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Oxytocin injections in the postpartal period affect mammary tight junctions in sows[1]

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ABSTRACT: The potential impacts of injecting oxytocin (OXY) to sows in the early postpartum period on the quality of mammary tight junctions, milk composition, and immune status of sows and piglets were studied. Postparturient sows received i.m. injections of either saline (control [CTL]; $n = 10$) or 75 IU of OXY ($n = 10$). Injections were given twice daily (0800 and 1630 h) starting on d 2 of lactation (i.e., between 12 and 20 h after birth of the last piglet), totaling 4 injections. Milk samples were obtained before the first injection (d 2 morning [AM]), before the second injection (d 2 afternoon [PM]), and on d 4 PM and d 5 PM. Blood samples were obtained from sows before milking on d 2 AM, d 2 PM, and d 5 PM. On d 5 of lactation, a blood sample was obtained from 3 piglets per litter. Circulating concentrations of prolactin, IGF-I, lactose, and IgA in sows did not differ between treatments at any time ($P > 0.10$), but OXY sows had less IgG than CTL sows ($P < 0.01$) on d 2 PM before the second OXY injection. There were differences in milk composition on d 2 PM, with OXY sows having more IGF-I ($P < 0.01$), solids ($P < 0.05$), protein ($P < 0.01$), energy ($P < 0.05$), and IgA ($P < 0.01$) and a greater Na:K ratio ($P < 0.01$) than CTL sows. These differences were not seen in the next 2 milk samples, except for protein and IgA that still tended ($P < 0.10$) to be greater in OXY vs. CTL sows on d 4 PM (for protein) and on d 5 PM (for IgA) after the last injection. Milk lactose content was lower in OXY vs. CTL sows on d 5 PM ($P < 0.01$). Values for immunoglobulin immunocrit, IgG, IgA, and IGF-I in piglet blood did not differ between treatments ($P > 0.10$). Injecting OXY to sows in the early postpartum period increased leakiness of the mammary tight junctions, improved composition of early milk, and may potentially affect immune status of neonatal piglets.

Key words: mammary gland, oxytocin, postpartum, sow, tight junctions

INTRODUCTION

Newborn piglets rely solely on colostrum as a source of energy for thermoregulation and body growth (Herpin et al., 2005; Le Dividich et al., 2005; Quesnel et al., 2012) and as a source of passive immunity for protection against pathogens (Rooke and Bland, 2002). Colostral cells can also influence the innate and specific immune responses of neonatal piglets (Bandrick et al., 2014). Colostrum is, therefore, essential for piglet survival (review by Le Dividich et al., 2005; Devillers et al., 2011; Declerck et al., 2016), and also contains components with potentially beneficial effects, such as growth factors that stimulate intestinal growth and maturation (Xu, 2003). Through bioactive components, colostrum participates in maternal lactocrine programming of postnatal development, with potential long-term impacts (Bartol et al., 2017). The composition of colostrum is affected by the status of tight junctions between mammary epithelial cells (Nguyen and Neville, 1998), and it changes as lactation advances. Colostrum contains more proteins (mainly immunoglobulins), less lipid, and less lactose than transient or mature milk (Theil et al., 2014). Providing as little as 15 mL of supplementary colostrum to small piglets within 4 h of farrowing improves their IgG blood concentrations and reduces mortality (Muns et al., 2014). The ability to

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manipulate mammary tight junctions in the late colostral phase may allow immunoglobulin concentrations to be maintained at higher levels for a longer period. Indeed, although IgG and IgA are transferred into colostrum via receptor-mediated intracellular routes (Salmon et al., 2009), they also enter colostrum, in part, via the paracellular route (Delouis et al., 2001). High doses of oxytocin (**OXY**) can alter the permeability of mammary tight junctions in goats (Linzell and Peaker, 1974), rodents (Nguyen and Neville, 1998), and cattle (Jonsson et al., 2013), leading to alterations in the composition of lacteal secretions. It is expected that OXY holds the same function on tight junctions in swine. The goal of the present study was to establish whether injections of OXY in the early postpartum period can prolong the colostral phase in sows through alteration of the mammary tight junctions, hence improving the quality of lacteal secretions and the immune status of neonatal piglets.

MATERIALS AND METHODS

Animals were cared for according to a recommended code of practice (Agriculture and Agri-Food Canada, 1993), and procedures were approved by the institutional animal care committee.

Sows and Treatments

Second-parity sows (Yorkshire × Landrace) were bred with semen from a pool of Duroc boars and allowed to farrow. Farrowings were induced in all sows with 1 mL (87.5 µg) of a prostaglandin analog (cloprostenol; Merck Santé Animale, Intervet Canada Corp., Kirkland, Québec, Canada) in the perivulvar region on the afternoon of d 114 of gestation, and sows were not given any OXY during parturition. Only 20 sows that finished farrowing between 1200 and 2000 h were used in the study, and this was considered as d 1 of lactation. They were divided into 2 treatment groups whereby they either received i.m. saline injections in the neck (controls [**CTL**], $n = 10$) or injections of 75 IU (3.75 mL) of OXY ($n = 10$) in the postparturient period. Injections were given twice daily (0800 and 1630 h) starting on d 2 of lactation (i.e., between 12 and 20 h after birth of the last piglet), totaling 4 injections. The interval between the end of farrowing and the first OXY injection was similar for both treatment groups ($P > 0.10$) and averaged 16.0 ± 2.4 h. Litters were standardized to 11 ± 1 piglets on d 2 of lactation at 1630 h by cross-fostering within treatment. Piglets were weighed twice on d 2 of lactation (0705 and 1525 h), on d 7, 14, and 21 of lactation (1330 h), and on d 35 postpartum. No creep feed was distributed to pigs until weaning (d 22), and they had no access to sow feed. Preweaning mortalities of live-born piglets were recorded.

During gestation, sows were fed a commercial corn-soy diet containing 13.3% CP, 13,690 kJ/kg DE, and 0.56% lysine according to their body condition at mating. Sows received 1 meal daily of 3.1, 2.9, 2.7, or 2.6 kg for backfat thicknesses of 9 to 11, 12 to 14, 15 to 17 m, and >18 mm, respectively. As of d 100 of gestation, all sows received an extra 1 kg of feed. During lactation, sows were fed a 19.7% CP commercial corn-soy diet (14,736 kJ/kg DE and 1.00% lysine) in 2 meals at 0800 and 1500 h daily. Sows received 1.6 kg of this diet on the day of farrowing, and from d 2 to weaning this same diet was fed ad libitum. Refusals were weighed daily to obtain feed intakes. Sows were weighed and had their backfat thicknesses measured ultrasonically at P2 of the last rib (WED-3000; Shenzhen WELLD Medical Electronics Co., Ltd., Shenzhen, China) at 1300 h on d 110 of gestation and on d 3 and 21 of lactation. Sows were housed in individual stalls (0.6 by 2.1 m) during gestation and were transferred to farrowing crates on d 110 of gestation. At weaning, litters were transferred to 1.9 by 1.9-m pens and pigs were fed, consecutively, 3 commercial diets ad libitum. The first diet, containing 18.5% CP, 15,598 kJ/kg DE, and 1.4% of total lysine, was fed to piglets until they all had received an average of 1.5 kg. The second diet, containing 18.6% CP, 16,464 kJ/kg DE, and 1.3% of total lysine, was fed to piglets until they all had received an average of 5.0 kg. The last diet, containing 20.6% CP, 14,410 kJ/kg DE, and 1.21% of total lysine, was fed to piglets until d 35 of age.

Blood and Milk Sampling

On d 80 of gestation, all sows were injected i.m. with a 1-mL solution containing 2 mg of ovalbumin (**OVA**; Sigma, Oakville, ON, Canada) diluted in PBS and emulsified with an equal part of incomplete Freund's adjuvant (Sigma). A second injection of a solution of 1 mg of OVA mixed with incomplete Freund's adjuvant was given on d 95 of gestation. Jugular blood samples were collected from sows (10 mL) on d 80 (before injection of OVA) and 110 of gestation to measure concentrations of IgG and antibody titers against OVA. Representative milk samples were obtained twice on d 2 (0735 and 1600 h) and once on d 4 and 5 (1600 h) of lactation by hand milking. They were collected from 3 functional glands (anterior, middle, and posterior) that were emptied following an i.v. injection of 1.0 mL of OXY (20 IU/mL; P.V.U., Victoriaville, QC, Canada) and were then pooled. Pigs were separated from their dam for 35 min before OXY was injected. Standard milk composition was determined (solids, fat, protein, lactose) as well as concentrations of IgA, IgG, Na, K, and IGF-I. Jugular blood samples were collected from sows (40 mL) on d 2 of lactation just prior to the morning and the afternoon milkings and on d 5

prior to the afternoon milking to measure concentrations of IgA, IgG, IGF-I, prolactin, lactose, and antibody titers against OVA. Jugular blood samples were also obtained from 3 piglets (10 mL) of average BW within litter on d 5 of lactation to determine the immunocrit value as well as concentrations of IgA, IgG, IGF-I, and antibody titers against OVA. The experiment was performed from November 2014 to July 2015.

Assays in Blood

Blood samples for prolactin, IgG, IgA, and antibody titer against OVA (20 mL) were collected into Vacutainer tubes without anticoagulant (Becton Dickinson, Franklin Lakes, NJ) and were left at room temperature for 3 h, stored overnight at 4°C, centrifuged for 12 min at 1,800 × g at 4°C the following day, and serum was then harvested. Samples for IGF-I and lactose measurements (20 mL) were collected into EDTA tubes (Becton Dickinson) and were put on ice and centrifuged within 20 min for 12 min at 1,800 × g 4°C, and plasma was immediately recovered. Serum and plasma samples were frozen at −20°C until assayed. Immunoglobulin immunocrit values were determined in triplicate serum samples using the technique described by Vallet et al. (2013). Intra- and interassay CV were 0.96 and 0.62%, respectively. A previously described RIA was used to determine concentrations of prolactin (Robert et al., 1989). The radioinert prolactin and the first antibody to porcine prolactin were purchased from A. F. Parlow (U.S. National Hormone and Peptide Program, Harbor UCLA Medical Centre, Torrance, CA). Parallelism of a serum pool from lactating sows was 101.9%. Average recovery calculated by addition of various doses of radioinert prolactin to 50 μL of a pooled sample was 100.0%. Sensitivity of the assay was 1.5 ng/mL. The intra- and interassay CV were 3.84 and 2.75%, respectively. Concentrations of IGF-I were measured with a commercial RIA kit for humans (Alpco, Salem, NH) with small modifications as detailed previously (Plante et al., 2011). Validation for a plasma pool from lactating sows was demonstrated, with parallelism being 101.2% and average mass recovery being 101.3%. Sensitivity of the assay was 0.10 ng/mL. For sow plasma, intra- and interassay CV were 4.27 and 0.69%, respectively, and for piglet plasma intra- and interassay CV were 5.05 and 2.94%, respectively. Lactose, providing a measure of tight junction integrity, was measured by enzymatic analysis using a validated commercial kit (Megazyme Int., Wicklow, Ireland). Intra- and interassay CV were 0.62 and 3.48%, respectively. Concentrations of IgG and IgA in serum were measured by ELISA. The following commercial kits were used in accordance with manufacturer recommendations without any modifications: porcine IgG (Bethyl

Laboratories Inc., Montgomery, TX) and porcine IgA (Bethyl Laboratories Inc.). All samples were tested in duplicate. Parallelism and average mass recovery were, respectively, 99.2 and 90.9% for IgG and 97.8 and 97.3% for IgA. Intra- and interassay CV were, respectively, 4.07 and 6.90% for IgG and 5.64 and 4.53% for IgA. Antibody responses against OVA were measured by indirect ELISA and quantified by optical density measurements as described previously (Lessard et al., 2005). All samples and controls were tested in duplicate. Intra- and interassay CV were 5.40 and 4.14%, respectively.

Assays in Milk

Whole milk was analyzed for dry matter (solids), protein, fat, lactose, gross energy, Na, K, and IgA contents. Dry matter was measured according to AOAC (2005) Method 947. Gross energy was measured using an adiabatic bomb calorimeter (C 5000; IKA, Staufen, Germany). Nitrogen was determined according to the method of Dumas (Method 7024; AOAC, 2005) based on sample pyrolysis and direct determination of N_2 using LECO FP-528 (Leco, St Joseph, MI. Crude protein was estimated to be N × 6.38 (Gordon and Whittier, 1965). Total lipids were measured using a Soxtec apparatus (Avanti 2050; Foss France, Nanterre, France). Lactose was assayed using an enzymatic method (Enzytec E1213, Lactose/d-galactose test combination; R-Biopharm, Darmstadt, Germany). The Na and K contents were also determined in milk using an atomic absorption spectrometer (SpectraAA-220 FS model; Varian, Les Ulis, France). The Na:K ratio in colostrum, which is known to be inversely correlated with mammary epithelium integrity during lactation (Sørensen et al., 2001), was used to evaluate the mammary epithelium permeability. Concentrations of IgA were determined with the commercial ELISA kit described previously for serum and validated in milk. Parallelism was 97.8% and average mass recovery was 97.3%. The intra- and interassay CV were 5.78 and 5.14%, respectively.

Concentrations of IgG, antibody titer against OVA, and IGF-I were measured in lactoserum obtained by centrifuging fresh milk twice for 60 min at 51,500 × g at 4°C and harvesting the middle phase. The same commercial ELISA kits described for serum samples were used for IgG after validation in lactoserum. Parallelism and average mass recovery were 101.0 and 95.9%, respectively. Intra- and interassay CV were, respectively, 4.71 and 4.27% for IgG. Antibody titer against OVA and concentrations of IGF-I were measured as described for serum or plasma. The commercial ELISA kit for IGF-I (Alpco) was validated for lactoserum, with parallelism being 101.1% and average mass recovery being 96.8%. The intra- and interassay CV were 1.29 and 2.28%, re-

spectively. The intra- and interassay CV for antibody titer against OVA were 3.88 and 4.71%, respectively.

Statistical Analyses

The MIXED procedure of SAS (Release 9.4; SAS Inst. Inc., Cary, NC) was used for statistical analyses. The 1-way factorial design used for blood variables in piglets (average per sow) included the fixed effect of treatment tested with the residual error. Repeated measures ANOVA with the fixed effect of treatment (the error term being sow within treatment) and day (the residual error being the error term) as well as the treatment × day interaction were performed on average weekly sow feed intake during lactation. Separate ANOVA for each sampling time or BW time were performed on all other variables. A logistic analysis (using odds ratio) was used to look at treatment effect on mortality rate. Data in text and tables are presented as least squares means ± SEM unless stated otherwise.

RESULTS

There were no differences in BW or backfat thickness of sows due to treatments at any of the measurement days ($P > 0.10$) nor were there differences in losses of BW or backfat during lactation (data not shown; $P > 0.10$). On d 3 of lactation, CTL and OXY sows weighed 259 and 259 ± 3.8 kg, respectively, and had backfats of 20.0 and 19.5 ± 1.2 mm. On d 21 of lactation, CTL and OXY sows weighed 237 and 238 ± 4.1 kg, respectively, and had backfats of 15.5 and 15.3 ± 1.0 mm. Sow feed intake during lactation was not affected by treatment (data not shown; $P > 0.10$) but increased with advancing weeks of lactation (data not shown; $P < 0.01$). Average feed intake on wk 1 of lactation was 4.68 and 4.12 ± 0.32 kg for CTL and OXY sows, respectively, and on wk 3 was 7.10 and 7.69 ± 0.33 kg, respectively. There was a treatment × week interaction ($P = 0.05$) showing a greater increase in feed intake for OXY than for CTL sows. The number of live piglets in OXY and CTL litters, respectively, was 15.9 and 16.4 ± 1.05 at birth and 13.3 and 13.2 ± 0.4 on d 2 in the morning (**AM**), and it did not differ between treatments ($P > 0.10$). Piglet BW as well as BW gain between 0705 and 1525 h on d 2 were not affected by treatment (Table 1; $P > 0.10$). However, there was a tendency ($P < 0.10$) for the incidence of preweaning mortalities to be greater in CTL than in OXY sows, with means (±SD) of 6.1 ± 5.1% and 2.3 ± 3.8%, respectively.

During gestation, all sows showed a drastic increase in antibody titers against OVA between d 80 ($7.32 \times 10^3 \pm 1.23 \times 10^3$ arbitrary units) and 110 ($691.12 \times 10^3 \pm 107.77 \times 10^3$ arbitrary units; $P < 0.01$),

Table 1. Average BW of piglets born from sows that received 4 injections of saline (control [CTL]; $n = 10$) or oxytocin (OXY; $n = 10$) in early lactation. The first injection was given 12 to 20 h after birth of the last piglet (d 2) and the others twice daily at 0800 and 1630 h

Item[1]	Treatment		
	CTL	OXY	SEM
BW, kg			
Birth	1.48	1.58	0.07
d 2 AM	1.55	1.67	0.05
d 2 PM	1.56	1.68	0.06
BW gain on d 2	0.007	0.013	0.008
d 7	2.69	2.86	0.09
d 14	5.00	5.27	0.16
d 21	7.37	7.63	0.22
d 35	11.31	11.52	0.34

[1]AM, morning; PM, afternoon.

and there was no change due to sampling time during lactation (Table 2; $P > 0.10$). Concentrations of IgG in sow blood decreased between d 80 and 110 of gestation (31.89 ± 1.67 mg/mL and 22.47 ± 1.13 mg/mL, respectively; $P < 0.01$). Concentrations of measured variables in sow blood on d 2 AM, d 2 in the afternoon (**PM**), and d 5 of lactation are shown in Table 2. There were no treatment effects ($P > 0.10$) on concentrations of prolactin, IGF-I, lactose, IgA, or on antibody titers against OVA, but IgG concentrations were 18% lower in OXY than CTL sows on d 2 PM ($P < 0.01$).

Milk composition on d 2 AM, d 2 PM, d 4 PM, and d 5 PM is shown in Table 3. On d 2 PM, milk from OXY sows contained 17% more energy ($P < 0.05$), 14% more solids ($P < 0.05$), 29% more protein ($P < 0.01$), 110% more IGF-I ($P < 0.01$), 51% more IgA ($P < 0.01$), 184% more IgG ($P < 0.01$), 264% greater antibody titer against OVA ($P < 0.01$), and had a 71% greater Na:K ratio ($P < 0.01$) than milk from CTL sows. Some of these effects lasted longer with the percentage of protein in milk tending to be greater on d 4 PM and IgA tending to be greater on d 5 PM in milk from OXY vs. CTL sows ($P < 0.10$). The effect on lactose was seen on d 5 PM with milk from OXY sows containing 15% less lactose than that from CTL sows ($P < 0.01$).

Concentrations of measured variables in blood from piglets on d 5 of lactation are shown in Table 4. There was no effect of treatment on immunoglobulin immunocrit, IGF-I, IgG, IgA, and antibody titer against OVA ($P > 0.10$).

DISCUSSION

Current findings demonstrate that 1 OXY injection given to sows in the early postpartum period increased the permeability of mammary tight junctions as indicated by the greater Na:K ratio in milk

Table 2. Hormone, lactose, and immunoglobulin concentrations in sows that received 4 injections of saline (control [CTL]; $n = 10$) or oxytocin (OXY; $n = 10$) in early lactation. The first injection was given 12 to 20 h after birth of the last piglet (d 2) and the others twice daily at 0800 and 1630 h

Item[1]	Treatment		SEM
	CTL	OXY	
Prolactin, ng/mL			
d 2 AM	31.4	25.6	2.6
d 2 PM	34.1	34.8	2.2
d 5 PM	27.8	26.1	1.6
IGF-I, ng/mL			
d 2 AM	87.4	84.4	5.1
d 2 PM	87.5	80.6	6.0
d 5 PM	123.8	132.9	11.1
lactose, µg/mL			
d 2 AM	40.4	42.8	2.1
d 2 PM	42.0	44.7	2.4
d 5 PM	45.3	50.0	2.7
IgG, mg/mL			
d 2 AM	9.68	8.27	0.62
d 2 PM	11.09[a]	9.11[b]	0.45
d 5 PM	21.32	21.45	1.14
IgA, mg/mL			
d 2 AM	1.60	1.47	0.25
d 2 PM	2.19	1.74	0.40
d 5 PM	2.08	1.81	0.35
Antibody against ovalbumin, arbitrary units $\times 10^3$			
d 2 AM	333.1	425.1	80.6
d 2 PM	398.7	394.7	78.3
d 5 PM	383.7	413.0	81.8

[a,b]Means within a row without a common superscript differ ($P < 0.01$).
[1]AM, morning; PM, afternoon.

Table 3. Milk composition in sows that received 4 injections of saline (control [CTL]; $n = 10$) or oxytocin (OXY; $n = 10$) in early lactation. The first injection was given 12 to 20 h after birth of the last piglet (d 2) and the others twice daily at 0800 and 1630 h

Item[1]	Treatment		SEM[2]
	CTL	OXY	
Energy, kJ/g			
d 2 AM	5.15	5.12	0.22
d 2 PM	4.90[c]	5.74[d]	0.27
d 4 PM	5.69	5.89	0.23
d 5 PM	5.20	5.14	0.22
Solids, %			
d 2 AM	19.74	19.68	0.67
d 2 PM	18.73[c]	21.32[d]	0.71
d 4 PM	20.95	21.59	0.59
d 5 PM	19.65	19.46	0.58
Fat, %			
d 2 AM	6.73	6.62	0.90
d 2 PM	7.10	8.15	0.27
d 4 PM	8.96	9.25	0.73
d 5 PM	7.89	7.59	0.71
Protein, %			
d 2 AM	7.83	7.81	0.69
d 2 PM	6.07[a]	7.86[b]	0.28
d 4 PM	5.92[e]	6.35[f]	0.17
d 5 PM	5.74	5.67	0.20
Lactose, %			
d 2 AM	2.18	1.97	0.09
d 2 PM	2.30	2.10	0.09
d 4 PM	2.55	2.69	0.09
d 5 PM	2.88[a]	2.44[b]	0.10
Na:K			
d 2 AM	0.33	0.35	0.02
d 2 PM	0.28[a]	0.48[b]	0.04
d 4 PM	0.29	0.29	0.01
d 5 PM	0.29	0.31	0.03
IGF-I, ng/mL			
d 2 AM	70.3	68.3	9.9
d 2 PM	37.2[a]	78.1[b]	9.0
d 4 PM	18.3	17.5	2.0
d 5 PM	13.5	15.5	1.4
IgG, mg/mL			
d 2 AM	23.44	24.63	5.89
d 2 PM	6.62[a]	18.78[b]	1.86
d 4 PM	2.27	1.78	0.38
d 5 PM	1.46	0.79	0.42
IgA, mg/mL			
d 2 AM	3.75	4.16	0.53
d 2 PM	2.71[a]	4.09[b]	0.32
d 4 PM	3.13	3.39	0.23
d 5 PM	2.89[e]	3.48[f]	0.22
Antibody against ovalbumin, arbitrary units $\times 10^3$			
d 2 AM	408.2	467.1	141.2
d 2 PM	101.7[a]	370.0[b]	78.5
d 4 PM	23.9	35.2	11.7
d 5 PM	12.6	16.1	5.9

[a,b]Means within a row without a common superscript differ ($P < 0.01$).
[c,d]Means within a row without a common superscript differ ($P < 0.05$).
[e,f]Means within a row without a common superscript tend to differ ($P < 0.10$).
[1]AM, morning; PM, afternoon.
[2]Maximal SEM.

of OXY sows compared to that of CTL sows on d 2 PM. Concentrations of IGF-I, IgG, IgA, and antibody titers against OVA were also increased in milk of treated sows at that time, thereby suggesting passive transfer from the dam's circulation to the milk. There seemed to be no effect of the 3 subsequent injections of OXY on mammary tight junctions as suggested by the similar Na:K ratio in milk on d 4. Responses to the first injection were expected because of the role of tight junctions on milk composition. Tight junctions form a seal that surrounds each mammary epithelial cell at the apical border, thereby regulating movement of material through the paracellular pathway. They are leaky in pregnancy and undergo closure around parturition to become impermeable in lactation (Linzell and Peaker, 1974; Nguyen and Neville, 1998). Many factors, both local and systemic, can influence the status of mammary tight junctions. Numerous hormones, such as prolactin, progesterone, and glucocorticoids, can play a role in the regulation of tight junctions, but OXY is the hormone harboring the most drastic effects (Nguyen and Neville, 1998; Kobayashi et al., 2016).

Table 4. Mean immunocrit values, hormone, and immunoglobulin concentrations on d 5 of lactation for 3 piglets of average BW per litter born from sows that received 4 injections of saline (control [CTL]; $n = 10$) or oxytocin (OXY; $n = 10$) in early lactation. The first injection was given 12 to 20 h after birth of the last piglet (d 2) and the others twice daily at 0800 and 1630 h.

Item	Treatment CTL	OXY	SEM
Immunocrit	0.112	0.122	0.006
IgG, mg/mL	46.5	55.3	5.9
IgA, mg/mL	3.81	4.83	0.56
Antibody against ovalbumin, arbitrary units $\times 10^3$	623.43	960.31	158.43
IGF-I, ng/mL	56.8	66.1	4.9

Large doses of OXY were shown to alter milk composition in a fashion consistent with an increase in permeability of tight junctions. Linzell et al. (1975) demonstrated that OXY must be injected in supraphysiological concentrations (10 times the physiological dose) in rabbits to elicit changes in milk sodium and potassium levels. Allen (1990) also reported increases in milk sodium and plasma lactose following the administration of supraphysiological doses of OXY to dairy cows, and Jonsson et al. (2013) injected 100 IU of OXY i.m. to elicit a response in cattle. More recently, Wall et al. (2016) showed an increased transfer of IgG into milk following intravenous administration of 100 IU of OXY in dairy cows. Oxytocin concentrations in sows on the day of farrowing are approximately 23 pg/mL (Yun et al., 2015). The supraphysiological dose of OXY used in the present study increased the milk Na:K ratio within 8 h of the injection. However, this effect was transient because it was no longer apparent 48 h later, even though injections were still given on d 3. There was a prolongation of the colostral period with exogenous OXY, and findings suggest that there is a maximal time period during which OXY can alter the permeability of tight junctions because most effects were no longer present on d 4 PM. However, the increased protein content in milk was still present on d 4 PM and that of IgA was seen on d 5 PM, showing a longer effect of OXY for some milk constituents. Interestingly, milk lactose content was only reduced later, on d 5 PM, and lactose in the blood of sows was not significantly affected, likely indicating that a longer treatment period would be necessary for it to be altered.

The lower IgG concentrations in sow serum concomitant with the increased IgG in milk on d 2 PM provide a clear indication of transfer of IgG from the sow circulation to the milk. This was not the case for IGF-I and IgA because values were not affected in sow blood. Yet, it does not preclude the fact that there may have been some transfer of these components from the sow circulation to milk. The greater milk concentrations of IGF-I, IgG, IgA, and antibody titers against OVA on d 2 PM did not translate into significantly increased circulating concentrations of these variables in piglets on d 5. This may be linked to the gut closure phenomenon that occurs approximately 24 to 36 h following the birth of a piglet (Weström et al., 1984). However, taking into account the fact that the OXY was injected 12 to 20 h after the end of farrowing, it seems more likely that changes in the circulation of piglets may have occurred sooner and been transitory and were, therefore, no longer apparent on sampling day (i.e., d 5). On the other hand, of the numerous studies using nutrition as a tool to increase immunoglobulin concentrations in sow colostrum (see review by Farmer and Quesnel [2009]), only 2 reported effects on IgG concentrations in piglets (Krakowski et al., 2002; Bontempo et al., 2004) even though colostral immunoglobulin concentrations were often increased. Nevertheless, the tendency for greater survival rate of piglets from OXY sows suggests that alterations in milk composition may have played a role in development factors that are linked with survival. Of importance also is the likely beneficial impact of greater IgA consumption on local immunity (Salmon et al., 2009) and the potential improvement in gastrointestinal tract (GIT) development of piglets due to an increased consumption of colostral IGF-I (Woliński et al., 2012).

The absence of treatment effects on IgG, IgA, antibody against OVA, and immunoglobulin immunocrit values in piglets on d 5 supports the findings of Vallet et al. (2013) that immunoglobulin immunocrit may be a quick and easy way to assess the immune status of piglets. Oxytocin injections did not alter piglet growth in the present study, and this may be due to the fact that an increase in permeability of mammary tight junctions is generally accompanied by a decrease in milk secretion rate (Nguyen and Neville, 1998). Therefore quality, but not quantity, of lacteal secretions would have been altered. On the other hand, when farrowings were induced in sows using a prostaglandin analog on d 113 of gestation, colostrum composition was transiently altered but its yield was not affected (Foisnet et al., 2011). Furthermore, no significant correlation was found between colostrum yield and the Na:K ratio in colostrum (Quesnel, 2011). It may, therefore, be that the quantity of colostrum produced in sows from the present study was not altered by treatment but that the compositional changes were not large enough to affect piglet growth. Another reason for the lack of treatment effect on piglet growth may be that the number of animals was not adequate to detect differences in so variable a factor.

Current findings demonstrate that injecting a supraphysiological dose of OXY to sows in the early postpartum period delays the occurrence of tightening of mam-

mary tight junctions, thereby having beneficial effects on the composition of early milk. Such a treatment appears to prolong the colostral phase, which may be advantageous for the survival of newborn piglets. Beneficial effects of this prolonged colostral phase may be via improved immune status of piglets or via greater development of the GIT linked with the various bioactive peptides, growth factors, and hormones that are present in colostrum (Woliński et al., 2012). It may also be that this prolonged intake of colostral-like early milk can help in establishing future development of reproductive tissues in swine as proposed by the lactocrine hypothesis (Frankshun et al., 2012). Further studies are needed to determine an optimal treatment protocol that would be beneficial for the growth and development of suckling piglets without having any detrimental effects on the sow.

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