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## INFLUENCE OF BIRTH PREMATURITY ON COLOSTRUM COMPOSITION AND SUBSEQUENT IMMUNITY OF PIGLETS

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### Abstract

Two groups of seven pregnant sows were farrowing either naturally after 111-114 days of gestation or prematurely after 109 days following an injection of 125 µg/animal of a prostaglandin analogue. Colostrum intake was controlled individually on piglets during the first 24 h of life. Measurements of IgG, IgA and IgM were performed on 3 samples of colostrum after 0, 12 and 24 h following the birth of the first piglet, then in the serum of the piglets, sampled at 4, 12 and 42 days of age. Then, we calculated the total amount of Ig intake during the first 24 h of life and an estimation of the Ig stores of each piglet on the basis of weight, blood volume, and hematocrit at different ages.

Results showed a marked average difference in the birth weight in favour of mature piglets as well as an increased colostrum intake: 315 vs an average of 213 g for premature piglets. Considerable variations between sows were found in the initial level of colostral IgG, independently of the gestation length. Two thirds of the IgG intake by piglets occurred during the first 12 h. Total Immunoglobulin intake in the first day was respectively 15.8 g vs 25.1 g for premature or mature piglets. Total estimated Ig content in the serum of 4 day-old piglets was also lower for premature ones (2.55 vs 3.2 g/animal), representing 10 to 27 % of the total amount of Igs ingested during the first day of life. However, concentrations or levels of Igs in the serum of piglets were not significantly different between the two groups at any age, nor were total stored Igs differences at 12 and 42 days of life.

Linear and curvilinear regressions were calculated between different parameters on the one hand for premature and on the other hand for mature piglets. In general, curvilinear regressions did not bring more information than linear ones. IgG levels at 4 days of age appeared to be poorly correlated with individual birth weight, but was strongly correlated with the amount of colostrum suckled. Strong correlations were also found between total amounts of IgG or IgG + IgA + IgM in the blood at different ages and total amounts of the respective immunoglobulin intake through colostral route during the first day.

These results are further demonstrating the well-known great importance for the piglet to suckle a high amount of colostrum during the first day, even the first 12 h of life, for an efficient transfer of passive maternal immunity.

Passive transfer of maternal humoral immunity is of peculiar importance for the survival of newborn animals, specially during days following birth, when neonates are submitted to high risk of ente-

ric aggressions (bacterial, viral or protozoal enteritis). This was shown more than 20 years ago by Brambell (1958). At birth, the piglet is considered as nearly "immunologically virgin" since virtually

no, or at least very few immunoglobulins (Igs) are present in its serum (Sterzl *et al.*, 1960; Kim *et al.*, 1966; Prokesova *et al.*, 1970, 1979; Metzger *et al.*, 1978). Contrary to primates and rodents, transfer of maternal antibodies do not occur by trans-placental route in normal conditions as epithelio-chorial mode of placentation (Brambell, 1970) is totally impermeable to sow's seric proteins.

Thus, colostrum and milk routes appear to be essential to confer to the piglet both general and local passive immunity of maternal origin. In swine, as stressed by Curtis and Bourne (1971), colostrum proteins, specially immunoglobulins, are absorbed through the immature intestinal mucosa without degradation during the first day of life while sow's milk, which is rich in secretory IgA, provides local immunity to the intestinal tract until weaning. Both types of transfer are decisive factors in protecting young piglets against colibacillosis (Porter and Hill, 1970), Transmissible Gastroenteritis (Bohl *et al.*, 1972) or Aujeszky's disease (McFerran and Dow, 1973) and probably against all the main types of neonatal diseases.

This paper will deal with colostrum transmission of general immunity in pig. Large differences in the immunological status of piglets may occur in relation to different parameters (Hendrix *et al.*, 1978). Some of these parameters such as the amount of colostrum produced, or its quality expressed as its Ig content (or more specifically as its antibody content) are associated with the sow. Some other parameters depend upon the piglet itself. They include the amount of colostrum intake during the first day of life. That amount depends itself upon numerous other events like prematurity at birth, birth weight, amount of colostrum suckled and amount of Ig transferred through the gut mucosa, or timing of the suckling event in relation to the sharp fall of proteins in colostrum whey and to the fastening of the piglet's immature intestinal mucosa within few hours after birth.

No definitive evidence has appeared about the respective importance of these parameters. The experiment reported herein has been undertaken to see if premature birth was an important parameter that might impair passive transfer of general immunity from the sow to the neonates.

## Materials and Methods

### Animals

Two groups of seven pregnant sows were selected according to their ages and litter parities. They were farrowed under a permanent regime either naturally after 111-114 days of gestation or prematurely after 109 days following an injection of 125 µg/animal of a prostaglandin analogue. Each

piglet was carefully dried, weighed and removed from the sow until the birth of the last animal. Newborns were kept on a deep litter of straw, under an extra heat source provided by an infrared lamp of 250 watts. Animals were allowed to suck at regular intervals of about 80 min during a period of 24 h after the first suckling. Weak piglets were helped to suckle the first time. Colostrum intake was controlled by weighing piglets individually before and after each suckling.

### Samples

Three colostrum samples were taken at birth of the first piglet, then 12 and 24 h after the first suckling. Blood samples were collected from piglets by anterior cava vein puncture at 4 days (when all passive transfer is over), 12 days (weaning), and 42 days of age (when piglets were supposed to reach their own adult like immune status). Additional blood samples were collected on 30 piglets before any colostrum intake.

### Measurement of IgG, IgA and IgM concentrations

In all these samples of colostrum whey and

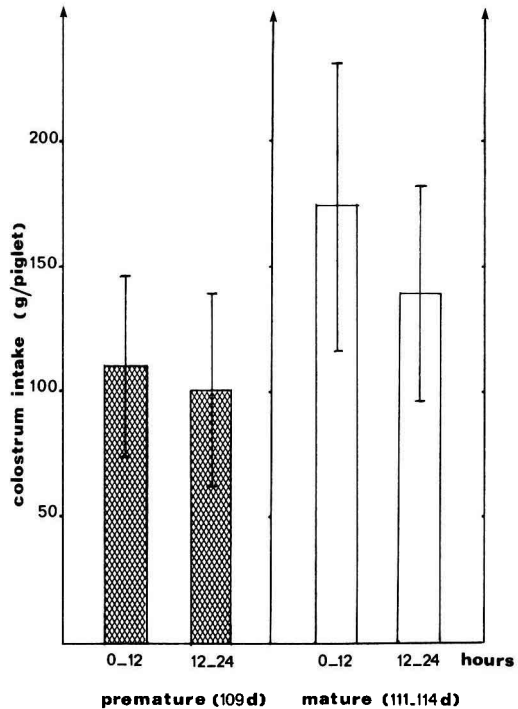


Fig. 1. — Effect of prematurity on colostrum intake by piglets during the first 24 h of life.

blood serum, IgG, IgA and IgM concentrations were measured with a competitive immunoenzymatic test already described (Franz and Corthier, 1981). Briefly, microplates wells (Linbro polystyrene plates) were treated for 1 hour at + 4 °C with 1 % glutaraldehyde, rinsed six times with distilled water and coated with an appropriate dilution of rabbit sera Ig fractions specific for porcine IgG, IgA or IgM heavy chains in 0.05 M carbonate buffer pH 9.6. After incubation overnight at + 4 °C, the plates were washed three times with phosphate buffered saline containing 0.05 % Tween 20 and 0.02 % sodium azide (PTA). Then, samples diluted in PTA mixed with an equal volume of known

amount of porcine IgG, IgA or IgM conjugated to alkaline phosphatase (Sigma type VII) were added to the coated wells and incubated for two hours at 37 °C. Finally, the plates were washed 3 times with PTA and the enzyme substrate (p-nitrophenyl phosphate 1 mg/ml: Sigma 104 tablets in diethanolamine buffer pH 9.8) were added. After incubating for 1 to 2 h at 37 °C, optical density was measured directly in the wells using a Titertek Multiskan (Flow Laboratories). In each experiment, standard samples of purified IgG, IgA or IgM or standard serum in serial dilutions were tested as above and gave a standard calibration curve which was then computer adjusted. From these calibration

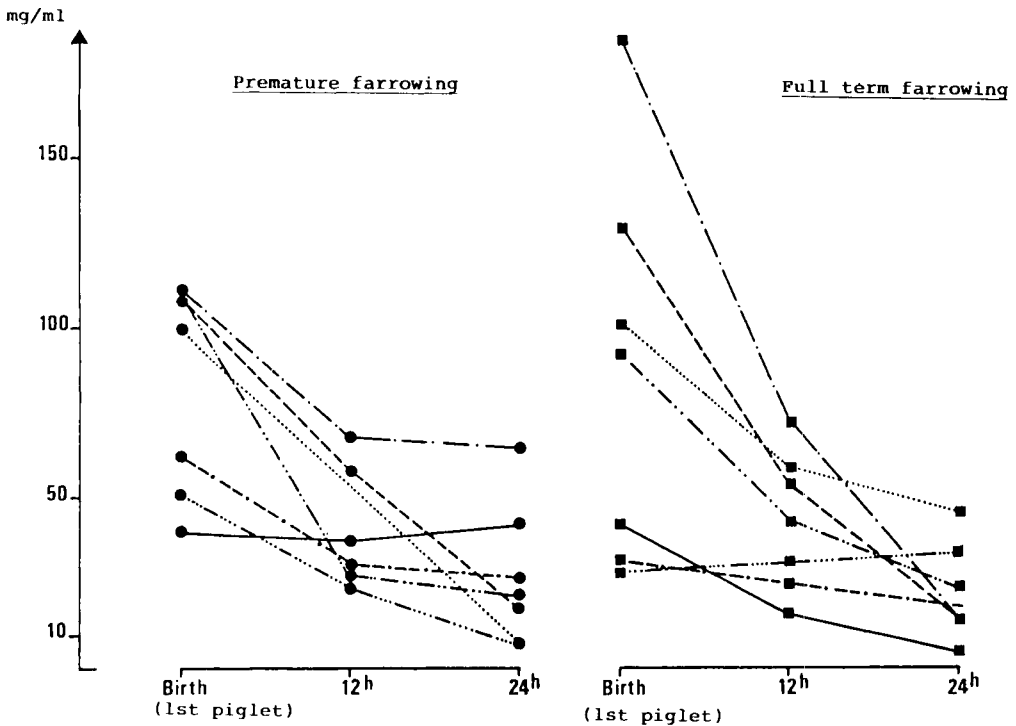


Fig. 2. — Variation between sows and delay after farrowing in the level of IgG in the colostrum.

curves, values of unknown Ig concentration samples were calculated by computer.

*Estimation of amounts of Ig ingested and stored by piglets*

Amounts of Ig ingested by each piglet were estimated for the first 12 or 24 h of life using values of colostrum ingestion and Ig concentrations in colostrum.

Amounts of Ig stored in the piglet blood at different ages were estimated on the basis of Ig concentrations and blood volume calculation corrected for the hematocrit as described by Ramirez *et al.* (1963).

**Results and Discussion**

*Piglet weight and colostrum ingestion*

The birth weight of full term piglets averaged 1150 g and was 10% higher than that of piglets born preterm. Colostrum ingestion was higher in mature than in immature piglets. Taken as a whole, as can be seen in figure 1, the total colostrum intake for the first 24 h was 48% higher in mature piglets (315 ± 91 g) than in immature ones (213 ± 61 g). Despite large variations observed between piglets and litters, the average amount of colostrum ingested was higher during the first 12 h than during the 12 following hours of life. This result

Table 1. — Total average amount of Ig intake and subsequent amount in the blood serum at 4 days of age (expressed in mg/piglet).

Treatment	Immunoglobulin intake			Amount in blood serum at 4 days	
	0-12 h	12-24 h	Total 24 h	(% of 24 h mg intake)	(% of 24 h intake)
<i>Premature</i>					
IgG	6 908	3 957	10 865	1 906	(17.5)
IgA	2 678	1 795	4 473	510	(11.4)
IgM	262	247	509	136	(26.7)
<i>Mature</i>					
IgG	11 098	4 948	16 046	2 241	(14.0)
IgA	4 876	3 024	7 900	786	(10.0)
IgM	718	432	1 150	160	(13.9)

Table 2. — Effect of premature farrowing on the postnatal IG levels in the serum of piglets (mg/ml) on average and standard deviation<sup>a</sup>.

	Age (days)		
	4	12	42-44
<b>IgG</b>			
Premature		15.4 (5.9) n=47 <sup>b</sup>	10.2 (3.8) n=33
Mature		15.1 (5.5) n=60	6.5 (2.2) n=44
<b>IgA</b>			
Premature		5.1 (3.0)	0.3 (0.2)
Mature		5.3 (2.1)	0.5 (0.3)
<b>IgM</b>			
Premature		1.2 (0.6)	0.9 (0.2)
Mature		1.1 (0.4)	1.05 (0.35)

a : The effect of prematurity is not significant.

b : n=number of piglets.

Table 3. — Immunoglobulin concentrations in neonate sera before ingestion of colostrum.

µg/ml (S.D.)	IgG	IgA	IgM
<i>Mature</i> (pregnancy 111-114 d.) n. 14	< 0.05	43.8 (14.8)	374 (150)
<i>Immature</i> (pregnancy 109 d.) n. 16	< 0.05	47.1 (14.3)	460 (310)

agrees with those of De Passille (1982), indicating a tendency for piglets to increase naturally the suckling interval from birth to 24 h of life.

*Ig concentrations in colostrum whey*

Immunoglobulin content was maximum in the first colostrum, independently of prematurity, for the three classes of Ig. In agreement with Bourne (1969), an important fall from 85 to 28 mg/ml in the average concentration of IgG was observed within 24 h after the end of farrowing (fig. 2). No effect of premature farrowing was noticed: the initial IgG concentrations were 83 and 87 mg/ml of colostrical whey for sows which farrowed respectively before and at term. However, these values appeared to be somewhat higher than that of 63 mg/ml found by Bourne (1973). IgA levels were also high:  $29.7 \pm 8.2$  mg/ml at birth and the decrease was consistently slower than that of IgG ( $16.5 \pm 7.9$  mg/ml at 24 h). IgM content was lower and rather constant in the first day ( $2.4$  mg/ml) in colostrum of sows which farrowed before term and fell from 4.5 to 2.4 mg/ml in colostrum of sows farrowing at full term.

*Total Immunoglobulin intake by piglets*

Estimated values of Ig intake per piglet are given in table 1. These values varied similarly to the total amount of colostrum ingested. The total amount ingested by mature piglets was higher for the three classes of Ig and the intake was more important during the first 12 h of life than during the following 12 h. IgG intake during the first 12 h represented 62 to 69 % of the total intake during the first day.

*Levels and amounts of Igs in the serum of piglets*

Despite large variations in the total amount of colostrum ingested within the first day of life, no significant differences appeared in the mean levels of IgG, IgA or IgM in the blood at different ages between the two groups of piglets (table 2). This sample timing allows the observation of the well-known decrease of seric IgG concentration between

4 and 12 days as well as the established adult like concentration at 42 days. It avoids the minimum values usually observed around 3 to 4 weeks of age (Bourne *et al.*, 1978). The minimum values observed at 12 days for IgA and IgM are related to the short half-lives of these passively transferred Ig in the piglets serum (Curtis and Bourne, 1973).

Conversion of these concentrations to estimated total amounts stored in the blood of piglets at a given age is of further interest. Though quite large differences have been noticed between the two groups in the amount of colostrum and Ig intake, the amount of Ig stored in the serum of 4-day-old piglets showed only slighter differences (around 25 % for all classes as a whole) (table 1). Depending on Ig class, storage at four days represented

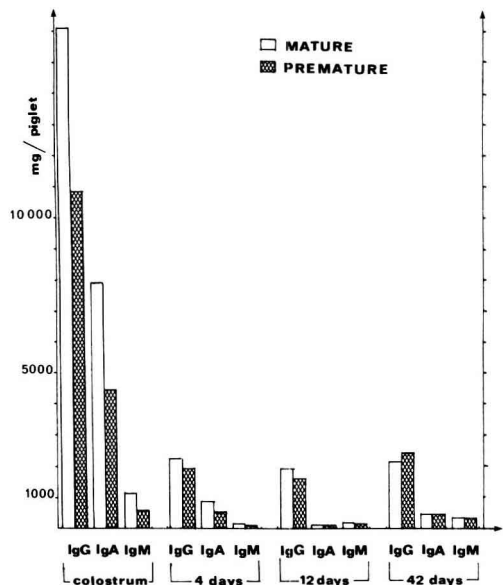


Fig. 3. — Estimation of the total amount of immunoglobulin classes ingested with colostrum (first 24 h) and stored in blood of piglets at different ages.

only 10 to 27 % of the amount ingested during the first day of life. Several remarks must be done. We have chosen to measure 4th day storage because at this time we could be sure that no more passive transfer could occur but, as half-life of IgA and IgM in serum is short, part of transferred IgM and IgA might already have disappeared, so that estimation may be underevaluated for these two classes. On the other hand, in these estimations, all Ig are supposed to come from colostrum transfer. In fact, if active synthesis of IgG and IgA is practically negligible in the newborn (table 3), that is not the case for IgM which has been found to give rise to average serum concentrations of 0.3 to 0.4 mg/ml before any colostrum ingestion by the piglet. Thus, part of storage of IgM evaluated at 4 days of age may be due to active synthesis by the newborn's own immune system.

Keeping this in mind, it is interesting to note that lighter immature piglets, able to suckle a smaller amount of colostrum, seem to store a higher proportion of ingested Igs. This is perhaps

due to a longer phase of immaturity of the gut mucosa.

Figure 3 shows that average IgG stores were not consistently altered between 4 and 12 days of age. The same feature was seen for IgM, for which the higher level of active synthesis as well as the early response to environmental antigens might balance the short half-life. On the contrary, IgA disappearance may be explained both by low active synthesis and short half life of transferred material.

In conclusion, though average birth weight and average colostrum ingestion are higher in mature than in immature piglets, no significant differences between the two groups are observed in levels or stores of IgG, IgA or IgM at 4, 12 or 42 days of life.

*Relationships between Immunoglobulin levels and stores and piglets performances*

The total correlation calculated between IgG level at 4 days and birth weight appeared to be

Table 4. — Relationship between IgG level at four days and birthweight or colostrum intake.

Treatment and R.S. <sup>a</sup>	Premature		Mature	
	1st degree	2nd degree	1st degree	2nd degree
<i>IgG level at four days of age</i>				
Individual birthweight	0.095	0.131	0.050	0.051
Colostrum intake (0-12 h)	0.233	0.265	0.204	0.207
Colostrum intake (0-24 h)	0.231	0.374	0.160	0.171
IgG intake (0-24 h)	0.432	0.445	0.356	0.359

a : Value of R<sup>2</sup> for the first and second degree of regression. Y = aX + bX<sup>2</sup> + c.

Table 5. — Relationship between total amount of Igs in the blood at different ages and total Ig intake in the first 24 h of life.

Treatment and R.S. <sup>a</sup>		Premature		Mature	
Y	X	1st degree	2nd degree	1st degree	2nd degree
Blood total IgG	Colostrum IgG intake				
4 days		0.695	0.701	0.356	0.356
12 days		0.370	0.417	0.590	0.591
42 days		0.407	0.434	0.209	0.238
Blood total Ig (G+M+A)	Colostrum G+M+A intake				
4 days		0.644	0.652	0.397	0.397
12 days		0.113	0.388	0.484	0.486
42 days		0.330	0.352	0.165	0.175

a : as in table 4.

rather low and more significant for immature piglets (table 4). Nevertheless, only 5 to 13 % of the total variation of the IgG level could be explained by birth weight, in agreement with Hendrix *et al.* (1978). Removal of piglets immediately after birth, helped first suckling of the weaker piglets as well as general controlled suckling might explain partially this low relationship. On the contrary, high correlations were found between IgG level and amount of colostrum intake either between 0-12 h or between 0-24 h of life, which is consistent with the results of Blecha and Kelley (1981).

The effect of colostrum quality expressed as its IgG concentration showed a significant correlation between IgG level at 4 days of age and the total amount of IgG intake by piglets within the first day (table 4). This observation agreed with the results of Fallon (1978) obtained on calves fed artificially on colostrum at the level of 4 to 11 % of the initial body weight.

A further demonstration of the close relationship between intake and stores was provided by calculating the regression between total immunoglobulins in the blood and amount ingested through colostrum (table 5). Highly significant correlation was found — particularly for immature piglets — between amount of IgG in the body fluid and amount suckled during the first 24 h. Curvilinear regression appeared to be generally not significant for both categories of animals. The relation showed that 21 to 43 % of the variation of the IgG stores at 42 days could be explained by the initial intake of IgG in the colostrum. This goes against the belief that passive immunity might suppress the onset of active immunity. In fact, a good colostrum intake and the consecutive transfer of large amounts of Igs does not seem to impair or delay the development of active Ig synthesis. Similar figures are given by calculations integrating the sum of the three classes of Ig. Our results indicate that the immunity of premature piglets is closely related to colostrum intake.

#### *Survival and growth rate of the piglets*

Mortality rate of piglets between 4 and 42 days was respectively 12.8 and 13.3 % for immature and full termed animals. This relatively high proportion could be explained by an important weaning stress applied at 12 days of age. The levels of IgG at 4 days for piglets lost during this period, i.e.  $18.36 \pm 5.51$  for immature vs  $15.29 \pm 5.91$  mg/ml for mature ones, were not significantly different from the average values presented in table 2. However these results seemed to have a limited significance. In this respect data of Hendrix *et al.* (1978) showed that piglets which died before weaning had lower levels of Igs at 24 h of age.

Total correlations as well as partial correlations for a constant weight were calculated between average daily gains and IgG levels at 4 days of age in piglets serum (table 6). Rather low relationships were found between growth rate and IgG level measured at 4 days for mature piglets: only 5 % the variation of average daily gain between 0 and 42 days could be explained by the variation in the IgG level at 4 days.

#### Conclusions

This experiment dealt entirely with the passive aspect of the neonatal immunity in the piglets. Level of Igs in the colostrum and colostrum intake appeared the two most important keys of immunity. Slight prematurity at birth did not seem to significantly impair the transmission of this immunity. Though piglets born preterm were 10 % less heavy than piglets born at term and ingested less colostrum, Ig levels and stores at different ages were not significantly decreased as compared to those of piglets born at term.

In addition, no differences were seen in mortality or performances between the two groups of piglets.

Table 6. — Relationship between growth rate and IgG levels at four days; total and partial correlation coefficients.

Treatment	Premature <sup>a</sup>		Mature <sup>b</sup>	
	r <sub>xy</sub>	r <sub>xyz</sub> <sup>c</sup>	r <sub>xy</sub>	r <sub>xyz</sub>
X: IgG level at four days				
Y: average daily gain				
0 - 12 days	0.100	-0.010	-0.042	-0.134
0 - 42 days	-0.042	-0.011	0.216	0.280

a : birth weight:  $1\ 058 \pm 213$  g; A.D. gain 0 - 12 days: 136 g; 0 - 42 days: 145 g.

b : birth weight:  $1\ 150 \pm 196$  g; A.D. gain 0 - 12 days: 157 g; 0 - 42 days: 138 g.

c : partial correlation independently of birthweight (z).



IgG constitutes at least 80 % of the colostrum Igs in sows and a sharp fall occurs within the first day *post-partum*. Our results agree with those of Bourne (1969) and Silim (1982) in this respect.

Our experiment stresses again the importance of colostrum intake as soon as possible within the first day of life to assure a good transmission of passive humoral immunity to piglets. It is well known too that colostrum does not play a unique immunological role in the life of piglets. Its nutritional importance has already been clearly demonstrated (Aumaitre and Seve, 1978), particularly to provide dietary energy and to maintain the thermo-

regulatory process (Le Dividich and Noblet, 1982). Practical recommendations can be derived from these results. They indicate that conditions to provide a maximum amount of early colostrum must be assured, particularly to prematurely born or weak piglets, in order to supply them with sufficient immunological and nutritional conditions.

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