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Effects of supplemental oxytocin or prostaglandin $F_{2\alpha}$ analogue in extended boar semen on piglet productivity of gilts and sows artificially inseminated in summer

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We determined the effects of oxytocin (OT) and prostaglandin $F_{2\alpha}$ analogue (PG) added to extended boar semen on the duration of artificial insemination (AI) and reproductive performance of pigs bred in July and August (temperate climate of Central Europe). Eighty gilts and second parity sows (G+SP) and sixty-four multiparous sows (M) were divided into three groups. Group OT (11 G+SP and 37 M) and group PG (20 G+SP and 28 M) were artificially inseminated twice (at the onset of estrus and 22–24 h later) using extended semen supplemented with 20 IU of OT or 5 mg of PG, respectively. Thirty-three G+SP and 15 M served as controls (C) inseminated with non-supplemented semen. The mean duration of the first AI was shorter (P<0.05) in M compared with G+SP females inseminated with PG-supplemented semen (80±22 s *vs*. 191±26 s, respectively), whereas the second AI was shorter (P<0.05) in M than in G+SP artificially inseminated with OT-supplemented semen (93±15 s *vs*. 192±28 s). The mean pregnancy rate was lower (P<0.05) in C G+SP (26/33; 85%) compared with OT G+SP females (11/11; 100%). The OT M females had more (P<0.05) stillborn piglets per litter compared with their G+SP counterparts (0.8±0.1 *vs*. 0.1±0.3). In summary, the addition of PG was associated with shorter first AI times in multiparous sows compared with G+SP, but with lower farrowing rates in younger animals. Oxytocin supplementation was associated with a shorter second AI and higher pregnancy rates in young females, but more stillborn piglets per litter in older sows.

Key words: pig, oxytocin, prostaglandin, artificial insemination, summer

Introduction

Seasonality is a primary cause of non-infectious subfertility and abortions in swine herds [21]. Seasonal subfertility is defined as a decrease in piglet productivity of gilts and sows during summer and autumn [1]. The manifestations of seasonal subfertility include delayed onset of puberty in gilts, prolonged weaning-to-estrus intervals, reduced farrowing rates, increased numbers of stillbirths and abortions, and fewer live born piglets per litter [1].

Additionally, circulating progesterone concentrations are lowest in sows during late summer/early autumn, which may indicate a propensity for early pregnancy loss in pigs [34]. Consequently, there is a noticeable decline in net piglet productivity of summer-breeding gilts and sows.

In the wild pig, decreasing day length in late summer and autumn provides a physiological cue to indicate that it is not the optimal time for breeding; this is because after the ~115-day pregnancy, the piglets would be born midwinter and had a lower chance of survival [24]. The domestic pig is a year-round breeder; however, the *Sus scrofa domesticus* has clearly retained some of the reproductive characteristics of its wild ancestor, as there is a sharp atavistic decline in fertility during the late summer and early autumn [34]. In commercial settings, photoperiodic cues are less pronounced or absent, leading to variable responses to the decreasing day length in breeding pigs [4, 3]. Summertime, however, is still the least favorable season for swine reproduction due mainly to heat stress [29]. Elevated temperatures impinge negatively on the reproductive system of sows and boars, manifesting in debilitated uterine and gonadal function [4, 3].

Implementation of reproductive technologies have influenced the way the pigs are raised for pork production [5, 27, 2, 10, 15]. The use of artificial insemination (AI) is a practice that has become widespread in the pig industry [2]. While there are various types of AI used (e.g., intracervical or intrauterine semen deposition), the ultimate goal is to deliver a sufficient number of viable sperm into the oviduct [2, 31, 11]. Any event that can reduce this reservoir of sperm can compromise swine fertility. The problem of seasonal subfertility in swine is further exacerbated by the dilution of ejaculates in semen extenders prior to liquid storage and AI (a.k.a. dilution factor) leading to the reduction in the level of hormones influencing the rate of sperm transport in the female reproductive tract [24]. Therefore, hormonal treatments administered during or through AI may be useful in reducing the effects of seasonal subfertility, and employed to develop strategies aimed at boosting fertility and reproductive health of pigs and their offspring.

Oxytocin has been found to be associated with the mounting reflex, fertilization and the contractions of myometrium; the latter suggesting it is involved in sperm transport in domestic animals. Progesterone and stress factors, including heat stress, block the release of oxytocin, which could be at least partly responsible for the decline in fertility observed in the summer months [12]. Prostaglandins play an important role in the control of ovulation, luteal function, maternal recognition of pregnancy, implantation, maintenance of gestation, parturition, and microbial-induced infections [25]. Addition of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to extended boar semen has slightly increased the reproductive parameters of sows, specifically the conception rate and total number of live born piglets [33, 16]. The mechanisms whereby periovulatory $PGF_{2\alpha}$ affects these parameters have not yet been fully elucidated, but it is possible that the sperm transport after insemination with sufficient amount of $PGF_{2\alpha}$ is accelerated.

The main goal of this experiment was to determine and compare, in a single study, the effects of oxytocin and $PGF_{2\alpha}$ analogue added to boar semen extender on the duration of insemination and reproductive performance of gilts and pigs bred in July and August.

Materials and Methods

All procedures described in this section of the manuscript followed the guidelines contained in the EU Directive 2010/63/EU for animal experiments (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm), albeit the present study conducted in a commercial facility did not require, in compliance with the EU regulations, any additional permits or animal utilization protocols. This experiment utilized gilts and sows of the Polish White Landrace × Polish Large White crosses housed in a commercial facility (maximum capacity of 700 breeding females) situated in the Poland's Łódzkie region. The buildings in farm were mechanically ventilated in response to changes in temperature readings taken by the climate-controlling computer (*Wesstron*, Augustowo, Poland). All animals received fully balanced dry mixes adjusted to their physiological condition. During the period from insemination to gestation day 104 (gd104; transfer to the farrowing unit), the mixes contained 13.5% of crude protein, 6.0% of fiber, 5.3% of ash, 2.3% of oil and crude fats, 0.7% of lysine, 0.22% of methionine, 0.8% of calcium, 0.17% of sodium, and 0.6% of phosphorus. During the period from gd104 until the ensuing weaning or culling, the mixes contained 15.6% of crude protein, 4.5% of fiber, 5.7% of ash, 4.5% of oil and crude fat, 1% of lysine, 0.3% of methionine, 0.9% of calcium, 0.16% of sodium, and 0.7% of phosphorus. For 28 days after the second AI, the animals were kept in individual stalls and then transferred to the group pens of 7–10 animals each, in which they stayed until gd104 (i.e., approximately 10 days before the expected farrowing).

Reproductive management in the farm followed the stringent weekly cycles of operation. Farrowing induced with an injection of bioactive prostaglandin F_{2a} analogue (*Alfaprostol*[a]; *Grabrostim*, Ceva, Polska) typically occurred on Thursday and Friday. Piglets were weaned on Thursday, approximately 28 days *post-partum*, and breeding sows were relocated to the AI sector of the facility. Artificial insemination procedures were then performed on Tuesday and repeated after 22–24 h on Wednesday. Throughout the entire duration of housing in the AI sector, breeding females were exposed to a teaser boar for at least 1.5 h each day and maintained under the 12-h *per diem* light regimen. Ultrasonographic pregnancy check was routinely performed on gestation days 28 and 42 (day 0=second insemination day) using an ultrasound scanner *SonoFarm mini* (*Dramiński*[a] *Ultrasound Scanners*, Olsztyn, Poland).

The research sample consisted of 144 female pigs. The animals were allocated by age to one of the three groups (each n=48):

1) control group (C) (33 gilts and second parity sows (G+SP) and 15 multiparous sows (M)): pigs were inseminated with non-supplemented semen doses;

2) oxytocin group (OT) (11 G+SP and 37 M): pigs were inseminated with inseminates containing 20 IU of oxytocin (*Oxitocinum*; *Biowet*, Puławy, Poland); and

3) prostaglandin $F_{2\alpha}$ analogue (PG) group (20 G+SP and 28 M): pigs were inseminated using semen supplemented with 5 mg of prostaglandin $F_{2\alpha}$ analogue (PG; *Dinolytic*[a]; *Zoetis*, Warsaw, Poland).

Both hormones were added to the plastic bags containing inseminates just before AI, at the doses recommended by manufacturers. The present experiment involved a series of AIs conducted during the summer months of July and August (from 12 July to 17 August). Twenty-four animals were inseminated every week (8 randomly selected females from each hormone supplementation group) using a foam-tipped insemination catheter (*Golden Pig*; *IMV*, L'Aigle, France) (intracervical semen deposition, gravity flow only). Inseminate doses (100 ml; containing 2.8×10^9 of spermatozoa) from Hypor Maxter boars, were purchased from the Boar Utilization Center (*Insefarm*; Śmiłowo, Poland, http://insefarm.pl). The average internal temperatures on the days of AI were 24.6°C in July and 23.6°C in August. The numbers and physical condition of piglets were recorded at farrowing. Live piglets with the birthweight <1 kg were classified as weak piglets.

The proportions were analyzed by χ^2-test (Brandt and Snedecor formula). The remaining numerical data were subjected to normality (Shapiro-Wilk) and equal variance tests, and then analyzed by two-way analysis of variance (ANOVA), with the main effects of animal parity (G+SP *vs.* M) and hormonal supplements used (C, OT or PG), followed by the least significance difference (LSD) test to determine the differences between individual mean values (*SigmaPlot*[a]; *Systat Software Inc.*, San Jose, CA, USA). The level of significance was set at $P<0.05$. All results are given as mean ± standard error of mean (SEM) unless otherwise indicated in table columns or the text of the manuscript.

Results and Discussion

The effects of addition of either PG or OT to the semen doses on the duration of AIs are summarized in table 1. There was a significant main effect of parity (G+SP *vs.* M) and an interaction between parity and hormonal supplement used (PG or OT) on the duration of the first AI. The mean duration of the first AI was shorter ($P<0.05$) in M sows compared with G+SP animals; this difference was seen ($P<0.05$) in PG groups but not in OT or control animals. There was a significant main effect of parity on the duration of the second AI; it was shorter in M than in G+SP animals due mainly to a difference recorded in animals AI'd with OT-supplemented semen.

Table 1. Mean (±SEM) durations of inseminations in summer-bred gilts and sows after the addition of 20 IU of oxytocin (OT) or 5 mg of prostaglandin F_{2a} analogue (PG) to 100-ml inseminate doses

Groups / Variable	G+P				M			
	C	OT	PG	Overall	C	OT	PG	Overall
Duration of 1st AI (s)	160±20	132±35	191±26 a	161±16 a	115±30	114±19	80±22 b	103±14 b
Duration of 2nd AI (s)	138±16	192±28 a	125±21	152±13 a	133±24	93±15 b	93±18	106±11 b

Note. G+SP: gilts and second parity sows; M: multiparous sows; AI: artificial insemination; ab $P<0.05$ (within rows, between G+SP and M animals allotted to the same groups or overall).

Table 2. Reproductive performance of summer-bred gilts and sows after the addition of 20 IU of oxytocin (OT) or 5 mg of prostaglandin F_{2a} analogue (PG) to 100-ml inseminate doses

Groups / Variable	G+SP				M			
	C	OT	PG	Overall	C	OT	PG	Overall
Pregnancy rate	78.8% (26/33) A	100% (11/11) B	90.0% (18/20)	85.9% (55/64)	93.3% (14/15)	97.3% (36/37)	96.4% (27/28)	96.25% (77/80)
Farrowing rate*	69.7% (23/33)	90.1% (10/11)	70.0% (14/20) a	73.4% (47/64)	86.7% (13/15)	91.9% (34/37)	92.9% (26/28) b	91.25% (73/80)
Farrowing rate**	88.5% (23/26)	90.1% (10/11)	77.8% (14/18)	85.4% (47/55)	92.9% (13/14)	94.4% (34/36)	96.3% (26/27)	94.8% (73/77)
Litter size	10.2±0.7	11.2±1.0	11.9±0.9	11.1±0.5	11.8±0.9	12.3±0.6	12.8±0.6	12.3±0.4
Live born piglets/litter	10.0±0.6	11.1±0.9	11.7±0.8	10.9±0.4	11.5±0.8	11.5±0.5	12.3±0.6	11.8±0.4
Stillborn piglets/litter	0.2±0.2	0.1±0.3 a	0.1±0.2	0.1±0.1 a	0.4±0.2	0.8±0.1 b	0.5±0.2	0.6±0.1 b
Weak piglets/litter	0.4±0.2	0.5±0.3	1.2±0.3	0.7±0.2	0.6±0.3	0.6±0.2	1.1±0.2	0.8±0.1

Note: C — control group (animals inseminated with non-supplemented semen). Farrowing rate was calculated as the proportion of all inseminated animals (*) or of the females with ultrasonographically confirmed pregnancy (**). G+P: gilts and second parity sows; M: multiparous sows; ab $P<0.05$ (within rows, between G+SP and M animals allotted to the same groups or overall); AB: $P<0.05$ (between C and OT for G+SP).

The results of the addition of hormones to inseminate doses on the reproductive outcomes in the gilts and sows of the present study are given in table 2. Oxytocin G+SP animals exhibited greater (P<0.05) pregnancy rates compared with their control counterparts (100% *vs*. 78.8%). Mean farrowing rates (expressed as the percentage of all inseminated animals) were greater (P<0.05) in M sows than in G+SP animals inseminated with PG-supplemented semen (92.9% *vs*. 70.0%). The supplementary hormones and/or parity also had an effect on certain aspects of swine fecundity. There was a significant main effect of hormone supplement used on the number of weak piglets per litter. Overall, PG females of all ages had more (P<0.05) weak piglets per litter (1.2±0.2) compared with C (0.5±0.2) and OT groups (0.6±0.2). Lastly, there was a significant main effect of parity on the number of stillborn piglets per litter. Overall, M females had more (P<0.05) stillborn piglets per litter than G+SP group (0.6±0.1 *vs*. 0.1±0.1) and this difference was due mainly to significantly more stillborn piglets per litter in OT M females compared with OT G+SP group (0.8±0.1 *vs*. 0.1±0.1).

Conclusion

The only "direct" effect of the hormones added to the inseminate doses on the fertility parameters of summer-bred pigs was observed in G+SP animals (an increase in pregnancy rates in the OT G+GP group relative to controls inseminated with non-supplemented semen). However, the use of PG and OT may have also "accentuated" the age-related differences in farrowing rates and numbers of stillborn piglets per litter, respectively; within the treatment groups, both these parameters were greater in multiparous sows compared with their younger counterparts.

Oxytocin in the inseminate dose may have stimulated cervical and myometrial contractions and had a positive impact on the movement of spermatozoa in the female reproductive tract [30]. During natural mating, stimulation of the oxytocin-producing hypothalamic neurons occurs because of movements of the boar's penis in the vagina and cervix. The copulation lasts 5–10 min and oxytocin concentrations in the mated sow rise dramatically within 2 min of the ejaculation. This increased oxytocin release results in easier and faster transport of spermatozoa to the oviducts. An earlier arrival of spermatozoa and their accumulation in the uterine tube are associated with better capacitation, fertilization rates and ensuing embryo development [30]. Therefore, this would be the logical reasoning behind the greater pregnancy rates in the OT group when compared the control group of G+SP animals [12]. However, the duration of the first and second AI did not vary significantly between those two subsets of animals. The duration of AI depends on the variation in intensity of uterine contractions [26]; therefore, gilts often take longer to inseminate than sows, which was confirmed in the present study. Similarly, the shorter duration of AI did not impinge on the improvement of reproductive efficacy in M sows compared with the G+SP group in this study, although depositing the semen too rapidly may cause a backflow of semen out of the cervix and vagina [14]. Collectively, the durations of the two AIs within the ranges recorded in this study did not seem to be a factor influencing the fertility of summer-bred gilts and sows.

The addition of PG to inseminate doses was associated with greater farrowing rates of M compared with G+SP animals. This difference appeared to be due mainly to a lower degree of early pregnancy loss as fertility rates calculated for gestating sows did not very significantly among various subsets of pigs studied. Preovulatory LH surges stimulate endometrial and intra-ovarian prostaglandin F_{2a} (PGF_{2a}) release, which in turn stimulates collagenase and elastase for follicular rupture [6]. Prostaglandin F_{2a} administration at the time of insemination leads to advanced ovulation suggesting that the exogenous PGF_{2a} may also advance the time of ovulation through intra-follicular mechanisms. This effect would ensure an ovulation to occur during the insemination period, resulting in a better chance of pregnancy. Prostaglandin F_{2a} was also found to affect sperm motility independently of uterine contractions, therefore it can increase the rate of sperm transport even after applying small amounts of the hormone [6]. Additionally, high concentrations of progesterone, possibly associated with the presence of persistent luteal tissue, have been reported in post-partum sows [13]. In a Dutch field study, 7.9% of sows were found to have serum concentrations of progesterone >3 ng/mL at weaning [13]. Some sows may retain semi active *corpora lutea* even after farrowing, but an association between high progesterone concentrations at weaning and subsequent litter size has not been confirmed. However, a study by [23] indicated that the administration of PGF_{2a} after farrowing improved the performance of sows during the subsequent pregnancy; the treated sows delivered larger litters with more viable piglets than untreated controls.

Diluted semen doses contain lower amounts of seminal plasma and hence reduced amounts of all seminal hormones including estrogens. Estrogens from boar semen increase the myometrial contractility by stimulating $PGF_{2\alpha}$ release [7; 8; 9], but they can also influence the timing of the preovulatory LH release surge and ovulations relative to natural mating or artificial insemination [7, 32, 35].

Gilts typically exhibit a 10 to 15% lower farrowing rate than multiparous sows [28]. Primiparous sows also have a 3 to 5% lower farrowing rates than multiparous sows. Therefore, we cannot rule out the possibility that a lack of differences in major fertility indices between M and G+SP in this study might be due to the presence of equal numbers or even more subfertile females in the M group than in lower-parity groups.

Any stress throughout pig gestation may result in loss of pregnancy and reduced litter size [17, 22]. However, animals selected for their reproductive potential (i.e., not culled due to subfertility) are often less tolerant to the heat than animals with lower reproductive potential [17]. By this logic, the animals with higher reproductive potential would have been the M group animals, with the younger gilts and

sows being less sensitive to the heat and hence retaining more embryos/conceptuses than their multiparous counterparts. However, based mainly on the breeding farm production records, reproductive performance of sows generally increases over the first three to four parities and then begins to decline as they reach the seventh or eighth parity. Therefore, even if OT supplementation resulted in a higher number of ovulations and fertilized eggs, the limited space for the development of fetuses to term may explain, at least partly, a greater number of stillborn piglets in the M group. Since mean litter sizes did not vary significantly between older and younger females in this study, a greater number of stillborn piglets in M compared with G+SP was less likely caused by prolonged farrowing [18, 19, 20], although such a possibility cannot be completely ruled out either.

To summarize, the summer-bred gilts and sows of the present study showed dissimilar reactions to OT and PG added to inseminate doses. The addition of PG to extended boar semen was associated with a shorter first insemination and greater farrowing rates in the M sows compared to G + SP animals. The supplementation of inseminate doses with OT was associated with a shorter second insemination but more stillborn piglets per litter in the M sows compared with G-SP group. Additionally, OT-supplemented semen yielded higher pregnancy rates in G+SP animals compared with their respective controls. This study was not conducted in a controlled laboratory setting but in a commercial facility. Thus, there may have been limitations in that the findings are biased due to standard farm management practices that deviate from artificially controlled environments, but that fact would only increase their applicability for use in practice. Both hormones tested can be used in the breeding and production facilities. Multiparous sows had positive responses to treatment with PG, whereas OT proved to be the most effective treatment for the G+SP group. Interesting questions not addressed in the present experiment would be if OT, PG and/or estrogens used in combination would improve the reproductive capacity of gilts and sows, and if the breed of the boar and semen dilution factor impinge on the effects of exogenous hormones added to boar semen.

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Вплив додавання окситоцину або аналога простагландину $F_{2\alpha}$ до розрідженої сперми кнурів на відтворювальну здатність свинок і свиноматок, штучно осіменених влітку

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Досліджували вплив окситоцину (ОТ) і аналога простагландину $F_{2\alpha}$ (PG), доданих до розрідженої сперми кнурів, на тривалість штучного осіменіння (AI) і репродуктивні показники свиней, осіменених у липні та серпні (помірний клімат Центральної Європи). Вісімдесят свинок і свиноматок другого опоросу (G+SP) і шістдесят чотири свиноматки третього і наступних опоросів (M) розділили на три групи. Групі ОТ (11 G+SP і 37 M) і групі PG (20 G+SP і 28 M) двічі проводили AI (на початку тічки та через 22–24 год.) з використанням розрідженої сперми з додаванням 20 МО ОТ або 5 мг ПГ відповідно. Тридцять три G+SP і 15 M слугували контролем (C), осімененим спермою без добавок. Середня тривалість першого AI була коротшою ($P<0,05$) у M порівняно з G+SP самками, запліднених спермою з додаванням PG (80±22 с проти 191±26 с відповідно), тоді як друге AI було коротшим ($P<0,05$) у M, ніж у G+SP, осіменених спермою з додаванням ОТ (93±15 с проти 192±28 с). Середня запліднюваність була нижчою ($P<0,05$) у C G+SP (26/33; 85%) порівняно з самками ОТ G+SP (11/11; 100%). Самки ОТ M мали більше ($P<0,05$) мертвонароджених поросят на приплід порівняно з їхніми аналогами G+SP (0,8±0,1 проти 0,1±0,3). Отже, додавання PG до розрідженої сперми кнурів призводить до зменшення тривалості першого AI у багатоплідних свиноматок порівняно з G+SP, але знижує запліднюваність у молодих тварин. Додавання окситоцину до розрідженої сперми кнурів скорочує тривалість другого AI та підвищує запліднюваність у молодих самок, але збільшує кількість мертвонароджених поросят на приплід у старших свиноматок.

Ключові слова: свині, окситоцин, простагландин, штучне осіменіння, літо

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