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ANIMAL REPRODUCTION

Risk factors associated with stillbirth in sows

Vamiré Luiz Sens Junior[1,2] Emanoelle Regina Rosa[3] Deivison Pereira Fagundes[4] Vanessa Peripolli[2*] Rafael da Rosa Ulguim[4] Bruna Pereira Siqueira[5] Hérica da Silva Messias[5] Fernando Pandolfo Bortolozzo[4] Ivan Bianchi[2]

[1]SEARA Alimentos LTDA, Itajaí, SC, Brasil.
[2]Programa de Pós-graduação Profissional em Produção e Sanidade Animal (PGPSA), Instituto Federal Catarinense (IFC), 89245-000, Araquari, SC, Brasil. E-mail: vanessa.peripolli@hotmail.com. *Corresponding author.
[3]Instituto Federal Catarinense (IFC), Araquari, SC, Brasil.
[4]Setor de Suínos, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brasil.
[5]Instituto Federal do Norte de Minas Gerais (IFNMG), Januária, MG, Brasil.

ABSTRACT: In this study, the risk factors associated with stillbirth in sows were identified and their odds ratio assessed. For this purpose, 587 farrowings on Farm A and 929 on Farm B were monitored, and the sow parity, body condition score, farrowing duration, total number of piglets born, numbers of live births, stillbirths, and mummified piglets, obstetric interventions, and piglet sex and weight were recorded. At the end of farrowing, piglets classified as stillborn were necropsied to confirm the diagnosis. Consequently, 5.49% of the piglets on Farm A and 5.10% of those on Farm B were stillborn. On both farms, sows with a high parity, prolonged farrowing, and a large litter size had the highest odds ratio of stillbirths. On Farm B, farrowing intervention through the use of vaginal palpation and oxytocin increased the odds of stillbirth by 1.7 and 2.5 times, respectively. Heavy litters increased the odds of stillbirth by 1.4 times. Additionally, low-birth-weight piglets were 2.3 and 3.1 times more likely than their medium-birth-weight and high-birth-weight counterparts, respectively, to be stillborn. In conclusion, on both farms, the risk factors associated with stillbirth were a high parity, a large litter size, and prolonged farrowing.
Key words: parity, farrowing duration, litter size, farrowing intervention.

Fatores de risco associados à natimortalidade em fêmeas suínas

RESUMO: O objetivo deste estudo foi avaliar os fatores de risco associados ao nascimento de natimortos em fêmeas suínas e a razão de chance para a sua ocorrência. Foram acompanhados 587 partos na granja A e 929 na granja B onde foram registrados: ordem de parto, escore de condição corporal, duração do parto, total de nascidos, nascidos vivos, natimortos, mumificados, intervenções ao parto, peso e o sexo dos leitões. Ao final do parto foi realizada necropsia dos leitões classificados como natimortos a fim de confirmar o diagnóstico. A ocorrência de leitões natimortos foi de 5,49% e 5,10% na granja A e B, respectivamente. Fêmeas de maior ordem de parto, com partos prolongados e leitegadas mais numerosas apresentaram maior chance da ocorrência de leitões natimortos em ambas as granjas. Na granja B a necessidade de intervenção ao parto através do uso de ocitocina e palpação vaginal aumentaram a chance da presença de leitões natimortos em 1,7 e 2,5 vezes, respectivamente. Leitegadas pesadas aumentaram em 1,4 vezes as chances de ocorrência de leitões natimortos. No entanto, leitões com menor peso ao nascer aumentam em 2,3 vezes as chances de natimortos, quando comparadas a leitões de peso intermediário, e 3,1 vezes em relação a leitões com maior peso de nascimento. Os fatores de risco associados à ocorrência de natimortos nas duas granjas foram a ordem de parto elevada, leitegadas numerosas e partos prolongados.
Palavras-chave: ordem de parto, duração do parto, tamanho da leitegada, intervenção ao parto.

INTRODUCTION

In industrial pig farming, the annual number of piglets weaned per female is commonly used to express the reproductive efficiency index of the herd, which is mainly influenced by the number of live births and rate of pre-weaning mortality. Considering these factors, genetic selection through the introduction of hyperprolific sows (UDOMCHANYA et al., 2019) has been practiced to increase the number of piglets born, and hence weaned, per female (KOKETSU et al., 2017). However, the number of stillbirths increases with increasing litter size (BORGES et al., 2005).

Stillbirths are considered one of the main causes of reduced piglet numbers, reaching rates of 3–8% (CHRISTIANSON, 1992; RANGSTRUP-CHRISTENSEN et al., 2017; VANDERHAEGHE et al., 2013) and accounting for up to 25% of piglet losses between the farrowing and weaning stages (CUTLER et al., 1992). Although, stillbirths may result from an infection, they are caused mainly by maternal, environmental, and piglet factors (LE

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COZLER et al., 2002). Large litters prolong the farrowing duration, increasing the number of stillborn, especially due to asphyxia (ALONSO-SPILSBURY et al., 2005; BORGES et al., 2005; MOTA-ROJAS et al., 2006; NAAM & SUKON, 2020a). An increase in the incidence of stillbirths was also reported in sows with a high parity number (BORGES et al., 2005), with the sows showing energy deficit at farrowing (LE COZLER et al., 2002) and a high body condition score (VANDERHAEGHE et al., 2013), factors that prolong farrowing. Additionally, the lack of incorrect supervision during farrowing increases the rate of stillbirths (HOLYOAKE et al., 1995; LE COZLER et al., 2002; MOTA-ROJAS et al., 2006).

Given the importance of knowing the risk factors for stillbirth and the impact of sows on herd efficiency, data on stillbirths in hyperprolific sows must be updated to define strategies for mitigating such occurrences in production systems. This study identified the risk factors associated with stillbirth in sows and to assess their odds ratio.

MATERIALS AND METHODS

Animals and recorded parameters

This study was conducted on two commercial pig farms located in the southern region of Brazil. Farm A (24°55′S,50°05′W) houses 5,500 F1 Landrace × Large White sows (Camborough®, Agroceres PIC®, Patos de Minas, Minas Gerais, Brazil) in raised farrowing crates with a plastic floor (1.6 × 2.2 m) in air-conditioned negative-pressure rooms. On this farm, the study was performed between January and February 2018, with 587 farrowings monitored in total. Farm B (27°03′58.9″S, 52°19′16.6″W) houses 2,500 F1 Landrace × Large White sows (TN70, Topigs Norsvin, Videira, Santa Catarina, Brazil) in raised farrowing crates with a plastic floor (1.6 × 2.2 m) in rooms fitted with the Ducto Fan ventilation system. On this farm, the study was performed between March and December 2019, with a total of 929 farrowing monitored.

Data on the age, breed, and parity of the sows were recorded once they had been transferred to the farrowing room. The body condition score of between 1 and 5 (1 = thin; 5 = fat), Caliper score, and ultrasound-assessed back fat thickness at P2 (Renco®) were recorded on Farm A only.

The farrowing was all supervised, with data on the sow parity, total farrowing duration, obstetric interventions (vaginal palpation and use of pharmacological drugs), total number of piglets born, numbers of live births, stillbirths, and mummified piglets, and piglet sex recorded. The farrowing duration was considered to have started with the birth of the first piglet and ended with fetal annexes expulsion. Obstetric interventions were performed when necessary, following the protocol: vaginal palpation was initially used when the sow had already farrowed at least one piglet and the interval between subsequent births was longer than 20 min. If uterine contractions were still absent despite the initial intervention, then 20 IU of oxytocin was administered intramuscular (Oxytocin, Biofarm, Brazil). On Farm B, the individual weight of each piglet and the mean litter weight were also recorded. The piglet sex and weight were recorded within 12 hours after birth. The stillborn piglets were necropsied for their classification as prepartum (piglets with organs showing variable autolysis, intracavitary fluid, and bluish corneas), intrapartum (piglets with meconium on the skin and colostrum in the stomach and without aerated lungs), or postpartum (piglets with aerated lungs, but no colostrum in the stomach, and variable stomach contents) stillbirths, according to the methodology proposed by LEENHOUWERS et al. (2003). On Farm B, the stillborn piglets were subjected to the lung test and classified as antepartum (without aerated lungs) or postpartum (with aerated lungs) stillbirths.

Statistical analysis

The data were analyzed using Statistical Analysis System software (v.9.4, SAS Institute Inc., Cary, NC, USA). Descriptive statistics (mean and standard deviation) and normality and frequency distributions were generated to characterize the occurrence of stillbirths (UNIVARIATE and FREQ procedures). Spearman's rank correlation analysis (CORR procedure) was performed to determine whether the variables under study were associated with stillbirths. All variables that correlated significantly with stillbirths were then categorized and included in the logistic regression model; namely, parity (1, 2–4, and 5–6 on Farm A; and1, 2, and 6–7 on Farm B); use of oxytocin during farrowing (yesor no), vaginal palpation (yes or no), and obstetric interventions (yes or no); farrowing duration (< 300 min and ≥ 300 min); total number of piglets born (<12 piglets and ≥12 piglets on Farm A; < 14 piglets and ≥ 14 piglets on Farm B); and litter weight (< 20.3 kg and ≥ 20.3 kg) and mean piglet weight (<1.308 kg, 1.309–1.625 kg, and ≥ 1.626 kg) on Farm B. Logistic regression analysis was performed using the GLIMMIX procedure with binary distribution. The categorized variables were considered fixed effects. In another approach, the occurrence of stillbirths was

analyzed using the GLIMMIX procedure with binomial distribution, with continuous variables categorized by quartiles. Significance was defined as $P < 0.05$ for all tests, and differences with P-values between 0.05 and 0.1 were considered significant.

RESULTS

The percentages of stillbirths were similar between the farms under study, reaching 5.49%and 5.10% on farms A and B, respectively (Table 1). Of the total percentage of stillbirths on Farm A, 1.73% were classified as prepartum, 3.30% as intrapartum, and 0.45% as postpartum (Table 1). On Farm B, the percentage of postpartum stillbirths (0.41%) was similar to that observed on Farm A. There was no difference between the percentages of antepartum and intrapartum stillbirths on Farm A, with the two categories combined accounting for 5.03% stillbirths. On Farm B, all live (1.46 ± 0.38 kg) and stillborn (1.20 ± 0.41 kg) piglets were weighed, where up on the stillborn were found to be 260 g lighter at birth ($P < 0.001$).

The variables significantly associated with stillbirth on both farms were identified using Spearman's rank correlation method. Some variables, such as parity, farrowing duration, and total number of piglets born, were significantly correlated with stillbirth ($P \leq 0.05$) on both farms. Variables such as oxytocin use, vaginal palpation, and a high litter weight were also significantly ($P < 0.01$) associated with stillbirth on Farm B, reaching 50.76%, 60.00%, and 43.99%, respectively. Broadly speaking, the correlations were weak. Nonetheless, the significant variables were analyzed in detail to understand their effect on stillbirth (Table 2).

On both farms, sows with a parity number higher than 5 and between 6 and 7 were respectively 1.6 and 1.8 times more likely to farrow stillborn piglets than sows with parity ranging from 2 to 4.On Farm B, oxytocin use, obstetric interventions, and vaginal palpation increased the likelihood of piglets being stillborn by 1.7, 1.8, and 2.5 times, respectively. However, the effects of those interventions on the percentage of stillbirth were not identified on Farm A (Table 3). Sows with prolonged farrowing (≥300 min) were 1.8 times more likely to birth stillborn piglets than sows with short farrowing (<300 min). Large litters (≥12piglets on Farm A and ≥14 piglets on Farm B) increased the likelihood of stillbirths by 3.9 and 3.3 times, respectively. On Farm B, heavy litters (≥20.3 kg) were 1.4 times more likely to include stillborn piglets. However, for piglets with a low birth weight (<1.308kg), the risks of being stillborn were 2.3 and 3.1 times higher than for those with a medium (1.309–1.625 kg) and high birth weight (≥1.626 kg), respectively (Table 4). The frequencies of farrowing with stillbirths on farms A and B were 49.40% (parity 5–6) and 48.00% (parity 6–7), respectively. The percentages of stillbirths from farrowing lasting 300 min or longer were 56.00% on Farm A and 48.52%

Table 1 - Description of sow parameters, farrowing performance, and litter characteristics (mean ± SD) in the farms under study.

Parameter	Farm A (587 farrowing)	Farm B (929 farrowing)
----------Sows----------		
Parity	2.94 ± 1.85	2.86 ± 2.07
Caliper score (caliper units)	14.67 ± 2.23	-
Body condition score (1 to 5)	3.82 ± 0.54	-
Backfat thickness (mm)	15.41 ± 3.88	-
----------Piglets----------		
Live births (*n*)	12.11 ± 3.82	13.04 ± 3.43
Stillbirths (%)	5.49 ± 7.94	5.10 ± 8.80
Antepartum stillbirths (%)	1.73 ± 4.76	-
Intrapartum stillbirths (%)	3.30 ± 5.60	-
Postpartum stillbirths (%)	0.45 ± 2.05	0.41 ± 2.2
Mummified piglets (%)	2.38 ± 5.96	2.90 ± 8.15
Total number of piglets born (*n*)	13.27 ± 4.26	14.26 ± 3.53
Litter weight (kg)	-	20.02 ± 4.64
Mean piglet weight (kg)	-	1.48 ± 0.25
Farrowing duration (min)	261.34 ± 155.32	295.43 ± 149.28
Gestation length (days)	115.70 ± 1.41	115.28 ± 1.30

Table 2 - Spearman's rank correlation coefficient of stillbirths by sow parameter, farrowing performance, and litter characteristics in the farms under study.

Variable	Percentage of stillbirths			
	Farm A		Farm B	
	r	P-value	r	P-value
Parity	0.113	0.006	0.085	0.0088
Caliper score (caliper units)	–0.105	0.011	-	-
Body condition score (visual)	–0.081	0.048	-	-
Back fat thickness (mm)	–0.056	0.181	-	-
Gestation length (days)	–0.063	0.129	0.017	0.5907
Farrowing induction	-	-	–0.043	0.1829
Use of oxytocin	-	-	0.106	0.0011
Vaginal palpation	-	-	0.160	<0.0001
Obstetric interventions*	–0.003	0.937	0.140	<0.0001
Farrowing duration (min)	0.163	0.0003	0.183	<0.0001
Total number of piglets born	0.287	<0.0001	0.294	<0.0001
Litter weight (kg)	-	-	0.123	0.0002
Mean piglet weight	-	-	–0.191	<0.0001

*Use of oxytocin and/or vaginal palpation during farrowing.
Supervised farrowings: Farm A (587); Farm B (929).

on Farm B, where as 60.00% and 49.65% stillbirths were from large litters on farms A and B, respectively.

On Farm A, the distribution of the stillbirth-related variables by quartile (Table 5) showed an increased percentage of stillbirths associated with parity (2–4 = 4.10% vs. 6 = 7.29%, P = 0.0002), farrowing duration (161–230 min = 4.31% vs. > 365 min = 8.46%, P < 0.0001), and total number of piglets

Table 3 - Percentage of sows with stillborn piglets, and results from the logistic regression analysis of the association of risk factors with stillbirth (Farm A).

Variable	Sows with SB, n/n (%)	Comparisons	OR	CI (95%)	Adjusted P-value
Parity					
1	42/93 (45.16)	2–4: 1	0.729	0.433–1.225	0.4559
2–4	60/160 (37.50)	5–6: 1	1.186	0.747–1.882	0.7499
5–6	165/334 (49.40)	5–6: 2–4	1.627	1.106–2.294	0.0361
Use of oxytocin					
Yes	40/93 (43.01)	Yes: No	0.888	0.567–1.389	0.6017
No	227/494 (45.95)				
Vaginal palpation					
Yes	53/122 (43.44)	Yes: No	0.897	0.600–1.342	0.5974
No	214/465 (46.02)				
Obstetric interventions*					
Yes	76/177 (42.94)	Yes: No	0.863	0.604–1.232	0.4159
No	219/410 (46.59)				
Farrowing duration(min)					
<300	169/412 (41.02)	≥300: <300	1.830	1.279– .618	<0.0010
≥300	98/175 (56.00)				
Total number of piglets born					
<12	72/262 (27.48)	≥12: <12	3.958	2.786–5.624	<0.0001
≥12	195/325 (60.00)				

SB: stillbirths; OR: odds ratio; CI: confidence interval.
*Use of oxytocin and/or vaginal palpation during farrowing.

Table 4 - Percentage of sows with stillborn piglets, and results from the logistic regression analysis of the association of risk factors with stillbirth (Farm B).

Variable	Sows with SB, n/n (%)	Comparisons	OR	CI (95%)	Adjusted P-value
----------Parity----------					
1	113/296 (38.18)	2: 1	0.817	0.592–1.126	0.4317
2	120/358 (33.52)	6–7: 1	1.495	1.071–2.087	0.0477
6–7	132/275 (48.00)	6–7: 2	1.831	1.325–2.529	0.0007
Use of oxytocin					
Yes	67/132 (50.76)	Yes: No	1.726	1.191–2.500	0.0039
No	298/797 (37.39)				
----------Vaginal palpation----------					
Yes	48/80 (60.00)	Yes: No	2.517	1.575–4.024	0.0001
No	317/849 (37.34)				
----------Obstetric interventions*----------					
Yes	86/167 (51.50)	Yes: No	1.838	1.311–2.577	0.0004
No	279/762 (36.61)				
----------Farrowing duration (min)----------					
<300	201/591 (34.01)	≥300: <300	1.829	1.392–2.403	<0.0001
≥300	164/338 (48.52)				
----------Total number of piglets born----------					
<14	80/355 (22.54)	≥14: <14	3.390	2.516–4.568	<0.0001
≥14	285/574 (49.65)				
----------Litter weight (kg)----------					
<20.3	160/463 (34.56)	≥20.3: <20.3	1.487	1.141–1.939	0.0034
≥20.3	205/466 (43.99)				
----------Piglet weight(kg)----------					
≤1.308	132/233 (56.65)	≤1.308: 1.309–1.625	2.352	1.705–3.246	<0.0001
1.309–1.625	165/462 (35.71)	≤1.308: ≥1.626	3.190	2.174–4.682	<0.0001
≥1.626	68/234 (29.06)	1.309–1.625: ≥1.626	1.356	0.965–1.907	0.1857

SB: stillbirths; OR: odds ratio; CI: confidence interval.
*Use of oxytocin and/or vaginal palpation during farrowing.

born (12–14 = 5.21% vs. > 16 = 7.80%, P < 0.0001) from the second to the last quartile, respectively. However, in the first two quartiles, the percentages of stillbirths were similar for the same variables. Similarly, on Farm B, the percentage of stillbirths increased with increases in parity (2 = 4.42% vs. 6 = 6.93%), farrowing duration (211–262 min = 4.80% vs. > 335 min = 8.25%), total number of piglets born (13–14 = 3.81% vs. > 17 = 9.21%), and litter weight (17.7–20.3 kg = 4.90% vs. >22.8 kg = 6.56%) from the second to the last quartile, respectively. As on Farm A, no significant difference in the percentages of stillbirths was found in the first two quartiles for the same variables. The percentage of stillbirths associated with birth weight (P < 0.001) decreased from 7.61% for the first quartile (light piglets, < 1.308 kg) to 3.57% for the last quartile (heavy piglets, > 1.625 kg). On Farm B, sows that received obstetric interventions (vaginal palpation or oxytocin) also had a higher percentage of stillbirths (8.31%) than those farrowed without interventions (4.89%).

DISCUSSION

Despite that the farrowing performance indicators of farms A and B were similar in this study, the total number of piglets born was higher on Farm B, which increased the mean farrowing duration but not the percentage of stillbirths. Although, characteristics related to farrowing supervision were not evaluated, Farm B probably demanded a greater need for obstetric interventions (positively correlated to stillbirths) owing to the higher total number of piglets born and longer farrowing. The need for oxytocin and vaginal palpation was associated with stillbirths on Farm B only. However, we cannot categorically state whether those interventions resulted in stillbirth or whether dystocia was the

Table 5 - Stillbirth rates on farms A and B, according to different variables classified by quartile distribution.

Variable	----------Farm A (587 farrowing)----------				SEM	P-value
Parity	1	2–4	5	6		
	5.68^{ab}	4.10^{b}	6.67^{a}	7.29^{a}	0.443	0.0002
Farrowing duration (min)	<161	161–230	231–365	>365		
	5.41^{cb}	4.31^{c}	6.16^{b}	8.46^{a}	0.473	<0.0001
Total number of piglets born	<12	12–14	15–16	>16		
	3.40^{c}	5.21^{bc}	6.61^{ab}	7.80^{a}	0.473	<0.0001
Variable	----------Farm B (929 farrowing)----------				SEM	P-value
Parity	1	2	6	-		
	5.32^{b}	4.42^{b}	6.93^{a}	-	0.295	<0.0001
Farrowing interventions*	Yes	No	-	-		
	8.31^{a}	4.89^{b}	-	-	0.207	<0.0001
Farrowing duration (min)	<211	211–262	263–335	>335		
	4.22^{b}	4.80^{b}	4.62^{b}	8.25^{a}	0.343	<0.0001
Total number of piglets born (n)	<13	13–14	15–17	>17		
	3.14^{c}	3.81^{c}	5.65^{b}	9.21^{a}	0.327	<0.0001
Litter weight (kg)	<17.6	17.7–20.3	20.3–22.8	>22.8		
	5.73^{ab}	4.90^{b}	4.75^{b}	6.56^{a}	0.360	0.0022
Piglet weight (kg)	<1.310	1.310–1.470	1.470–1.625	>1.625		
	7.61^{a}	5.28^{b}	4.76^{bc}	3.57^{c}	0.358	<0.0001

SEM: standard error of the mean.
*Use of oxytocin and/or vaginal palpation during farrowing.
Means followed by different lower-case letters in the row differ at 5% ($P < 0.05$).

cause. Thus, when farrowing require obstetric interventions, good practices should be observed. The 5.49% and 5.10% stillbirth rates of farms A and B, respectively, are within the 5.2–7.4% range observed in previous studies (NAAM & SUKON, 2020a; RANGSTRUP-CHRISTENSEN et al., 2017; UDOMCHANYA et al., 2019). However; although, the percentages of stillbirths by number of farrowing on farms A (45.5%) and B (39.3%) corroborated the findings of previous studies conducted in Brazil (BORGES et al., 2005; LUCIA JR et al., 2002), they are lower than those observed in other countries, such as Denmark (RANGSTRUP-CHRISTENSEN et al., 2017), Vietnam (NAAM & SUKON, 2020a), Thailand (UDOMCHANYA et al., 2019), and Japan (TANI et al., 2016).

UDOMCHANYA et al. (2019) observed that stillborn piglets were 173 g lighter at birth than live-born piglets, which was corroborated in our study. A low piglet body weight was also associated with a higher risk of stillbirth, suggesting that small piglets may be less vigorous and at higher risk of asphyxia during farrowing (LEENHOUWERS et al., 1999). Litters with a high mean weight (≥ 20.3 kg) were also associated with stillbirths, because large litters tend to have piglets with a low birth weight, which affects the stillbirth rate (NAAM & SUKON, 2020a).

The total number of piglets born also significantly affected the risk of stillbirth, as described in previous studies in which sows with a litter of more than 9 (NAAM & SUKON, 2020a) or 12 (BORGES et al., 2005; LUCIA JR et al., 2002) piglets had a higher risk of farrowing stillborn. The increased occurrence of stillbirths in larger litters is mainly due to the longer farrowing duration (NAAM & SUKON, 2020a), as farrowing lasting longer than 300 min subject the piglets to unfavorable conditions (ALONSO-SPILSBURY et al., 2005). In this study, prolonged farrowing (≥ 300 min) increased the risk of stillbirth by 1.8 times. This may be related to the farrowing process itself, as intense uterine contractions may promote rupture of the umbilical cord prior to its expulsion, cutting off the blood flow to the placenta and leading to piglet asphyxia and hypoxia (MOTA-ROJAS et al., 2006).

Sows with a parity number ranging from 5 to 7 had a higher risk of farrowing stillborns than those with parities ranging from 2 to 4. Older sows tend to have lower muscle tone (LE COZLER et al., 2002), resulting in poor expulsion of the fetuses and

a prolonged farrowing duration (VANDERHAEGHE et al., 2013; NAAM & SUKON, 2020a).

Obstetric intervention, whether through the use of oxytocin or vaginal palpation, increased the risk of stillbirth. Although, oxytocin is a drug used to promote uterine contractions in sows, its use remains controversial with regard to the rate of stillbirths (NAAM & SUKON, 2020b). Oxytocin administration has been shown to reduce the farrowing interval between piglets and the farrowing duration, decreasing the risk of stillbirth (UDOMCHANYA et al., 2019). However, if the dose exceeds a specific amount or is administered with insufficient cervical dilation, then the risk of farrowing problems and the number of stillborn piglets increase (LE COZLER et al., 2002). In this study, the use of oxytocin increased the likelihood of stillbirth by 1.7 times, possibly as a result of the drug activity, which increases the frequency of uterine contractions but consequently also reduces blood flow through compression or tearing of the umbilical cord, thereby resulting in intrauterine piglet hypoxia (MOTA-ROJAS et al., 2006). Farrowing supervision provides the help needed in cases of dystocia (LE COZLER et al., 2002; VANDERHAEGHE et al., 2013). However, HOLM et al. (2004) observed that prolonged or dystocic farrowing were associated with a greater need for farrowing supervision and a higher percentage of stillbirths. In the present study, the risk of stillbirth increased 2.5 times when vaginal palpation was applied. However, the procedure was performed only when farrowing assistance was required, suggesting that it may not be the causative factor of stillbirth.

CONCLUSION

High parity, a large litter size, and prolonged farrowing were the risk factors associated with stillbirths in sows on two farms in Brazil. Among these risk factors, a large litter size had the highest odds ratio for stillbirths.

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BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

All animal research and experimental procedures of this study were approved by the Comitê de Ética no Uso de Animais of Instituto Federal Catarinense (IFC), Araquari Campus (Protocol No. 265/2018).

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to the design and writing of this manuscript. All authors have critically reviewed the manuscript and approved the final version.

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