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Study on Preservation of Boar Semen and Bacteria Contamination in Kandal Province

Sophorn Kimsan[1,3], Bunthon Chea[1], Manay Ith[1], Kouch Theng[1], Kroesna Kang[1] and Songchhang Kang[2]

1. Faculty of Veterinary Medicine, Royal University of Agriculture, Phnom Penh 12400, Cambodia

2. National Institute of Education, Phnom Penh 120101, Cambodia

3. Graduate School, Royal University of Agriculture, Phnom Penh 12400, Cambodia

Abstract: The breeding program is progressing steadily, with increasing the awareness among farmers regarding the its critical role in improving productivity, promoting rapid growth, enhancing disease resistance, and boosting household income. At the same time, the dissemination of AI (Artificila Insemination) technique among farmers has been active and effective. For the application of the AI, the quality of boar semen is extended in BTS (Belts Ville Thawing Solution) for more longer preservation period. The study purpose is to evaluate sperm survival time and bacterie contamination in boar semen. Six boars with different breed around 3 years old were used for this study as two Durocs, two Landraces and two Yorkshires. In evaluation sperm survival time in extender on each boar was designed by CRD (Completely Randomized Design) and calculating the survival time of spermatozoa was done after semen dilution and the sperm mobility was determined. The experimental BTS compositions are glucose, sodium bicarbonate acid, trisodium citrate, ethylenediaminetetraacetic acid, potassium chloride and distill water. In the same time, eighteen sample semen ware collected and used for detection of bacterial contamination. The results showed that the survival time of spermatozoa with its straight mobility more than 50% between the commercial BTS and BTS-KH was from 52 to 56 h. They are not significantly different ($p > 0.05$). And the total survival time of spermatozoa was from 92 to 98 h, they are also not significantly different ($p > 0.05$). The survival time of spermatozoa with its straight mobility more than 50% between the Yorkshire, Landrace and Duroc was 55 h 5 min, 54 h 25 min and 50 h 18 min respectively. They are significantly different ($p > 0.05$). The total survival time of spermatozoa between the commercial BTS and BTS-KH was 97 h 19 min, 91 h 29 min and 92 h 26 min respectively. They are also not significantly different ($p > 0.05$). The main bacteria isolated from the diluted semen were gram-negative bacteria, *Escherichia coli* 27.78% was contaminated with an average 6.4×10^6 CFU/mL, and the family *Pseudomonaceae* was 11.11% with 8.3×10^6 CFU/mL. In conclusion, the comparative results of the survival time of spematozoa during the breeding period ($t = 0.5$) between the breeds and extender are similar. For bacteria contamination isolate from the semen all breeds may be due to direct and environmental factors. It is a rainy season with heavy rains that can cause infections.

Key words: Pig breed, BTS, survival time, bacteria contamination.

1. Introduction

Cambodia is a country in mainland Southest Asia. It borders Thailand to the northwest, Laos to the north, Vietnam to the east, and has a coastline along the Gulf of Thailand on the southwest. It cover a total area 181,035 km^2. The country is situated in its entirety inside the tropical Indomalayan realm and the Indochina zone.

In 2021, approximately 63 percent of Cambodia's people are farmers with 23 percent of agiculture land. Crop activity was reported to be 92 percent, while 72 percent reported livestock and poultry raising. And estimated 21 percent was involved in fishery activity and 4 percent in aquaculture [1-3].

Swine production is important for food supply. In 2022, the number of pigs was 2.02 million thousand heads, local pork supply was at level of 109 thousand tonnes in 2021, up from 107 thousand tonnes in 2020, this is a change of 1.87 percent but swine value chain faces numberous challenges to its competitiveness that is well documented in previous study as high swine mortality limited factors effectiency, aggravated by

Corresponding author: Kouch Theng, Ph.D., research field: animal science and veterinary medicine.

poor breed, natural breeding, feeding option [1]. Currently, breeding program is aslo progressing steadity as most farmers are becoming more aware of animal breeding as a key factor in high yield, rapid growth, disease resistance and high household income. At the same time, the dissemination of AI (Artificila Insemination) technique among farmers has have been active and effective [4].

For application of AI, the quality of boar semen is extended in BTS (Belts Ville Thawing Solution) for more longer preservation period [5]. The purpose of this study is to evaluate sperm survival time and bacterie contamination in boar semen.

2. Material and Method

2.1 Study Area

The study was conducted in at pig breeding farm, located in Takhmao city, Kandal province, 12 km south of Phnom Penh City during rainy season from June to august, 2023.

2.2 Animal and Study Method

Six boars with different breed around three years old were used for this study as two Durocs, two Landraces and two Yorkshires. In evaluation sperm survaval time in extender on each boar was designed by CRD (Completely Randomized Design) (Table 1).

2.3 Parameters of the Study

Method of calculating the survival time of spermatozoa was done after semen dilution and the sperm mobility was determined with the fallowing formular as:

$$Sa = \sum at$$

where:

Sa: Index of survival time of spermatozoa in maximum level.

a: interval of activity or mobility of spermatozoa; and

t: real time of spermatozoa activity or mobility follow by:

$$t_n = \frac{t_{n+1} - t_{n-1}}{2}$$

where:

t_{n-1}: time of semen storage in initial time;

t_{n+1}: time of semen storage in last time.

The experimental BTS compositions are glucose, sodium bicarbonate acid, trisodium citrate, ethylenediaminetetraacetic acid, potassium chloride and distill water.

In the same time, eighteen sample semen were collected for detection of bacterial contamination.

2.4 Statistical Analysis

The collected data were recorded and analyzed. All data are shown as the mean ($\bar{X}$) standard deviation ($S\pm$). ANOVA (Analysis of Variance) was performed using SPSS (Statistical Package for the Social Sciences).

3. Result

3.1 Survival Time of Sperm

Table 2 shows that the survival time of spermatozoa with its straight mobility more than 50% between the commercial BTS and BTS-KH was 55 h 14 min and 52 h 19 min, respectively. They are not significantly different ($p > 0.05$).

Table 3 shows that the total survival time of spermatozoa between the commercial BTS and BTS-KH was 98 h 19 min and 92 h 13 min, respectively. They are also not significantly different ($p > 0.05$).

Table 1 Disign of study by completely rendomized design.

D-C	Y-C	L-C	D-C	Y-Kh	L-Kh
Y-C	L-C	D-C	Y-C	L-Kh	D-Kh
L-C	D-C	L-C	L-C	D-Kh	L-Kh

D: Duroc breed, L: Landrace breed, Y: Yorkshire breed, C = Commercial BTS, Kh: Experimental BTS (BTS-KH).

Table 2 Survival time (h) of sperm depending on semen extender ($t = 0.5$).

Parameters	Commercial BTS	BTS-KH
n	9	9
$\bar{X}$	55.23	52.32
$S\pm$	15.25	14.30
p	> 0.05	

Table 3 Survival time (h) of sperm depending on semen extender (t = total).

Parameters	Commercial BTS	BTS-KH
N	9	9
$\bar{X}$	98.32	92.21
S±	15.52	18.31
p	> 0.05	

Table 4 Survival time (h) of sperm depending on breeds (*t* = 0.5)

Parameters	Boar breeds		
	Yorkshire	Landrace	Duroc
n	6	6	6
$\bar{X}$	55.09	54.41	50.30
S±	10.37	15.51	12.49
p-value	> 0.05		

Table 5 Survival time (h) of sperm depending on breeds (t = total).

Parameters	Boar breeds		
	Yorkshire	Landrace	Duroc
N	6	6	6
$\bar{X}$	97.31	91.49	92.43
S±	14.53	19.51	12.28
p-value	> 0.05		

Table 6 Bacteria contaminated in semen.

Species	CFU/mL
Escherichia coli	6.4×10^6
Pseudomonas spp.	8.3×10^6

The survival time of spermatozoa with its straight mobility more than 50% between the Yorkshire, Landrace and Duroc was 55 h 5 min, 54 h 25 min and 50 h 18 min respectively. They are significantly different ($p > 0.05$) (Table 4).

The total survival time of spermatozoa between the commercial BTS and BTS-KH was 97 h 19 min, 91 h 29 min and 92 h 26 m respectively. They are also not significantly different ($p > 0.05$) (Table 5).

3.2 Bacteria Contamination Isolated

The main bacteria isolated from the diluted semen were gram-negative bacteria, *Escherichia coli* 27.78% was contaminated with an average 6.4×10^6 CFU/mL, and the family *Pseudomonas* was 11.11% with 8.3×10^6 CFU/mL (Table 6).

4. Discussion

The result of analyzing indicated a positive of Escherichia coli and Pseudomonas spp. This research is consistent with many previous studies of artificial insemination practice [6-9] which reported that the occurrence of bacteria in semen, is normal in daily practice and may be associated with controlling the level of bacterial growth during semen storage. Considering the changes observed in the microbiological colonies form, it can be further confirmed that a certain number of bacteria have the ability to develop rapidly in certain samples of semen examined. These can contribute to an increase in the number of bacteria that need total air at different storage times. These findings seem to be in line with the potential for bacterial growth in semen devices described in scientific articles written by several authors [6, 10].

It seems consistent with our research as well, according to Úbeda et al. [9]. A shorter period for interaction between bacteria and sperm may lead to less speculation of the negative effects of some bacteria on sperm in artificial insemination. Other authors have suggested that the presence of bacteria in AI samples did not have a significant effect on fertility rates or total fertility [11]. This suggests that the use of antibiotics in semen does not seem to be necessary. On the other hand, there are many studies that show that bacterial infection can affect the quality and longevity of semen during storage, and high levels of bacterial infection can lead to unwanted side effects, at the level of reproduction and fertility results, especially when the proportion of sperm to bacterial is about 1:1 or for some bacteria 100: 1 [10, 12]. The negative effects of bacteria on semen stored at 15-177 (after more than 24-48 h from fertilization) were mainly demonstrated by experimental studies showing high concentrations of some bacterial species [13-15]. The predominance of bacteria on the surface of the sperm, along with the accumulation of sperm in the presence of bacteria, was detected. Bacterial strains found can have different

origins in animals (e.g. *E*. coli, *Enterobacter* spp. and *Staphylococcus* spp.) and non-animals (e.g. *Pseudomonas*, *Bacillus* and other species) [16]. These results can be explained by errors in semen collection and by differences between pigs in terms of their original semen properties. The bacterial of fresh boar semen is about 82.41 $\times 10^3$ CFU/mL [17]. Almost all of the ejaculation (99%) included in the previous study was transmitted by various bacteria previously reported in pig semen [7, 8, 10, 16, 18, 19]. Our research broadly confirms the general view that gram-negative bacteria, especially from the family *Enterobacteriaceae*, are most prevalent in semen [9, 12, 16]. We also isolated *E. coli* bacteria and *Pseudomonadaceae* and *Staphylococcus* spp. These results are consistent with other authors compiled by Maroto Martin et al. [8], which concluded that at least 25 different bacteria were identified in the semen of *Pseudomonas*, *Staphylococcus*, *Proteus* and *E. coli*.

5. Conclusion

In conclusion, the comparative result of spermatozoa survival time during the breeding period (t = 0.5) across different breeds and extenders shows no significant different. Bacterial contamination detected in semen samples from all breeds may be attributed to both direct and environmental factors. The rainy season, characterized by heavy rainfall, could contribute to increased susceptibility to infections.

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