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Evaluation of Extended Boar Semen after Glass Wool Filtration

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Abstract : The purpose of this study was to select high-quality boar semen after the glass wool filtration of extended boar semen. After collecting boar semen, its concentration, morphology, viability, and motility were examined according the glass wool's height and time. After glass wool filtration, the sperm concentration decreased, but the proportion of normal sperms and the sperm viability increased. Nevertheless, the sperm motility showed no changes. The above results showed that the glass wool filtration of boar semen is a method of obtaining sperms with relatively low abnormal rates and high viabilities.

Key words **:** boar semen, glass wool filtration, artificial insemination.

Introduction

Artificial insemination (AI) is applied to all animals bred for industrial purposes. In particular, it is a highly important technology for the breeding of cattle and swine. This method is already producing a number of calves in cattle through AI using frozen semen. However, in the case of swine, AI has rapidly increased since the mid-1990s. In Europe, 98% of swine are produced via AI (8). In the US, 80 to 90% of swine are produced via AI (13). The rates of pregnancy after AI vary ranging from 45 to 80% (13,19). This can be explained by various factors including the duration of AI, the quality of sperms, the skills of AI technicians, and hygienic conditions (22,23).

The selection of sperms with good fertilizing capacity is essential for AI. Techniques used to obtain such sperms include swim-up (14,17), glass wool (7,15,18), sephadex filtration (3,6) and sperm separation using Percoll (9). These methods may result in a wide variety of semen qualities according to differences in species and specific ways of using the methods. Bussalleu *et al*. (4) researched the qualities of boar semen using various types of column matrix such as glass wool, glass bead, and ion sephadex. While these studies examined the sperm motility and abnormal rate after filtration, a limited number of studies have evaluated the conditions of sperms according to their storage time. In addition, using columns is time-consuming and costly. Therefore, the purpose of this study was to evaluate and verify the conditions of boar semen with time using the glass wool filtration technique which is inexpensive and prompt.

Materials and Methods

Semen samples

Semen used in this study were obtained by collecting only concentrated semen from 14 to 18-month-old Hampshire swine using the hydraulic method once a week, which were bred at a farming house in Jeju-do. The collected semen was first diluted using the Beltsville thawing solution, and then was moved to a laboratory and underwent an experiment

Glass wool filtration

Glass wool was inserted into a 50 ml disposable syringe at heights of 1 cm and 1.5 cm to separate boar sperms. The glass wool was repeatedly washed with ultra-pure distilled water to remove pieces of glass inside it, and then was dried and used after Ethylene Oxide (EO) sterilization. Each 50 ml of the extended semen was led to flow into the syringe filled with glass wool of 1 cm and 1.5 cm height, respectively, and then was filtrated for 5 to 10 minutes. After glass wool filtration, a test on the sperm vitality with time was performed immediately, three hours, and six hours after the process, respectively.

Semen evaluation

The sperm concentration was counted using a hemocytometer. A sperm morphology test was performed by employing the eosin-nigrosine staining technique. On the test day, each sample of 5 μl liquid semen was mixed with 5 μl eosin-nigrosine on a slide, and then the mixture was dried for 30 seconds and observed using an optical microscope. The shapes of the sperms were divided into three parts: head, mid-peace, and tail. Their signs of abnormality were then observed. The sperm viability test was conducted using the same method as that used for the morphology test, and the sperms' survival was determined by whether or not their head was stained. Each slide contained 200 sperms. Their motility was evaluated by measuring only total spermatozoa motility (MOT), average path velocity (VAP), straight velocity (VSL), and curvilinear velocity (VCL) using the computer assisted sperm analysis (CASA, Sperm 2.1, VideoTesT, Russia).

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Statistical analysis

The statistical significance ($p < 0.05$) of the sperm motility and viability measured by group in this experiment was verified using paired T-tests (Excel, Microsoft).

Results

The results of examining the concentration of the boar semen with time after glass wool filtration are shown in Table 1. The glass wool filtration group showed an overall statistically significant lower concentration than the control group.

Table 1. Semen concentration after glass wool filtration

	Control	Glass wool filtration (depth)	
		1 cm	1.5 cm
Concentration ($\times 10^6$/ml)	1276.6 ± 423.9[a]	873.2 ± 51.4[b]	819.3 ± 41.8[b]

[a,b]Values with different superscripts are significantly different ($p < 0.05$). Data are shown as mean ± SD.

Table 2. Morphology of sperm after glass wool filtration with different depth

Morphological parameter	Control	Glass wool filtration (depth)	
		1 cm	1.5 cm
Normal sperm (%)	70.3 ± 4.7[a]	88.6 ± 4.3[b]	89.9 ± 2.6[b]
Abnormal head (%)	2.2 ± 2.8	0.2 ± 0.3	0.3 ± 0.3
Abnormal midpiece (%)	21.5 ± 6.7[c]	8.7 ± 5.0[d]	7.8 ± 2.8[d]
Abnormal tail (%)	5.9 ± 2.9	2.5 ± 1.5	2.0 ± 1.5

[a,b,c,d]Values in the same row with different superscripts are significantly different ($p < 0.05$). Data are shown as mean ± SD.

Table 3. Viability of sperm after glass wool filtration with different depth

Time	Control	Glass wool filtration (depth)	
		1 cm	1.5 cm
0 h (%)	75.5 ± 4.3[a]	88.5 ± 1.3[b]	87.1 ± 2.0[b]
3 hrs (%)	73.2 ± 2.4[a]	87.4 ± 2.1[b]	85.6 ± 1.5[b]
6 hrs (%)	72.7 ± 1.5[a]	86.9 ± 1.1[b]	85.2 ± 0.9[b]

[a,b]Values with different superscripts are significantly different ($p < 0.05$). Data are shown as mean ± SD.

Moreover, the glass wool filtration group exhibited that the 1 cm-high column filtration group yielded a slightly higher concentration of sperms than the 1.5 cm-high column filtration group. However, no statistically significant differences were detected between the two groups.

The results of the sperm morphology test after glass wool filtration are shown in Table 2. The proportion of normal sperms was statistically significantly higher in the glass wool filtration group than in the control group ($p < 0.05$). Differences according to the height of glass wool were not found. In addition, the control group showed an overall statistically significant higher abnormal rate than the glass wool filtration group in the mid-piece of sperms. While the control group exhibited higher abnormal rates than the glass wool filtration group in the head and tail, this result was not statistically significant.

The results of examining the viability of the boar sperms with time after glass wool filtration are shown in Table 3. The comparison of the sperm viability with time revealed that the glass wool infiltration group yielded an overall statistically significant higher level of viability than the control group.

The results of the CASA on the sperm motility with time after glass wool filtration are shown in Table 4. While the control group showed an overall higher level of motility than the wool filtration group, the result was not statistically significant. Both groups showed gradual declines in motility with time.

Discussion

The selection of sperms with good fertilizing capacity is essential for the AI of swine. Various techniques are currently used to obtain such high-quality sperms. The purpose of this study was to evaluate and verify the conditions of boar semen with time by employing the glass wool filtration technique, which is inexpensive and prompt, to improve the quality of boar semen. This study created 1 cm and 1.5 cm-high columns of glass wool using syringes and examined the sperm concentration, morphology, viability, and motility according to the time consumed for the sperms to pass each column of glass wool.

The glass wool filtration group showed statistically significant decreases in sperm concentration compared to the control group. This is a similar result to those reported by other studies (2,5,11). In addition, while statistically significant dif-

Table 4. CASA movements of sperm after glass wool filtration with different depth

	Control			Glass wool filtration (depth)					
				1 cm			1.5 cm		
	0 hr	3 hrs	6 hrs	0 hr	3 hrs	6 hrs	0 hr	3 hrs	6 hrs
MOT (%)	75.3 ± 12.9	66.7 ± 10.7	52.5 ± 12.8	70.8 ± 14.1	61.4 ± 13.4	55.4 ± 12.5	72.2 ± 13.4	62.5 ± 15.5	58.4 ± 11.3
VAP (μm/s)	55.1 ± 13.0	53.4 ± 16.2	49.5 ± 15.6	52.6 ± 11.0	49.3 ± 9.2	45.1 ± 18.1	59.8 ± 17.5	51.5 ± 11.9	48.2 ± 18.5
VSL (μm/s)	51.5 ± 14.2	51.3 ± 15.8	51.8 ± 18.8	51.3 ± 12.5	48.7 ± 11.1	49.4 ± 11.8	50.3 ± 14.6	49.5 ± 13.8	48.8 ± 16.1
VCL (μm/s)	86.8 ± 5.6	74.2 ± 17.5	65.4 ± 14.5	75.9 ± 14.3	65.7 ± 13.2	55.7 ± 15.3	78.8 ± 13.0	68.2 ± 19.2	67.1 ± 18.1

MOT; total motility, VAP; average path velocity, VSL; straight line velocity, VCL; curvilinear velocity.

ferences according the height of glass wool were not detected, the 1.5 cm-high filtration group showed an overall slightly lower concentration of sperms than the 1 cm-high filtration group.

The results of the sperm morphology test exhibited that the glass wool filtration group had a statistically significant higher proportion of normal sperms than the control group ($p < 0.05$), but this group showed no differences according to the height of glass wool. In addition, in terms of abnormal rates, the control group exhibited a statistically significant higher abnormal rate than the glass wool filtration group in the midpiece of the sperms. This group also showed higher abnormal rates than the glass wool filtration group in the head and tail, but this result was not statistically significant. This outcome is probably because sperms with abnormalities were screened through the filtration due to their low motility. Other studies reported differences according to the filtration column's ingredients (1,10). A study noted that the use of glass beads lowered the frequency of abnormalities in the head of sperms (4). On the other hand, another study reported that filtration using the column with Sephadex-50 reduced the frequency of abnormalities in the tail of sperms (16).

As this shows, the sperms' frequency of abnormality was differently exhibited depending on the column's ingredient. This study, which used glass wool, also showed a similar result to those reported by previous researchers.

In this study, when the viability of boar semen was assessed immediately, three hours, and six hours after glass wool filtration, the glass wool filtration group showed statistically significant higher viabilities than the control group with time. This result may have been due to the screening of dead sperms which had already lost their motility during the filtration process. However, for both group, declines in sperm viability with time could not be verified. This result differed from the pre-test prediction that as the semen would be slightly damaged during filtration, the grass wool filtration group's viability would decrease at higher degrees than the control group with time. As this shows, no differences found between the two groups may imply that while damage to the sperms during the filtration may have influenced reduction in their vitality, this process did not lower their viability within a short period of time. This also coincides with the result of Samper (21) who reported in his study that after the glass wool filtration of boar semen, its viability was reduced more than that of the control group. In terms of the thickness of glass wool, while a thicker column of glass wool resulted in a corresponding lower abnormal rate and a slightly higher viability, this result was not statistically significant. Therefore, when selecting the column's thickness, even the sperm vitality and filtration time should be considered. The boar sperm motility is largely affected by external temperatures, and thus unlike many other animals, it cannot be considered an appropriate indicator for fertilizing capacity. Roldan (20) and Bussalleu *et al*. (5) reported in their studies that after filtration, the sperm motility declined, but the sperm membrane permeability and mitochondrial activity increased or did not change. The present experiment showed no differences in sperm motility between the glass wool filtration and control groups after the filtration. However, unfortunately, the present study could not measure the sperm membrane integrity or mitochondrial activity, and direct comparisons of the sperm motility resulting from this study with the results of other studies may be problematic.

The above results showed that the glass wool filtration of boar semen can be a method of obtaining sperms with relatively low abnormal rates and high viabilities.

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Glass Wool Filtration 후 돼지정액의 평가

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요 약 : 돼지의 희석정액을 glass wool filtration 한 후 양질의 정자를 선별하고자 실시하였다. 돼지의 정액을 채취한 후 glass wool 높이와 시간에 따른 정자의 농도, 기형율, 생존율, 그리고 운동성을 조사하였다. Glass wool 여과 후 정자의 농도는 줄었으나, 정상 정자비율과 생존율은 상승하였고, 운동성의 변화는 나타나지 않았다. 이상의 결과들을 통해 돼지 정자의 glass wool 여과는 상대적으로 낮은 기형율과 높은 생존율을 가진 정자를 얻을 수 있는 방법임을 알 수 있었다.

주요어 : 돼지정액, glass wool filtration, 인공수정