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Effect of Different Inoculation Concentration of *Escherichia coli* on Boar Sperm Quality and Reproductive Performance in Sow

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ABSTRACT

The objective of this study was to determine the effects of *E. coli* on boar sperm quality and reproductive performance in sows after artificial insemination. Three different levels of *E. coli* were artificially inoculated to semen with following concentrations; Control, 500, 5,000 and 50,000 colony forming unit (cfu)/ml. Semen samples were preserved at 17℃ for 5 days. Sperm motility was significantly decreased ($p<0.05$) on day 3 in the group inoculated with 5,000 cfu/ml compared to control groups. In all treatment groups, sperm motility was gradually decreased as storage time increased, but the decline pattern was more drastic in the groups inoculated with 5,000 and 50,000 cfu/ml groups from day 3 ($p<0.05$) compared to control group. After 3 day of storage at 17℃, sperm viability in sample inoculated with the highest concentration (50,000 cfu/ml) of bacteria was less ($p<0.05$) than that of control group. The pH of semen sample pH was maintained 7.2～7.5 in all groups during the experimental period. No differences ($p>0.05$) were found for both storage time and bacterial concentration. The pregnancy rate and live born piglets tend to decrease by increasing the concentration of *E. coli* in semen. In particular, the rate of pregnancy was lower in the group inoculated with 50,000 cfu/ml (58.3%) compare to the other groups (81.8, 75.0, 76.5%). These results suggest that the contamination of *E. coli* in boar semen negatively affects fertilizing ability of boar sperm and the reproductive performance obtained from sows after artificial insemination.

(Key words: Boar, *Escherichia coli*, Semen quality, Reproductive performance)

INTRODUCTION

Artificial insemination (AI) is the technology of reproduction widely used in the swine industry. Therefore, the quality control of the boar fresh and extended semen is fundamental for successful swine AI programs. Bacterial contamination of boar semen can be occurred commonly during routine collection, processing and storage (Althouse and Lu, 2005; Waberski *et al*., 2010). High levels of bacterial contamination in the extended semen are associated with a high incidence of poor sperm motility, damaged acrosomes, sperm agglutination, and decreased sperm longevity (Althouse *et al*., 2000; Althouse and Lu, 2005). Maes *et al*. (2008) also reported that it could result in embryonic or fetal death, endometritis and/or disease in the recipient female.

E. coli is one of the most frequently isolated bacteria in boar semen (Dagnall, 1986; Sone *et al*., 1989; Arredondo *et al*., 2001; Althouse and Lu, 2005) and the presence of *E. coli* in semen might affect semen quality and longevity during preservation and storage (Kuster and Althouse, 1997; Althouse *et al*., 2000; Althouse and Lu, 2005). The diminution of sperm quality by bacterial contamination can result in reductions in reproductive performance and may lead to major economic losses for the swine industry. Although correlations have been reported between *E. coli* contamination and boar sperm quality, the relationship between *E. coli* contamination and reproductive performance after AI have not been determined in Korea.

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Therefore, the aim of this study was to the effect of different concentration of *E. coli* on boar sperm quality during semen storage and reproductive performance in sow after AI.

MATERIALS AND METHODS

Semen Collection and Preparation

Semen samples from Duroc boars raised in National Institute of Animal Science were collected. Boars were used and were average of 18 months of age and about 170 kg body weight. Boars were housed individually to a combination of concrete and plastic flooring pen and fed according to the guideline for breeder boars of National Institute of Animal Science.

Semen samples collected by gloved-hand technique from 6 boars of proven fertility were extended in equal volumes of BTS extender that did not contain antibiotics. The sperm suspension was diluted to a concentration of 3×10^7 cells/ml with extender.

Bacterial Inoculation

Bacteria used in this study was hemolytic and enteroaggregative *E. coli* heat-stable enterotoxin 1 (EAST-1)-positive. *E. coli* was isolated from fresh boar semen collected at dedicated boar studs. The prepared semen samples were split into four fractions. Three samples were inoculated with *E. coli* and one sample used as a control that did not contain any bacterial contamination. Bacterial suspensions of the following initial concentrations were used in the experiment with *E. coli*: 500, 5,000 and 50,000 cfu/ml and a control with extender. Semen samples were incubated at 17℃ during 5 days.

Analysis of Sperm Motility

emen samples were stored at 17℃ for 5 days. Semen sample was incubated for 30 min at 37℃ water bath and 10 μl of semen were applied to pre-warmed makler counting chamber (Sefi-Medical, Israel). Sperm motility was analyzed by CASA system (Medical Supply Co. Ltd., Korea) connected with CCD camera (Veltek, Korea) on microscope (Olympus, Japan) with a warm plate (37℃). Result was expressed as percentage of total motile spermatozoa on the total number of evaluated sperm cells. At least five non-consecutive, randomly selected microscopic fields per sample were scanned by recording at least 400 motile sperm.

Analysis of Sperm Viability

The viability was measured by SYBR-14/Propidium iodide stain. Ten μl of diluted spermatozoa were mixed with 5 μl of the working solution of SYBR-14 and 10 μl of propidium iodide. After incubation at 37℃ for 15 min, a total of 300 spermatozoa were assessed under fluorescence microscope at magnification of × 400. The nuclei of spermatozoa with intact plasma membrane stained green with SYBR-14, while those with damaged membranes stained red with propidium iodide.

Analysis of pH

The pH of each sample was measured using a pH meter (SevenMulti S-47K, Metter-Toledo, Schwerzenbach, Switzerland). To avoid contamination between treatments, the electrode was thoroughly washed before and after each measurement with distilled water. Three measures per treatment were performed in each time point.

Reproductive Performance in Sows after Artificial Insemination

Fifty-six herds of weaned Duroc sows were artificially inseminated. Semen inoculated with *E. coli* was stored at 17℃ for 3 days before artificial insemination (AI). The possible impact of *E. coli* contamination of the semen on reproductive performance was evaluated by calculating the parameters, such as pregnancy rate, farrowing rate, total piglets born and live born piglets.

Statistics

Data were analyzed by ANOVA using the General Linear Models procedure of the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). Differences among treatment means were determined by using the Duncan's multiple range tests. A probability of $p<0.05$ was considered statistically significant.

RESULTS

This study was shown that the effect of different concentration of *E. coli* on boar sperm quality and reproduction performance of sows after artificial insemination (AI). Sperm motility was significantly decreased ($p<0.05$) on day 3 in the group inoculated with 5,000 cfu/ml compared to control groups. In all treatment groups, sperm motility was gradually decreased as storage time increased, but the decline pattern was more drastic in the groups inoculated with 5,000 and 50,000 cfu/ml groups from day 3 ($p<0.05$) compared to control group (Table 1). Similar to the results for sperm motility, sperm viability was an effect of both storage time and bacterial concentration ($p<0.05$). After 3 day of sto-

Table 1. Effect of different infective concentration of *E. coli* on sperm motility (%) over 5 days of storage at 17℃

Storage time (days)	Concentration of *E. coli* (cfu/ml)			
	Control	500	5,000	50,000
0	84.4±4.5^{a,A}	82.5±4.5^{a,A}	81.8±4.6^{a,A}	81.9±2.8^{a,A}
1	81.6±3.0^{a,AB}	82.1±4.9^{a,A}	81.0±2.5^{a,A}	82.1±5.7^{a,A}
3	80.8±4.8^{a,AB}	75.5±3.1^{a,AB}	68.0±2.3^{b,B}	65.4±5.0^{b,B}
5	74.9±4.3^{a,B}	70.7±3.4^{ab,B}	65.3±3.7^{bc,B}	62.2±2.6^{c,B}

^{A,B} Means with different superscripts in same column differ significantly *(p*<0.05*)*.
$^{a\sim c}$ Means with different superscripts in same row differ significantly (*p*<0.05).
Values are expressed as Mean±SD.

Table 2. Effect of different infective concentration of *E. coli* on sperm viability (%) over 5 days of storage at 17℃

Storage time (days)	Concentration of *E. coli* (cfu/ml)			
	Control	500	5,000	50,000
0	86.2±1.4^{a,A}	85.4±2.0^{a,A}	84.4±2.9^{a,A}	81.0±3.9^{a,A}
1	83.7±2.8^{a,A}	82.7±4.9^{a,AB}	81.6±2.1^{ab,A}	75.8±1.8^{b,AB}
3	77.8±2.8^{a,B}	78.2±1.9^{a,BC}	75.2±2.0^{ab,B}	71.3±2.8^{b,B}
5	73.9±4.2^{a,B}	72.7±4.0^{a,C}	69.2±1.0^{ab,B}	65.6±2.9^{b,C}

^{A,B} Means with different superscripts in same column differ significantly *(p*<0.05*)*.
$^{a\sim c}$ Means with different superscripts in same row differ significantly (*p*<0.05).
Values are expressed as Mean±SD.

Table 3. Effect of different infective concentration of *E. coli* on semen pH over 5 days of storage at 17℃

Storage time (days)	Concentration of *E. coli* (cfu/ml)			
	Control	500	5,000	50,000
0	7.5±0.2	7.5±0.2	7.4±0.2	7.3±0.1
1	7.3±0.1	7.4±0.1	7.3±0.1	7.4±0.2
3	7.5±0.1	7.3±0.1	7.2±0.2	7.4±0.1
5	7.4±0.2	7.5±0.2	7.5±0.1	7.5±0.2

No differences (*p*>0.05) were found between treatments.
Values are expressed as Mean±SD.

Table 4. Effect of different infective concentration of *E. coli* on reproductive performance in sows after artificial insemination

Variables	Concentration of *E. coli* (cfu/ml)			
	Control	500	5,000	50,000
Pregnancy rate, % (n/n)	81.8 (9/11)	75.0 (12/16)	76.5 (13/17)	58.3 (7/12)
Farrowing rate, % (n/n)	81.8 (9/11)	68.8 (11/16)	70.6 (12/17)	50.0 (6/12)
Total piglets born*	11.0±3.7	10.5±2.8	9.3±1.7	10.7±2.9
Live born piglets*	8.1±2.9	9.4±2.4	8.1±2.4	7.7±2.3

No differences (*p*>0.05) were found between treatments.
Values are expressed as Mean±SD.

rage at 17℃, the percentage of viable spermatozoa in samples inoculated with the highest concentration of bacteria was less ($p<0.05$) than that of control group (Table 2). The pH of semen sample pH was maintained 7.2~7.5 in all groups during the experimental period. No differences ($p>0.05$) were found for both storage time and bacterial concentration (Table 3). The pregnancy rate and live born piglets tend to decrease by increasing the concentration of *E. coli* in semen (Table 4). In particular, the rate of pregnancy was lower in the group inoculated with 50,000 cfu/ml (58.3%) compare to the other groups (81.8, 75.0, 76.5%).

DISCUSSION

Bacterial contamination of raw boar semen can be occurred commonly during routine collection and processing from boar studs (Althouse and Lu, 2005; Waberski *et al*., 2010). *E. coli* is one of the most frequently isolated bacteria in boar semen (Dagnall, 1986; Sone *et al*., 1989; Arredondo *et al*., 2001; Althouse and Lu, 2005). Several studies have shown that bacterial contamination might affect semen quality and longevity during preservation and storage (Maes *et al*., 2008; Sepulveda *et al*., 2013).

Results of this study showed that the semen quality such as sperm motility and viability decreased by increasing the concentration of *E. coli* in semen samples. Kaur *et al*. (1986) reported that the attachment of *E. coli* to sperm cells might cause reduction of motility and agglutination of sperm cells. In our previous study, we also reported that *E. coli* contamination in extended semen reduced the sperm motility and agglutinated sperm. Also, sperm motility and viability in the experiment done at 17℃ was significantly reduced even day 0 from the group inoculated with 50,0000 cfu/ml *E. coli* (So *et al*., 2011).

Office of International Epizooties (OIE) has established the standard that 5.013×10^3 cfu/ml as a limit for the bacterial contamination in semen samles (2001). Maroto Martin *et al*. (2010) reported that litter size was reduced significantly when semen used for AI is contaminated with *E. coli* above a threshold value of 3,500 cfu/ml. In present study, the pregnancy rate and live born piglets tend to decrease by increasing the concentration of *E. coli* in semen samples. In particular, the rate of pregnancy was lower in the group inoculated with 50,000 cfu/ml compare to the other groups. This result is similar to those obtained by Mirjyn (1999) who reported that the presence of *E. coli* contamination in semen samples is able to produce a significant reduction of sperm quality and affects its fertilizing ability.

Our data showed that the contamination of *E. coli* in boar semen negatively affects fertilizing ability of boar sperm and the reproductive performance obtained from sows after artificial insemination. Thus, strict hygienic management should be considered in boar studs during semen production process to minimize the bacterial contamination of commercial semen doses and the economic losses for the swine industry.

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