UNIVERSITI PUTRA MALAYSIA

EFFECT OF EXTENDERS STORED AT DIFFERENT TEMPERATURES PRIOR TO PREPARATION ON THE QUALITY OF BOAR SEMEN

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EFFECT OF EXTENDERS STORED AT DIFFERENT TEMPERATURES PRIOR TO PREPARATION ON THE QUALITY OF BOAR SEMEN

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Effect Of Extenders Stored At Different Temperatures Prior To Preparation On The Quality Of Boar Semen", by Teh Yi Jie and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project

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DEDICATIONS

This write-up is dedicated to:

My family,

Father

Mother

Brothers

Friends

All my lecturers, farm and faculty staff who have committed themselves towards the noble cause of education

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With my deepest appreciation and gratitude, I thank those who helped in making this project paper a reality.

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LIST OF ABBREVIATIONS

%	Percentage
µl	Microliter
ml	Milliliter
°C	Degree Celsius
g	Gram
BTS	Betsville Thawing Solution
CASA	Computer Assisted Sperm Analysis
SQA	Sperm Quality Analyzer
AI	Artificial Insemination
BSE	Breeding Soundness Examination
IACUC	Institutional Animal Care and Use Committee
WHO	World Health Organization
SD	Standard Deviation
FS	Formal Saline
NS	Normal Saline

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

KESAN PELANJUT YANG DISIMPAN PADA SUHU YANG BERBEZA SEBELUM PENYEDIAAN KE ATAS KUALITI AIR MANI BABI JANTAN

Oleh

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2017

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ABSTRAK

Dalam permanian beradas, pelanjut air mani adalah penting untuk mengekalkan kualiti dan kemandirian sperma. Kajian ini bertujuan untuk menentukan kesan suhu penyimpanan yang berbeza bagi serbuk pelanjut KRUUSE Betsville Thawing Solution (BTS) terhadap ciri-ciri pergerakan, peratusan hidup dan morfologi air mani babi. Tujuh sampel air mani telah dikumpulkan dari tujuh individu yang berbeza untuk kajian ini. Setiap sampel dicampur dengan pelanjut

BTS yang telah disimpan pada suhu 4°C, 16°C dan suhu bilik sebelum penyediaan pelanjut. Semua sampel air mani yang telah dilanjutkan kemudiannya disimpan pada suhu 16°C dan dinilai setiap 24 jam selama 3 hari. Data dianalisis dengan ANOVA sehala (SPSS 22) dengan $P < 0.05$ dianggap ketara secara statistik. Motiliti am dan peratusan hidup spermatozoa menurun secara beransur-ansur mengikut masa bagi semua suhu. Walau bagaimanapun, tidak ada perbezaan yang ketara terhadap motiliti am, peratusan hidup dan morfologi tidak normal bagi semua suhu. Terdapat korelasi yang positif ($P < 0.05$) antara motiliti am dan peratusan hidup sperma-sperma. Terdapat pula korelasi negatif ($P < 0.05$) bagi kedua-dua motiliti am dan peratusan hidup mati apabila dibandingkan dengan peratusan morfologi yang tidak normal. Kesimpulannya, penyimpanan serbuk pelanjut pada suhu bilik sebelum penyediaan boleh mengekalkan kualiti tertinggi untuk air mani babi yang dilanjutkan. Walau bagaimanapun, tiada perbezaan statistik bagi kemandirian sperma dalam air mani babi yang telah dilanjutkan dengan pelanjut yang disimpan di dalam suhu-suhu yang berlainan .

Kata kunci: Betsville Thawing Solution (BTS), pelanjut, air mani babi, motiliti am, peratusan hidup, morfologi tidak normal

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Project

EFFECT OF EXTENDERS STORED AT DIFFERENT TEMPERATURES PRIOR TO PREPARATION ON THE QUALITY OF BOAR SEMEN

By

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2017

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ABSTRACT

In artificial insemination, semen extenders are important to maintain the quality and survivability of sperm. This study aimed to determine the effects of different storage temperatures of KRUUSE Betsville Thawing Solution (BTS) powder on motion characteristics, live percentage and morphology of extended boar semen. Semen samples were collected from seven different boars. Each sample was mixed with 3 different BTS extenders that were stored at 4°C, 16°C and room temperature prior to preparation of the extender. All the extended semen samples were kept at 16°C and evaluated at 24 hour intervals for 3 days. Data was analysed

with one-way ANOVA (SPSS 22) with $P < 0.05$ considered statistically significant. General motility and live percentage of spermatozoa decreased gradually over time for all temperatures. However, there was no significant difference in the general motility, live percentage and abnormal morphology between all temperatures. There was a positive correlation ($P < 0.05$) between general motility and live percentage of the sperm. Negative correlation ($P < 0.05$) was observed between both general motility and live percentage with the abnormal morphology percentage. In conclusion, storing the extender in room temperature prior to preparation can maintain the highest quality of extended boar semen. However, statistically there was no difference in the survivability of sperm in extended boar semen for all storage temperatures.

Keywords: Betsville Thawing Solution (BTS), extender, boar semen, general motility, live dead percentage, abnormal morphology

1.0 INTRODUCTION

Artificial insemination (AI) is widely used in swine breeding farms. In order to have a successful AI program, the quality of semen is very important as the fertilizing potential of sows is inherently linked to it (Vyt *et al*., 2008). Boar semen used for AI are collected, analyzed, and extended in a variety of commercially available diluents or extenders – which are an aqueous solution used to increase the volume of ejaculate until the required dose while preserving the functional characteristics of sperm cells (Gadea, 2003). Diluents can be divided into two major groups which are short-term preservation (1 – 3 days) and long term preservation (over 4 days). Betsville Thawing Solution (BTS) is one of the most widely used semen extenders throughout the world (Alvarez and Storey, 1982). It contains small amounts of potassium and serves to preserve the sodium potassium pump of sperm cells and thus avoid intracellular potassium depletion which is related to reduced sperm motility (Alvarez and Storey, 1982). Currently, no study has been done to determine the effect of different storage temperatures of BTS extenders on boar semen. Additionally there is a variety of storage temperatures used by Malaysian swine farmers to store extenders in the farm. The most common storage area is in a 4°C refrigerator which is also the temperature to store drugs and vaccines, 16°C refrigerator which is the optimum temperature to keep extended boar semen and also in room temperature. According to manufacturer's guidelines, the extender (in powder form) should be kept at 4°C to maintain the quality of the powder for a long period. Storage of the BTS powder at improper temperatures may affect its composition and ultimately the quality of extended boar semen.

The objectives of this study are:

i. To determine the survivability and quality of sperm in extended boar semen using extenders that were stored at 3 different temperatures prior to preparation.

ii. To determine the optimum temperature to store the BTS extender in order to maintain a high quality of extended semen.

The hypotheses of this study are:

i. The survivability and quality of sperm in extended boar semen will differ when using extenders stored at different temperatures.

ii. The optimum storage temperature of BTS extender is 4°C in order to maintain the best quality of extended boar semen.

2.0 LITERATURE REVIEW

2.1 Duroc pigs

The Duroc breed originated from the United States of America. According to Taylor and Roese (2005), the modern Duroc breed is crossed from the Jersey Red of New Jersey and the Duroc of New York.

It ranges in colour from dark, brick red to several shades lighter. It has small drooping ears, medium in length and a slight dish of the face. The forequarters, particularly the head and neck are light (Klober, 2009).

Durocs are commonly used as a terminal-cross male in crossbreeding programs due to its characteristics of muscling, meat quality and hardiness that are excellent for heavy carcass production (Klober, 2009). In Malaysia, Durocs are crossed with Large White/Landrace cross sows in commercial breeding programs (Moktir and Rachel) because Duroc or Duroc crosses do not have good maternal characteristic and its litter size is also lower than other breeds (Taylor and Roese, 2005).

2.2 Semen Collection

As artificial insemination is widely used in swine breeding programs, boars need to be trained to mount dummy sows to facilitate semen collection. A well trained boar will start mounting the dummy within a few minutes, followed by pelvic thrusting and exteriorizing the penis for ejaculation.

One of the most popular methods for boar semen collection is through the application of digital pressure to the penis using the gloved hand method (Youngquist *et al*., 2007) using non-spermicidal glove such as vinyl and nitrile. During the collection, the semen collector grasps the penis with one hand while applying pressure while the other hand will hold a pre-warmed container covered with a filter paper to collect the ejaculated semen.

The boar ejaculate is comprised of multiple fractions. The initial pre-sperm fraction is clear and sperm free and is not collected. This is followed by the sperm-rich and sperm-poor fractions which are collected. According to Rodríguez (2012), the sperm-rich fraction contains 80-90% of all sperm cells in the ejaculate. A gel-fraction that originates from bulbourethral gland will be filtered as there is minimal value in collecting it. Lastly, the collected semen needs to be protected from any chemical, temperature change and ultraviolet light during transportation to the laboratory in order to maintain its quality.

2.3 Semen Quality and Evaluation

Semen quality is a very important part of Breeding Soundness Examination (BSE) assessments. The evaluation of boar semen needs to include both physical and cellular assessment. For the physical assessment, the semen's colour, turbidity, odour and volume need to be recorded. Normal semen should be grey-white to white in colour and have a milky to

creamy consistency. In the cellular assessment, motility, sperm morphology and concentration need to be determined with a microscope. To evaluate the concentration, haemocytometers are considered as the gold standard (World Health Organization, 2010).

2.3.1 Sperm Motility

Motility is known to be an important characteristic in predicting the fertilizing potential of sows and an increase in 1% of motility in diluted semen is related to an increase of 0.14 piglets per litter (Vyt *et al*., 2008). In AI, semen motility needs to be more than 70% in order to be used (Shipley, 1999). One of the simplest ways to assess motility is through visual assessment under a microscope. However, this method is subjective and it needs to be performing consistently with the same preparation and same experienced technician in order to reduce variabilities (Vyt *et al*., 2004b). Other methods to assess the motility are Computer Assisted Sperm Analysis (CASA) and Sperm Quality Analyzer (SQA).

2.3.2 Sperm Morphology

Sperm morphology is another important parameter that needs to be evaluated in semen evaluation. Poor sperm morphology results in lower pregnancy rates and reduced litter size when used for AI insemination (Alm *et al.,* 2006; Tsakmakidis *et al*., 2010). Abnormal sperm morphology can be

classified as primary, secondary or tertiary abnormalities (Donadeu, 2004). Primary abnormality comprises abnormalities in the shape of the head which damages the genetic material or abnormalities of the mitochondrial sheet that would impair flagella function. Secondary abnormalities include proximal and distal cytoplasmic droplets. Morphological anomalies acquired by inappropriate handling of semen (e.g. coiled tails) are considered as tertiary abnormalities. Secondary and tertiary abnormalities can be compensated by increasing the number of sperm per dose (Donadeu, 2004). In a fertile dose of 2 x 10^9 spermatozoa per dose in AI, at least 80% of normal morphology is considered acceptable (Shipley, 1999).

2.4 Boar semen processing and storage

After evaluation, the boar semen will be diluted with an extender. A fertile dose of diluted semen should contain at least 2 – 3 x 10^9 spermatozoa (Alm *et al*., 2006). During the dilution process, it is preferable to have a two steps dilution. Firstly, the raw semen is diluted with a small volume of preheated extender followed by another large volume dilution of the same preheated extender that was kept at room temperature. Lastly, the extended semen are stored between 15°C – 18°C which is the optimum storage temperature for extended boar semen because fast cooling of the ejaculate from body temperature to below 15ºC will result in a lipid phase separation that will alter the sperm membrane permeability with subsequent loss of sperm vitality (Johnson et al., 2000).

2.4.1 Semen Extender

Semen extender or diluent is an aqueous solution to increase semen volume to the required dose while preserving the functional characteristics of sperm cells (Gadea, 2003). There are 2 groups of semen extenders which are for short term preservation (1 – 3 days) and long term preservation (over 4 days). Depending on the extender capacity, the dilution ratio is between 1:4 to 1:25 (Youngquist *et al.,* 2007). The Betsville Thawing Solution (BTS) is one of the most widely used semen extenders in the world (Alvarez and Storey, 1982) and contains potassium, glucose, sodium citrate, sodium bicarbonate, EDTA and antibiotics. It contains potassium to prevent the potassium loss from inside the cells and loss of motility due to sodium potassium pump inefficacy (Maes *et al*., 2011). The glucose will act as an energy source for the spermatozoa. Sodium citrate and sodium bicarbonate are buffers to control the pH (7.2) of the extended semen (Gadea, 2003). EDTA serves as a chelating agent to block the action of calcium as mediator of sperm capacitation and acrosome activity. Lastly, antibiotic is added to inhibit bacterial growth.

3.0 MATERIALS AND METHOD

3.1 Animals and Location

Semen were collected from seven sexually matured adult Duroc boars between 1 to 5 years of age and reared at 2 swine farms in Tanjung Sepat, Selangor. A total of 7 samples (one sample from each animal) were acquired over a period of two days using the gloved hand method by allowing the boar to mount a dummy sow. This project was approved by the Institutional Animal Care and Use Committee (IACUC), with reference number: FYP.20161FPV.27.

3.2 Semen Collection

Seven semen samples from seven boars were collected within two days using the gloved handed method carried out by the farm staff. Before collection, the prepuce the boar was washed with tap water. Once the boar started mounting the dummy sow with the penis exteriorized, the penis was held by the collector in his hand and pressure was applied to the penis. The pre-sperm fraction was discarded while the sperm rich fraction was collected into a beaker covered with a filter paper to filter out the gel component of the semen. The collected semen was placed in an insulated thermal box to prevent exposure to sunlight and immediately brought to the farm laboratory for further analysis and processing.

3.3 Fresh Semen Evaluation

After collection, approximately 1 ml of fresh boar semen was placed into a test tube held in a 37ºC water bath. The semen was evaluated through visual inspection for its volume and colour (watery, thin milky, milky and creamy).

3.3.1 Sperm Motility

A drop of fresh semen was placed on a warmed glass slide and its wave pattern (Table 1) was observed under a light microscope at 40x magnification. Following that, 20 µL of fresh semen was diluted with 1.8 ml normal saline (NS) with a dilution ratio of 1:100 and a drop of that diluted semen was placed on a warmed glass slide and examined for general motility under 100x magnification. Individual motility was then examined under 400x magnification.

Table 1: Wave pattern

Grade	Description	Remark
5	Strong, wave movement	Strong
4	Dark, distinct waves moving rapidly	Very good
3	Waves apparent, but with moderate motion	Good
2	Waves barely distinguishable	Fair
1	No waves, but motile sperm are present	Poor
0	No waves and no sperm motility	Very poor

(Abaigar *et al*., 2001)

3.3.2 Sperm Viability and Morphology

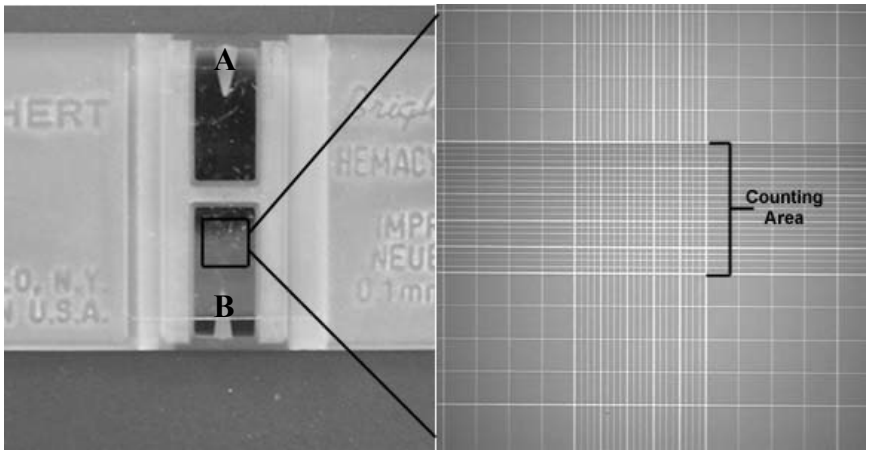
To examine the morphology of sperm and live percentage, a drop of raw semen was added to three drops of eosin-nigrosin stain on a glass slide and mixed

well. After that, a small droplet of the mixture was placed at the end of a glass slide and a cover slip was placed in front of the droplet at a 45° angle. It was then drawn back till it touched the droplet and pushed forward to make a smear. The glass slide was air dried before being examined under 400x magnification for live percentage and abnormal sperm morphology.

3.3.3 Sperm Concentration

For the determination of concentration, the diluted semen (1:100 NS) was further diluted with formal saline (FS) with a ratio of 1:4 to give a final dilution of 1:500. By using a haemocytometer, a 10 µl droplet of the diluted semen was added into each chamber (A and B) and covered with a cover slip (Figure 1). The number of spermatozoa touching the top or right lines of 5 large squares in each chamber was counted under a light microscope at 400x magnification. The mean of the 2 chambers was calculated and the concentration was calculated using the formula: "n" x 50,000 x 500 (dilution factor) with the unit sperm/ml.

Figure 1: Sperm counting chamber A and B in haemocytometer. (Source: Rouge, 2002)



3.4 Semen processing and Storage

Each pack of Kruuse BTS Plus boar semen extender stored at different temperatures (4°C, 16° and room temperature – placed at least 48 hours prior to preparation) was prepared by diluting 31 g of each extender powder with 1 L of demineralized water and placed in a 37°C water bath for at least 30 minutes prior to its use. The raw semen was then split into 3 portions and placed back into the water bath. The extender was added into the fresh semen at a ratio of 1:6 when the temperature difference was no more than 2°C. 100 ml of extended semen were packed into identical bottles of (3 bottles for each semen sample for 3 different storage temperatures of the BTS powder). All the extended semen were stored at 16°C.

3.5 Extended Semen Evaluation

All chilled extended semen were evaluated every 24 hours for 3 days (24, 48 and 72 hours post-extension of semen). 1 ml of extended boar semen was aspirated from the bottle and placed in a test tube held in a 37°C water bath for at least 2 minutes to thaw. The remaining extended boar semen was placed back in the 16°C refrigerator. General motility of the spermatozoa was examined by placing a drop of extended semen on a warm glass slide and under 100x magnification while the individual motility of spermatozoa was examined under 400x magnification. The live percentage and abnormal morphology of extended boar semen's spermatozoa

was evaluated by staining the extended boar semen with eosin-nigrosin stain and observed under 400x magnification.

3.6 Statistical Analysis

All statistical analyses were done using IBM SPSS 22 with $P < 0.05$ considered statistically significant. The relationship between the temperature groups and general motility, live percentage and abnormal morphology of spermatozoa were analyzed using one-way ANOVA. Comparisons of the progression of general motility, live percentage and abnormal morphology of spermatozoa over time were done using Repeated Measures one-way ANOVA. Spearman's correlation test was used to determine the relationship between general motility, live percentage and abnormal morphology of spermatozoa.

4.0 RESULTS

The mean volume and concentration of the 7 boar semen samples were 275.71 ± 91.31 ml and 2.64 ± 1.48 x 10^9 sperm/ml. All semen samples were normal milky to creamy in colour. Wave pattern ranged from a score of 2 to 4. Table 2 shows the mean percentage of fresh boar semen parameters.

Table 2: Mean percentage of general motility, live spermatozoa, and abnormal morphology of 7 fresh boar semen samples. All values are expressed as mean percentages ± SD.

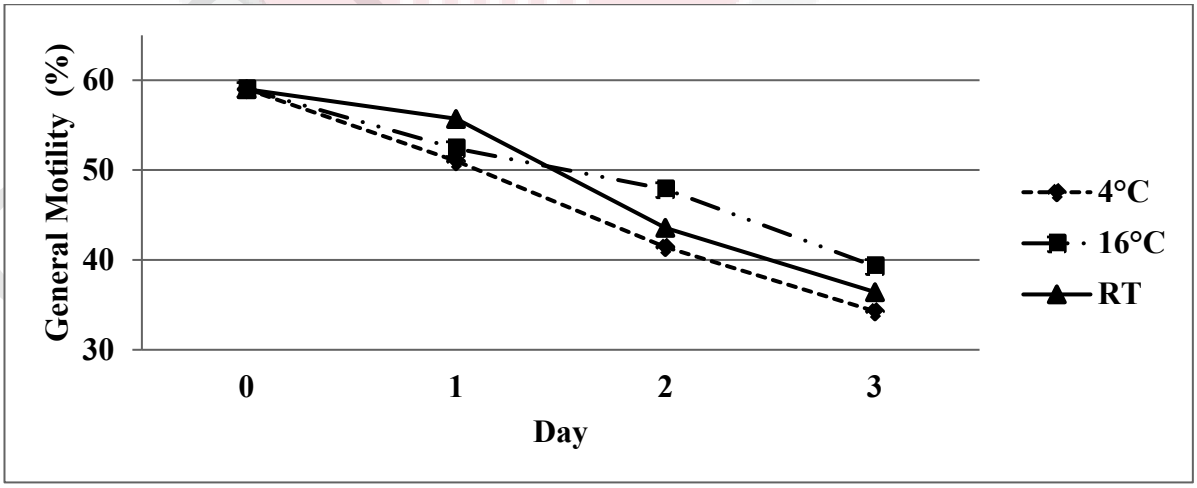
Parameter	Mean ± SD (%)
General Motility	59.28 ± 15.66
Live Spermatozoa	87.93 ± 7.94
Abnormal Spermatozoa	7.64 ± 5.41

The mean general motility for all temperatures decreased gradually over time (Table 3, Figure 2). The 16°C and room temperature group did not show any significant difference ($P > 0.05$) in the drop of general motility over the 3 days. Conversely, the 4°C temperature group showed a significant decrease in the general motility ($P = 0.018$) on Day 3 compared to Day 2. There was no significant difference ($P > 0.05$) in the general motility between all 3 temperature groups from Day 1 to Day 3.

Table 3: General motility of boar spermatozoa extended with BTS powder stored at 4°C, 16°C and room temperature (27°C – 29°C). All values are expressed as mean percentages ± SD.

General Motility			
Storage Temperature of Extender	**Day 1**	**Day 2**	**Day 3**
4°C	51.43 ± 16.76	44.43 ± 18.65	34.29 ± 17.90
16°C	52.14 ± 19.12	47.86 ± 25.31	36. 43 ± 15.20
Room Temperature	55.71 ± 16.44	43.57 ± 21.26	36.67 ± 17.84

Figure 2: Effect of BTS powder stored at 3 different temperatures on general motility of extended boar spermatozoa (n = 7).



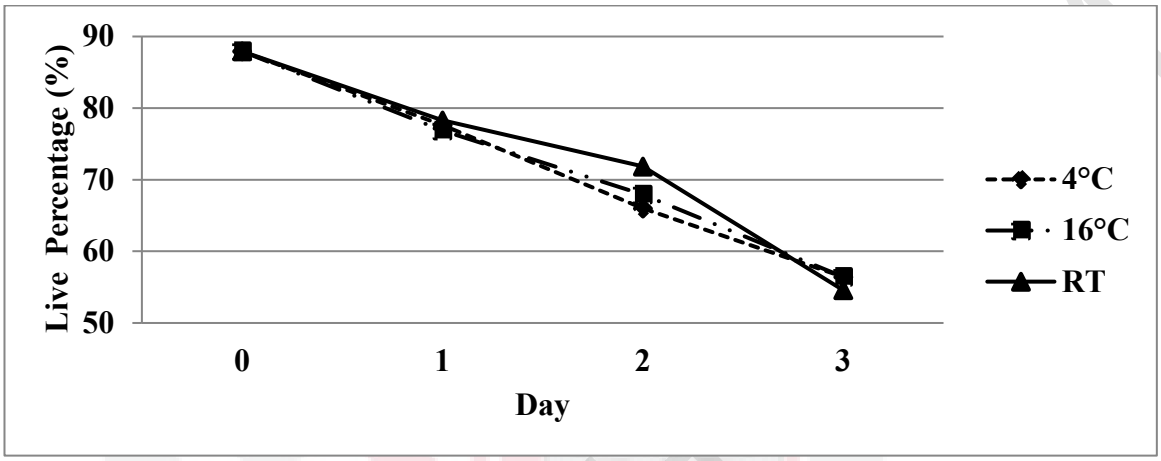
The live percentage of the spermatozoa also decreased gradually over time in all 3 temperature groups (Table 4, Figure 3). The 4°C temperature group showed a

significant drop (P = 0.013) in live percentage on day 2 compared with day 1. On the other hand, the 16°C temperature group showed a significant drop (P = 0.012) of live percentage on day 1 compared with day 0. However, there were no significant decreases on day 2 and day 3. In the room temperature group, there was a significant reduction (P = 0.005) of live percentage on day 3 compared to day 2. Comparing between the 3 temperature groups, there was no significant difference (P > 0.05) in the live percentage of spermatozoa for days 1, 2 and 3.

Table 4: Live percentage of boar spermatozoa extended with BTS powder stored at 4°C, 16°C and room temperature (27°C – 29°C). All values are expressed as mean percentages ± SD.

	Live Percentage of Spermatozoa		
Storage Temperature of Extender	**Day 1**	**Day 2**	**Day 3**
4°C	77.64 ± 6.83	66.00 ± 10.31	56.43 ± 16.59
16°C	76.86 ± 8.00	67.93 ± 9.75	56.43 ± 15.05
Room Temperature	78.29 ± 7.74	71.86 ± 10.76	54.57 ± 13.28

Figure 3: Effect of BTS powder stored at 3 different temperatures on live percentage of extended boar spermatozoa (n = 7).

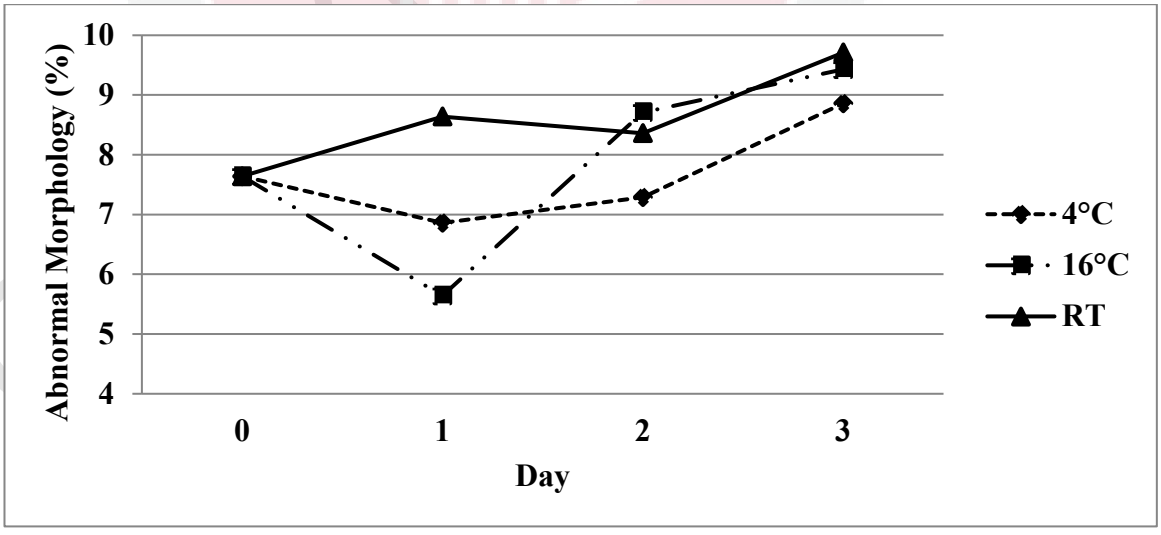


For temperature groups of 4°C and 16°C, the percentage of abnormal spermatozoa morphology decreased slightly on day 1 compared to day 0 but increased gradually on day 2 and day 3 (Table 5, Figure 4). For the room temperature group, the percentage of abnormal morphology increased slightly on day 1, decreased on day 2 and increased on day 3. All temperature groups showed no significant change of abnormal morphology percentage for the 3 days ($P > 0.05$). There was also no significant difference ($P > 0.05$) in abnormal spermatozoa morphology between all 3 temperature groups from Day 1 to Day 3.

Table 5: Abnormal morphology of boar spermatozoa extended with BTS powder stored at 4°C, 16°C and room temperature (27°C – 29°C). All values are expressed as mean percentages ± SD.

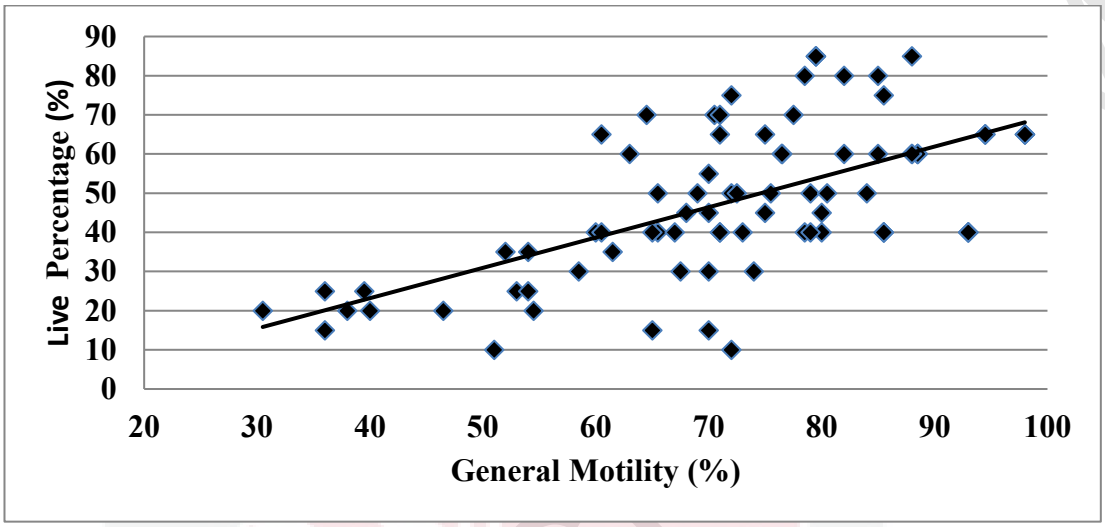
Abnormal Morphology of Spermatozoa			
Storage Temperature of Extender	**Day 1**	**Day 2**	**Day 3**
4°C	6.86 ±3.67	7.29 ± 4.49	8.86 ± 3.66
16°C	5.64 ± 2.66	8.71 ± 5.09	9.43 ± 4.37
Room Temperature	8.64 ± 6.96	8.36 ± 4.06	9.71 ± 2.96

Figure 4: Effect of BTS powder stored at 3 different temperatures on abnormal morphology of extended boar spermatozoa (n = 7).



There is a strong positive correlation (r = 0.595) between the general motility and live percentage of spermatozoa with the correlation being significant at the 0.01 level (Figure 5).

Figure 5: Relationship between general motility and live percentage of spermatozoa (r = 0.595).



There are significant moderate negative correlations for both general motility with abnormal morphology of spermatozoa (r = -0.414; Figure 6) and live percentage with abnormal morphology of spermatozoa (r = -0.499; Figure 7). The correlation is significant at the 0.01 level.

Figure 6: Relationship between general motility and abnormal morphology of spermatozoa (r = -0.414).

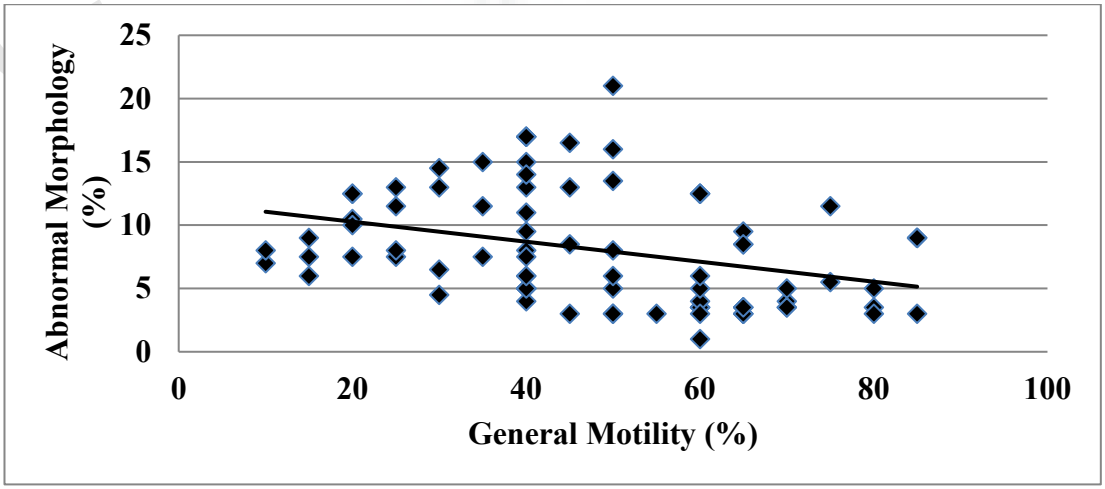
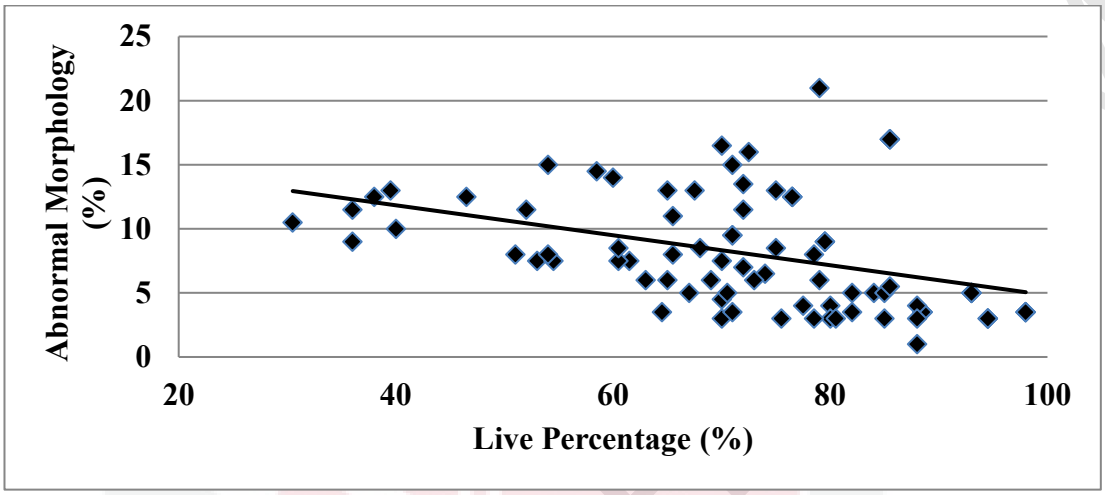


Figure 7: Relationship between live percentage and abnormal morphology of spermatozoa (r = -0.499).



5.0 Discussion

During evaluation of raw semen, the average quality of collected raw semen was considered as moderate. According to Shipley (1999), semen motility used in AI needs to be more than 70%. Only 1 out of 7 fresh semen samples that was collected had a general motility of more than 70% and this could be due to a temperature fluctuation during transportation. Besides that, sperm motility decreased when the time between ejaculation and analysis increased (Chomsrimek *et al*., 2008).

In the present study, the general motility of extended semen decreased over time and this could be due to the detrimental effect of reactive oxygen species (ROS) secondary to bacterial overgrowth or metabolic activity of spermatozoa leading to depletion of nutrients (Chomsrimek *et al*., 2008). The agglutination of spermatozoa was observed in all extended semen during the evaluation which can be suggestive of the presence of anti-sperm antibodies, temperature fluctuations during transport and bacterial contamination (Rozeboom, 2000; WHO, 2010). Severe agglutination can affect the assessment of sperm motility and concentration (WHO, 2010) while bacterial culture and antibiotic sensitivity test can be done to find out the reason for the presence of agglutination.

The live percentage of spermatozoa was observed to decrease over time. In extended semen, spermatozoa need energy which is crucial for the maintenance of sperm motility and viability (Machebe *et al*., 2014). As the energy source (glucose) of the BTS extender is limited, it will become depleted over time. Since there is no significant difference in the live percentage of spermatozoa between the 3

temperature groups, it could mean that glucose was not affected by the storage temperatures.

Less than 10% abnormalities were observed in all extended semen. Maximum percentages of primary and secondary abnormalities in boar semen were determined as 10% and 20%, respectively (Shipley, 1999). Most of the abnormalities of spermatozoa found in this study were bent or looped tail as well as proximal and distal cytoplasmic droplet. These 2 abnormalities are considered as secondary abnormalities and it can be compensated by increasing the number of sperm per dose (Donadeu, 2004). The presence of bent tails could be due to poor temperature control and hypotonic stress because of contaminated water, improper seminal pH and testicular degeneration of the animal (Noakes *et al*., 2009).

A strong positive correlation was found between general motility and live percentage of spermatozoa. It shows that motility is one of the most important semen characteristic in predicting the fertilizing potential of an ejaculate. While for the relationship for both general motility with abnormal morphology of spermatozoa and live percentage with abnormal morphology of spermatozoa, it showed moderate negative correlations. By having an abnormal morphology such as bent tail and cytoplasmic droplet, the spermatozoa may consume more energy that leads to an early death. All correlation results are supported by the study done by Churchil *et al*. (2014), which had the same findings.

6.0 Conclusion

In conclusion, storing the extender in room temperature prior to preparation can maintain the highest quality of extended boar semen. However, statistically there was no difference in the survivability of sperm in extended boar semen for all storage temperatures. Additionally, the storage length of extended boar semen relies on the initial quality of the fresh semen.

7.0 Recommendation

Further studies need to be conducted to determine the fertilizing capability of the extended boar semen that was used in this study which includes observing the reproductive performance of sows such as the conception rate, number of live piglets born and lives piglet weight.

Similar studies can be done to determine the effects of different types or brands of extenders on semen quality as they may contain differing compositions that may be affected by storage temperature and thus affect the quality of boar semen.

Larger sample sizes are also recommended to efficiently compare between the different storage temperatures. Besides that, the extenders could also be stored at various temperatures for a longer duration prior to its preparation.

8.0 References

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