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Influence of transcervical infusion of seminal plasma on the farrowing rate and litter size in artificially inseminated sows

[*Influência da infusão transcervical de plasma seminal na taxa de partos e tamanho da ninhada em porcos inseminados artificialmente*]

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ABSTRACT

Recent studies have focused on the use of seminal plasma to increase sow fertility after classical intracervical artificial insemination (AI). The aim of the present study was to investigate the influence of seminal plasma infusion, prior to the application of conventional AI dose, on the fertility rate in sows. A total of 114 sows were treated with intrauterine infusion of 30ml seminal plasma (SP), while 114 control sows were infused by physiological solution (PS), immediately before the application of conventional AI dose. The experiment was conducted at one commercial pig farm in Serbia, which is comprised of 1,500 sows in the breeding herd. Intrauterine infusion of seminal plasma produced significantly ($P<0.05$) higher farrowing rate (93.8%) and significantly ($P<0.01$) more live-born piglets per litter (12.27), compared with the control sows (83.33% farrowing rate and 10.48 piglets). The present results show that intrauterine infusion of seminal plasma can be a useful tool for increasing the fertility rate in artificially inseminated sows, under the conditions of practical intensive pig production.

Keywords: seminal plasma, infusion, AI, fertility, sows

RESUMO

Estudos recentes concentraram no uso de plasma seminal para aumentar a fertilidade de porcos após inseminação artificial intracervical clássica (AI). O objetivo do presente estudo foi investigar a influência da infusão de plasma seminal, antes da aplicação da dose de AI convencional, na taxa de fertilidade de porcas. 114 porcas foram tratadas com infusão intrauterina de 30ml plasma seminal, e 114 porcas de controle receberam infusão de solução fisiológica (PS) imediatamente antes da aplicação da dose convencional de AI. O experimento foi realizado em uma fazenda de porcos comercial na Serbia, que é composta de 1.500 porcas no rebanho de reprodução. A infusão intrauterina de plasma seminal produziu uma taxa de fertilidade (93,8%) significativamente maior ($P<0.05$), e significativamente mais ($P<0.01$) leitões nascidos vivos por ninhada (12,27) comparado com as porcas de controle (83,33% taxa de fertilidade e 10,48 leitões). Os resultados mostram que infusão intrauterina com plasma seminal pode ser uma ferramenta útil para aumentar a taxa de fertilidade em porcas inseminadas artificialmente, sob as condições de prática de produção intensiva de porcos.

Palavras-chave: plasma seminal, infusão AI, fertilidade, porcas

INTRODUCTION

In most European countries, diluted liquid semen in the dose volume of 80ml, with average 3×10^9 motile spermatozoa are used for conventional intracervical artificial insemination (Roca *et al*., 2001; Stančić *et al*., 2009; Khalifa *et al*., 2014). On average, a single boar produces about 20 doses per ejaculate, or 1,200 of such insemination doses annually. This means that a certain number of ejaculates must be overdiluted (Singelton, 2004; Stančić *et al*., 2009).

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However, using overdiluted AI doses is frequently recognized as a reason for reduced fertility in artificially inseminated sows (Gadea, 2005; Alm *et al*., 2006). Namely, it has been found that over dilution of seminal plasma, for example in overdiluted AI doses, leads to significant reduction of spermatozoa fertility (Kommisrud *et al*., 2002; Maxwell *et al*., 2007; Stančić *et al*., 2012). In addition, ejaculate over dilution decreases the concentration of bioactive components in the seminal plasma, which influences the physiological processes important for successful transport and function of spermatozoa, as well as successful fertilization and embryo development in the female reproductive tract (Robertson, 2005, 2007; Rodríguez-Martínez *et al*., 2011; Jalali *et al*., 2014).

Recently, it has been shown that intrauterine infusion of natural (Waberski *et al*., 1996; Capitan *et al*., 2006, Kirkwood *et al*., 2008; Okazaki *et al*., 2012) or synthetic seminal plasma (Predil MR-A®) (Lyczynski *et al*., 2000; Martin Rillo *et al*., 1996; Garcia Ruvalcaba *et al*., 2009; Dimitrov, 2012; Stančić *et al*., 2014), prior to the application of conventional AI dose, increases the farrowing rate and litter size in artificially inseminated sows. Therefore, the objective of this study was to evaluate the influence of natural seminal plasma infusion – prior to the application of conventional AI dose – on the fertility rate in sows in the conditions of Serbian intensive pig production.

MATERIAL AND METHODS

Farm and animals. The experiment was conducted at one commercial pig farm in Serbia, with 1,500 Swedish Landrace sows in the breeding herd, in terms of the regular production cycle. In the year 2014/2015, the average farrowing rate on the farm was 78.6%, with 10.57 live-born piglets per liter. Until AI, the weaned sows were housed in open group pens. Classical artificial insemination (AI) was performed. After AI, the sows stayed in crates for 30 days, and then they were moved to open group pens. Detections of rebreeding (return to estrus after AI) were performed daily, by full contact with a teaser boar, starting on day 14 after AI. About 5 to 7 days before the expected farrowing, the pregnant sows were relocated to individual farrowing pens. Lactation lasted for 28 days.

Since this is research in which only biological material was used, an Ethics Committee was not taken into consideration since the animals used in the research were in the regular intensive production process, from which only written results at end of the reproductive cycle were used.

Experimental sows. The sows used in the experiment included 228 second to fifth farrowing parity Swedish Landrace sows, in which the estrus was detected on the fourth or fifth day after weaning. A total of 228 sows were divided into two groups (114 in each group): treated sows and untreated sows, i.e. the control group. Both groups were equal in parity, body condition, health status and the number of weaned piglets per litter in the previous reproductive cycle.

Seminal plasma preparation. Two Swedish Landrace boars, with a similar fertility rate (litter size and farrowing rate) in the previous exploitation period, were used for seminal plasma obtained and sow insemination. The ejaculates were collected once a week, within the experimental period. The volume, concentration, progressive motility and total sperm number were detected for each ejaculate, immediately after the collection. The ejaculates used for the experiment were only those with minimal 120ml volume, minimal 200×10^6 sperm concentration/ml and minimal 65% progressive motility. After quality evaluation, ejaculates were centrifuged at $3000\times g$ for 15 minutes, at 4°C. The obtained seminal plasma was divided into aliquots of 30ml, placed in plastic flasks with caps, and stored in a freezer at -20°C.

Experimental procedure. Estrus was detected by full contact with a teaser boar, two times a day (07h and 17h), starting about 24h after weaning. After collection and evaluation of standard semen parameters, fresh ejaculates were diluted with *BTS* medium extenders (Minitübe, Germany), in an average 1:4 proportion. On average, 15 AI doses (80ml with 3×10^9 progressive motile spermatozoa) were obtained per ejaculate. Average ejaculate volume was 250ml, total spermatozoa number was 60×10^9, and progressive motility was at least 75%. The

boars were equally used for insemination of the treated sows and the control group of sows.

The frozen seminal plasma doses were thawed in a refrigerator at 4ºC, starting about 24 hours before the start of AI. Immediately before the start of AI, the thawed 30ml seminal plasma doses, conventional liquid AI doses and physiological solution were warmed at 35ºC in a water bath for 30 minutes. Conventional intracervical AI was performed in the 114 experimental sows (treated with seminal plasma) and 114 control (untreated) sows. Insemination of all sows was done with catheters *SafeBlue ClearGlide with PC Cannula*- (Minitübe, Germany).

Insemination was performed by the same boars whose ejaculates were used for preparation of seminal plasma. Immediately before the conventional application of AI dose, the experimental (treated) sows were treated with intrauterine infusion via intracervical catheter for post cervical insemination with 30ml seminal plasma (both at first and second AI). The control sows were infused with 30ml physiological solution (both at first and second AI). The sows were first inseminated 12 hours after estrus detection, and secondly 24 hours after estrus detection. Return to estrus (rebreeding) was detected twice a day by full contact with a teaser boar, starting on day 14 after AI.

Statistical analysis. All data were analyzed using Group *T*-test (statistical analysis systems, package version "Statistics 12"), and presented as mean values ± standard deviations (SD). Farrowing rates (%) were analyzed using the Kruskal-Wallis Test (non-parametric). Average live-born piglets per litter were analyzed by using the Group *T*-test. A value of $P< 0.05$ was considered to be statistically significant.

RESULTS

The relationship between the intrauterine seminal plasma and the farrowing rates is shown in Table 1. The sows treated with intrauterine infusion of 30ml seminal plasma, prior to conventional AI dose application, have significantly ($P<0.05$) higher farrowing rate (93.8%), compared with the control (untreated) sows (83.33%). A significantly ($P<0.01$) larger number of pigs born alive per litter (12.27) were obtained in the sows infused by seminal plasma than in the control sows (10.48) (Table 2).

Table 1. Farrowing rate in the sows after infusion of seminal plasma* ($\bar{x}$ ± SD)

Sows group	Treatment	Sows inseminated (n)	Sows farrowed (n)	Farrowing rate (%)
Treated	30ml seminal plasma + AI dose	114	107	93.80±0.24[a]
Control	30ml physiological solution + AI dose	114	95	83.33±0.37[b]

*Seminal plasma (treated sows) or physiological solution (control sows) were infused immediately before conventional AI dose. AI dose: volume 80ml with 3×10^9 progressive motile spermatozoa.
[ab] Within a column, means without a common superscript differ ($P<0.05$).

Average number of dead-born piglets per litter was not significantly different ($P>0.05$) between the treated (0.71) and the control sows (0.85) and, consequently, total born piglets per litter was significantly ($P<0.01$) different between the treated (12.98) and the control sows (11.33) (Table 2).

Table 2. Litter size in the sows after transcervical infusion of seminal plasma ($\bar{x}$ ± SD)

Group	Treatment	Sows inseminated (n)	Litter size at farrowing (n)		
			live	dead	total
Treated	30 ml seminal plasma + AI dose	114	12.27±2.49[A]	0.71[a]	12.98[A]
Control	30 ml physiological solution + AI dose	114	10.48±1.56[B]	0.85[a]	11.33[B]

[AB,ab] Within a column, means without a common superscript differ ([AB]$P<0.01$; [ab]$P<0.05$).

DISCUSSION

Our results clearly show that intrauterine infusion of 30ml of natural seminal plasma, immediately prior conventional AI dose (volume of 80ml with 4×10^9 spermatozoa) application, provides significantly (P<0.05) higher farrowing rate (93.8%) and live-born piglets per litter (12.27) (P<0.01), compared with the control (untreated) sows (83.33% farrowing rate and 10.48 live-born piglets). Thus, in comparison with the control sows, intrauterine infusion of seminal plasma produced significantly (P<0.05) higher farrowing rate by 10.47%, and significantly (P<0.01) more (aver. 1.79) live-born piglets per litter.

The findings of the present study confirm the results of previous studies in that intrauterine infusion of natural seminal plasma increases the fertility rate in sows (Waberski *et al*, 1997; Capitan *et al*., 2006; Kirkwood *et al*., 2008; Okazaki *et al*., 2012; Waberski *et al*., 1996). An improvement has been reported in both litter size and farrowing rate in gilts with transcervical infusion by seminal and spermatozoa antigens (Murray *et al*., 1983) or by boar semen with killed spermatozoa, prior to regular breeding (Riley, 1999).

Certain authors (Capitan *et al*., 2006) found significantly (P<0.05) higher average number of piglets born alive (12.75 piglets/litter) compared to the control gilts (10.50 piglets), after intrauterine infusion of boar seminal plasma with killed spermatozoa before insemination. Intrauterine infusion of seminal plasma at the beginning of estrus results in significantly (P<0.05) higher farrowing rate (93%) and live-born piglets per litter (12.72), compared with the control sows (88.8% and 10.41 piglets) (Bortolozzo *et al*., 2000). The results of other authors (Stahlberg *et al*., 2001) also demonstrate that transcervical intrauterine infusion of seminal plasma produces significantly higher (P<0.05) farrowing rate in sows (100%) and live-born piglets per litter (13.03), compared with the control sows (93.3% and 12.20 live-born piglets) (Stahlberg *et al*., 2001). These results are very similar to the results in the present study.

Compared with natural mating, when full ejaculate is deposited into the female reproductive tract, artificial insemination involves the deposition of diluted AI dose, with a greatly reduced spermatozoa number and seminal plasma amount (Okazaki *et al*., 2012). Unfortunately, it has been shown that using preserved extensively diluted boar semen for artificial insemination of sows often results in lower fertility rates, compared with natural mating (Tummaruk *et al*., 2000; Gadea, 2005; Alm *et al*., 2006; Stančić *et al*., 2009; Tanavots *et al*., 2012). This evidence suggests that bioactive substances in seminal plasma play an active role in the physiological processes important for sperm function *in vitro* and *in vivo*, fertilization, and embryo development in the female reproductive tract (Strzežek *et al*., 2005; Nasrin *and* Calogero, 2012; Okazaki *et al*., 2012).

Namely, it has been found that overextended seminal plasma reduces the progressive motility of sperm and increases the number of spermatozoa with damaged acrosome and/or disintegrated acrosomal membrane (Kommisrud *et al*., 2002; Maxwell *et al*., 2007; Novak *et al*., 2010; Stančić *et al*., 2012). It has also been demonstrated that seminal plasma influences the spermatozoa transport, survival and fertilization capacity in the female reproductive tract (Waberski *et al*., 2000; Rozeboom *et al*., 2000; Strzežek *et al*., 2005; Chutia *et al*., 2014).

Seminal plasma suppresses the immune response of the uterus against spermatozoa antigens (Waberski *et al*., 2000; Robertson *and* Sharkey, 2001; Langendijk *et al*., 2002; O'leary *et al*., 2004, 2006). Furthermore, some component(s) of seminal plasma advance ovulation and thus improve the chance for successful fertilization (Waberski *et al*., 1997; Madej *et al*., 2013). And finally, seminal plasma affects important physiological mechanisms in uterus for embryo-maternal interactions and establishment of successful pregnancy (Waberski *et al*., 2000; Robertson *and* Sharkey, 2001; O'leary *et al*., 2002; Robertson, 2005, 2007; Jalali *et al*., 2014).

It has been shown that specific proteins of seminal plasma (particularly spermadhesins) play a key role in the above-mentioned processes (Töpfer-Petersen *et al*., 1998; Bortolozzo *et al*., 2000; Centurion *et al*., 2003; Jonáková and Tichá, 2004; Caballero *et al*., 2008; Garcia *et al*., 2009; Kaczmarek *et al*., 2013). It has been found that ejaculates with the highest protein

levels in seminal plasma exhibited the highest fertility rates, compared with ejaculates with lower protein levels (Flowers, 2001; Mogielnicka-Brzozowska and Kordan, 2011)

Some results demonstrate positive correlations between the fertility rate and concentrations of the two specific seminal plasma proteins (Mogielnicka-Brzozowska and Kordan, 2011). Namely, ejaculates with the highest concentration of these proteins produce the highest farrowing rates (86.7%) and the largest number of live-born pigs (11.2), compared to those with lower concentration of these proteins (78.4% and 9.5 piglets born alive). According to these data, the author concluded that quantification of these two proteins in seminal plasma can be used for development of semen fertility tests for boars.

CONCLUSION

The obtained results in the present study demonstrate that transcervical intrauterine infusion of seminal plasma, prior to application of conventional AI dose, improves sow fertility (farrowing rate and litter size), under the conditions of practical Serbian pig production. Additionally, the results of the present study support the opinions of other authors that by using this procedure it is possible to decrease the negative impact of insemination with overdiluted AI doses. This provides an opportunity to increase the number AI doses per ejaculate and per boar anually. Consequently, reproductive exploitation of genetically superior boars can be significantly improved.

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