



Salivary oxytocin changes and effect of the season in sows kept in different farrowing systems: Farrowing crate and farrowing pen with temporary crating



Marina López-Arjona ^{a,*}, Heng-Lun Ko ^b, Pol Llonch ^a, Déborah Temple ^c, J.J. Cerón ^b, Janni Hales ^d, Xavier Manteca ^a

^a Department of Animal and Food Science, School of Veterinary Science, Universitat Autònoma de Barcelona, 08193 Cerdanyola del Vallès, Barcelona, Spain

^b Interdisciplinary Laboratory of Clinical Analysis, Interlab-UMU, Regional Campus of International Excellence Campus Mare Nostrum, University of Murcia, 30100 Murcia, Spain

^c SEGES Innovation, Agro Food Park 15, 8200 Aarhus N, Denmark

^d AWEC Advisors S.L., Parc de Recerca de la UAB, 08193 Cerdanyola del Vallès, Barcelona, Spain

ARTICLE INFO

Keywords:

Animal welfare
Farrowing
Oxytocin
Saliva
Sow

ABSTRACT

Alternative farrowing systems that have been developed in recent years could have a positive effect on the welfare of sows during farrowing and lactation. Oxytocin measurements in saliva may provide information about positive animal welfare status. The purpose of this study was to evaluate the changes in salivary oxytocin concentrations in sows during the lactation period in three different farrowing systems and in two different seasons. Crossbred Duroc sows ($n = 34$, average parity = 3.6 ± 1.80) were housed in conventional farrowing crates (FC) ($n = 10$) or in farrowing pens with temporary crating (TC), including SWAP ($n = 12$) and JLF15 ($n = 12$) in two different seasons: summer and winter. Saliva samples were collected for six days during lactation: days 2, 4, 12, 23, 25 (i.e., 1-day post-weaning) and 26 (i.e., 2-day post-weaning) after farrowing. Moreover, behavioral data from sows was recorded on days 2, 4, 12 and 23 after farrowing, using a 30-s scan sampling method for 3 min per pen to record the behaviors which were assessed by the same observer. The results showed that the salivary oxytocin concentrations were 472.5 pg/mL and 399.4 pg/mL higher in both TC (SWAP and JLF15, respectively) than in the FC in early-lactation period, and these differences were more pronounced in summer and at the end of lactation in winter. In terms of behavior, higher number of mother-young interactions were observed in TC than FC in early- and mid-lactation period. In conclusion, TC is associated to a higher salivary oxytocin concentration that could indicate an increased mother-young interaction, although oxytocin concentration can be influenced by other factors, such as season or day of lactation.

1. Introduction

The behavior of sows before and during farrowing, as well as during lactation, can be influenced by the farrowing systems used. In recent years, different authors have tried to explore a farrowing environment that allows the sows reflect their behavior during farrowing and lactation (Hales et al., 2016; Singh et al., 2017). Some elements, such as accessibility to nesting materials and available space, can affect the metabolic status of the sow and improve that the piglets ingest colostrum. This could decrease piglet mortality and optimize their growth performance during the lactation period (Yun et al., 2014). Using free farrowing pens could increase the activity, enrich behavioral pattern,

decrease abnormal behavior of sows and increase the weaning weight and activity level of piglets (Zhang et al., 2020).

The utilization of farrowing crates may decrease piglet crushing in comparison to other alternative systems with farrowing pens (Weber et al., 2007). However, it might also result in a higher mortality attributed to other factors, including the farrowing process, body length of the sows, and individual birth weight (Marchant et al., 2000; Pedersen et al., 2011). Recently, farrowing pens have been designed to address welfare considerations by offering increased space, enabling sows to rotate and engage more interactively with their piglets (Bradshaw and Broom, 1999). Sows in farrowing pens improve the activity and express a greater behavioral repertoire compared to sows in crates,

* Corresponding author.

E-mail address: marina.lopez.arjona@uab.cat (M. López-Arjona).

as well as perform more reciprocal mother-young interactions (Chidgey et al., 2016). Temporary crating system improved sow interactions with the piglets and explorative behavior, and they did not alter some salivary stress biomarkers in sows, like cortisol and chromogranin A (Ko et al., 2022a).

Oxytocin can be related to maternal affiliation in humans (Scatliffe et al., 2019) and plays a role in the establishment and maintenance of social bonds (Crockford et al., 2014). Oxytocin concentration, serving as a regulator of maternal traits (Scatliffe et al., 2019), influenced the attentiveness of sows to their offspring both when standing and lying down in the initial stages of lactation (Yun et al., 2013). Moreover, the use of less-invasive samples, such as saliva, presents an advantage, since it can be collected without causing stress (Gröschl, 2008).

Currently, there are not many studies in which the physiological changes related to well-being in sows kept in different lactation systems can be objectively evaluated through non-invasive biomarkers such as the measurement of oxytocin in saliva. The proposed hypothesis of this study would be that the oxytocin concentrations in saliva could change depending on the farrowing system and that can provide information about the effect of farrowing system in animal welfare. Therefore, the objective of this study was to evaluate the changes in behaviors and salivary oxytocin concentration in sows during farrowing and lactation in three different farrowing systems (farrowing crate (FC) and farrowing pen with temporary crating (TC): which were SWAP and JLF15). This study was made in two different seasons (summer and winter) to study the seasonal effect.

2. Materials and methods

2.1. Ethical approval and inclusion criteria of the animals

The ethical approval number was FUE-2016-00441221 by the ethical committee of Autonomous University of Barcelona. Sows were included in the study with the following criteria: (1) they were clinically healthy and (2) did not have any evident sign of disease during the study. Two sows were excluded due to savaging or excessive crushing, and it was decided by the research team, as these behaviors were not expected prior to the experiment, and also, they had too less piglets per litter, which was very different from the rest of the pens. The other excluded sow was for practically reason, because she had a delay of the farrowing.

2.2. Housing and experimental design

Pigs were raised on a farrow-to-finish commercial farm in Girona, Spain. Three farrowing systems were used during lactation, one was a FC and the other two were TC. The TC were the "Sow Welfare and Piglet Protection Pen" (SWAP) and JLF15 (both produced by SKIOLD A/S, Ikast, Denmark).

The study was carried out in two seasons, in winter (January–February, outdoor temperature: 7.6 ± 2.3 °C) and in summer (June–July, outdoor temperature: 24.4 ± 2.6 °C), one batch each season in 2019. In each batch, there were five FC pens in one farrowing unit, and in another farrowing unit, there were six SWAP pens and six JLF15 pens ($n = 34$). Sows were transferred to the farrowing pens a week before the expected farrowing date. The day of the farrowing of each pen was designated as day (D) 0. In terms of the crating period, FC sows were crated from entry to weaning, whereas TC sows were crated from 1 day before expected farrowing to 3 days after farrowing. In total, 34 crossbred Duroc sows (the average parity of these 34 sows was 3.6 ± 1.80) were followed from entry to 2 days after weaning. Piglets were weaned on day 24 (D24). At weaning, piglets were moved to a nursery and sows were moved to six group pens, having the sows from the same farrowing system being kept

together (FC, SWAP, and JLF15 pens each in winter and in summer). Technical details (i.e., creep areas, dimension of the pens, and crates) of the three farrowing systems and animal management have been previously described in Ko et al. (2022a), and data about the post-weaning pens for sows and piglets are reported in Ko et al. (2022b).

2.3. Saliva collection and oxytocin analysis in sows

This present study used the saliva samples previously described in Ko et al. (2022a). Salivette tubes (Sarstedt, Aktiengesellschaft and Co., Nümbrecht, Germany) were used for saliva obtention. Sows were sampled between 09:00 and 10:00. Sows chewed on the cotton swabs attached to a clamp for 1 min. The clamp for TC pens were extended with a long stick to facilitate saliva sampling from SWAP and JLF15 sows, in which they had had previously trained for. After collection, the cotton swabs were introduced back to the Salivette tubes and transported refrigerated (4 °C) to the laboratory where they were centrifuged (3000 rpm for 10 min) (Heraeus™ Labofuge™ 200 Centrifuge, Thermo Fisher Scientific GmbH, Dreieich, Germany). The obtained volume of saliva was transferred to 1.5 mL tubes (Eppendorf Ibérica, Spain) to be stored at -80 °C until analysis.

Six sampling points were chosen for determination of salivary oxytocin, considering D0 as the farrowing day: D2 (1D before opening the crate of the SWAP and JLF15 sows, FC sows remained crated), D4 (1D after opening the crate of the SWAP and JLF15 sows, FC sows remained crated), D12 (mid lactation), D23 (end of lactation and 1D pre-weaning), D25 (1D post-weaning), and D26 (2D post-weaning).

Oxytocin concentrations were measured in saliva samples by a immunoassay based on AlphaLISA technology previously validated in pig saliva samples (López-Arjona et al., 2020).

2.4. Behavioral observations

Behavioral observation was conducted directly on farm by one observer on D2, D4, D12, and D23, after saliva sampling was completed. Behavioral observation was scheduled for six sessions each day: three in the morning (10:00–13:30) and three in the afternoon (14:00–17:30). All the pens were observed in each session. The observer used the 30-s scan sampling method for 3 min per pen to record the behaviors. Behaviors can be divided into two categories: social interaction and non-social interaction. Behaviors in social interaction category include social behavior in piglets (SB), naso-naso contact by piglet towards sow (NNC), piglet resting with sow contact (RSC), sow towards piglet social interaction (MYI), and sow towards sow social interaction (MMI); behaviors in non-social interaction category include locomotor/object play/exploration in piglet (PPE), and exploration in sow (SEB). The description for each behavior can be found in Ko et al. (2022a).

2.5. Statistical analysis

GraphPad Prism software Inc. (GraphPad Prism, version 8 for Windows, Graph Pad Software Inc., San Diego, CA, USA) was used to analyze the salivary oxytocin data. The Shapiro-Wilk test was employed to assess the distribution of the data, revealing a nonparametric distribution for the salivary oxytocin concentrations in the saliva of sows.

To examine the impact of the day on salivary oxytocin concentrations and the influence of the farrowing system, the oxytocin values were log transformed. A two-way analysis of variance (ANOVA) was then performed, and post hoc analysis was carried out using the Fisher LSD test (with a level of significance of 0.05). Given the non-normal distribution of these data, they were presented using the median (interquartile range).

Behavioral records were processed using RStudio version 2023.06.1. The six observation sessions on each observation day were aggregated for each behavioral category. The proportion of each behavioral category was determined by dividing the number of occurrences of each behavior by the total count of all registered behaviors. All the behaviors except RSC are presented as percentage. RSC for each observation day was the average of all the six numbers of RSC during the observation day. It is presented as the number of piglets. SB and PPE were analyzed by linear mixed models (LMM). NNC, SC and MYI were $\log(1 + x)$ transformed and analyzed by LMMs. The MMI and SEB values were changed to either 1 or 0 (i.e. Yes or No) (due to high frequency of 0) and analyzed using general linear mixed models (GLMM) with a binomial distribution. RSC was analyzed using a GLMM with a Poisson distribution. All models included behavior as the response variable, with farrowing system, day, and their interaction as fixed effects. Covariates included litter size, and random effects were represented by batch and pen.

3. Results

In the summer batch, one multiparous FC sow was excluded from the study because she exhibited savaging behavior towards her piglets after farrowing. Additionally, one multiparous SWAP sow from the same batch was excluded due to the occurrence of excessive piglet crushing, resulting in a low number of live piglets in one litter. Another multiparous FC sow in the summer batch was excluded due to a week-long delay in farrowing. In total, there were 31 sows included for data analysis (FC: 8 sows, SWAP: 11 sows, and JLF15: 12 sows). Average crating period for FC sows was 34.64 ± 1.6 days, and for SWAP and JLF15, it was 5.9 ± 1.4 days.

3.1. Changes in salivary oxytocin concentrations of sows in different farrowing systems

When the different farrowing systems were compared regardless of season, the oxytocin concentrations were higher in the SWAP and JLF15 than in the conventional system on D2 ($P = 0.0091$ and $P = 0.0208$, respectively). Moreover, when the oxytocin concentrations were

compared between different days within the same farrowing system, only significant differences were found in conventional system, being lower on D2 in comparison to D4, D12, D25 and D26 ($P = 0.0164$, $P = 0.0112$, $P = 0.0090$ and $P = 0.0145$).

3.2. Changes in salivary oxytocin concentrations in different days during lactation and in different seasons

In summer, in the SWAP, oxytocin concentrations were higher on D2 and D4 than on D26 ($P = 0.0100$ and $P = 0.0451$, respectively), also on D2, oxytocin concentrations were higher than on D25 ($P = 0.0236$). In the JLF15, oxytocin concentrations were higher on D2 than on D23 ($P = 0.0447$). Finally, in the FC, oxytocin concentrations on D4 were higher than on D23 ($P = 0.0170$) and on D12 were higher than on D23 ($P = 0.0216$). Moreover, when the oxytocin concentrations were compared between the same days of different farrowing systems, SWAP sows had higher oxytocin concentrations than FC sows on D2 ($P = 0.0218$).

Overall, during the summer season, oxytocin concentrations were higher at early lactation stage than later regardless of the farrowing system, with the SWAP system showing more days in which significant increases were found in early lactation.

In winter, when different sample points were compared within farrowing system, oxytocin concentrations were higher on D26 than on D2, D4 and D12 ($P = 0.0398$, $P = 0.0277$ and $P = 0.0246$, respectively) in the SWAP. In case of the JLF15, oxytocin concentrations were higher on D26 than on D12 ($P = 0.0477$). In the FC, no significant differences were found in salivary oxytocin concentrations. Overall, during the winter season, the oxytocin concentrations were higher at later lactation stage than earlier, especially in the SWAP system. When the oxytocin concentrations were compared between days of different farrowing systems JLF15 sows had higher oxytocin concentration than FC sows on D26 ($P = 0.0469$).

Fig. 1 shows the comparison between oxytocin concentrations of different days within each season.

All the results (both social interactions and oxytocin concentrations) are shown in Tables 1 and 2.

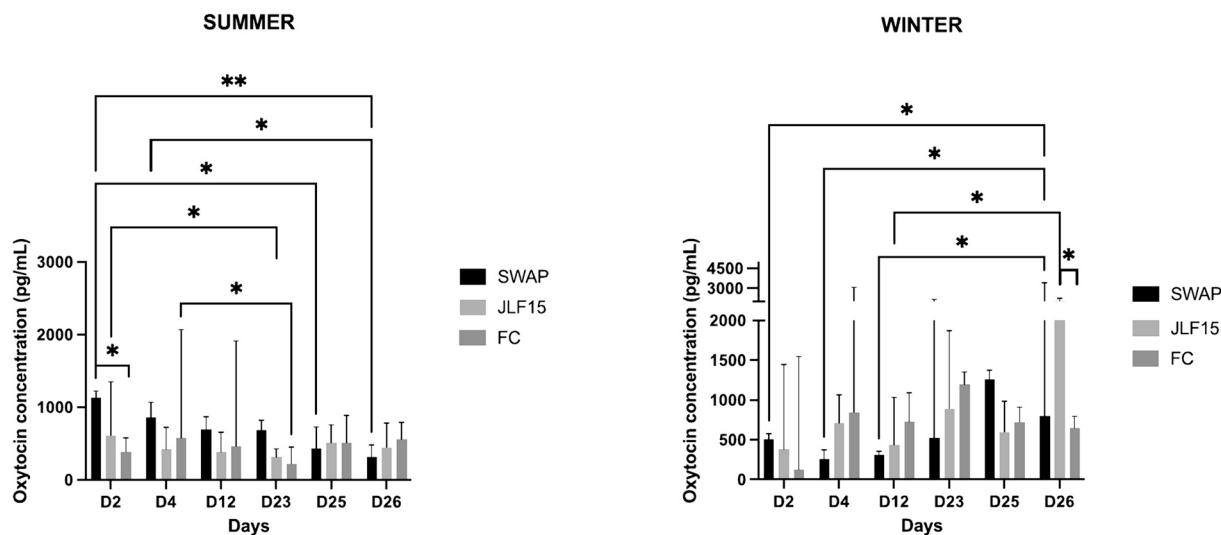


Fig. 1. Oxytocin concentration in saliva of sows in different days along lactation and after weaning with the three farrowing systems (SWAP, JLF15 and FC) during summer (left) and winter (right) season. The rectangles colored in different shades of gray indicate the medians and the asterisk indicate significant differences (* $P \leq 0.05$; ** $P \leq 0.01$).

Table 1

Salivary oxytocin concentrations of sows (pg/mL) and behavioral data from sows and piglets regardless of season during different days of lactation.

	Social interaction								Salivary oxytocin (pg/mL) in sows		
	Piglet towards piglet(s)		Piglet towards sow			Sow towards piglet(s)		Non-social interaction			
	SB ¹	NNC ²	SC ²	RSC ³	MYI ²	MMI ⁴	Piglet	Sow			
Day 2											
FC	44.4 ± 5.3	3.4 ± 1.4 ^x	12.5 ± 4.4	1.6 ± 0.5	2.5 ± 1.3	-	34.8 ± 3.7	2.4 ± 2.1	281.4 ± 248.1 ^{x,a}		
SWAP	31.9 ± 4.6	7.7 ± 1.7 ^{xy}	16.2 ± 2.9	1.1 ± 0.4	5.1 ± 2.0	-	38.9 ± 2.4	0.3 ± 0.3	753.9 ± 393.8 ^y		
JLF15	30.7 ± 5.6	14.0 ± 4.0 ^{y,a}	15.7 ± 2.5	1.3 ± 0.4	7.9 ± 1.9	-	28.7 ± 5.0	3.0 ± 1.7	680.8 ± 582.9 ^y		
Day 4											
FC	38.8 ± 5.2	3.2 ± 1.1	14.0 ± 3.7	1.0 ± 0.1	3.2 ± 1.1 ^x	-	35.2 ± 1.9	5.5 ± 3.3	831.1 ± 729.0 ^b		
SWAP	23.3 ± 3.5	9.2 ± 2.0	20.3 ± 6.3	0.9 ± 0.2	10.8 ± 2.7 ^y	0.6 ± 0.3	28.7 ± 4.2	7.1 ± 2.2	646.7 ± 359.7		
JLF15	30.9 ± 4.5	9.7 ± 3.4 ^{ab}	12.4 ± 2.8	0.8 ± 0.2	13.2 ± 5.3 ^y	0	30.6 ± 4.7	3.3 ± 1.8	603.2 ± 301.3		
Day 12 (mid-lactation)											
FC	31.3 ± 6.3	1.3 ± 0.7 ^x	13.2 ± 4.9	0.7 ± 0.2	1.0 ± 0.5 ^x	-	38.5 ± 5.9	2.3 ± 0.9	795.8 ± 683.5 ^b		
SWAP	24.1 ± 3.3	7.1 ± 1.2 ^{xy}	25.0 ± 5.5	0.8 ± 0.2	9.9 ± 2.3 ^y	0.4 ± 0.3	30.4 ± 3.9	3.1 ± 1.3	530.4 ± 256.2		
JLF15	26.7 ± 2.7	8.5 ± 2.4 ^{y,ab}	16.1 ± 2.4	1.0 ± 0.2	7.3 ± 2.3 ^{xy}	0.2 ± 0.2	38.8 ± 4.4	2.4 ± 1.1	504.4 ± 364.6		
Day 23 (end of lactation)											
FC	29.7 ± 2.5	5.4 ± 1.3	20.4 ± 3.6	0.8 ± 0.2	4.6 ± 1.2	-	38.8 ± 2.5	1.0 ± 0.4	564.4 ± 521.3		
SWAP	24.1 ± 1.8	5.6 ± 0.9	24.4 ± 4.1	1.7 ± 0.4	7.0 ± 1.3	0	33.8 ± 2.9	5.2 ± 3.1	838.0 ± 845.5		
JLF15	24.6 ± 1.9	5.4 ± 1.6 ^b	22.4 ± 4.1	1.6 ± 0.3	4.2 ± 0.8	0	39.5 ± 2.8	3.9 ± 1.8	753.0 ± 781.7		
Day 25 (1D after weaning)											
FC									645.2 ± 258.2 ^b		
SWAP									822.3 ± 487.3		
JLF15									606.1 ± 227.3		
Day 26 (2D after weaning)											
FC									595.4 ± 208.5 ^b		
SWAP									934.9 ± 1339.0		
JLF15									944.5 ± 729.9		
Global P-value											
Farrowing system	0.01	<0.0001	0.09	0.99	0.002	0.55	0.18	0.62			
Day	0.13	0.50	0.08	0.05	0.13	0.57	0.46	0.12			

Behaviors initiated by the piglets: SB = Social interactions between piglets. / NNC = Piglet initiated naso-naso contact with the sow. / SC = Piglet initiated physical contact (except the snout and the udder) with the sow. / RSC = Piglet resting in physical contact with the sow. / PPE = Piglet locomotor or object play, and exploration of the pen.

Behaviors initiated by the sow: MYI = Sow initiated physical contact with the piglet. / MMI = Mother-mother interactions. / SEB = Sow exploration of the pen.

¹ SB and PPE were normally distributed, so they were analyzed by linear mixed models.

² NNC, SC and MYI were log(1 + x) transformed and analyzed by linear mixed models.

³ RSC was analyzed by a general linear mixed model with a passion distribution.

⁴ Values of MMI and SEB were changed to 1/0 (i.e. Yes/No) and analyzed by general linear mixed models with a binomial distribution.

Table 2

Salivary oxytocin concentrations of sows (pg/mL) and behavioral data from sows and piglets during different days of lactation in both seasons (summer and winter).

	Social interaction						Non-social interaction		Salivary oxytocin (pg/mL) in sows
	Piglet towards piglet(s)		Piglet towards sow		Sow towards piglet(s)	Sow towards sow	Piglet	Sow	
	SB ¹	NNC ²	SC ²	RSC ³	MYI ²	MMI ⁴	PPE ¹	SEB ⁴	
Day 2									
FC									
Summer	51.2 ± 3.1	5.1 ± 3.1	7.9 ± 4.2	3.0 ±	3.5 ± 2.0	-	26.7 ±	5.6 ±	412.8 ± 37.3 [×]
Winter	40.3 ± 8.0	2.3 ± 1.4	15.3 ±	0.7	1.8 ± 1.8	-	6.2	5.6	80.1 ± 62.3 ^y
			6.6	0.8 ±			39.7 ±	0.5 ±	
				0.3			3.4	0.5	
SWAP									
Summer	34.7 ± 6.9	5.5 ± 3.0	18.6 ±	1.8 ±	2.3 ± 1.5	-	38.9 ±	0	1021 ± 264.6 ^{y,a}
Winter	29.6 ± 6.7	9.5 ± 1.6	4.6	0.4	7.4 ± 3.2	-	4.9	0.5 ±	420.4 ± 230.8 ^a
			14.2 ±	0.6 ±			38.9 ±	0.5	
			3.7	0.5			2.3		
JLF15									
Summer	35.2 ± 9.7	9.9 ± 3.2	13.4 ±	2.0 ±	8.8 ± 3.6	-	32.4 ±	0.3 ±	670.7 ± 524.7 ^a
Winter	26.1 ± 6.2	18.1 ±	4.2	0.6	7.0 ± 1.8	-	5.5	0.3	693.4 ± 733.9
		7.4	18.0 ±	0.7 ±			25.1 ±	5.6 ±	
			2.7	0.3			8.6	3.1	
Day 4									
FC									
Summer	48.3 ± 6.1	2.1 ± 2.1	12.3 ±	1.0 ±	2.1 ± 2.1	-	35.2 ±	0	960.5 ± 977.5 ^a
Winter	33.2 ± 6.7	3.9 ± 1.3	8.7	0.2	3.9 ± 1.3	-	4.7	8.8 ±	1445 ± 1416
			15.0 ±	0.9 ±			35.3 ±	4.9	
			3.9	0.2			1.7		
SWAP									
Summer	23.2 ± 6.5	5.6 ± 2.3	32.0 ±	1.4 ±	11.6 ± 5.9	0.3 ± 0.3	24.6 ±	2.7 ±	848.1 ± 230.5 ^a
Winter	23.4 ± 4.0	12.2 ±	12.3	0.3	10.1 ± 2.0	0.7 ± 0.5	7.6	2.0	243.9 ± 139.8 ^a
		2.6	10.6 ±	0.6 ±			32.2 ±	10.8 ±	
			1.5	0.2			4.6	3.1	
JLF15									
Summer	36.9 ± 6.3	3.8 ± 1.9	13.9 ±	1.1 ±	5.6 ± 2.4	0	34.8 ±	5.0 ±	471.1 ± 241.4
Winter	25.0 ± 6.1	15.5 ±	4.1	0.2	20.8 ± 9.8	0	6.1	3.4	735.4 ± 315.5
		5.7	10.8 ±	0.5 ±			26.3 ±	1.7 ±	
			4.2	0.3			7.2	1.2	
Day 12 (mid-lactation)									
FC									
Summer	39.8 ± 11.6	1.4 ± 1.4	14.9 ±	1.0 ±	0.5 ± 0.5	-	43.4 ±	0	870.6 ± 1004 ^a
Winter	26.2 ± 7.4	1.3 ± 0.8	10.0	0.4	1.3 ± 0.8	-	3.0	3.6 ±	735.9 ± 410.4
			12.1 ±	0.6 ±			35.6 ±	1.0	
			6.1	0.2			9.5		
SWAP									
Summer	26.6 ± 5.1	6.7 ± 2.1	18.0 ±	0.7 ±	11.3 ± 4.5	0	34.6 ±	2.8 ±	675.5 ± 231.9
Winter	22.0 ± 4.6	7.4 ± 1.6	5.4	0.3	8.7 ± 2.1	0.8 ± 0.5	6.7	2.8	312.8 ± 44.9 ^a
			31.0 ±	0.9 ±			26.8 ±	3.4 ±	
			8.6	0.3			4.4	1.2	
JLF15									
Summer	27.7 ± 5.0	9.9 ± 4.6	14.9 ±	1.3 ±	8.1 ± 4.5	0	38.8 ±	0.5 ±	449.9 ± 348.2
Winter	25.7 ± 2.7	7.1 ± 1.7	4.2	0.4	6.5 ± 1.6	0.4 ± 0.4	8.5	0.4	586.2 ± 426.6 ^a
			17.4 ±	0.7 ±			38.7 ±	4.3 ±	
			2.7	0.3			3.7	1.8	
Day 23 (end of lactation)									
FC									
Summer	25.8 ± 6.1	6.4 ± 3.2	19.9 ±	1.4 ±	3.5 ± 2.2	-	43.3 ±	1.0 ±	250.4 ± 202.9 ^b
Winter	32.0 ± 1.4	4.9 ± 1.2	8.8	0.2	5.3 ± 1.5	-	0.5	1.0	1192 ± 228.1
			20.7 ±	0.5 ±			36.0 ±	1.1 ±	
			3.5	0.3			3.4	0.5	
SWAP									
Summer	22.4 ± 3.0	6.3 ± 1.8	24.0 ±	1.7 ±	5.6 ± 2.0	0	34.2 ±	7.5 ±	634.5 ± 274.5
Winter	25.5 ± 2.4	5.0 ± 1.0	6.0	0.6	8.1 ± 1.8	0	5.6	6.8	1082 ± 1248
			24.7 ±	1.8 ±			33.4 ±	3.3 ±	
			6.1	0.6			3.2	1.8	
JLF15									
Summer	26.5 ± 3.0	4.2 ± 1.2	17.2 ±	2.0 ±	3.4 ± 0.4	0	45.1 ±	3.6 ±	277.6 ± 160.6 ^b
Winter	22.8 ± 2.6	6.6 ± 3.0	4.8	0.4	4.9 ± 1.5	0	2.8	2.7	1228 ± 881.0
			27.5 ±	1.1 ±			34.0 ±	4.2 ±	
			6.2	0.3			3.6	2.5	
Day 25 (1D post-weaning)									
FC									
Summer								613.8 ± 266.1	
Winter								670.3 ± 280.1	

(continued on next page)

Table 2 (continued)

	Social interaction				Non-social interaction		Salivary oxytocin (pg/mL) in sows	
	Piglet towards piglet(s)	Piglet towards sow		Sow towards piglet(s)	Sow towards sow	Piglet	Sow	
	SB ¹	NNC ²	SC ²	RSC ³	MYI ²	MMI ⁴	PPE ¹	SEB ⁴
SWAP								
Summer							432.4 ± 326.0 ^b	
Winter							1212 ± 205.6	
JFL15								
Summer							543.2 ± 193.8	
Winter							700.4 ± 269.7	
Day 26 (2D post-weaning)								
FC								
Summer							549.2 ± 261.6	
Winter							632.4 ± 178.3 ^x	
SWAP								
Summer							347.7 ± 131.5 ^b	
Winter							1522 ± 1802 ^b	
JFL15								
Summer							551.4 ± 305.7	
Winter							1731 ± 711.7 ^{y,b}	
Global P-value								
Farrowing								
system	0.01	<0.0001	0.09	1.00	0.001	0.46	0.19	0.98
Day	0.12	0.49	0.05	0.05	0.13	0.92	0.53	0.46
Season	0.03	0.04	0.67	0.003	0.19	0.29	0.19	0.11

Behaviors initiated by the piglets: SB = Social interactions between piglets. / NNC = Piglet initiated naso-naso contact with the sow. / SC = Piglet initiated physical contact (except the snout and the udder) with the sow. / RSC = Piglet resting in physical contact with the sow. / PPE = Piglet locomotor or object play, and exploration of the pen.

Behaviors initiated by the sow: MYI = Sow initiated physical contact with the piglet. / MMI = Mother-mother interactions. / SEB = Sow exploration of the pen.

¹ SB and PPE were normally distributed, so they were analyzed by linear mixed models.

² NNC, SC and MYI were log(1 + x) transformed and analyzed by linear mixed models.

³ RSC was analyzed by a general linear mixed model with a passion distribution.

⁴ Values of MMI and SEB were changed to 1/0 (i.e. Yes/No) and analyzed by general linear mixed models with a binomial distribution.

3.3. Mother-young interactions, social interactions between piglets, and exploration in piglets and sows in different farrowing systems

There was a significant effect of farrowing system globally in SB, NNC, and MYI ($P \leq 0.01$). When looking into each behavioral category in details, we saw higher mother-young interactions expressed in SWAP and JLF15 than FC in early- and mid-lactation period. To be precise, JLF15 piglets initiated more NNC than FC piglets on D2. SWAP and JLF15 sows interacted with their piglets more than FC sows on D4. In mid-lactation, JLF15 piglets again initiated more NNC than FC piglets, and SWAP sows again interacted with their piglets more than FC sows. No significant behavioral differences were found between farrowing systems in late-lactation period.

4. Discussion

In this study, higher salivary oxytocin concentrations, which is a hormone that increase in situations of positive emotions (López-Arjona et al., 2020b; MacLean et al., 2017), were found in both TC (SWAP and JLF15) compared to the FC at early-lactation period. The oxytocin in saliva was measured by an immunoassay based on AlphaLISA technology previously validated in saliva of pigs (López-Arjona et al., 2020). To our knowledge, this study represents the first time that salivary oxytocin concentrations have been associated to mother-young interaction in sows in different farrowing systems along the lactation period.

Oxytocin was selected as biomarker since immediately after parturition, maternal attachment and behaviors aimed at caring, nursing, and protecting the newborn are highly influenced by this hormone. In domestic species, low levels of oxytocin can be associated with deficient dam-young bonding (Mota-Rojas et al., 2023). Sows in loose-house pens with enriched environment tended to have higher concentrations of serum oxytocin, while the concentration of cortisol was lower, suggesting an increase in maternal behavior and a reduction of stress (Wang

et al., 2020). Oxytocin has been also related with uterine activity (Alonso-Spilsbury et al., 2004; Marcat-Rius et al., 2023; Mota-Rojas et al., 2006); although it depends on the different dosages and oxytocin administration timing (Mota-Rojas et al., 2005, 2006).

The fact that oxytocin concentrations in saliva were higher at the beginning of lactation in the SWAP and JLF15 systems could be due to the greater maternal behavior at the beginning of lactation in these systems compared to the conventional, since this hormone can increase in maternal behavior in different species (Algers et al., 1990; Uvnäs Moberg and Prime, 2013). Also, in this study, some maternal behaviors between sow and piglets were higher at early stages of lactation than at the end in these alternative systems, which could be related to the oxytocin concentrations. Also, oxytocin can be affected by other factors that can be associated to lactating period, such as the oxytocin role in postpartum physiology, especially the process of milk ejection during suckling (Algers et al., 1990).

SWAP and JLF15 implement a crating period extending from 1 day before the expected farrowing date to 3 days after farrowing, whereas FC entails a more extended crating period, spanning from entry to weaning, lasting 24 days in total. These alternative systems are crafted with the intention of enhancing pig welfare in comparison to conventional farrowing crates. They offer sows some degree of freedom of movement while ensuring the well-being of piglets is not compromised (Goumon et al., 2022), whereas in FC there is a space restriction that can lead to stress and altered behavior (Baxter et al., 2012; Wischner et al., 2009). This improvement in welfare of sows could be reflected in the increase in oxytocin concentrations observed in our report in the alternative systems in comparison with the conventional system.

Previously, other studies have shown increases in salivary oxytocin of pigs during situations of positive emotions (López-Arjona et al., 2020b) or decrease in stress situations (López-Arjona et al., 2020a). In addition, the increase in oxytocin found specially in SWAP system could be linked to an improvement of maternal behavior (Scatliffe et al.,

2019), since in SWAP system the creep area is positioned near the head of the sow to promote a 'nest-like' environment and encourage sow-piglet interaction (Damm et al., 2006) and in general, this alternative farrowing system can promote positive interactions or positive emotional states. In a previous report, Ko et al. (2022a) concluded that farrowing pens incorporating a temporary crating system contributed to the enhancement of sow-piglet interactions and the promotion of sow explorative behavior, although without significant differences in stress biomarkers like cortisol and chromogranin A when used these systems, so the increase in oxytocin concentrations in temporary crating in our study could indicate that oxytocin could be a welfare biomarker more sensitive than these stress biomarker in this situation.

In summer, oxytocin concentrations were found to be higher in early lactation compared to the end in all three farrowing systems, with SWAP being highest. It has been described that SWAP sows exhibit approximately 10 times more exploration of the pens compared to FC sows (Ko et al., 2022a). Furthermore, this system is favored by the sows when they are in a resting position (Damm et al., 2006) and because there are different zones in which the sows' biological needs, such as urine and feces, can be separated from the resting zone. Also, this system can allow more naso-nasal contacts between piglets and sows (Jarvis et al., 2004). Along this line, Scatliffe et al. (2019) concluded that oxytocin plays a significant role in fostering the development of attachment between infants and parents, facilitated by early contact and interaction. The increase in oxytocin found on day 4 (one day after the sows were released from crating) in SWAP could be caused by the greater freedom of movement and behavior enjoyed by sows once they were released (Chidgey et al., 2016). The loose-sow housing, characterized by higher floor space from days 3 to 28 of lactation, leads to enhanced maternal behavior in sows and improved social behavior in piglets. This improvement occurs without elevating the risks to piglet mortality during this period (Singh et al., 2017).

In winter, oxytocin concentrations were higher at the later lactation stages than at early stages. In conventional farrowing crates, the sow's ability to regulate her own suckling frequency is decreased, leading to an abrupt weaning process. However, opened crates that incorporate a sow-only area have demonstrated a more gradual weaning process, allowing for a reduction in suckling frequency as lactation advances (Pajor et al., 1999). This approach has the potential to benefit sow welfare by mitigating the effects of confinement (Berkeveld et al., 2009) and could be the cause of a higher oxytocin concentration at this stage in winter season in TC farrowing system in comparison to conventional system. Overall values of oxytocin were higher in winter compared to summer; this would be in line with the findings of Renaudeau et al. (2001) who observed that lactating sows kept in a thermoneutral environment increased nursing time compared to sows kept under heat stress. The detection of increased oxytocin values at the first days in the summer period in the TC systems, could be related to a reduction of heat stress due to the increase in space of the sow, showing an improvement in welfare, while the heat stress could mask the possible increases in oxytocin at weaning like in winter season.

Additional studies involving a large number of sows should be performed to corroborate our findings. In addition, it would be of interest to compare the changes in oxytocin with other stress biomarkers in saliva samples, such as cortisol, salivary alpha-amylase, chromogranin-A or total esterase activity (Cerón et al., 2022), in order to gain more knowledge about the mechanisms involved in these variations.

5. Conclusions

Salivary oxytocin could be a welfare indicator of sows during lactation in alternative farrowing systems, although changes in oxytocin concentrations also depend on other factors, such as the season or the day of lactation. More studies should be undertaken to confirm the results of this study and elucidate the potential of oxytocin as a biomarker of welfare in sows at farrowing and lactation.

Funding

The study was funded by Proyectos I + D + I Convocatoria RETOS 2015 (AGL2015-68373-C2-1-R) supported by the Spanish Ministry of Economy, Industry and Competitiveness (MINECO), and the Technological Transfer of the Rural Development Program 2014–2020 (56 30062 2017 2A) by the Catalan Government. Marina López-Arjona received funding from a post-doctoral fellowship "Juan de la Cierva Formación" supported by the Spanish Ministry of Science and Innovation (FJC2021-047105-I), Spain. Pol Llonch received funding from the "Ramón y Cajal" programme of the Spanish Ministry of Science (RYC2020-029067-I), Spain.

CRediT authorship contribution statement

Marina López-Arjona: Writing – original draft, Methodology, Investigation, Conceptualization. **Heng-Lun Ko:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Pol Llonch:** Writing – review & editing, Visualization, Investigation. **Déborah Temple:** Writing – review & editing, Methodology. **J.J. Cerón:** Writing – review & editing. **Janni Hales:** Writing – review & editing, Methodology. **Xavier Manteca:** Writing – review & editing, Investigation, Conceptualization.

Declaration of competing interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgements

We would like to acknowledge all the contributors who made this research achievable: Gerfam Rubirola, L'Ekip, Jyden and Rotecna.

References

- Algers, B.O., Rojanasthien, S., Uvnäs-Moberg, K., 1990. The Relationship between Teat Stimulation, Oxytocin Release and Grunting Rate in the Sow During Nursing. *Alonso-Spilsbury, M., Mota-Rojas, D., Martínez-Burnes, J., Arch, E., López Mayagoitia, A., Ramírez-Necoechea, R., Olmos, A., Trujillo, M.E., 2004. Use of oxytocin in penned sows and its effect on fetal intra-partum asphyxia. Anim. Reprod. Sci.* 84, 157–167. <https://doi.org/10.1016/j.anireprosci.2003.11.002>.
- Baxter, E.M., Lawrence, A.B., Edwards, S.A., 2012. Alternative farrowing accommodation: welfare and economic aspects of existing farrowing and lactation systems for pigs. *Anim. Sci.* 6, 96–117. <https://doi.org/10.1017/S1751731111001224>.
- Berkeveld, M., Langendijk, P., Soede, N.M., Kemp, B., Taverne, M.A., Verheijden, J.H., Kuijken, N., Koets, A.P., 2009. Improving adaptation to weaning: effect of intermittent suckling regimens on piglet feed intake, growth, and gut characteristics. *J. Anim. Sci.* 87, 3156–3166.
- Bradshaw, R.H., Broom, D.M., 1999. Behaviour and performance of sows and piglets in crates and a Thorstensson system. *Proc. Brit. Soc. Anim. Sci.* 1999 <https://doi.org/10.1017/s1752756200003343>, 179–179.
- Cerón, J.J., Contreras-Aguilar, M.D., Escrivano, D., Martínez-Miró, S., López-Martínez, M.J., Ortín-Bustillo, A., Franco-Martínez, L., Rubio, C.P., Muñoz-Prieto, A., Tvarijonaviciute, A., López-Arjona, M., Martínez-Subiela, S., Tecles, F., 2022. Basics for the potential use of saliva to evaluate stress, inflammation, immune system, and redox homeostasis in pigs. *BMC Vet. Res.* 18, 81.
- Chidgey, K.L., Morel, P.C.H., Stafford, K.J., Barugh, I.W., 2016. Observations of sows and piglets housed in farrowing pens with temporary crating or farrowing crates on a commercial farm. *Appl. Anim. Behav. Sci.* 176, 12–18. <https://doi.org/10.1016/j.applanim.2016.01.004>.
- Crockford, C., Deschner, T., Ziegler, T.E., Wittig, R.M., 2014. Endogenous peripheral oxytocin measures can give insight into the dynamics of social relationships: a review. *Front. Behav. Neurosci.* 8, 68. <https://doi.org/10.3389/fnbeh.2014.00068>.
- Damm, B.I., Moustsen, V., Jørgensen, E., Pedersen, L.J., Heiskanen, T., Forkman, B., 2006. Sow preferences for walls to lean against when lying down. *Appl. Anim. Behav. Sci.* 99, 53–63. <https://doi.org/10.1016/j.applanim.2005.09.014>.
- Goumon, S., Illmann, G., Moustsen, V.A., Baxter, E.M., Edwards, S.A., 2022. Review of temporary crating of farrowing and lactating sows. *Front. Vet. Sci.* <https://doi.org/10.3389/fvets.2022.811810>.
- Gröschl, M., 2008. Current status of salivary hormone analysis. *Clin. Chem.* 54, 1759–1769. <https://doi.org/10.1684/abc.2009.0357>.
- Hales, J., Moustsen, V.A., Nielsen, M.B.F., Hansen, C.F., 2016. The effect of temporary confinement of hyperprolific sows in sow welfare and piglet protection pens on sow

behaviour and salivary cortisol concentrations. *Appl. Anim. Behav. Sci.* 183, 19–27. <https://doi.org/10.1016/j.applanim.2016.07.008>.

Jarvis, S., Reed, B.T., Lawrence, A.B., Calvert, S.K., Stevenson, J., 2004. Peri-natal environmental effects on maternal behaviour, pituitary and adrenal activation, and the progress of parturition in the primiparous sow. *Anim. Welf.* 13, 171–181.

Ko, H.L., Temple, D., Hales, J., Manteca, X., Llonch, P., 2022a. Welfare and performance of sows and piglets in farrowing pens with temporary crating system on a Spanish commercial farm. *Appl. Anim. Behav. Sci.* 246 <https://doi.org/10.1016/j.applanim.2021.105527>.

Ko, H.L., Temple, D., Hales, J., Manteca, X., Llonch, P., 2022b. Welfare and performance of post-weaning sows and piglets previously housed in farrowing pens with temporary crating on a Spanish commercial farm: a pilot study. *Animals* 12, <https://doi.org/10.3390/ani12060724>.

López-Arjona, M., Mateo, S.V., Manteca, X., Escribano, D., Cerón, J.J., Martínez-Subiela, S., 2020. Oxytocin in saliva of pigs: an assay for its measurement and changes after farrowing. *Domest. Anim. Endocrinol.* 70, 106384 <https://doi.org/10.1016/j.domend.2019.106384>.

López-Arjona, M., Escribano, D., Mateo, S.V., Contreras-Aguilar, M.D., Rubio, C.P., Tecles, F., Cerón, J.J., Martínez-Subiela, S., 2020a. Changes in oxytocin concentrations in saliva of pigs after a transport and during lairage at slaughterhouse. *Res. Vet. Sci.* 133, 26–30. <https://doi.org/10.1016/j.rvsc.2020.08.015>.

López-Arjona, M., Padilla, L., Roca, J., Cerón, J.J., Martínez-Subiela, S., 2020b. Ejaculate collection influences the salivary oxytocin concentrations in breeding male pigs. *Animals* 10, 1–12. <https://doi.org/10.3390/ani10081268>.

MacLean, E.L., Gesquiere, L.R., Gee, N.R., Levy, K., Martin, W.L., Carter, C.S., 2017. Effects of affiliative human-animal interaction on dog salivary and plasma oxytocin and vasopressin. *Front. Psychol.* 8, 1606. <https://doi.org/10.3389/fpsyg.2017.01606>.

Marcat-Rius, M., Bienboire-Frosini, C., Lezama-García, K., Domínguez-Oliva, A., Olmos-Hernández, A., Mora-Medina, P., Hernández-Ávalos, I., Casas-Alvarado, A., Gazzano, A., 2023. Clinical experiences and mechanism of action with the use of oxytocin injection at parturition in domestic animals: effect on the myometrium and fetuses. *Animals*. <https://doi.org/10.3390/ani13040768>.

Marchant, J.N., Rudd, A.R., Mendl, M.T., Broom, D.M., Meredith, M.J., Corning, S., Simmins, P.H., Terrington, A., 2000. Timing and causes of piglet mortality in alternative and conventional farrowing systems. *Vet. Rec.* 147, 209–214.

Mota-Rojas, D., Martínez-Burnes, J., Trujillo, M.E., López, A., Rosales, A.M., Ramírez, R., Orozco, H., Merino, A., Alonso-Spilsbury, M., 2005. Uterine and fetal asphyxia monitoring in parturient sows treated with oxytocin. *Anim. Reprod. Sci.* 86, 131–141. <https://doi.org/10.1016/j.anireprosci.2004.06.004>.

Mota-Rojas, D., Trujillo, M.E., Martínez, J., Rosales, A.M., Orozco, H., Ramírez, R., Sumano, H., Alonso-Spilsbury, M., 2006. Comparative routes of oxytocin administration in crated farrowing sows and its effects on fetal and postnatal asphyxia. *Anim. Reprod. Sci.* 92, 123–143. <https://doi.org/10.1016/j.anireprosci.2005.04.012>.

Mota-Rojas, D., Marcat-Rius, M., Domínguez-Oliva, A., Martínez-Burnes, J., Lezama-García, K., Hernández-Ávalos, I., Rodríguez-González, D., Bienboire-Frosini, C., 2023. The role of oxytocin in domestic Animal's maternal care: parturition, bonding, and lactation. *Animals*. <https://doi.org/10.3390/ani13071207>.

Pajor, E.A., Weary, D.M., Fraser, D., Kramer, D.L., 1999. Alternative housing for sows and litters 1. Effects of sow-controlled housing on responses to weaning. *Appl. Anim. Behav. Sci.* 65, 105–121.

Pedersen, L.J., Berg, P., Jørgensen, G., Andersen, I.L., 2011. Neonatal piglet traits of importance for survival in crates and indoor pens. *J. Anim. Sci.* 89, 1207–1218. <https://doi.org/10.2527/jas.2010-3248>.

Renaudeau, D., Quiniou, N., Noblet, J., 2001. Effects of exposure to high ambient temperature and dietary protein level on performance of multiparous lactating sows. *J. Anim. Sci.* 79, 1240–1249.

Scatliffe, N., Casavant, S., Vittner, D., Cong, X., 2019. Oxytocin and early parent-infant interactions: a systematic review. *Int. J. Nurs. Sci.* 6, 445–453. <https://doi.org/10.1016/j.ijnss.2019.09.009>.

Singh, C., Verdon, M., Cronin, G.M., Hemsworth, P.H., 2017. The behaviour and welfare of sows and piglets in farrowing crates or lactation pens. *Animal* 11, 1210–1221. <https://doi.org/10.1017/S1751731116002573>.

Uvnäs Moberg, K., Prime, K., 2013. Oxytocin effects in mothers and infants during breastfeeding. *Infant* 9, 201–206.

Wang, C., Han, Q., Liu, R., Ji, W., Bi, Y., Wen, P., Yi, R., Zhao, P., Bao, J., Liu, H., 2020. Equipping farrowing pens with straw improves maternal behavior and physiology of min-pig hybrid sows. *Animals* 10. <https://doi.org/10.3390/ani10010105>.

Weber, R., Keil, N.M., Fehr, M., Horat, R., 2007. Piglet mortality on farms using farrowing systems with or without crates. *Anim. Welf.* 16, 277–279.

Wischner, D., Kemper, N., Stamer, E., Hellbruegge, B., Presuhn, U., Krieter, J., 2009. Characterisation of sows' postures and posture changes with regard to crushing piglets. *Appl. Anim. Behav. Sci.* 119, 49–55. <https://doi.org/10.1016/j.applanim.2009.03.002>.

Yun, J., Swan, K.M., Viennola, K., Farmer, C., Oliviero, C., Peltoniemi, O., Valros, A., 2013. Nest-building in sows: effects of farrowing housing on hormonal modulation of maternal characteristics. *Appl. Anim. Behav. Sci.* 148, 77–84. <https://doi.org/10.1016/j.applanim.2013.07.010>.

Yun, J., Swan, K.M., Viennola, K., Kim, Y.Y., Oliviero, C., Peltoniemi, O.A.T., Valros, A., 2014. Farrowing environment has an impact on sow metabolic status and piglet colostrum intake in early lactation. *Livest. Sci.* 163, 120–125. <https://doi.org/10.1016/j.livsci.2014.02.014>.

Zhang, X., Li, C., Hao, Y., Gu, X., 2020. Effects of different farrowing environments on the behavior of sows and piglets. *Animals* 10. <https://doi.org/10.3390/ani10020320>.