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Improving maternal welfare during gestation has positive outcomes on neonatal survival and modulates offspring immune response in pigs

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ABSTRACT

Improving the housing of pregnant sows by giving them more space and access to deep straw had positive effects on their welfare, influenced their maternal behavior and improved the survival of their offspring. The present study aimed at determining whether these effects were actually due to environmental enrichment and whether the provision of straw pellets and wood can partly mimic the effects of straw bedding during gestation. Three graded levels of enrichment were used, that were, collective conventional pens on slatted floor (C, $n = 26$), the same pens with manipulable wood materials and distribution of straw pellets after the meals (CE, $n = 30$), and larger pens on deep straw litter (E, $n = 27$). Sows were then housed in identical farrowing crates from 105 days of gestation until weaning. Decreased stereotypies, blood neutrophils, and salivary cortisol, and increased behavioral investigation indicated that health and welfare of sows during gestation were improved in the E environment compared with the C environment. The CE sows responded as C or E sows depending on the trait. Piglet mortality rate in the first 12 h after birth was lower in E and CE litters than in C litters, but enrichment level during gestation had only small effects on lactating sow behavior and milk composition postpartum. On days 2 and 3 of lactation, E sows interrupted less often their nursing sequences than C and CE sows. On day 2, milk from both E and CE sows contained more minerals than that from C sows. In one-day-old piglets, the expression levels of genes encoding toll-like receptors (TLR2, TLR4) and cytokines (interleukin-1, -6 and -10) in whole blood after 20-h culture, were greater in E piglets than in CE or C piglets. In conclusion, housing sows in an enriched environment during gestation improved early neonatal survival, probably via moderate and cumulative positive effects on sow behavior, milk composition, and offspring innate immune response. The gradation in the effects observed in C, CE and E housing environment reinforced the hypothesis of a causal relationship between maternal environmental enrichment, sow welfare and postnatal piglet traits.

1. Introduction

In mammals, stressful situations for pregnant females can have consequences on the physiology and the disease risk of their offspring later in life [1,2]. In the current farming systems, mortality and morbidity of neonates are still an issue for all livestock species [3–5]. In addition, a large number of farm animals, including reproductive females, are experiencing challenging husbandry conditions. For example, pregnant sows are often facing the cumulative effects of feed restriction, social stress due to space restriction and competition for access to the feeder, leg disorders, boredom due to the lack of cognitive and sensorial stimuli, and sometimes fear from humans [6–8]. Providing environmental enrichment, including bedding materials, and increasing space allowance, are major elements that meet the behavioral needs and comfort of sows [9–11]. Thus, the possibility that enriching the sow's environment may have a positive effect on the survival rate, health and welfare of their newborns must be tested.

Prenatal stress occurs when maternal stress directly influences the development of the fetus. In the porcine species, prenatal stress induces post-natal changes in the corticotropic axis of the piglets [12], their behavior and emotiveness [13,14], and their immune response [15]. It is accepted that most of prenatal stress effects result from fetal exposure to

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higher maternal concentrations of cortisol, a hormone which influences fetal development [16,17], but placental insufficiency could also be involved [18]. However, other pathways related to maternal stress during gestation are likely to influence the offspring during and after birth, such as alterations in maternal immunity, progress of farrowing, quality and quantity of lacteal secretions, and post-natal maternal care [19]. Most of the studies investigating the effects of the stress of pregnant females have used extreme stress models, using as a control poor environments that were close to the living conditions encountered in conventional farming systems [15,20,21]. These studies have rarely been able to demonstrate that maternal gestational stress influenced neonatal survival [19]. The complementary strategy, testing the possibility of improving piglet health and survival in the postnatal period by improving mothers' living environment during gestation has just started to be explored [22,23]. These studies showed that enriching the environment of sows by straw litter and more space during gestation improved the immediate welfare of the mothers, and this was also associated to a better survival of their newborns in the first days after birth.

In the present study, we tested the existence of a causal link between improved maternal well-being due to environmental enrichment and neonatal survival. We hypothesized that graded levels of environmental enrichment of the maternal environment during gestation would have graded positive effects on neonatal survival, and on the physiological and behavioral mechanisms underlying better survival. For this purpose, we compared three housing environments for gestating sows, that is, a French conventional group-housing system on slatted floor, the same housing environment but moderately enriched with wood material in the pen and distribution of straw pellets after the meals, and pens with enlarged available space and deep straw bedding. Characteristics known to be important for piglet neonatal survival, both at maternal (maternal stress, immunity and behavior, milk composition) and neonate (immune function) levels, were evaluated.

2. Materials and methods

2.1. Experimental design leading to graded levels of enrichment

The experiment was carried out in the experimental farm of the Chambre Régionale d'Agriculture de Bretagne (CRAB) at Crécom (Saint-Nicolas-du-Pélem, France). The experiment encompassed three experimental groups of sows, housed in pens corresponding to three different levels of environmental enrichment during gestation. Sows were introduced into the gestating pens a few days before insemination. Pens were designed for 12 females maximum each. They were divided into an open loafing area, where the group of sows had space to move and have social interactions, and a row of free-access individual stalls equipped with feeding troughs. The enriched (E) gestating pens were on deep straw and offered a minimum of 3.5 m^2 of space per sow with high ceilings (the minimum space requirement according to European rules is 2.25 m^2 per sow). The conventional (C) gestating pens were on a concrete slatted floor with two metal chains per pen as a minimal enrichment material, and offered a minimum of 2.4 m^2 of space per sow with a lower ceiling height (Table 1). To provide an increased grade of enrichment, a group of sows was housed in conventional pens with enrichments (CE) in the form of 3 pieces of oak attached to a chain and wheat straw pellets distributed in the trough after each meal to mimic spontaneous ingestion of straw in E sows. Pellets with a diameter of 6 mm were made of dry chopped straw that took up water when distributed in the trough and disintegrated into wisps of straw. Sows were transferred to farrowing pens at approximately 105 days of gestation (DG 105, DG 0 being the day of the first insemination, and the mean gestation length being of 115 days), where they remained with their litter until weaning. The farrowing pens (4.91 m^2) were on slatted floor and identical for all sows, and complied with European 2008/120/CE standards regarding maternal and piglet welfare. In these pens, sows were confined in conventional individual farrowing crates (0.62 × 2.10 m) during the whole period they spent in the maternity rooms. Management practices from late pregnancy to weaning were similar for the three groups of sows and their piglets. During the whole experiment, C and CE sows were housed in different pens but in the same gestating and farrowing rooms, while E sows were housed in an adjacent independent farrow-to-finish unit. Thus, C and CE sows and piglets were exposed to the same ambient parameters (sound, light, temperature, dust, gas, etc), while E gestation and maternity rooms possibly differed. These parameters were not measured and these possible differences were considered as being part of the experimental model. Ambient temperatures were recorded daily in each maternity room, and did not differ between rooms. On the morning of expected farrowing, when heat lamps were on, temperature of each farrowing crate was recorded at 10 cm from the floor at 4 locations. The temperature was lower for each of the locations in the C (C and CE groups) versus E unit, respectively: 23.9 ± 0.2 and 24.4 ± 0.2°C behind the sows ($P = 0.002$), 25.7 ± 0.5 and 26.4 ± 0.5°C under the lamp ($P = 0.022$), 24.6 ± 0.3 and 25.4 ± 0.3°C near the lamp ($P < 0.001$), 23.7 ± 0.2 and 24.3 ± 0.2°C ($P < 0.001$).

Table 1
Description of the gestation pens, and number and parity rank of sows in the conventional (C), conventional enriched (CE) and enriched (E) treatment groups in the three replicates of the experiment.

Treatment group	C	CE	E
Replicate	1 / 2 / 3	1 / 2 / 3	1 / 2 / 3
Number of sows	8 / 10 / 8	10 / 10 / 10	8 / 9 / 10
Mean parity	3.6 / 3.9 / 2.1	3.6 / 3.9 / 4	3.9 / 3.3 / 3.8
Individual feeding stalls	Yes	Yes	Yes
Pen surface	28.8 m^2	28.8 m^2	42 m^2
Floor	Concrete slatted	Concrete slatted	Full concrete + accumulated straw bedding
Other enrichments	2 metal chains	2 metal chains, 3 wood pieces, straw pellets	

2.2. Sows and litter management

The experiment was carried out in three successive replicates of Landrace x Large White sows and their litters. Sows of various parities (mean parity rank of 3.3 ± 2.1 [1,8] for C, 3.8 ± 2.1 [1,9] for CE and 3.7 ± 1.8 [1,7] for E sows) were inseminated with mixed semen from Piétrain boars. In total, 83 sows had confirmed pregnancies and were raised throughout gestation and lactation (C: $n = 26$, CE: $n = 30$, E: $n = 27$, Table 1). On DG 114, parturition was induced by an intramuscular injection of cloprostenol (2 mL of Planate®, MSD Santé Animale, Beaucouzé, France). Then, piglets received standard care interventions (tooth resection and tail docking for all, surgical castration of males). In litters with more than 15 piglets 24 h after parturition, the extra-piglets were transferred for adoption to a sow with a smaller litter. Cross-fostering was limited as much as possible and was done within treatment. Weaning occurred approximately 28 days after farrowing.

Sows and piglets had free access to water throughout the experiment. During gestation, commercial standard gestation feed containing 6.9% of crude fibers was provided to each sow in the form of soup at 0830 and 1630 h in two equal meals. All sows received the same amount of feed per day during gestation (3.0 kg from insemination to day 28, 2.6 kg from day 29 to 60, 3.0 kg from day 61 to 80, 3.6 kg from day 81 to 104, and 3.6 kg from day 105 to the term). These amounts were below the ad libitum level even though it covered the nutritional needs of pregnant sows, and there were no feed refusals. At the end of each meal, straw pellets (First pellets, Maisonneuve, France) were given to CE sows only, at a rate of 200 g/d between DG 3 and DG 30 and 400 g/d between day

DG 31 and DG 104. In the farrowing rooms, sows were individually fed the same lactation diet through an automatic dispensing system (1 more kg daily from farrowing day to the 3rd day of lactation, and then the amount was adapted to litter size and sow appetite). Piglets had also access to creep feed from day 10 of lactation. The nutritional composition of the diets and straw pellets is described elsewhere [24].

Sows were weighed a few days before artificial insemination, on DG 105 and on the day of weaning. On those same days, their backfat thickness was measured at the level of the last rib on each side, 65 mm from the midline (P2 site).

2.3. Measurement of the behavior of sows

2.3.1. Behavioral measurements in the gestation pens

On DG 101, the behavioral activity of each sow was recorded by two observers. The day before, sows were marked with a fatty chalk with a number on the back and flanks for identification. Focal recordings were made after the morning meal (0830–1030 h) and before the afternoon meal (1330-1530 h). In the morning, the observation started after the first trough provided with feed (and straw pellets for CE sows) was empty. In the afternoon, the recordings started after the sows were warned of the presence of the observer talking to the sows and walking around the pen. The two observers alternated between C/CE and E rooms in the morning and afternoon to limit the observer effect. During these 2 h periods, any occurrence of agonistic behavior and investigative behavior towards available substrates was continuously and individually recorded. A 5 sec interval without any new acts, was chosen to identify two distinct sequences. In addition, every 7 min (17 scans per 2-h period), the posture (lying, standing, sitting) and the behavioral activities were registered for each sow. These behavioral activities were mutually exclusive and included the following items (as defined in ATOL ontology, 2012): resting in the feeding stall, resting in the pen, investigation towards each available substrate, negative and positive social behaviors according to the reaction of the receiver, stereotyped activity (sham chewing, repetitive biting or licking trough or floor), mobility, ingestion behavior if feed, straw pellets or water were still present in the trough, eliminative behavior, and other behaviors.

On the same day (DG 101), at the end of the morning observation, the reactivity to human approach was assessed. The observer entered the home pen, and, in a random order, slowly approached each sow and tried to put his hand on its back. The individual response of each sow was scored from 0 to 2 (score 0: the sow does not avoid the human approach and can be touched, score 1: the sow does not avoid the human approach but cannot be touched, score 2: the sow avoids the human approach and cannot be touched) as adapted from [25].

2.3.2. Behavioral measurements at the transfer to maternity rooms and around farrowing

At transfer on DG 105, all sows were individually observed between the exit of the weight scale and the entrance into the farrowing crate. The quality of gait was observed to detect lameness (score 0: no lameness vs. score 1: lameness) as adapted from Welfare Quality Protocol [26]. The ease to move the sow with or without human intervention was scored (score 0: the sow moves spontaneously, score 1: limited human intervention with one or two pushes on the back of the sow; score 2: the sow stops moving or does not move at all despite human intervention) as adapted from [25].

During the peripartum period, video cameras were installed and programmed to record continuously the behavior of four sows per group and per replicate during a one week period starting on a Tuesday (farrowing being expected to be centered on the Thursday). The aim was to analyze records by 24 h periods, starting from 24 h prepartum to 72 h postpartum. Because the farrowing process started in various time depending on the sows, full records were finally obtained for 5-4 multiparous sows per replicate and per maternity room, generating data for 6 C sows (2 per replicate) and 7 CE sows (2, 2 and 3 in replicate 1, 2 and 3) in one system, and for 12 E sows (4 per replicate) in the other system. Records were analyzed by 24 h periods, starting from 24 h prepartum to 72 h postpartum. The analysis of sow behavior focused on the mutually exclusive items of postures (standing, ventral and lateral lying, and sitting) and the mutually exclusive items of activities (nursing, resting, investigation towards the environment, and other behaviors such as ingestion and eliminative behaviors). Additionally, it was recorded when sow's body was in contact with half or more of the piglets resting at the udder without suckling. For each postural or activity item, the generated variables concerned the total duration of expression of the item (expressed as a percent of the 24 h period), the number of sequences of expression and the mean duration of a sequence (in sec). The duration of farrowing was determined as the interval between the first and the last born piglet. The duration of a nursing sequence was calculated as the time period between the start of piglet gathering, defined by half of the litter present at the udder, and the end of the massage period (massage after milk let-down), defined by less than half of the litter still massaging the udder. The interruption of the nursing sequence at any step was also recorded because it is a risk factor for piglet mortality.

2.4. Neonatal mortality assessment and collection of blood, saliva and milk samples

Sows were weighed a few days before artificial insemination, on DG 105 and on the day of weaning. Piglets born alive and stillborn were individually weighed within 12 h after birth. Then piglets were weighed on day 4 and at weaning. Numbers of piglets at birth and at weaning were recorded. Piglet mortality, date and cause, were registered throughout lactation. Piglets dead at birth and dying during the first 72 h after birth were weighed and conserved in plastic bags at +5 °C until necropsy. The cause of death at birth (death before farrowing due to infection in utero, other causes or mummified piglets, death during farrowing with asphyxia or septicemia) and after birth (malformation, killed by the sow, anemia, crushing, starvation, birth weight < 800 g, dehydration due to enteritis) were determined using a diagnostic tool described previously [5].

Saliva samples were collected from sows on DG 14, and before (DG 105) and after (DG 107) the transfer to farrowing rooms at 0900 h using cotton buds (Salivette®, Sarstedt 51588 Nümbrecht, Germany). Blood samples were collected from all sows on DG 73 and DG 102, before the morning meal. The sampling was performed at the jugular vein of sows restrained by snaring, using a vacutainer system and 10 mL EDTA and heparin tubes. On the day after farrowing (24 to 36 h), blood samples were collected on one middle-sized female piglet per litter ($n = 83$). The 4 mL samples were collected using a vacutainer system at the jugular vein of piglets that were manually held in a supine position on the knees of the sampler, head and neck straightened out. On that same day, milk from all sows ($n = 83$) was manually collected from several functional teats per sow, after an intramuscular injection of 20 IU of oxytocin (Ocytovem, Céva Santé Animale, Libourne, France).

2.5. Biochemical and immuno-assays

Salivary cortisol concentration was assessed using a luminescence immunoassay kit (LIA, IBL, Hamburg, Germany). The detection threshold was 0.15 ng/mL, intra- and inter-assay coefficients of variation (CV) were 6% and 8% at 2.1 ng/mL. Milk concentration of immunoglobulins (Ig) A was assessed using an ELISA porcine kit (Bethyl Laboratories, Montgomery, Texas, USA). The limit of sensitivity was 7.8 ng/mL, intra- and inter-assay CVs were 9 and 15%. Ash, dry matter, gross energy, crude protein, lipids and lactose were assayed as previously described by Loisel et al [27].

The concentration of hydroperoxides (H_2O_2), generated by the peroxidation of lipids, proteins or nucleic acids, and the total blood antioxidant potential (BAP), resulting from the combined effects of many

antioxidants such as ascorbic acid, proteins, alpha-tocopherol or bilirubin, were assayed on heparin plasma from sows and piglets by analytical methods using commercial kits (d-ROM and PAT assays, H&D srl, Parma, Italy), and a spectrophotometer (Konelab20i, Thermo Fisher Scientific, Cergy-Pontoise, France) as previously described [28].

Sow haptoglobin concentration was assayed in EDTA plasma using commercial kit (Tridelta Ltd, Maynooth, Ireland) and a spectrophotometer. The intra- and inter-assay CVs were 7 and 24%, respectively and the limit of sensitivity was 0.03 mg/mL. The total numbers of lymphocytes, monocytes and polymorphonuclear cells were measured in EDTA-blood of sows with a hematology automatic cell counter (MS-9®, Melet Schloesing laboratories, 95520 Osny, France).

2.6. Milk and blood cell analyses by flow cytometry

All antibodies were purchased from Clinisciences (Nanterre, France), except CD172a (Santa Cruz Biotechnology, Dallas, TX). The total numbers of polymorphonuclear cells together with macrophages, monocytes and non-monocyte mononuclear cells of milk were quantified by cytometry as previously described [23]. Briefly, cells obtained from 10 mL fresh milk samples were divided in two samples stained with the anti-porcine CD172a antibody or with the corresponding isotypic control. Cells were suspended in 0.5 mL PBS with 1 μL of the DNA-selective Vybrant® DyeCycle™ Ruby stain (Thermo Fisher Scientific, Waltham, MA). Total events were acquired from 100 μL cell suspension using the MACSQuant flow cytometer (Miltenyi Biotec), analyzed with the MACSQuantify software, and the absolute numbers of cells contained per mL of milk were backward calculated.

To estimate the total numbers of monocytes, T- and B-lymphocytes in blood of one-day-old piglets, 50 μL of heparinized blood were incubated for 15 min with a 20-μL mix of antibodies directed against swine CD3, CD21 and CD172a or the corresponding mix of isotypic controls. Samples were incubated for 15 additional min in 450 μL of a lysis solution according to manufacturer instructions (Becton, Dickinson, Le Pont de Claix, France). Cells were washed, suspended in 500 μL PBS, and 50 μL total numbers of cells per samples were analyzed by the flow cytometer.

2.7. Whole blood cell cultures and determination of gene expressions

We investigated mRNA expression of toll-like receptors (TLR)-2, -4 and -9, and of 5 cytokines (interleukin (IL)-1β, -6, -10, interferon (IFN)-α, Transforming Growth Factor (TGF)-β) in cultured whole blood cells. These cells were incubated in resting conditions (medium alone), or after activation with agonists stimulating TLR-4 (O55:B5 lipopolysaccharide, LPS, Sigma-Aldrich) or TLR-9 (CpG oligonucleotide, ODN2007, Invivogen). Briefly, whole heparinized blood samples were diluted 1:5 in supplemented RPMI, distributed in 24-well culture plates (0.4 mL/well), and completed in triplicates by 0.6 mL of either complete medium alone or medium supplemented with LPS (10 μg/mL in the well), or ODN2007 (10 μg/mL in the well). After 20 h of incubation, cells were collected, triplicates were pooled, spined down, suspended in 0.5 mL of guanidine thiocyanate DL buffer (Macherey Nagel, Hoerdt, France), and stored at -80°C. Total RNA was extracted using a commercial kit (Nucleospin blood kit, Macherey Nagel) according to the manufacturer's instructions. After a DNase treatment (DNA-free kit, Applied Biosystems, Foster City, CA, USA) in the presence of an RNase inhibitor (ThermoFisher Scientific, Illkirch, France), the quality and amount of extracted RNA were estimated using a Denovix spectrophotometer (Clinisciences, Nanterre, France). After a concentration step performed with a speed-vac concentrator (ThermoFisher Scientific), only samples reaching the minimal required RNA concentration of 111 ng/μL were used. The quality criteria of A260/280 and A260/230 ratios greater than 1.6 were met for 170 samples out of 249. The integrity of isolated RNA was assessed using the Agilent RNA 6000 Nano kit with an Agilent 2100 Bioanalyzer (Agilent Technologies France, Massy, France). Average RNA integrity numbers were 7.6 ± 0.5 (mean ± SD). Altogether, 106 RNA samples (n = 13-16 per mitogen condition for the C group, n = 11 per condition for the CE group, and n = 9-10 per condition for the E group) were generated for quantitative PCR analyses.

First-strand cDNA synthesis was performed with 1 μg of total RNA, by using High Capacity RNA to cDNA Kit (Applied Biosystems, Foster City, USA). Primers were designed from porcine sequences available in Ensembl or NCBI databases using Primer Express® v3.0 software (Applied Biosystems). Amplification reactions and disassociation curves were carried out on a Step One Plus TM real-time PCR system (Applied Biosystems). The 4 tested house-keeping genes (HRPT1, PPIA, TBP1, GAPDH) had their expression unaffected by culture condition or treatment group. Among them, HPRT1 and PPIA were identified as the most stable house-keeping genes by the GenNorm algorithm and were used for normalization.

2.8. Statistical analyses

Analyses were performed using SAS software (SAS Inst. Inc., Cary, NC). Discrete variables were analyzed by Generalized Linear Models using the GENMOD procedure, with the treatment (C, CE, or E) and the replicate (1, 2, or 3) as fixed effects. A binomial law with a Logit link function was used for mortality rates, scores of lameness and reactivity to human, and the use of manipulable material at DG 101. A Poisson law with a log link function was used for numbers of piglets per litter and numbers of lactation bouts after farrowing. Other data were analyzed by linear models (ANOVA) using the MIXED procedure, with the treatment as a fixed effect and the replicate as a random effect. Normality of the distribution and equality of variance of continuous variables were checked visually, and adequate transformations were performed when needed: cortisol concentration and gene expression data were square root transformed before analysis, milk cell proportions and behavioral data expressed in ratio were transformed using the arcsinus square root transformation. For serial data, day-related or mitogen-related effects and their interactions with the treatment effect were analyzed using repeated measures analyses, with the animal (sow or piglet) considered as experimental unit. Then, the two-by-two comparisons of means were done by Tukey tests with a threshold P-value < 0.05. In tables and figures, values are expressed as raw means and standard errors of the means (SEM) when data were analyzed by the GENMOD procedure, and as least squares means and SEM when data were analyzed using the MIXED procedure.

3. Results

3.1. Sow performance, litter mortality and growth data

Gestation length and body weights of sows before insemination, on DG 105 or at weaning did not differ between the 3 treatments ($P > 0.10$, data not shown). Backfat thickness did not significantly differ between treatments before insemination and on DG 105, but was greater for E than for C and CE sows at weaning ($P < 0.05$, data not shown).

The total number of piglets per litter did not differ between treatments ($P > 0.10$, Table 2) but the number of piglets that died very early (during and within 12 h following birth) was greater in C than in CE ($P = 0.073$) and E litters ($P < 0.05$). As a consequence, the rate of very early mortality was greater in C than in CE and E litters ($P < 0.05$). Overall, mortality from birth to weaning did not differ significantly among treatments (Table 2).

Average piglet weight at birth was not influenced by treatment ($P > 0.10$). Nevertheless, the proportion of piglets weighing more than 1.8 kg at birth was greater in E than in C and CE litters ($P < 0.05$, Table 2). Piglet weight at weaning and average daily growth rate during suckling period did not differ between treatments, indicating no differences in lactation performance of sows. Piglets that died during birth or before weaning weighed less at birth than piglets that were alive at weaning (1.12 ± 0.04 vs. 1.49 ± 0.03 kg, $P < 0.001$). No significant difference

Table 2
Litter size, litter mortality rates and body weights of piglets born from sows housed during gestation in collective conventional pens (C), in C pens enriched with manipulable materials and straw pellets (CE), and in larger pens on straw bedding (E).

	Treatment			SEM[1]	P-value[1]
	C	CE	E		
Number of litters	26	30	27		
Numbers of piglets / litter					
Born	15.4	15.7	15.2	-	0.857
Born alive	13.8	14.7	14.2	-	0.625
Stillborn and dead within 12 h	1.7	1.1	1.0	-	0.053
Weaned	11.7	12.4	12.1	-	0.713
Mortality rates, %					
Very early (birth -12 h pp)[2]	11.1[a]	6.6[b]	6.3[b]	-	0.034
Early (12 h–72 h pp)	11.1	10.2	10.0	-	0.931
Late (72 h pp–weaning)	3.0	3.0	3.9	-	0.930
Overall [3]	23.2	19.1	19.3	-	0.357
Piglet body weights, kg					
At birth (all piglets)	1.46	1.43	1.53	0.1	0.417
At weaning	9.07	8.85	8.99	0.3	0.733
Proportion of piglets at birth, %					
With body weight < 1.0 kg	15.3	16.3	12.6	-	0.147
With body weight > 1.8 kg	16.3[a]	18.7[a]	28.2[b]	-	< 0.001
Average daily growth rate during lactation, g	267	261	260	11	0.622

[1] SEM = greatest standard error of the least-squares means. P-values of the treatment effect. a, b: values with different letters differ with $P < 0.05$.
[2] Proportion of piglets that died during birth and within 12 h after birth (post-partum, pp).
[3] Proportion of piglets that died from birth to weaning, including stillbirth.

among treatments was observed in the causes of mortality within 72 h of birth (mummified: 11%, dead before parturition: 4%, asphyxia: 15%, infection in utero: 14%, septicemia: 6%, dehydration due to enteritis: 3%, birth weight < 800 g: 16%, starvation: 10%, crushed: 17%, anemia: 1%, killed by the sow: 1%, deformities: 1% of the total number of dead piglets).

3.2. Behavior of gestating sows

On DG 101, scan sampling revealed that the percentage of time spent in the standing posture was greater in the two enriched groups (CE and E, 29% and 30% on average) than in the C group C (23%, $P < 0.05$). Regardless of the treatment, sows were resting in the feeding stalls more often than in the other locations of the pen, but CE sows were characterized by a longer occupational time than the other groups ($P < 0.05$, Fig. 1A). Interactions between sows were scarce. The number of sows observed at least once involved in positive (5 C, 7 CE and 10 E sows) or negative interactions (3 C, 3 CE and 5 E sows) was not influenced by treatment ($P > 0.1$), but the frequency of positive interactions in the group was greater in E group (3.0 %) than in CE and C groups (1.5 % in both groups, $P < 0.05$). Continuous focal observations showed that the investigative behavior towards the available manipulable substrates was lower in C and CE sows than in E sows ($P < 0.01$, Table 3), although highly variable between sows. The C sows explored more pen walls than the metallic chains ($P < 0.001$), CE sows explored more the chains and pieces of wood than the pen walls ($P < 0.001$), and E sows explored much more often straw than pen walls ($P < 0.001$). The C sows exhibited a higher proportion of stereotypies (34% of the scans) compared with sows of the CE (22%) and E groups (7%, $P < 0.05$, Fig 1A).

On DG 101, the reactivity of sows to a human approach and physical contact was not affected by the treatment (score 2: 54% and 57% of C and CE sows, respectively, 38% of E sows, $P > 0.10$). On the day of transfer to maternity rooms (DG 105), the majority of sows moved voluntarily from the weight scale into the farrowing crates. There was a significant treatment effect ($P < 0.05$) on the ease to move the sow, but no clear difference of responsiveness could be detected between treatments: no intervention of the animal keeper was required for 88% of E and C sows, and 97% of CE sows. Three E (12%) and two C (8%) sows

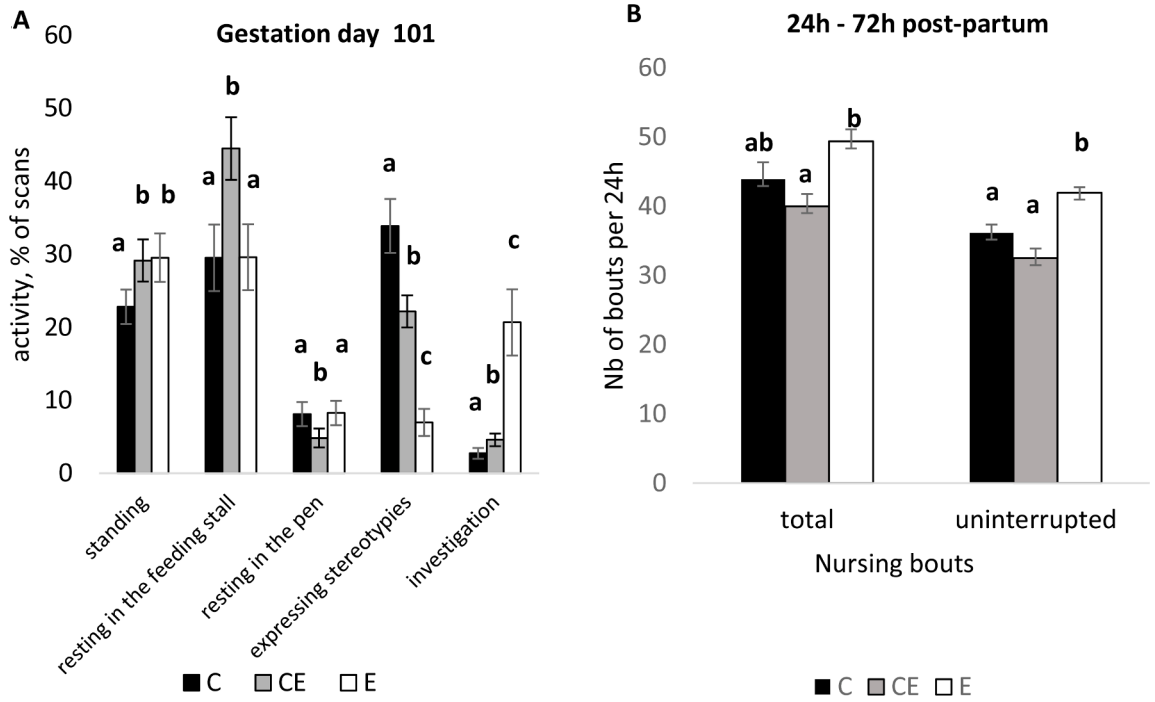

Fig. 1. Behavioral activities at 101 days of gestation (1A) and number of nursing sequences per 24 h during the 24-72 h post-partum (1B) of sows housed from insemination to 105 days of gestation in collective conventional pens (C), C pens enriched with manipulable materials and straw pellets (CE), and in larger pens on straw bedding (E). At 101 days of gestation, behavior was recorded during a 4 h period with scan samples of 7-min intervals. The total number of nursing bouts (including complete sequences and the sequences interrupted before milk ejection) and the number of completed nursing sequences were counted from 24 to 72 h after farrowing. Means (± SEM) with different letters (a, b, c) are statistically different ($P < 0.05$).

Table 3
Investigative behavior of sows housed during gestation in collective conventional pens (C), in C pens enriched with manipulable materials and straw pellets (CE), and in larger pens on straw bedding (E). Posture frequency was recorded by 7 min interval scan sampling and investigative behaviors were recorded by continuous focal observations over a 4 h period on day 101 of gestation.

	Treatment			SEM[5]	T effect[5]
	C	CE	E		
Number of sows	26	30	27		
Number of investigating sows[1]	17	29	26		
Occurrences / investigating sow[2]	2.7	4.6	20.7		
Targets of the investigative behavior					
Pen facilities (%)[3]	62^{ax}	16^{by}	10^{cy}	41	< 0.001
Chain (%)[3]	38^{ay}	42^{bx}	-	42	< 0.01
Wooden objects (%)[3]	-	42^{x}	-	29	
Straw (%)[3]	-	-	90^{x}	20	
Substrate effect[4]	< 0.001	< 0.001	< 0.001		

[1] Number of sows exhibiting at least one investigative behavior during the 4 h observation period.
[2] For each sow displaying some investigative behavior, the number of occurrence of investigating behavior over the 4 h observation period was recorded.
[3] Values represent the mean ratio of occurrences of investigation directed toward a substrate divided by the total number of investigative occurrences per sow.
[4] P-value of the substrate effect within treatment; x, y: means in a column differ at $P < 0.05$.
[5] SEM: greatest standard error of the means presented in a raw, and P-value of the treatment (T) effect within substrates; a, b, c: means in a raw differ at $P < 0.05$.

required a moderate human intervention. One C and one CE sow resisted to the animal keeper. On DG 105, 96% of sows were free from lameness in all treatments, and only one C sow was scored lame.

3.3. Behavior of parturient and lactating sows

The behavior during parturition and lactation was recorded for a sub-group of 6 C sows, 7 CE sows and 12 E sows. The farrowing duration (4.8 ± 2.2 h) and the time spent in the different postures during farrowing did not differ between treatments ($P > 0.10$).

During the 24 h preceding and the 72 h following farrowing, the gestational environment had almost no influence on the total time spent in the recorded postures and activities ($P > 0.5$, data not shown). However, the analysis of the repartition of these behaviors during the 24 h period revealed differences between groups of sows (Table 4). The duration and the frequency of standing bouts ($P = 0.036$ and $P = 0.052$) and of lateral lying bouts ($P < 0.01$ and $P = 0.016$) were influenced by treatment. Regardless of the day, E sows performed or tended to perform shorter bouts of standing posture (compared with C sow, $P = 0.074$) and lateral lying posture (compared with C sows, $P = 0.014$, and CE sows, $P = 0.089$). But these bouts were or tended to be more numerous, compared with C sows for standing posture ($P = 0.051$) and compared with CE sows for lateral lying ($P = 0.02$). During the 0 - 24 h period, E sows also spent more sequences in contact with their piglets than CE sows (44 vs. 30 ± 5 bouts, $P = 0.021$).

There was nearly no synchronized nursing during the first 24 h after farrowing. From then on, sows regularly initiated nursing sequences, of which a small proportion was interrupted by the sow before milk ejection. From 24 to 72 h post-partum, E sows initiated more nursing sequences than CE sows (Fig. 1B, $P < 0.05$), with C sows having intermediate numbers. More importantly, E sows displayed more uninterrupted nursing sequences compared to C and CE sows ($P < 0.05$).

3.4. Salivary cortisol, and blood immune and oxidative status of gestating sows

There was a significant treatment x day interaction for salivary cortisol concentration ($P < 0.01$). At DG 14, cortisol concentration was greater in C and CE than in E sows ($P < 0.001$). At DG 105, cortisol concentration was greater in C than E sows ($P < 0.001$), and CE sows presented intermediate concentrations (Fig. 2A). At DG 107, i.e., 2 days after the transfer of sows into the farrowing crates, cortisol concentration no longer differed between the three treatments. For both C and CE sows, concentration was lower after transfer than during gestation ($P < 0.05$).

The lymphocyte number in the blood of sows was unaltered by the treatments ($P > 0.10$, Table 5). The C sows had a greater number of polymorphonuclear cells than CE and E sows whatever the day ($P < 0.01$, Fig. 2B), and the monocyte number tended to be higher in C than CE sows ($P = 0.07$). Haptoglobin concentration was not influenced by treatment ($P > 0.10$). Regarding the systemic redox status, the concentrations of hydroperoxides and BAP were not influenced by treatment ($P > 0.10$).

3.5. Milk composition 24 h after farrowing

On the day after farrowing, E sows tended to produce milk that contained less dry matter compared with C and CE sows ($P < 0.1$, Table 6). Their milk also contained less fat and energy than milk from C sows ($P < 0.05$) and CE sows ($P = 0.065$). In contrast, E and CE sows had more ash in their milk than C sows ($P < 0.05$).

Immunoglobulin A content was not influenced by treatments ($P > 0.10$). The absolute number of cells alive in milk and, among them, the proportions of monocytes and lymphocytes were not influenced by treatments. However, the percentages of macrophage and polymorphonuclear cells tended to be influenced by treatment ($P = 0.051$). The milk from E sows presented greater percentages of macrophage and polymorphonuclear cells than the milk from C sows ($P < 0.05$), CE sows displaying intermediate levels.

3.6. Blood immune cell numbers and response to TLR agonists in one-day-old piglets

At one day of age, piglets from the three treatment groups exhibited similar concentrations of IgG (30 ± 1 g/L, $P > 0.10$), hydroperoxides (175 ± 3 μg Eq H_2O_2/mL, $P > 0.10$), and BAP (1 941 ± 27 μM Fe2+, $P > 0.10$) in plasma. The total numbers of circulating leukocytes (6 094 ± 255 cells/mm^3 blood), and among them, the numbers of polymorphonuclear or macrophage cells (3 977 ± 222 cells/mm^3), monocytes (422 ± 21 cells/mm^3), T lymphocytes (1 394 ± 43 cells/mm^3) and B lymphocytes (2 702 ± 12 cells/mm^3), were not influenced by treatment.

In cultured whole blood cells collected from piglets, regardless of the mitogen in the well, the mRNA levels of IL-1beta, IL-6, IL-10, TLR-2 and TLR-4 were influenced by treatment ($P < 0.05$, Fig. 3). Unstimulated cells from E piglets expressed more IL-1beta, IL-10 and TLR-4 than those from CE piglets ($P < 0.05$), and more TLR-2 and -4 than those from C piglets ($P < 0.05$). In the ODN-stimulated condition, the mRNA expression of IL-6 in cells from E piglets was greater than in cells from CE piglets ($P < 0.05$) and tended to be greater than in cells from C piglets ($P = 0.071$). The expression of TLR-4 was greater in cells from E than C piglets ($P < 0.05$). Apart from TLR-2 expression which tended to be influenced by treatment in the LPS condition ($P = 0.063$), there were no differences in gene expression in LPS-stimulated conditions. The expressions of TGF-beta, IFN-alpha and TLR-9 were never influenced by treatment.

Table 4
Number and mean duration of postural and activity bouts around farrowing in sows housed during gestation in collective conventional pens (C), C pens enriched with manipulable materials and straw pellets (CE), and in larger pens on straw bedding (E).

	Standing [1]	Ventral lying [1]	Lateral lying [1]	Sitting [1]	Resting [1]	Investigation [1]	Lying in contact with piglet[1]	Other activities [1]
24 h before farrowing								
C ($n = 6$)	39	61	46	75	65	44	-	81
	(365)	(394)	(1141)	(144)	(929)	(137)		(319)
CE ($n = 7$)	57	66	42	71	62	33	-	79
	(352)	(479)	(889)	(139)	(949)	(155)		(380)
E ($n = 11$)	69	76	44	68	76	21	-	81
	(274)	(327)	(831)	(113)	(694)	(149)		(454)
0 to 24 h after farrowing								
C ($n = 6$)	7	23	21	17	38	0.8	35	21
	(429)	(841)	(3417)	(112)	(492)	(848)	(1276)	(495)
CE ($n = 7$)	10	26	21	24	34	2.4	30	24
	(419)	(714)	(2739)	(106)	(787)	(818)	(1195)	(776)
E ($n = 10$)	9	30	31	18	47	0.1	45	23
	(343)	(711)	(1947)	(68)	(480)	(756)	(818)	(461)
24 to 48 h after farrowing								
C ($n = 6$)	7	24	19	21	35	3.2	19	21
	(672)	(950)	(3562)	(123)	(1709)	(32)	(662)	(766)
CE ($n = 7$)	7	18	17	16	31	2.9	19	15
	(608)	(652)	(3945)	(134)	(1843)	(121)	(774)	(691)
E ($n = 10$)	11	26	30	17	39	0.2	17	21
	(376)	(636)	(2230)	(76)	(1515)	(5)	(388)	(522)
48 to 72 h after farrowing								
C ($n = 6$)	6	21	18	16	31	0.2	16	14
	(622)	(737)	(3765)	(92)	(2209)	(13)	(397)	(885)
CE ($n = 7$)	7	23	20	18	37	2.4	21	17
	(720)	(1013)	(3006)	(96)	(1833)	(44)	(469)	(731)
E ($n = 10$)	10	24	30	17	42	0.8	17	19
	(445)	(667)	(2123)	(81)	(1500)	(7)	(382)	(686)
SEM[2]	14	15	9	15	12	11.9	10	7
	(89)	(145)	(473)	(23)	(290)	(152)	(174)	(150)
P-value for the number of bouts								
Treatment [2]	0.052	0.574	0.016	0.864	0.112	0.088	0.503	0.647
Period [2]	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Treat. x Period[2]	0.176	0.893	0.255	0.942	0.773	0.029	0.002	0.032
P-value for the duration of bouts								
Treatment [2]	0.036	0.289	0.010	0.052	0.186	0.025	0.272	0.883
Period [2]	< 0.001	< 0.001	< 0.001	0.068	< 0.001	< 0.001	< 0.001	< 0.001
Treat. x Period[2]	0.330	0.194	0.306	0.841	0.853	0.217	0.105	0.993

[1] Bouts of postures and behavioral activities were recorded by continuous focal observations over 24 h periods. Numbers of behavioral bouts over the 24 h periods are presented and the mean durations of these bouts (sec) are indicated in brackets. The considered periods are from 24 h before to the birth of the first piglet, the first 24 h following the birth of the last piglet, and the two following 24 h periods. The activity "lying in contact with piglets" excluded nursing sequences.
[2] SEM: the greatest standard error of the means presented in the raw, and the P-values of the effects of treatment, period and their interaction.

4. Discussion

In the present study, we observed that the two treatment groups with enriched housing conditions for pregnant sows had better newborn survival during the first 12 h post-partum. By using graded levels of enrichment, we observed graded consequences on indicators of maternal well-being during gestation (more expression of investigation behavior, less stereotypies, lower salivary cortisol). Regarding characteristics possibly involved in neonatal survival, in comparison to the treatment with no enrichment (C), the best enriched treatment (E) led to a higher number of successful suckling bouts performed by the sows during the 24 to 72 h post-partum. It was also associated with increased frequency of standing bouts during that period, changes in milk composition, and tended to increase the frequency of polymorphonuclear cells in the milk. Regarding piglet characteristics, the E treatment was associated with a higher frequency of heavy piglets at birth and increased expression of some microbial recognition receptor and inflammatory genes in piglet blood immune cells. Contrary to our expectations, the intermediate enrichment of the CE environment had very few effects on the sow and piglet studied characteristics, and only ash milk content and milk polymorphonuclear cell percentages moved toward the levels observed in the most enriched system.

4.1. Enriching sow environment during gestation improved their welfare and health

At 101 days of gestation, sow activity differed between the three enrichment levels. Regardless of treatment, sows spent a lot of time resting, preferentially in the feeding stall, which may reflect a strong motivation for a physical support and having a protected area, as reported in previous studies [29,30]. In the conventional enriched (CE) environment, sows spent even more time resting in the feeding stall, which could reflect a stronger attraction for this area where straw pellets were provided in the trough at the end of each meal. Sows spent little time standing, less than 30% of their time, but the pen enrichment promoted their activity when compared with the conventional pen supplied with chains only. The enrichment of the environment by increasing the available space is known to increase the time spent in the standing posture and activity [9,31], mainly because of increased exploratory activities [9]. Other enrichments like the provision of a manipulable substrate on the floor also increased the exploratory behavior [32].

In the present study, we actually observed more frequent investigative behavior in the two enriched environments (CE and E) compared to the C system. This is assumed to be positive for sow welfare [9,11]. Nonetheless, the investigative activity of sows towards the manipulable substrates remained significantly lower in the conventional enriched

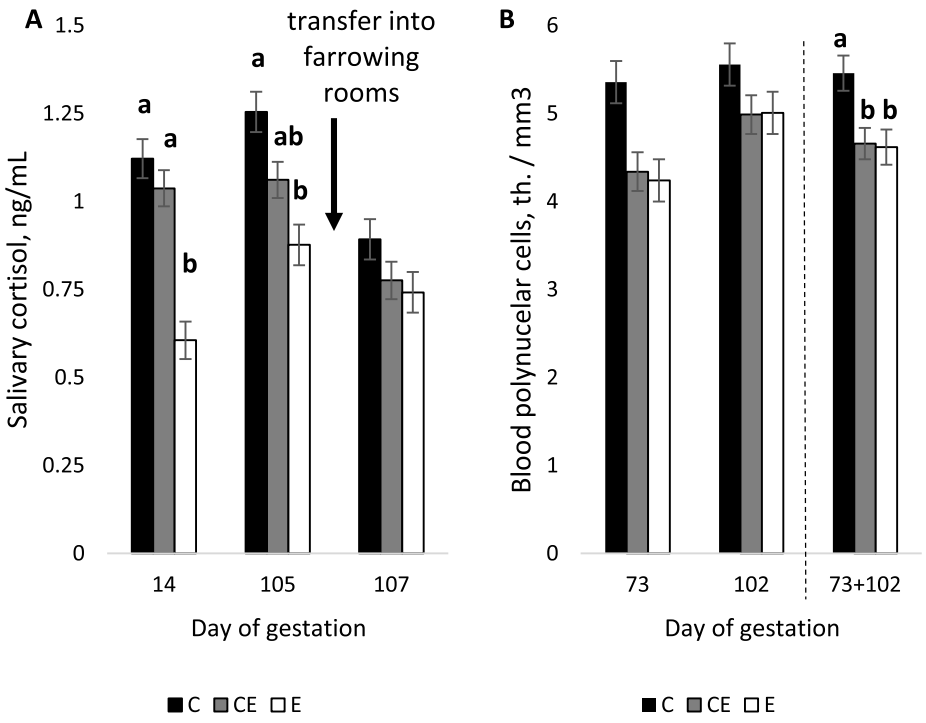

Fig. 2. Salivary cortisol (2A) and blood polymorphonuclear cell numbers (2B) in sows housed from insemination to 105 days of gestation in collective conventional pens (C), C pens enriched with manipulable materials and straw pellets (CE), and in larger pens on straw bedding (E). Means (± SEM) with different superscript letters (a, b) are statistically different ($P < 0.05$). For blood polynuclear cell numbers, the least-square means pooled for gestation days 73 and 102 are represented to illustrate the significant effect of treatment, while no day x treatment interaction was observed.

Table 5
Blood indicators of immune activation, inflammation and oxidative stress at 73 and 102 days of gestation (DG) in sows housed during gestation in collective conventional pens (C), in C pens enriched with manipulable materials and straw pellets (CE), and in larger pens on straw bedding (E).

	DG 73			DG 102			SEM[1]	P-value[1]		
	C	CE	E	C	CE	E		T	D	T x D
Number of sows	24	29	25	25	29	24				
Lymphocytes[2]	5.93	6.10	6.34	5.50	5.21	5.55	0.26	0.61	< 0.001	0.40
PMN cells[2]	5.36	4.34	4.24	5.56	4.99	5.01	0.23	< 0.01	< 0.001	0.31
Monocytes[2]	0.43	0.38	0.38	0.43	0.38	0.42	0.02	0.09	0.22	0.20
Haptoglobin[3]	1.49	1.29	1.13	1.54	1.50	1.38	0.11	0.17	< 0.01	0.48
Hydroperoxides[3]	631	719	632	543	600	566	29	0.13	< 0.001	0.55
BAP[3]	2348	2377	2362	2333	2316	2340	16	0.86	< 0.01	0.11

[1] SEM: greatest standard error of the least-square means, and P-values for the effects of treatment (T), gestation day (D), and their interaction.
[2] Blood lymphocyte, polymorphonuclear (PMN) cell, and monocyte numbers (thousand cells / mm^3).
[3] Plasma haptoglobin: mg/mL, hydroperoxides: µg Eq H_2O_2/mL, Blood Antioxidant Potential (BAP): µM Fe^{2+}.

(CE) pens than in the straw-enriched (E) environment, reflecting a strong preference for straw bedding over other forms of enrichment proposed to the sows. Straw bedding may be highly attractive because it allows rooting behavior, which appears to be a high priority behavior in pigs. It fulfills the needs of the sows to explore their surroundings by rooting, sniffing, biting and chewing both nutritive and indigestible items [33]. But the provision of straw bedding also satisfies the feeding motivation of sows that are submitted to feed restriction during gestation [34,35]. It is likely that feeding sows with straw pellets contributed to reduce hunger. The analysis of the use of the different substrates confirmed a preference for investigation towards the pen facilities rather than towards the chains, a material of only marginal interest according to the European recommendations for the housing enrichment (EU recommendation 2016/336 of 8 March 2016).

The occurrence of aggressive interactions was not affected by the enrichment, while the frequency of positive interactions was greater in the E treatment compared to CE and C groups. These effects must be considered cautiously because social interactions were scarce in this study. The literature indicates that the frequency of aggressive interactions between familiar sows was not influenced or tended to decrease with bigger space [7,9,10], probably because a less obstructed space favors more normal movements and social interactions, including a more efficient avoidance behavior [9]. The effect of enrichment on non-aggressive social behaviors has not been investigated so far in other studies but it could probably be interpreted as beneficial for E sow welfare [36].

Moreover, stereotypies, which usually highlight a signal of frustration [37], were less frequently observed in the two enriched environments than in the conventional pens. The enrichment of the environment has been shown to reduce stereotypies [38]. This was observed here, when providing manipulable objects and straw pellets, and even more significantly, when straw litter and an increased area were combined to enrich the environment. Effects on stereotypies were consistent with differences in the concentrations of cortisol at 105 days gestation, which were lower in sows housed in the more enriched pens (E), as previously reported [22,23], but intermediate in the sows housed in the conventional enriched pens (CE). Together, these results showed that enriching the conventional pens with wood material and straw pellets improved sow welfare, at least during late gestation, although the level of welfare was lower than that observed in the most enriched environment.

When sows moved to the maternity rooms, cortisol levels of C sows fell down to concentrations comparable to E and CE sows, indicating that the greater cortisol concentrations in C sows were directly related to

Table 6
Milk composition on the day after farrowing from sows housed in collective conventional pens (C), in C pens enriched with manipulable materials and straw pellets (CE), and in larger pens on straw bedding (E).

	Treatment[1]			SEM[1]	P-value[1]
	C	CE	E		
Number of sows	26	28-27	24-17		
Dry matter, g /100 g whole milk	21.0[a]	20.9[a]	19.5[b]	0.5	0.055
Ash, g /100 g whole milk	0.66[a]	0.71[b]	0.70[b]	0.1	0.013
Protein, g /100 g whole milk	8.1	7.9	7.8	0.4	0.842
Fat, g /100 g whole milk	8.8[a]	8.6[ax]	7.5[by]	0.5	0.079
Lactose, g/100 g whole milk	4.0	4.0	3.9	0.2	0.667
Energy, kJ/g whole milk	5.8[a]	5.8[ax]	5.4[by]	0.2	0.089
IgA, mg/mL	8.5	8.0	8.0	0.9	0.890
Total living cells, thousands / mL	65	65	67	22	0.992
% CD172a$^+$ PMN / macrophages[2]	54[a]	62[ab]	68[b]	4	0.051
% CD172a$^+$ monocytes[2]	0.9	0.9	1.1	0.1	0.541
% CD172a$^-$ lymphocytes and NK cells[2]	15	10	7	4	0.117

[1] SEM: greatest standard error of the least-squares means, and P values for the treatment effect are presented.
[2] Expressed in percent of total living cells / mL milk. PMN: polymorphonuclear cells (mainly neutrophils). NK: natural killer cells. Means with different superscript letters (a, b) are statistically different ($P < 0.05$) and means with letters x, y tend to differ ($0.5 < P < 0.10$).

the environment where they were kept during gestation. The stability in the concentration of salivary cortisol of sows around the transfer to farrowing crates has already been observed by us in published [23].and unpublished studies. The lack of change in this stress indicator despite restraining sows in isolated farrowing crates may be related to the fact that the crates physically separate the sows from each other at a physiological stage when they need to be socially isolated. Indeed, in natural conditions, sows isolate themselves from the group a few days before the onset of parturition to seek for a suitable nest site [39]. Although we are not aware of other studies reporting the cortisol response at the time of entering the farrowing crate, it has been shown that a few days later, at farrowing and at initiation of lactation, salivary cortisol is similar [40] or is even lower [41] in confined sows compared to loose sows. Cortisol concentrations, that can also be influenced by physical activity [42], might not be easily interpreted at this specific time point, when stress and physical activity vary at the same time.

Global indicators of health and immune activation such as acute phase proteins can be used to assess stress [43]. In the present study, the concentration of haptoglobin, an inflammatory protein, or systemic oxidative stress, which increase in pro-inflammatory situations [28,44,45], were not significantly different between C and E sows. In our previous experiment, C sows presented a higher concentration of hydroperoxide products in the blood at the end of gestation [23], that we interpreted as an indicator of higher inflammatory status due to a greater stress level. The present results indicated that the beneficial effect of environmental enrichment of the inflammatory status of sows might be fluctuating depending on uncontrolled environmental conditions (for example the season, or specific characteristics of the batch of animals).

Blood of E sows exhibited lower polymorphonuclear cell counts at 72 and 102 days of gestation, a difference already reported when comparing these two environments, and can be interpreted as an indicator of improved well-being [22,23]. Indeed, the number of blood polymorphonuclear cells can rise during microbial infections [46], or in response to a dirty sanitary housing conditions [47]. However, high blood polymorphonuclear cell numbers or percentages were also observed in situations of chronic stress, as a result of the redistribution of immune cells among blood, lymphoid and mucosal organs induced in response to cortisol and catecholamine release [48]. For example, such an increase has been observed in blood of sows exposed to long lasting stressful situations due to low space [49] or repeated social stress [50]. In growing pigs, this has been also observed in animals housed in a poor environment on slatted floor compared with those living on deep litter with access to an outdoor run [51]. In the present experiment, CE and E sows displayed comparable numbers of circulating polymorphonuclear cells. Together with cortisol and behavioral data, this confirms that the enrichment provided by straw pellets and wooden pieces in a conventional housing system had a beneficial effect on the welfare of sows.

4.2. Consequences of enriching sow environment during gestation on sow behavior during the peripartum period and on milk nutritional composition

The differences in environmental conditions during gestation had an effect on piglet survival. Indeed, the very early mortality within the first 12 h after farrowing was lower in E and CE litters compared with C litters. In our previous study, the difference in mortality rate between C and E sows was spread over a larger period, up to 72 h post-partum [23]. It is unlikely that this difference was the result of more difficult births in the C group, since the duration of farrowing and behavior of sows during farrowing were similar in the three groups of sows. During the peripartum period (- 24 to + 72 h after farrowing), E sows tended to be more active than other sows, showing more postural changes (more bouts of ventral lying and standing postures). Altered maternal behavior and postural time budget have been reported in other studies involving maternal stress during gestation, but theses alterations were not related to the frequency of postural changes [52]. Increased restlessness and lying down occurrences of the sows are expected to increase the risk of crushing piglets [53], but this was not observed here. After farrowing, E sows displayed more bouts of lying in contact with the piglets and when structured nursing sequences developed 24 h after farrowing, they performed more full nursing sequences (leading to milk ejection) than C sows. This is in agreement with the literature, indicating that maternal stress during gestation deteriorates maternal behavior, by decreasing social interactions with the piglets and frequency of postures favoring lactation [52]. These behavioral effects were not observed in CE sows, meaning that the lower piglet mortality during the first 12 h after birth in the CE and E litters could not be explained only by the differences in the behavior of sows around farrowing, and that other causes are at play.

Sow environment during pregnancy was associated with changes in fat and mineral contents of the milk produced in early lactation. The mechanism leading to the trend for less fat in milk of E sows than in the two other groups is unclear. The fat content in mammary secretions is influenced by diet, notably fat and fiber content, and mobilization of body lipids [54,55]. Here, sows were fed the same diets at the same level and had no access to straw since day 105 of gestation. Moreover, sows of the three groups did not differ for their body condition (weight and backfat thickness) at the end of gestation. Body lipid mobilization during the last 10 days of gestation cannot be estimated in the present study. Anyway, this reduction in fat and thus in energy content of milk produced one day after parturition should have been unfavorable to piglets survival [56], which was not the case in the present study. The greater content of minerals in milk of CE and E sows likely originated from an extra supply of minerals resulting from straw ingestion by the sows [24]. It would be interesting to investigate if the consumption of straw increases the concentrations of specific microelements in colostrum with potential beneficial impacts on health or survival of newborn piglets.

4.3. Consequences on piglet birth weight and immune protection

The improvement of piglet survival in the E and CE treatments was not related to the average birth weight of these piglets, which is in agreement with other studies on prenatal stress in pigs [19]. In our previous study, we observed a decreased frequency of very low birth weight piglets and suggested a better physiological maturity of E piglets compared with C piglets [57], which could confer them an advantage in

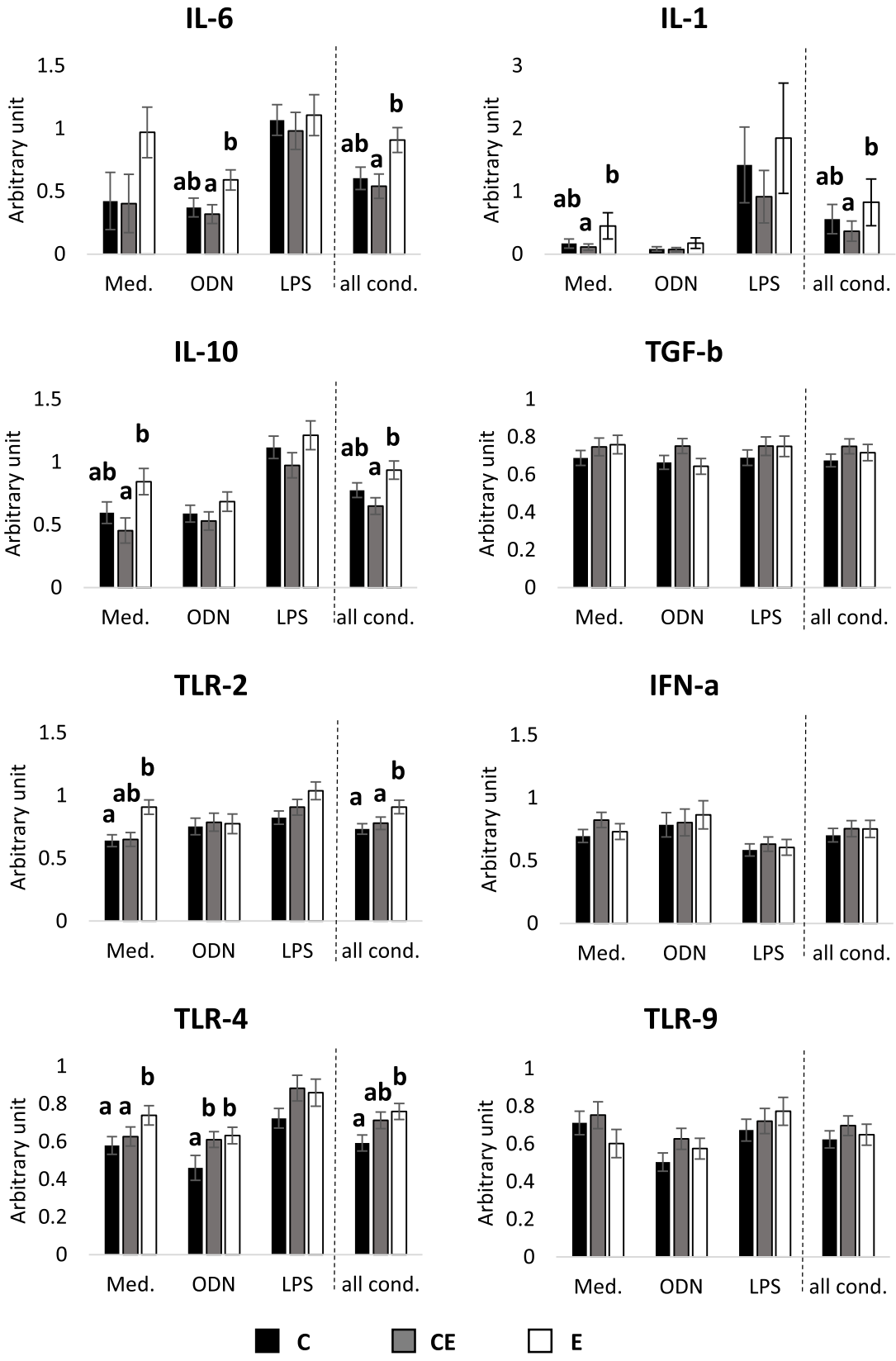

Fig. 3. Gene mRNA expression in whole blood cell cultures from one-day-old piglets born from sows housed from insemination to 105 days of gestation in collective conventional pens (C), in C pens enriched with manipulable materials and straw pellets (CE), and in larger pens on straw bedding (E). Mean expressions (± SEM) are presented for cells cultivated for 20 h in medium alone (Med.), with a CpG oligonucleotide (ODN) and lipopolysaccharide (LPS), and for the expression of all culture conditions pooled. Means with different letters (a, b) are statistically different ($P < 0.05$).

neonatal survival. This was not confirmed in the present study, however E sows produced higher numbers of heavy piglets than C and CE sows, which may also have a beneficial effect on neonatal survival [58]. We tested whether the differences in neonatal survival observed between treatments could be also related to differences in the immune protection of the neonates. The immune defense of neonatal piglets partly relies on the transfer of immune protection from their mother via lacteal secretions, that contain immunoglobulins, live immune cells, cytokines and anti-microbial peptides [59]. We found that IgG concentration in the blood of one-day old piglets was not influenced by treatment, indicating no effect of prenatal stress on the transfer of colostrum IgG to piglet bloodstream during the first hours after birth. The concentration of IgA in milk on day after birth, which contributes to protect the lumen after gut closure, was not influenced either. This confirms our previous results [23], as well as those from studies using factors of stress such as adrenocorticotropic hormone (ACTH) administration [60], repeated social stress [61] or poor rearing environment [23] showing no effect of prenatal stress on the transfer of colostrum IgG into the blood of piglets.

In contrast, we observed that mammary secretions of stressed C sows tended to have a lower proportion of polymorphonuclear cells / macrophages than that of E sows 24 h after farrowing. We previously reported a similar difference between the colostrum of C and E sows, that disappeared in milk four days later [23]. Therefore, the lower proportion of polymorphonuclear cells might be specific to the colostrum of stressed sows and progressively disappear when mammary secretion switches from colostrum to milk. The intermediate polymorphonuclear cell count in CE sows, who showed an intermediate welfare level between C and E sows, suggests a direct neuroendocrine effect of stress on the transfer of these cells through the mammary epithelium. Polymorphonuclear cells and macrophages present in the milk are responsible for the production of many cytokines, reactive oxygen species, and antimicrobial peptides, that could contribute to neonatal gut protection [62]. Thus, a greater number of these cells in the milk of E sows might be advantageous for piglet protection.

Besides passive immunity, prenatal stress is known to directly influence the immune development of the offspring. For example, lower numbers of lymphocyte and polymorphonuclear cell were reported in the blood of newborn prenatally-stressed piglets [61]. However, this effect on blood cell numbers was not systematically reported in prenatal stress studies in pigs [15,20], and was not observed either in the present study. The immune response of neonates mainly relies on the innate immune system, that recognizes microbes using specific receptors, among which the toll-like receptors (TLRs). We investigated the level of expression of three TLRs specialized in the recognition of Gram positive bacteria (TLR-2), Gram negative bacteria (TLR-4), and DNA-viruses (TLR-9) [63]. We previously reported that prenatal stress may decrease the *in vitro* inflammatory response of pig immune cells to lipopolysaccharides (LPS), a molecule of the membrane of Gram-negative bacteria and a TLR-4 agonist [61]. Apart from a study in calves, showing that heat stress of the cow during gestation altered the level of expression of TLR-2 and TLR-4 in blood immune cells of the neonates [64], the effect of prenatal stress on TLRs had not been investigated before. We found that the expressions of TLR-9 and interferon-alpha, which is secreted in response to TLR-9 stimulation, were not influenced by treatment, nor was the production of Tumor Growth Factor-beta (TGF-β), a cytokine playing a major role in the down-regulation of immune responses. The prenatal maternal enrichment seemed to have specifically increased the expression of TLR-2 and 4, and of the pro- and anti-inflammatory cytokines resulting from the activation of these receptors (IL-1, IL-6, and IL-10). This effect was observed in cells cultivated in medium alone, but was erased under stimulation of TLR-4 by the addition of LPS. We cannot say whether these immune differences had a role on neonatal survival, since these differences were observed at one day of age, after the window of time when the differences in neonatal mortality between treatments were observed.

Besides a direct prenatal effect, the immune differences in piglets might be driven by an indirect effect through microbiota. Indeed, microbiota contributes to the early postnatal innate immune development [65]. It can be modified in situations of maternal stress during gestation, as shown in other species [66,67]. In the present study, microbiota could have been influenced by the presence of deep straw, since housing on deep straw (E) or incorporation of a high level of fibers in the diet (CE) during gestation influenced the sow microbiota in feces or even in colostrum [68–70], and both maternal milk and fecal flora guide the development of neonate microbiota [71]. However, the gene mRNA expression in blood cells from CE piglets, whose mothers consumed straw pellets, was more often close to the level of expression observed in the cells from C piglets. Since C and CE animals were housed together in the same rooms, while E piglets were reared in an adjacent independent unit, the immune differences between C/CE pigs and E pigs might reflect the influence of other environmental characteristics (dust, local temperature, microbes, etc) than straw enrichment.

5. Conclusion

In conclusion, housing sows in an enriched environment during their gestation provided promising results on their immediate welfare. Subsequently, it improved the survival rate of newborns during the critical window of the first 12 h of extra-uterine life. This result might be obtained by the moderate but cumulative positive effects observed on some aspects of sow behavior (more nursing sequences, more contact with the piglets), milk composition (mineral content, macrophages and polymorphonuclear cell numbers), and on offspring physiology (innate immune response). Positive effects were also observed in the intermediate enrichment group on early piglet mortality and on some maternal factors potentially favourable to piglet survival (mineral content and macrophages and polymorphonuclear cell numbers in the milk), which reinforced the hypothesis of a causal relationship between maternal environmental enrichment and neonate survival.

Ethics approval

The experimental protocol was approved by the local Ethics Committee in Animal Experiment of Rennes, France, and by the French Ministry of Higher Education and Research, according to the EU Directive 2010/63/EU for animal experiments.

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Declaration of Competing Interest

None.

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