Comparative study on commercial semen extenders and storage times on the quality of boar semen

Yaovapol Chumpol[1], Weerachai Thongdee[1] and Rittichai Pilachai[2*]

ABSTRACT: The aim of this study was to compare the percentage of sperm life, motility, and abnormal morphology of boar spermatozoa extended in the four commercial extenders. Five ejaculated semen boars were stored (38 x 10^6 spermatozoa/ml) in the two commercial extenders devised for short-term preservation (BTS and Dilumax-BTS) and the two long-term preservation extenders (D-Max^6 and Gold life) with a randomized completely block design. The samples were stored for 9 days at 17 ºC. On day 0, 1, 3, 5, 7 and 9 of storage, sperm motility, sperm life and morphology of the stored semen were evaluated. Across storage day, the percentage of individual motile and total sperm life were significantly decreased (P<0.01) after storage. On day 5, 7 and 9 after storage, the percentage of sperm life in D-Max^6 and Gold life were significantly (P<0.01) higher compared to BTS and Dilumax–BTS diluted semen. The individual motile and sperm life reduction below 60 percentages were observed from days 5 to 9 of storage in BTS, Dilumax–BTS and Gold life, whereas the semen stored in D-Max^6 was observed the individual motile and sperm life above 60 percentages up to day 7 of stored. However, none of the semen extenders were associated with the morphology of spermatozoa. The data reveal that semen extended in D-Max^6 had the highest percentage of individual motile and sperm life throughout the 9-days storage period. It can be concluded that Gold life, BTS and Dilumax–BTS were preserved sperm quality for up to 3 days of storage, whereas D-Max^6 preserved the sperm quality for up to 7 days of dilution.

Keywords: extender, storage, quality, semen, boar

Introduction

Nowadays, more than 90% of artificial inseminations (AI) are used for breeding technique in swine industry worldwide including Thailand. Preservation of the fertilizing ability of boar semen for several days remains a major target for the breeding technique. Therefore, semen extenders have been developed to store semen for short- and long-term. The preservation media should supply energy for spermatozoa, protection against cold shock, buffering pH and inhibit the growth of bacteria (Gadea., 2003) The short-term preserved extender such as Beltsville thawing solution (BTS) and BTS+ contain a small amount of potassium in order to prevent the decrease of spermatozoa motility and maintain the optimal motility rate of semen stored for up to 3 days (Alexopoulos et al., 1996) whereas, the long-term preservation extenders can maintain a good sperm motility up to 5 days. The components of long-term diluent often contain high amount of active molecules, result in an extra protection to spermatozoa membranes and reduce the sperm cells energy expenditure (Gadea., 2003) Thus, the long-term extenders mostly use for preservation

[1] Bantad Research and Community Development Center, Faculty of Technology, Udon Thani Rajabhat University, Udon Thani, 41000, Thailand

[2] Program in Veterinary Technology, Faculty of Technology, Udon Thani Rajabhat University, Udon Thani, 41000, Thailand

* Corresponding author: r.pilachai@gmail.com

the semen for a long distance transport or storage for a few weeks.

Several short and long semen extenders are commercially available to preserve boar semen in Thailand. However, a comparison of the efficacy among the boar semen extenders on the sperm characteristics is not well document. Therefore, the objective of this study was to compare the percentage of sperm life, motility, and abnormal morphology of boar spermatozoa extended in short-term preservation (BTS and Dilumax-BTS) and long-term preservation extenders (D-Max^6 and Gold life). It was anticipated that the outcomes of this study can be applied to use and how long the semen can be preserved.

Materials and Methods

Materials

Five boars (2=Large white, 2=Duroc Jersey and 1=Landrace) with varying age (12 -24 months) were used in this study. They originate from Bantad Research and Community Development Center, Faculty of Technology, Udon Thani Rajabhat University. Each boar, sperm was collected using a hand technique and filtered through double layers of cotton gauze to remove the gel portions. Immediately after ejaculation, visually for percentage of progressive motility was evaluated using a phase contrast microscope and sperm concentration was estimated using a hemocytometer. Furthermore, sperm dead/life and morphologically abnormal spermatozoa were evaluated according to the eosin-nigrosin strained method (Dott and Foster., 1972) Thus, ejaculated semen with a normal sperm quality (e.g. >80% progressively motile and >80% morphologically normal sperm) was obtained in this study.

Methods

Each selected semen boar was diluted (38 $\times 10^6$ spermatozoa/ml) in two commercial extenders devised for short-term preservation (BTS and Dilumax-BTS) and two long-term preservation extenders (D-Max^6 and Gold life) with a randomized completely block design for 9 days at 17 ºC. On day 0, 1, 3, 5, 7 and 9 of storage, sperm motility, sperm dead/life and morphology of the stored semen were evaluated with 2 replicates assay.

All statistical analyses were performed using SPSS version 17 for windows. The data of sperm motility, sperm dead/life and morphology were subjected to repeat measuring analysis of variance to identify differences among boars and extenders in the different times of storage. Kruskal-Wallis test was used to test differences among sperm quality parameters. Differences among treatments were considered significant when $P<0.05$.

Results and Discussion

Semen quality

Semen quality parameters of 5 boars after collection were presented in **Table 1**. The semen volume, concentration of spermatozoa and total sperm cells per ejaculation ranged from 60 to 360 ml, 185 × 10^6 to 535 × 10^6 sperm cells/ml and 32 to 80 × 10^{10} sperm cells, respectively. Except volume of semen from boar no. 4 and the sperm concentrations of boar no. 5, all ejaculates semen

found to be normal value (i.e., semen volume, concentration of spermatozoa and total sperm per ejaculate ranging between 80 and 300 ml, 200 and 300 × 10^6 sperm cells/ml and 30 and 60 × 10^{10} sperm cells, respectively (Gadea., 2003). This is in line with the previous reported by (Duangjai et al., 2010).

Table 1 Semen quality of 5 boars after collection

Item	Boar				
	1	2	3	4	5
Semen volum (ml)	160	180	130	60	360
Density (score 0-5)	3	3	3	4	2
Colour (score 0-3)	3	2	3	3	2
Mass motility (score 0-4)	4	3	4	4	2
pH	7.3	7.4	7.5	7.8	7.2
Sperm concentration (x 10^6 cell/ml)	526	423	358	535	185

Although, the volume of semen from boar no. 4 (60 ml) and the sperm concentrations of boar no. 5 somewhat lower as compared to the normal range. However, both semen were observed the value of total spermatozoa per ejaculate within the standard ranges (32 and 66 sperm cells, respectively). Therefore, all ejaculated semen were obtained in this study.

Effect of semen extenders on sperm motility and sperm life

Sperm motility and sperm life were significantly decreased for all extenders during a period of 9 days storage time ($P<0.01$). The lowest decline was observed in D-max^6, whereas the highest decrease was found in Dilumax-BTS (**Tables 2** and **3**). The percentage of progressive motility preserved in long-term extenders seen higher compared to short-term preservation extenders. On day 5, 7 and 9 after storage, the percentage of sperm life in D-Max^6 and Gold life were significantly ($P<0.01$) higher compared to BTS and Dilumax–BTS diluted semen. None of the semen extenders were associated with the morphology of spermatozoa (**Table 4**).

Table 2 Percentage of sperm motility after storage in Dilumax-BTS, BTS, Gold life and D-max^6 for a time period up to 9 d.

Storage day	Extender					
	Dilumax-BTS	BTS	Gold life	D-max^6	S.E.	P-value
0	88.8	88.7	88.9	88.9	0.65	0.984
1	81.1	81.8	83.1	83.2	0.94	0.509
3	60.5^a	62.1^a	73.8^b	77.0^b	1.67	0.001
5	36.5^a	40.7^a	54.7^b	68.1^c	2.42	<0.001
7	19.9^a	23.6^a	38.6^b	59.6^c	2.92	<0.001
9	5.4^a	8.3^b	20.9^c	35.3^d	2.12	<0.001

$^{a, b, c, d}$ mean followed different superscripts within each day are significantly different ($P<0.05$).

Individual sperm motility parameter is considered to be an important factor related to fertility and number of piglets born (Colenbrander and Kemp., 1990). The minimum sperm motility should be >60% to give optimal fertility and little size when artificial insemination is used (Johnson et al., 2000). In the current study, the individual motile and sperm life reduction below 60 percentages were observed from days 5 to 9 of storage in BTS, Dilumax–BTS and Gold life, whereas the semen stored in D-Max[6] was observed the individual motile and sperm life above 60 percentages up to day 7 of stored. This is in line with another study (Eriksson at el., 2001; Ambrogi at el., 2006) that BTS extender showed lower motility in comparison with spermatozoa suspended in a long-term extender. However, Gold life extender as long-term preservation was maintained the motile of spermatozoa above 60% only 3 days after storage in this study. This was caused that long-term extender comprises higher amount of ingredients (i.e. antioxidant, capacitation inhibitor and protection against cold shock) compared to short-term diluent. However, the exactly composition of semen extender is not known due to a trade. Therefore, only D-Max[6] extender is suitable for a preservation period 7 days, but BTS, Dilumax–BTS and Gold life extender are not recommended for preserved semen exceeding 3 days.

Table 3 Percentage of sperm life after storage in Dilumax-BTS, BTS, Gold life and D-max[6] for a time period up to 9 d.

Storage day	Extender					
	Dilumax-BTS	BTS	Gold life	D-max[6]	S.E.	P-value
0	88.9	89.1	88.8	89.1	0.65	0.994
1	83.9	84.1	84.9	84.7	0.64	0.746
3	75.8	76.3	76.9	77.6	0.78	0.702
5	65.2[a]	66.6[a]	70.4[b]	73.5[b]	1.11	0.037
7	52.8[a]	54.6[a]	64.7[b]	69.1[b]	1.76	0.002
9	35.1[a]	37.3[a]	58.1[b]	62.6[c]	2.34	<0.001

[a, b, c, d] mean followed different superscripts within each day are significantly different (P<0.05)

Table 4 Percentage of sperm abnormality after storage in Dilumax-BTS, BTS, Gold life and D-max[6] for a time period up to 9 d.

Storage day	Extender					
	Dilumax-BTS	BTS	Gold life	D-max[6]	S.E.	P-value
0	16.1	15.1	14.2	15.5	0.89	0.987
1	17.5	17.3	16.7	17.1	0.79	0.970
3	22.0	21.7	21.6	21.6	0.79	0.986
5	28.1	27.8	27.4	26.8	0.94	0.464
7	33.3	31.9	31.5	30.9	0.79	0.607
9	35.7	35.7	35.5	34.9	0.57	0.800

Conclusion

Semen extended in D-Max[6] had the highest percentage of individual motile and sperm life throughout the 9-days storage period. It can be concluded that Gold life, BTS and Dilumax–BTS were preserved sperm quality for up to 3 days of storage, whereas D-Max[6] preserved the sperm quality for up to 7 days of dilution.

Acknowledgements

The study was financially supported by Udon Thani Rajabhat University, Thailand. We acknowledged the Bantad Research and Community Development Center, Faculty of Technology, Udon Thani Rajabhat University, Thailand, for providing research facilities and laboratory analyses. The cooperation of Thawatchai Sumongkon and Panupan Srikaew whom participated in this study, was highly appreciated.

References

Alexopoulos, C., C. Boscos, P. Saratsis, C. Caoulidis, and S. Kyriakis. 1996. The effect of storage time and number of spermatozoa per insemination dose on semen characteristics and fertilizing capacity of boar semen diluted with Beltsville Thaw Solution (BTS) extender. J. Anim. Sci. 62: 599–604.

Ambrogi, M.D., J. Ballester, F. Saravia, I. Caballero, A. Johannisson, M. Wallgren, M. Andersson, and H. Rodriguez-Martinez. Effect of storage in short- and long-term commercial semen extenders on the motility, plasma membrane and chromatin integrity of boar spermatozoa. Int. J. Androl. 29: 543–552.

Dott, H.M., and G.C. Foster. 1972. A technique for studying the morphology of mammalian spermatozoa which are eosinophilic in a differential live/dead stain. J. Reprod. Fertil. 29: 443-445.

Duangjai B., K. Saikhun, and P. Ratanaphumma. 2010. Effects of Extender and Storage Time on Motility and Ultrastructure of Cooled-Preserved Boar Spermatozoa. Kasetsart J. 44: 582–589.

Colenbrander, B., and B. Kemp. 1990. Factors influencing semen quality in pigs. J. Reprod. Fertil. Suppl. 40: 105–115.

Eriksson, B.M., J.M. Vazquez, E.A. Martinez, J. Roca, X. Lucas, and H. Rodriguez-Martinez. 2001. Effects of holding time during cooling and of type of package on plasma membrane integrity, motility and in vitro oocyte penetration ability of frozen-thawed boar spermatozoa. Theriogenology. 55: 1593–1605.

Gadea, J. 2003. Review: Semen extenders used in the artificial insemination of swine. Spanish J. Agri. Res. 1(2):17-27.

Johnson, L.A., K.F. Weitze, P. Fiser, and W.M.C. Maxwell. 2000. Storage of boar semen. Anim. Reprod. Sci. 62: 143–172.