration curve, values of unknown Ig concentration samples were calculated by the same computer programme. Ranges of the detection of ELISA kits were as follows: 15.62 – 1,000 ng/mL for IgA and IgM, and 7.8 – 500 ng/mL for IgG.

Statistical analysis. Data were analysed using Statistica 8.0 computer programme (StatSoft, Poland). Results from all groups were subjected to the W. Shapiro-Wilk’s test of normality and the Levene’a test of equal variances. In the case of lacking normality or unequal variances, the differences were analysed with a nonparametric Kruskal-Wallis test with post hoc multiple comparisons for comparison of all pairs. In the case of normal distribution and equal variances, the one-way ANOVA with HSD Tukey’s post-test were used.

Differences with α<0.05 were considered statistically significant.

For the analysis of correlation between the base 10 logarithm of the concentration of Ig and time of lactation, the Pearson correlation test was used. Logarithmic transformation was suggested by the distribution of data points.

Results

The concentration of IgG, IgM, and IgA were the highest at the first hour of lactation. The levels of these Ig subsequently decreased with time. The amount of IgG showed a dramatic 27-fold decline from 98.17 mg/mL at birth to 3.58 mg/mL at day 6 of lactation (Kruskal-Wallis, P=0.0032), followed by a slow, not significant, twofold decrease until the end of lactation.

Concentration of the remaining Ig, IgM and IgA, also declined with time, but the decrease was not so spectacular in the first 6 d of lactation. The concentration of IgM decreased over threefold (from 9.07 to 2.32 mg/mL, Kruskal-Wallis, P=0.0002) at above mentioned time. The IgA concentration was also high at birth and reached 23.30 mg/mL, but the decrease was consistently slower than that of IgG. At the 6th d of lactation the amount of this Ig was almost 4-fold lower than at the beginning (5.90 mg/mL, Kruskal-Wallis, P=0.0011). The levels of these Ig, 24 h from the start of parturition, were about 2-fold lower then at the beginning, and remained relatively stable from day 6 to the end of lactation. The concentrations of the IgG, IgM, and IgA in colostrum and milk are shown in Table 1.

The percentage of IgG decreased over threefold during lactation, from a mean value of 75% at the start of lactation to 20% at the 12th d of lactation, and remained stable until the end of the study (28th d).

The relative content of IgM remained stable during first 12 h of lactation and reached 6%-7% of total Ig. Twenty four hours after parturition, the percentage of IgM increased gradually to 13% and to about 20% at the 6th d of lactation. The highest percentage of IgM was observed at day 18 post farrowing (25%).

The percentage of IgA also was relatively stable during first 12 h of lactation (17%-18%), followed by a gradual increase from 28% at 24 h, by 50% at 6 d, to about 60% from the day 12 to the end of lactation (over threefold increase). The relative contribution of IgG and IgA to the total Ig content became reversed during lactation. The relative content of all classes of Ig during lactation are shown in Fig. 1.

The studies have revealed a strong, positive correlation between the concentration of respective Ig in colostrum or milk and the period of lactation. Correlation coefficients (r) were equal 0.97 (P=0.000) for IgG, 0.99 (P=0.000) for IgM, and 0.97 (P=0.000) for IgA.
Table 1
Mean (range) concentration of immunoglobulin (mg/mL) in sows’ colostrum and milk in different periods of lactation

<table>
<thead>
<tr>
<th>Time from the onset of farrowing</th>
<th>1 h</th>
<th>6 h</th>
<th>12 h</th>
<th>24 h</th>
<th>6 d</th>
<th>12 d</th>
<th>18 d</th>
<th>28 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>98.17 (48.4-134.9)</td>
<td>71.39 (39.8-88.8)</td>
<td>61.09 (38.3-87.1)</td>
<td>19.74 (6.4-37.0)</td>
<td>3.58 (1.1-6.1)</td>
<td>2.29 (1.1-4.3)</td>
<td>1.56 (0.4-2.7)</td>
<td>1.50 (0.4-3.2)</td>
</tr>
<tr>
<td>IgM</td>
<td>9.07 (5.39-13.97)</td>
<td>5.93 (3.28-8.95)</td>
<td>4.52 (2.60-6.63)</td>
<td>4.42 (2.9-5.9)</td>
<td>2.32 (1.9-3.4)</td>
<td>2.38 (1.9-3.6)</td>
<td>1.99 (1.2-2.9)</td>
<td>1.94 (0.7-3.6)</td>
</tr>
<tr>
<td>IgA</td>
<td>23.30 (14.3-36.2)</td>
<td>16.94 (13.2-22.9)</td>
<td>13.09 (4.3-24.9)</td>
<td>9.17 (2.7-14.3)</td>
<td>5.90 (3.6-9.1)</td>
<td>6.58 (4.1-10.3)</td>
<td>4.74 (3.8-6.1)</td>
<td>5.55 (4.5-6.4)</td>
</tr>
</tbody>
</table>

Fig. 1. Relative content of IgG, IgM, and IgA in sows’ colostrum and milk during whole lactation.

Discussion

Concentrations of Ig were the highest in the first colostrum, independently of sows and type of Ig. The Ig content of the colostrum of different sows showed marked variation, as it also has been shown by others (9, 17, 20). Season, parity, genotype, vaccination, section of the udder have been suggested as factors that influence colostrum Ig concentration (8, 20). In this study we found no evidence of the effects of lactation number or litter size, which is in agreement with previous reports published previously by Klobasa et al. (13) and by Markowska-Daniel et al. (16).

We found a strong correlation between IgG and IgM content in colostrum during first 6 h of lactation (r=0.83, P=0.004 and 0.88, P=0.01, respectively at 1 and 6 h of lactation). There were no significant correlation between IgG and IgA as well as IgM and IgA.

The result of this study confirm that pigs, like most other mammalian species, have both colostral and milk phases of lactation distinguished by the concentrations of IgG and IgA (7, 14, 22). The predominant Ig in colostrum was IgG, consistent with previous studies in caprine, cats, dogs, cattle, and horses (7, 10, 12, 14, 22). Similarly to cats, horses, or cows (7, 10, 12, 14, 22), the IgG concentration was at least five times higher in colostrum than in milk. Since the ability of the piglet to transfer IgG from the gut to blood disappears in the first 24 h to 36 h of life, the change of the main Ig from IgG to IgA after this period is in agreement with the need of the piglet to acquire IgG for passive humoral immune protection in the 1st d of life and then continuing protection from IgA in milk at mucosal surfaces thereafter (20). In agreement with Milon (17) and Klobasa (13), an important fall from 98.17 to 19.74 mg/mL in the average concentration of IgG in colostrum during first 6 h of lactation.
IgG was observed within 24 h after the end of parturition. However, these initial values appeared to be somewhat higher than those of 63 mg/mL found by Bourne (5) and of 48.26 mg/mL found by Frenyo et al. (9) at the beginning of lactation. Bland (3), using an electrophoresis for determination of IgG in tested samples, have shown that maximal IgG concentration in sows’ colostrum (at 0-4 h after farrowing) was only 61.0 mg/mL and decreased to 9.0 mg/mL after 24 h postpartum. These levels were lower than determined by us and by other authors (13, 17). It might result from different technique used for the evaluation of IgG concentration (electrophoresis by Bourne (5) and radial immunodiffusion method by Frenyo et al. (9)).

In the first 12 h of lactation the IgG accounted for over 75% of the total Ig. The concentration of IgG during first 12 h of lactation was significantly higher than that of IgM and IgA (P<0.05; Kruskal-Wallis). After 24 h from birth the concentration of IgG differed significantly only from IgM concentration (P=0.0001; Kruskal-Wallis), while difference between IgG and IgA concentration was not significant (P=0.0666; Kruskal-Wallis).

In experimental sows the transformation from colostrum to milk was accompanied by large changes in the amounts of respective Ig in mammary secretions. Starting from day 6th the dominant immunoglobulin of milk was IgA. Its levels were significantly higher than in case of the remaining Ig from day 6th to the end of lactation (P<0.01; Kruskal-Wallis). Immunoglobulin A accounted for at least 50% from 6th to 28th d of lactation. This is different to dogs and horses, but different from other mammals, such as cats or ruminants, in which IgG is the predominant immunoglobulin in milk (7, 11, 15, 22, 28).

The milk IgG and IgA concentrations significantly decreased within first 24 h of lactation. The concentration of IgM also decreased at the same time, but the decrease was not as dramatic as with respect to IgG and IgA. These findings are similar to previous studies in pigs (13), as well as horses and cats (7, 22).

Summarising, the transformation of colostrum to mature milk in pigs was characterised by dramatic decrease in total Ig content connected with the change of predominant immunoglobulin. The predominant immunoglobulin in colostrum was IgG, which plays an important role in systemic immunity of piglets, while in milk a major immunoglobulin was IgA, responsible for the protection at mucosal surface level. The mean quantitative values that we established for the three classes of Ig in mammary secretions of sows during whole lactation, may provide reliable data, useful in future experiments, including influence of variety of factors on Ig content in colostrum and milk, and those in which swine are used as models for human nutrition and maternal immuno-interactions with progeny. As concentrations of Ig in maternal colostrum are very variable, an improved understanding of factors influencing colostrum Ig concentration is now desirable.

Acknowledgments: This work is supported by Project No NN 308 275934 founded by the Ministry of Science and Higher Education.

References


