The Effect of Prostaglandin $F_2\alpha$ on Semen Collection Training in Boars

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ABSTRACT

The objective of this study was to determine the effect of administration of Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) on semen collection training and semen characteristics in sexually inexperienced boars. Boars were moved individually to a semen collection pen and were trained to mount dummy sow. During the first and second semen collection secessions, 4 out of 17 boars and 4 out of remaining 13 boars allowed collection of semen. The 9 boars that failed semen collection from first 2 attempts received immediately 15 mg of $PGF_2\alpha$ i.m. (intramuscular injection) upon entering the collection pen for semen collection resulted in successful semen collection from all 9 boars. Total numbers of spermatozoa were higher in $PGF_2\alpha$ treated boars but there was no significant difference in % motility kinematics characteristics between control and $PGF_2\alpha$ treated groups during 72 hr period. Overall, administration of $PGF_2\alpha$ in sexually inexperienced boars increased the sex drive and facilitated the mounting activity to the dummy sow for semen collection.

(Key words : $PGF_2\alpha$, Semen collection, Boar)

INTRODUCTION

Swine bred by artificial insemination (AI) in Korea has been increased nearly 80% in 2007, and approximately 1,549,000 doses of semen per year are needed for AI. Currently, there are 56 registered AI centers with a total number of 3,000 boars. In associate with changes in breeding management, number of stud boars and semen collection training frequency are being increased for AI. Semen collection training is needed to produce the high quality semen steadily and consistently in short period of time. In most case, semen collection is not a problematic in sexually inexperienced boars after initiation of few trails, but some boars are not allow semen collection and need to train (Szurop *et al.*, 1985). The reasons for unsuccessful semen collection would be adaptation for new environment, intimate relation with semen collector, reduced libido, and diseases (Wetteman *et al.*, 1992). To enhance the semen characteristics and expeditious semen collection in boars displaying decreased sex drive, contacting with other pigs and observing other boars being collected from were applied to stimulate the libido (Hemsworth *et al.*, 1979). Before semen collection, prostaglandin, oxytocin and GnRH were administered to stimulate the libido and influencing semen characteristics (Knight, 1974; Voglmayr, 1975; Ibrahim, 1988). Hashizume *et al.* (1984) reported that the efficient regime for high quality semen collection in studying the correlation between sexual behavior pattern and semen quality, semen collection procedure, and stimulation of sex drive by hormone injection on semen characteristics. Administration of prostaglandin increased the libido, semen quantity, and total number of sperm in pigs (Suzrop *et al.*, 1985). Collected semen after injection of prostaglandin and oxytocin did not affect the semen preservation and fertility in rabbit (Sorgen *et al.*, 1972) and did not affect frozen-thawing semen quality in buffalo (Narsimha *et al*, 1986; Ibrahim, 1988) and in bull (Marshall *et al.*, 1976). Therefore, the objective of this study was to investigate the effect of $PGF_2\alpha$ on training for semen collection and sperm parameters, and thus presents the proper semen collection training regime in sexually inexperienced boars.

MATERIALS AND METHODS

Animal

Seventeen terminal-line Duroc boars raised and selected in National Institute of Animal Science were used. Boars were average of 10 month of age and about 120 kg. Boars were housed individually to a combination of concrete and solid steel rod flooring pen and fed according to the guide line for breeder

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boars of National Institute of Animal Science.

Semen Collection Training

Boars were moved individually to semen collection room and if boars did not mount the dummy sow within 20 min, it scored as a fail. Training was given 3 times in every 4-day interval. Boars failed more than 2 times (9 out of 17), were treated with 15 mg of $PGF_{2\alpha}$ (Lutelyse, Pharmacia, Belgium) i.m. (intramuscular injection) before semen collection training. After semen collection, boars were assigned for next collection schedule and scored whether allowing semen or not. Collected semen samples were transferred immediately to the lab for the analysis of semen parameters.

Semen Dilution and Analysis of Semen Motility

Collected semen samples were evaluated for volume, gel weight, sperm number, and motility. Semen samples were diluted in BTS to achieve a 3×10^7/ml and were stored at 17℃ for 3 days. The percentage of sperm motility and velocity were measured using a computer assisted sperm analysis (CASA) system 3 times in 24 hr interval. CASA was determined as Zeng *et al.*, (2001, Table 1). In brief, 1.5 ml semen sample was incubated for 30 min at 37℃ water bath and 10 μ l of semen was applied to pre-warmed Makler counting chamber (Sefi-Medical, Israel) and were analyzed by SAIS II system (Medical Supply Co. Ltd., Korea) connected with CCD camera (Toshiba, Japan) on microscope (Olympus, Japan) with a warm plate (37℃).

Table 1. Parameters used in SAIS II system

Temperature		37℃
Analysis filter range		0.09
Threshold		1.00
Frame no. per filed		30
Maximum cell size(Pixels)		250
Minimum cell size(Pixels)		15
Minimum motile speed	Motility	5
	Velocity	10
	ALH	10
Sperm motion characteristics	VCL	80
	LIN	65
	ALH	6.5
VAP speed threshold	MEDIUM	50
	Show	20
Maximum velocity(μ m/s)		400
Threshold speed(μ m/s)		10

Statistical Analysis

The Duncan's new multiple range test (SAS Institute, Cary, NC) was used for the comparison of group mean differences. Unless otherwise stated, statistical significance was determined at $p<0.05$ level.

RESULTS

In boars without $PGF_{2\alpha}$ treatment, semen was successfully collected from 4 boars at the 1st and the 2nd training respectively, and remaining 9 boars that failed twice were treated with 15 mg of $PGF_{2\alpha}$ before semen collection training resulted in successful semen collection from all 9 boars (Fig. 1). To see if training was successful from boars that allowed semen previously, semen was collected again after 1 week and found that all boars expeditiously mounted the dummy sow and allowed semen. The percentage of motile sperm and sperm velocity such as VCL, VSL, VAP, LIN or ALH were similar for $PGF_{2\alpha}$ -treated and control boars (Table 2). There was no apparent differences on sperm motility and sperm velocity (VCL, VSL, VAP, LIN, or ALH) measured on same day or 3-day elapsed time after semen preservation in BTS for treated with or without $PGF_{2\alpha}$ (Table 3). Semen volume was higher in control group but cell concentration and total sperm numbers were higher in $PGF_{2\alpha}$

Table 2. Effects of $PGF_2\alpha$ treatment on sperm movement characteristics measured by CASA

Treatment	No. of boars	Mot. (%)	VCL (μ m/s)	VSL (μ m/s)	VAP (μ m/s)	LIN (%)	ALH (μ m)
Control.	8	91.0±4.5	183.3±28.7	75.8±22.1	95.3±24.0	40.9±7.2	7.8±1.4
$PGF_{2\alpha}$ inj.	9	90.8±11.2	208.5±24.6	91.9±24.0	113±19.0	43.8±8.9	8.7±1.3

Values are means±SD.
No significant differences for all parameters.
MOT : motility, VCL : curve linear velocity, VSL : straight line velocity, VAP : average path velocity, LIN : linearity. ALH : amplitude of lateral head displacement.

Table 3. Effects of $PGF_2\alpha$ treatment on sperm motility on elapsed time after semen storage measured by CASA

Treatment	No. of boars	Mot. (%)	VCL (μ m/s)	VSL (μ m/s)	VAP (μ m/s)	LIN (%)	ALH (μ m)
Control day 0	5	92.9±1.9	197.4±19.7	84.4±16.4	104.9±19.4	42.7±6.2	8.6±1.1
$PGF_{2\alpha}$ inj. day 0	8	94.7±2.7	209.4±24.4	84.2±12.8	108.5±14.3	40.2±4.2	9.0±1.2
Control day 3	5	85.4±3.1	189.5±21.6	82.1±12.6	98.6±9.9	43.4±6.7	7.6±0.6
$PGF_{2\alpha}$ inj. day 3	8	86.1±5.1	168.1±29.6	96.2±20.8	104.2±19.9	57.4±8.1	7.2±1.8

Values are means±SD.
No significant differences for all parameters.
MOT : motility, VCL : curve linear velocity, VSL : straight line velocity, VAP : average path velocity, LIN : linearity. ALH : amplitude of lateral head displacement.

Table 4. Effects of $PGF_2\alpha$ on semen characteristics in each training session.

Items		No. of boars	Volume (ml)	Concentration ($\times10^8$/ml)	Total sperm ($\times10^8$ sperm)
Training session	Control	8	108.5±34.8[a]	5.1±1.6[a]	550.8±55.0[a]
	$PGF_{2\alpha}$	9	78.3±35.9[b]	8.6±10.2[b]	673.4±360.1[b]
After training session	Control	8	104.5±29.5	4.2±1.9	444.0±252.6
	$PGF_{2\alpha}$	9	110.0±40.8	4.5±2.0	449.6±175.3

Values are means±SD.
[a, b] Means with different superscript in the column are significantly differ ($p<0.05$).

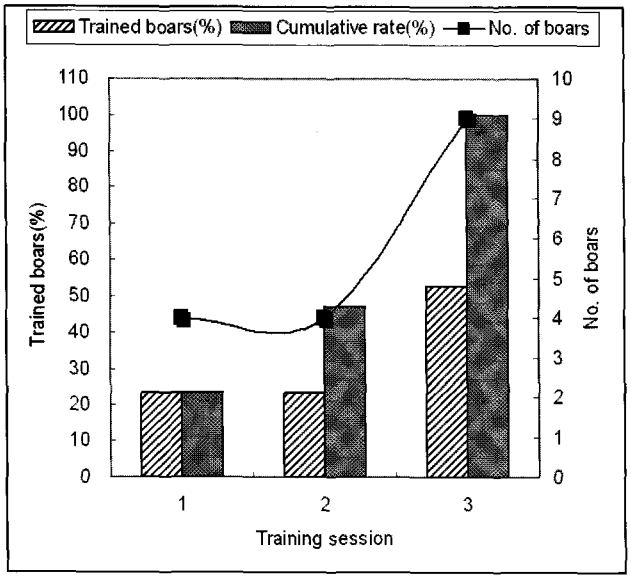

Fig. 1. Cumulative percentages of boars that allow semen collection with or without $PGF_2\alpha$ treatment. Boars were trained to mount a dummy sow for semen collection in each session (n=17). Boar received no treatment during 1st and 2nd session. Lutalyse (15mg) was administered only during 3rd session.

treated boars (Table 4, $p<0.05$).

DISCUSSION

In re-collected semen after completion of semen collection training, semen volume, sperm concentration, and total sperm numbers were not differ between two groups. In accordance with the report of Szurop *et al.* (1985), our study showed that $PGF_{2\alpha}$ treatment to boars that failed semen collection twice, made possible to collect semen, suggesting $PGF_{2\alpha}$ enhances libido and facilitates the dummy sow mounting activity. The exact mechanism by which $PGF_{2\alpha}$ increases sex drive was not known but it speculated that $PGF_{2\alpha}$ increases libido and hormone secretion from testis. However, Fonda *et al.*, (1981) reported that $PGF_{2\alpha}$ treatment increased serum concentrations of prolactin and cortisol, but had no effects on LH and testosterone levels. Although some exogenously administrated prostaglandin products enhance sexual behavior, it is not known if endogenous release of prostaglandin is necessary for libido in boars. Further research need to

find a role of $PGF_{2\alpha}$ in controlling libido and behavioral physiology in boars. In contrast to Sorgen *et al.* (1972) and Lubicz-Nawrocki *et al.* (1973) those who reported that general sperm parameters were not differ but sperm motility was increased in $PGF_{2\alpha}$ treated boars, our data showed that there was no significant difference in sperm motility on same day and 3-day time elapsed after semen collection. Hashizumeand *et al.* (1984) reported that $PGF_{2\alpha}$ treatment increased sex drive, semen volume, and sperm numbers. In contrast to Hemsworth *et al.* (1977; 1979) that reported no beneficial effect of $PGF_{2\alpha}$ on semen volume and total sperm numbers, our study for the effect of $PGF_{2\alpha}$ on sperm characteristics showed that sperm concentration and total sperm numbers were greater in $PGF_{2\alpha}$ treatment group. Estienne *et al.* (2004) reported that boars treated with $PGF_{2\alpha}$ for 16-wk showed decreased reaction time and tendency of gradual increase of semen volume, sperm concentration, and sperm motility. Nevertheless, administration of $PGF_{2\alpha}$ increases the total sperm numbers (Hashizume *et al.*, 2002) and no effect on sperm preservation and motility (Marshall *et al.*, 1976; Narasimha *et al.*, 1986; Ibrahim, 1988). There is no report on negative effects of $PGF_{2\alpha}$ on semen characteristics. In the study of side effect of i.m. injection of $PGF_{2\alpha}$, scratching of the face and neck with hind legs were observed in 10 mg of $PGF_{2\alpha}$ treated boars but soon calmed down. However, in 20 mg of $PGF_{2\alpha}$ treated boars showed severe scratching of the face and neck were observed and one boar showed vomiting activity (Kozink *et al.*, 2002), therefore further study will be needed to determine proper doses of $PGF_{2\alpha}$ for semen collection boars. Based on the findings from this study, we suggested that $PGF_{2\alpha}$ treatment would increase the libido and employ for useful regime when training sexually inexperienced boars. Further research is needed to find factors such as age, season, and breed differences on role of $PGF_{2\alpha}$ on sexual behavior and semen characteristics in boars.

REFERENCES

1. Estienne MJ, Harper AF (2004): Semen characteristics and libido in boars treated repeatedly with $PGF_{2\alpha}$. J Anim Sci 82:1494-1498.
2. Fonda ES, Diehl JR, Barb CR, Kiser TE, Kraeling RR, Rampacek GB (1981): Serum luteinizing hormone testosterone prolactin and cortisol concentrations after $PGF_{2\alpha}$. Prostaglandins 21:933-943.
3. Hashizume T, Niwa T (1984): Effect of administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on the properties of sperm rich fraction of boar semen. Jpn J Anim Reprod 30:182-185.
4. Hemsworth PH, Donnelly J, Findlay JK, Galloway DB (1977): The effects of prostaglandin $F_{2\alpha}$ on sperm output in boars. Prostaglandins 13:933-941.
5. Hemsworth PH, Galloway DB (1979): The effect of sexual stimulation on the sperm output of the domestic boar. Anim Reprod Sci 2:387-394.
6. Ibrahim MA (1988): Influence of oxytocin and prostaglandin on semen characteristics and process of ejaculation in buffalo bulls. Acta Vet Hung 36:3-10.
7. Knight TW (1974): The effect of oxytocin and adrenaline on the semen output of rams. J Reprod Fertil 39:329-336.
8. Kozink DM, Estienne MJ, Harper AF, Knight JR (2002): The effect of lutelyse on the training of sexually inexperienced boars for semen collection. Theriogenology 58:1039-1045.
9. Lubicz-Nawrocki CM, Saksena SK, Chang MC (1973): The effect of prostaglandins E1 and F2 alpha on the fertilizing ability of hamster spermatozoa. J Reprod Fertil 35:557-559.
10. Marshall CE, Hafs HD (1976): Sperm output and quality after $PGF_{2\alpha}$ in bulls. J Animal Sci 43:296-300.
11. Narasimha AV, Haranath GB, Visweswara C (1986): Effect of prostaglandin F2 alpha on libido, seminal quantity and quality of buffalo bulls. Theriogenology 25:689-694.
12. Sorgen CD, Glass RH (1972): Lack of effect of prostaglandin F_2alpha on the fertilizing ability of rabbit sperm. Prostaglandins 1:229-233.
13. Szurop A, Nagy A, Jochle W (1985): Stimulation of libido in pubertal and mature boars with prostaglandin $F_{2\alpha}$ analogs: clinical observations. Zuchthygiene 20:83-86.
14. Voglmayr JK (1975): Output of spermatozoa and fluid by the testis of the ram and its response to oxytocin. J Reprod Fertil 43:119-122.
15. Wettemann RP, Welty S, Bishop DK (1992): An attempt to stimulate sexual behavior of boars. Anim Sci Res Report, Oklahoma Agri Exp Station, Oklahoma State University, Stillwater, OK, 410-412.
16. Zeng WX, Terada T (2001): Protection of boar spermatozoa from cold shock damage by 2-hydroxypropyl-beta-cyclodextrin. Theriogenology 55:615-627.

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