EEFECTS OF DIFFERENT METHODS FOR MYOMETRIAL CONTRACTION STIMULATION PRIOR TO AI ON SOWS FERTILITY

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Abstract: These study compares farrowing rate and litter size in AI sows, treated with different methods for myometrial contraction stimulation, immediately around AI. The total of 249 sows, inseminated within 7 days after weaning, were divided into five groups. Group I: 10 IU oxytocine addition in semen dose immediately before AI (n=50), Group II: i/m vulvar injection of 5 IU oxytocin just prior to AI (n=50), Group III: cervix stimulation with AI catheter (n=49), Group IV: fence-line boar contact immediately around AI (n=50) and Group V: unstimulated AI (control) sows (n=50). Farrowing rate were significant higher (P<0.05) in all stimulated sows groups (92%, 88%, 90% and 84%), compared with unstimulated sows (78%). Subsequent litter sizes were not affected by treatment, ranged between 11.41 and 11.98 liveborn piglets. These results indicate that performed treatment for myometrial contractions stimulation can be useful method to improve sows fertility.

Key words: myometrium, contraction, stimulation, oxytocin, boar, AI, fertility, sow

Introduction

During mating or artificial insemination in sows, semen is deposited intra-cervically. From the site of deposition, sperm cells must be distributed over both horns and transported to the tubal end of the horns, i.e. utero-tubal junction, which serve as a sperm reservoir *(Hunter, 1981)*. The transport of sperm cells through the uterine horns is believed to be a passive process, in which intrinsic sperm cell motility plays no part *(Langendijk et al., 2005; Radović et al., 2006)*. This passive transport is probably driven by the flow of intrauterine fluid containing sperm cells,

due to gravitational force, movement of the sow and uterine contractions *(Scott and Glimpse, 2000)*. Inadequate stimulation of the sow during and after insemination result in reduced myometrial contractions *(Langendijk et al., 2002)* and a poorer sperm cell transport to the oviduct *(Langendijk et al., 2003; Stančić et al., 2006)*. It has been shown that uterine contractions is influenced by dramatically oxytocin concentration increases in the blood of sows, within 2 minutes of the onset of ejaculation by a mature boar *(Levis, 2000; Scott, 2000)*. This blood increasing of endogenous oxytocin is influenced by boar sexual stimuli (olfactory, visual, tactile and auditive) as well as direct stimulation of cervix by penis within the act of cupulation *(Langendijk et al., 2005)*.

An inadequate sperm transport within the uterus result in decreasing the sow fertility *(Langendijk et al., 2005)*. Namely, a sufficient number of spermatozoa in the oviductal sperm cell reservoir, ie. caudal isthmus in the 24-hour period preceding ovulation *(Hunter, 1981)*, is the ultimate factor for successful fertilization *(Soede et al., 1995)*. Any factors that reduce this reservoir may compromise fertility. In the AI, such reduction in the sperm cell reservoir may result of: (a) poor timing of semen deposition relative to time of ovulation *(Kemp and Soede, 1996; Stančić and Šahinović, 2001)*, (b) inadequate stimulation of the sow during and after insemination, resulting in reduced myometrial contractions *(Langendijk et al., 2002)* and a poorer sperm cell transport to the oviduct *(Langendijk et al., 2003; Stančić et al., 2006)*, and (c) excess semen reflux (backflow) during insemination *(Steverink et al., 1998)*. It has been shown that inadequate stimulation of sow during and immediately after insemination is the most common factor that affect the sows fertility rate *(Spronk et al., 1997)*. In farm practice, the reproductive performance of artificial inseminated sows is often lower than that achievable with natural breeding *(Spronk et al., 1997; Stančić, 2000)*. It is often result of inadequate myometrial stimulation, due to the small dose volume, high dilution rate of native ejaculate, as wel as an inadequate stimulation of the sow by boar presence and absence of mechanical stimulation of the cervix *(Langendijk et al., 2003; Beham and Watson, 2005; Kemp et al., 2005; Mezalira et al., 2005; Stančić et al., 2006)*. An adequat myometrial stimulation is most important in the intrauterine insemination technology with reduced dose volume and spermatozoa number per dose *(Roseboom et al., 2004; Mezalira et al., 2005; Stančić et al., 2010; Stančić et al., 2013)*.

The aim of the present study was to investigate whether farrowing rate and litter size can be enhanced by oxytocin addition in semen doses, sow injection by oxytocin, boar presence or by cervix stimulation, immediately around insemination, in the practical artificial insemination technology on pig farms in Serbia.

Materials and Methods

The study was conducted during September to November 2012 in an intensive pig farm, housing about 1,200 sows. Lactation length of herd was average 28 days. Average sows farrowing rate at the farm in 2011 were 76%, and average liveborn

piglets per litter were 10.68. Estrus detection of weaned sows involved full boar contact once daily starting on day 2 after weaning. Immediately after estrus detection, sows were inseminated with 4×10^9 sperm cells in 100 mL dose (BTS1-extender, Minitübe, Germany). Insemination was repeated 24 hours later if sows still exhibited estrous behavior, using disposable Safe Blue® AI catheters, lubricated and single wraped in protective sheaths, sterilized (Minitübe, Germany). Third estrus inseminations were not allowed. Age of semen at insemination was 4-6 hours to 1 day. AI doses were stored in thermo-box at $+17^o$C up to insemination.

At the time of AI (4-5 days after weaning), experimental sows (2 to 5 parity) were assigned to five groups. Group I: AI doses supplemented with 10 IU oxyticin (10 IU/mL wather solution, Oxytokel®, Kelan N.V. - Belgium), immediately before insemination (n=50), Group II: sows injected with 5 $IUmL^{-1}$ oxytocin in the mucosa of the vulvar lips just prior insemination (n=50), Group III: stimulation of cervix by moving the top of the catheter within the cervix, about 1 minute before and 1 minute after sperm deposition (n=49), Group IV: fence-line boar contact with sow immediately before, within and about 5 minutes after insemination (n=50) or Group V: insemination without stimulation, control group (n=50).

Data recorded were farrowing rate after first postlactational insemination and subsequent litter size (liveborn, stillborn and total born piglets).

Obtained data were analyzed by using software package "Statistica 12". Data for litter size were testing by General linear model (GLM) and by LSD test. Farrowing rate was analyzed by test of proportion.

Results and Discussion

Our results demonstrated that farrowing rate were significant higher (P<0.05) after oxytocin addition in AI dose (92%), vulvar oxytocin injection (88%), cervix stimulation (90%) or boar presence (84%), compared with untreated (control) sows (78%).

Table 1. Farrowing rate and litter size in treated and control sows

Group	Stimulation method	Fertility parameters			
		Farrowing rate (%)	Litter size (average ± SD)		
			Liveborn	Stillborn	Total
I	10 IU oxytocin in AI dose (n=50)	92[a] (46/50)	11.41[a]±2.37	0.93[a]±1.29	12.34[a]±2.69
II	5 IU oxytocin injection (n=50)	88[ab] (44/50)	11.52[a]±2.88	0.84[a]±1.14	12.36[a]±2.80
III	Cervix stimulation (n=49)	90[a] (44/49)	11.98[a]±2.96	0.66[a]±0.80	12.64[a]±3.17
IV	Boar presence (n=50)	84[b] (42/50)	11.86[a]±2.56	1.25[a]±1.14	13.12[a]±2.93
V	Without stimulation, control (n=50)	78[c] (39/50)	11.79[a]±2.75	0.77[a]±0.77	12.56[a]±2.78

[a,b,c]Values within a columns, with different superscripts differ (P<0.05). In parenthesis: No. farrowed/No. inseminated.

These value were not significant differ (P>0.05) between sows AI with oxytocin in dose, vulvar oxytocin injection or cervix stimulation. However,

farrowing rate were significantly lower (P<0.05) in sows stimulated by boar presence (84%) compared with oxytocin addition in AI dose (92%) or cervix stimulation (90%) and were not significantly lower (P>0.05) compared with sows injected with oxytocin (88%). Performed treatments has no significant effect (P>0.05) on litter size (Table 1).

The present results show that performed treatment with oxytocin, boar presence and cervix stimulation significantly increase farrowing rate, compared with untreated sows. The lower farrowing rate in sows stimulated by boar presence, compared with oxytocin treatment or with cervix stumualtion could have been caused due to absence of tactile boar stimuli *(Langendijk et al., 2005)*, because fence-line boar contact were performed in the present experiment. But, it is unclear which boar stimuli stimulate maximal uterine activity during estrus *(Gerritsen et al., 2005)*. Litter size was not affected by performed treatments, as it has been shown by other authors *(Gibson et al., 2004; Peláez et al., 2006; Stančić et al., 2006)*.

Establishment the optimal number of spermatozoa in the utero-tubal junction, caudal istmus and the site of fertilization (ampulo-isthmic junction of the oviduct) is the key factor for successful ovulated ova fertilization *(Hunter, 1981)*. Sperm cells have to be transported from the site of deposition to the utero-tubal junction within 15 minutes to 2 hours after deposition through the cervix. The rapid transuterine transport of spermatozoa to the utero-tubal junction and oviduct is extremely important for prevent spermatozoa to being phagocytized (killed) by leukocytes *(Levis, 2000)*. This passive transport is mainly driven by uterine contractions *(Scott, 2000; Umesiobi, 2010; Stančić and Dragin, 2011)*, influenced by dramatically oxytocin concentration increases in the blood of sows, within 2 minutes of the onset of ejaculation by a mature boar *(Levis, 2000)*. Elevation of plasmal endogenous oxytocin is induced by several boar sexual stimuli. These stimuli can be divided into sensory stimuli, i.e. tactile, olfactory, visual and auditory stimuli, on the one hand and seminal plasma-related stimuli (estrogen, oxytocin, prostaglandin $F_{2\alpha}$), on the other *(Langendijk et al., 2003; Langendijk et al., 2005)*. Additionally, the presence of a boar during estrus stimulate the estrus signs expression, particulary standing reflex *(Kemp et al., 2005; Stančić et al., 2008)*. Further more, the boar ejaculate contains high levels of estrogens *(Claus, 1990)*, which stimulates myometrial contractions *(Willenburg et al., 2004)* via an estrogen-induced local release of prostaglandin $F_{2\alpha}$ *(Claus, 1990; Willenburg et al., 2004)*. The synchronization of viable spermatozoa presence in oviduct and the time of ovulation is of extreme importance for successful fertilization. Whole boar semen or seminal plasma has been demonstrated to advance the time of ovulation *(Waberski et al., 2000)*. It is possible that semen-induced cytokines in the uterine lymph undergo counter-current transfer to the ipsilateral ovary and accelerate the final maturation of pre-ovulatory follicles *(O'Leary et al., 2004; Waberski et al., 2006)*.

If oxytocin was included in the semen, farrowing rate were higher (P =0.02) for weaned sows bred only once (84.9%) than for repeat sows (63.7%), but litter size was not affected *(Gibson et al., 2004)*. Authors conclude that inclusion of oxytocin in extended semen may benefit sow fertility when breeding management may otherwise result in a smaller sperm cell reservoir in the oviduct. Addition of 5 to 10 IUmL of oxytocin has no effect on boar sperm motility or morphology in the semen samples *in vitro* stored at +18°C for 56 hours *(Çiftçi, 2005)*. The farrowing rate was 5.7 percent greater for sows inseminated with oxytocin-treated semen (83%) compared to sows injected with oxytocin (77.3%) immediately before insemination. The litter size was 11.50 piglets for sows inseminated with oxytocin-treated semen and 10.97 piglets for sows injected with oxytocin at the time of insemination *(Peña et al., 1998)*. Hormone (estrogens, oxytocin or prostaglandin $F_{2\alpha}$) addition to semen increased numbers of embryos 25 to 30 days after AI. Therefore, in situations of lowered fertility, hormone addition could be a strategy to limit infertility in swine *(Willenburg et al., 2003; Peláez, et al., 2006)*. According to results obtained by other authors, the conclusions from review paper of *Levis (2000)* are: (1) Adding 4 to 5 IU's of oxytocin to a dose of semen improves farrowing rate and litter size, (2) Use of oxytocin treated semen is more effective in multiparous sows than in gilts, (3) During the summer months, oxytocin-treated semen significantly increased farrowing rate and litter size and (4) In most studies, the use of oxytocin at the time of insemination was profitable.

Releasing the endogenous oxytocin and myometrial contraction can also be induced with cervix stimulation by moving catheter within the cervix, immediately before and after AI dose deposition (*Fülöp et al., 1992; Steverink et al., 1998; Grafenau et al., 2005; Stančić et al., 2006*). It has been demonstrated *(Stančić et al., 2006)* that cervix stimulation by catheter immediately before and after insemination, significantly increase farrowing rate (83.3%), in comparison with unstimulated sows (71.1%).

According to mentioned facts, lower farrowing rate, after artificial insemination, my be the result of poorer uterine contraction caused by: (a) inadequate sow sexual stimulation, due to no full boar contact and act of coitus or (b) lower amount of semen oxytocin and estrogen in insemination dose, due to increase dilution rate of ejaculate.

Conclusion

Oxytocin addition to semen (10 IU per dose) immediately before AI, vulvar injection of 5 IU oxytocin just prior to AI, cervix stimulation with catheter or boar presence, significantly increase farrowing rate (92%, 88%, 90% and 84%) compared with untreated sows (78%). Subsequent litter sizes were not affected by treatment.

However, according to results of other authors, this method is controversial and the generalized recommendations for use should be made with caution, since the most profound effects occur in sub-fertile farms or groups of sows, seasonal infertility, and with sub-fertile boars. Nevertheless, in many cases, sows fertility rate are improved.

Uticaj različitih metoda za stimulaciju kontrakcije miometrijuma neposredno pre VO na fertilitet krmača

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Rezime

U istraživanju je izvršena komparacija vrednosti prašenja i veličine legla kod krmača tretiranih različitim metodama za stimulaciju kontrakcija miometriuma, neposredno pre VO. Ukupno 249 krmača, osemenjenih unutar 7 dana posle zalučenja, podeljeno je u 5 grupa. Grupa I: u inseminacionu dozu je dodato 10 i.j. oksitocina, neposredno pre VO (n=50), Grupa II: izvršena je i/m injekcija 5 i.j. oksitocina u vulvu krmača, neposredno pre VO (n=50), Grupa III: stimulacija cerviksa vrhom katetera, neposredno pre i nakon aplikacije inseminacione doze (n=49), Grupa IV: kontakt sa pono zrelim nerastom, neposredno pre, tokom i neposredno posle VO (n=50) i Grupa V: krmače su osemenjene bez ikakve stimulacije, kontrolna grupa (n=50). Vrednost prašenja je bila statistički značajno veća (P<0,05) kod svih stimulisanih krmača (92%, 88%, 90% i 84%), u poređenju sa krmačama koje nisu bile stimulisane (78%). Veličina legla se nije značajno razlikovala između stimulisanih i kontrolnih krmača. Ovi rezultati pokazuju da primenjeni tretmani za stimulaciju kontrakcija miometriuma mogu biti koristan metod povećanja fertiliteta krmača.

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