韓國受精卵移植學會誌(1996) 第 11 卷 第 2 號
Korean J. Emb. Trans. (1996) Vol. 11, No. 2 pp. 159~166

Study on the Preservation of Liquid Boar Semen with BF_5 and Bütschwiler Diluents

Y. M. Cheon, C. S. Park, K. W. Seo and K. S. Lee

College of Agriculture, Chungnam National University

희석액 BF_5와 Bütschwiler를 이용한 돼지 액상정액 보존에 관한 연구

천용민 · 박창식 · 서길웅 · 이규승

충남대학교 농과대학

요 약

본 연구는 돼지 액상정액을 인공수정용 100ml 플라스틱 병에 보존하면서 BF_5 희석액과 Bütschwiler 희석액 간에 보존 온도별 차이를 조사하고, BF_5 희석액에서의 글리세롤 농도의 효과를 조사하여 돼지 액상정액을 좀더 장기간 사용할 수 있는 방법을 찾고자 실시하였다.

돼지 액상정액을 5℃ 냉장고에 보존하면서 조사한 바에 의하면, 37℃에서 0.5 및 2시간 배양 후의 정자운동성은 전체 보존기간동안 BF_5 희석액이 Bütschwiler 희석액보다 유의하게 ($P<0.05$) 높게 나타났고, 정상첨체비율은 두 희석액간에 차이가 없었다.

돼지 액상정액을 15℃에 보존하면서 조사한 바에 의하면, 3일부터 7일 보존시 까지 정자운동성과 정상첨체비율에 있어서 Bütschwiler 희석액이 BF_5 희석액보다 유의하데 높게 나타났다. BF_5 희석액을 이용한 돼지 액상정액의 글리세롤 농도의 효과에 있어서는 최종 글리세롤 농도가 0, 2, 3 및 5% 보다 1%일 때 가장 높은 정자운동성과 정상첨체비율을 나타내었다.

분만율, 복당 생존자돈수 그리고 출생시 평균 생시체중은 BF_5 희석액과 Bütschwiler 희석액 간에 차이가 없었다.

이상의 연구 결과를 종합해 볼 때 BF_5 희석액을 5℃에서 Bütschwiler 희석액은 15℃에서 6~7일 동안 돼지 액상정액을 보존할 수 있었다.

(Key words : liquid boar semen, BF_5 diluent, Bütschwiler diluent, motility, acrosome)

INTRODUCTION

An important factor in artificial insemination of swine with liquid semen is how to prolong semen viability beyond the seventh day. Several diluents are used throughout the world by commercial artificial insemination centers. Among them, Modena(Moretti, 1981) is the most widely used in Korea. Summermatter(1984) modified Modena diluent by adding cysteine and BSA, and increasing the proportion of glucose, and used it as Bütschwiler. The above diluents have generally been the most successful within the storage temperature of 15 to 18℃. Chung *et al.* (1989), using BF_5 diluent, found that liquid semen can be stored at 5℃ for 9 days in 5 ml straw. However, the processing method of liquid semen in 5 ml straw was not simple for practical purpose.

The objectives of this study were to find out methods of long-term use of liquid semen in 100 ml plastic bottle for artificial insemination and to investigate differences between BF_5 and Bütschwiler diluents according to storage temperature, and effect of final glycerol concentration in BF_5 diluent.

MATERIAL AND METHODS

1. Semen collection

Semen was collected from three Large White boars twice weekly. The filtered sperm-rich fraction was collected by the gloved-hand technique into a 250 ml insulated vacuum bottle. The sperm-rich fractions of ejaculates with greater than 85% motile sperm and NAR acrosome were used in this experiments.

2. Semen evaluation

Sperm motility and NAR acrosome of ejaculated semen and diluted liquid semen were evaluated by microscopical examination. The sperm concentration was estimated with a hemocytometer. One ml semen was pipetted into test tube containing 4 ml of Beltsville Thawing Solution (BTS). An 1 ml of the aliquot of the diluted semen was then added to 2 ml of 1% glutaraldehyde in BTS for acrosome morphology evaluation. The remaining 4 ml of diluted semen was incubated at 37℃. After 0.5 and 2h incubation, 6㎕ aliquots were transferred onto glass slides and 18×18 mm cover-slips were applied.

The percentage of motile sperm was estimated at 37℃ by phase-contrast microscope at 250×. The acrosome morphology of 100 sperm per sample at 0.5 and 2h after incubation at 37℃ was evaluated by phase contrast microscopy at 1,000×. Acrosomes were differentially categorized into four morphological classes : normal apical ridge(NAR), damaged apical ridge(DAR), missing apical ridge(MAR) and loose acrosomal cap(LAC) as described by Pursel et al. (1972) and Pursel and Park(1987).

3. Semen processing

The compositions of BF_5 by adding BSA, Bütschwiler and BTS diluents are shown in Table 1.

1) Processing method of liquid semen preserved at 5℃

(1) Semen is slowly cooled to room temperature by 2h after collection.

(2) Plastic bottle of 100ml for artificial insemination(Mini tube, Germany) is filled with 10ml of semen (4~5 TIMES 10^8 sperm/ml) at room temperature.

(3) BF_5 or Bütschwiler diluent(first diluent) of 10ml are added in the plastic bottle with 10ml of semen at room temperature.

(4) The plastic bottle with the first diluted semen is cooled in a refrigerator to 5℃ over a 2h period and BF_5 or Bütschwiler + 3% glycerol diluent(second diluent) of 10ml are added to the first diluted semen.

(5) The diluted semen of 30ml in the plastic bottle is stored in the refrigerator until inseminated. The diluted semen is assessed in the laboratory for motility and acrosome using the method described the above.

(6) At insemination, the plastic bottle with 30ml of the diluted semen is rediluted with 50ml BTS diluent.

(7) The dilution method of liquid semen preserved at 5℃ is shown in Fig. 1.

2) Processing method of liquid semen preserved at 15℃

(1) Semen is slowly cooled to room temperature by 2h after collection.

(2) Plastic bottle of 100 ml for artificial in-

Table 1. The composition of diluents

Ingredient	BF_5 [1]	Bütschwiler [1]	BTS [3]
Glucose	3.2(g)	3.5(g)	3.7(g)
Sodium citrate		0.69	0.6
Sodium bicarbonate		0.10	0.125
TES	1.2		
EDTA, disodium		0.235	0.125
KCl			0.075
Hepes		0.95	
Egg yolk(ml)	20		
Tris, buffer	0.2	0.565	
Citric acid		0.315	
Cysteine		0.0054	
BSA	0.3	0.3	
Penicillin	0.01	0.01	0.01
Streptomycin	0.01	0.01	0.01
Distilled water(ml)	80	100	100
	BF_5 or Bütschwiler + 3% glycerol[2]		

[1] BF_5 and Bütschwiler = first diluent

[2] BF_5 or Bütschwiler + 3% glycerol = second diluent

[3] BTS = final diluent.

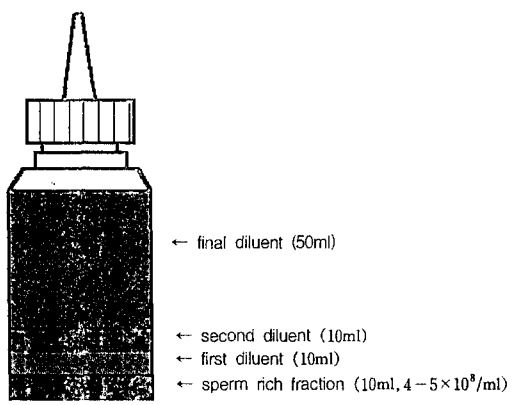

Fig. 1. Dilution method of liquid boar semen preserved at 5℃.

semination is filled with 10 ml of semen 4~5 TIMES 10^8 sperm/ml) at room temperature.

(3) BF_5 or Bütschwiler diluents(first diluent) of 20 ml are added in the plastic bottle with 10 ml of semen at room temperature.

(4) The diluted semen of 30 ml in the plastic bottle is stored at 15℃ until inseminated. The diluted semen is assessed in the laboratory for motility and acrosome using the methods described the above.

(5) At insemination, the plastic bottle with 30 ml of the diluted semen is rediluted with 50 ml BTS diluent.

4. Artificial insemination

Estrus was checked twice daily at 09:00 and 15:00h. The first insemination was done with liquid boar semen stored for 6 days at 22~26h after detection of standing estrus, using the spiral-tip catheter(Melrose and O'Hagan, 1961). The second insemination was done with the liquid boar semen stored for 7 days at 12h after the first insemination.

RESULTS AND DISCUSSION

1. Sperm viability in BF_5 or Bütschwiler diluents at 5℃

The percentages of motility on the liquid boar semen diluted with BF_5 or Bütschwiler diluents and preserved at 5℃ are presented in Table 2-1. The motility after 0.5 and 2h incubation at 37℃, respectively, was significantly higher for sperm preserved in BF_5 diluent than for sperm preserved in Bütschwiler diluent from 1 to 7 day of storage. The sperm motility in BF_5 diluent steadily declined from 85.0 and 81.3% at 1 day of storage to 73.1 and 67.8% after 7 day of storage at 0.5 and 2h incubation, respectively.

The percentages of NAR acrosome on the liquid boar semen diluted with BF_5 or Bütschwiler diluent and preserved st 5℃ are presented in Table 2-2. The NAR acrosome in BF_5 diluent steadily declined from 85.3 and 74.3% at 1 day of storage to 50.0 and 45.0% after 7 day of storage at 0.5 and 2h incubation, respectively. The NAR acrosome in BF_5 diluent after 0.5 and 2h incubation at 37℃, respectively, during preservation periods was similar to that in Bütschwiler diluent. The above results were similar to the report of Chung *et al.*(1989) using BF_5 diluent in 5ml maxi-straw at 5℃. So the diluted sperm with BF_5 diluent in 100ml plastic bottle will be stored for 7 day at 5℃. However, the sperm with Bütschwiler diluent was not used at 5℃ preservation temperature.

Table 2-1. Comparison of percentage of motility on the liquid boar semen diluted with two diluents and preserved at 5℃

Diluent	Incubation time(h)	Storage length of liquid semen(day)[1]			
		1	3	5	7
BF_5	0.5	85.0 ± 0.00 [a]	81.3 ± 1.86 [a]	80.0 ± 3.16 [a]	73.1 ± 1.83 [a]
Bütschwiler		35.0 ± 4.47 [b]	23.1 ± 1.86 [b]	22.6 ± 9.04 [b]	18.1 ± 3.65 [b]
BF_5	2	81.3 ± 1.86 [a]	76.3 ± 1.86 [a]	76.3 ± 1.86 [a]	67.8 ± 4.49 [a]
Bütschwiler		31.3 ± 7.96 [b]	22.4 ± 1.86 [b]	21.5 ± 9.20 [b]	15.0 ± 3.16 [b]

[1] Values were observed after 0.5 and 2h incubation in water bath of 37℃. Mean ± S.E. for 6 ejaculates from Large White boar.

[a,b] Means in the same column with different superscripts at 0.5 and 2h incubation time differ significantly($p<0.05$), respectively.

Table 2-2. Comparison of percentage of NAR on the liquid boar semen diluted with two diluents and preserved at 5℃

Diluent	Incubation time(h)	Storage length of liquid semen(day)[1]			
		1	3	5	7
BF_5	0.5	85.3 ± 2.06	70.0 ± 1.26	58.0 ± 2.28	50.0 ± 2.89
Bütschwiler		84.1 ± 2.63	77.0 ± 5.17	68.0 ± 8.36	57.3 ± 8.73
BF_5	2	74.3 ± 2.58	65.3 ± 5.16	53.0 ± 1.89	45.0 ± 3.66
Bütschwiler		75.3 ± 1.96	62.1 ± 5.07	59.1 ± 3.81	51.3 ± 5.12

[1] Values were observed after 0.5 and 2h incubation in water bath of 37℃. Mean ± S.E. for 6 ejaculates from Large White boar.

2. Sperm viability in BF_5 or Bütschwiler diluents at 15℃

The percentages of motility on the liquid boar semen diluted with BF_5 or Bütschwiler diluents and preserved at 15℃ are presented in Table 3-1.

The motility after 0.5 and 2h incubation at 37℃, respectively, was significantly higher for sperm preserved in Bütschwiler diluent than for sperm preserved in BF_5 diluent from 3 day of storage. The sperm motility in Bütschwiler diluent steadily declined from 85.0 and 81.8% at 1 day of storage to 66.8 and 58.6% after 7 day of storage at 0.5 and 2h incubation, respectively.

The percentages of NAR acrosome on the liquid boar semen diluted with BF_5 or Bütschwiler diluents and preserved at 15℃ are presented in Table 3-2. The NAR acrosome after 0.5 and 2h incubation at 37℃, respectively, was siginificantly higher for sperm preserved in Bütschwiler diluent than for sperm preserved in BF_5 diluent from 3 day of storage. The NAR acrosome in Bütschwiler diluent steadily declined from 89.0 and 88.1% at 1 day of storage to 63.1 and 58.8% after 7 day of storage at 0.5 and 2h incubation, respectively.

Weitze(1991) reported that the percentages of motility and NAR acrosome after storage for 144h at 15℃ were 75.0~80.8% and 87.6~89.6% in BW_{25} diluent, and 73.3~81.7% and 87.9~90.3% in Androhep diluent, respectively. The

Table 3-1. Comparison of percentage of motility on the liquid boar semen diluted with two diluents and preserved at 15℃

Diluent	Incubation time(h)	Storage length of liquid semen(day)[1]			
		1	3	5	7
BF_5	0.5	85.0 ± 0.00 [a]	70.0 ± 3.16 [a]	48.6 ± 1.86 [b]	8.6 ± 1.86 [b]
Bütschwiler		85.0 ± 3.15 [a]	80.0 ± 3.16 [a]	75.0 ± 3.16 [a]	66.8 ± 4.83 [a]
BF_5	2	78.1 ± 1.83 [a]	61.8 ± 6.58 [a]	26.8 ± 3.65 [b]	5.8 ± 2.04 [b]
Bütschwiler		81.8 ± 1.83 [a]	73.6 ± 1.86 [a]	65.0 ± 3.16 [a]	58.6 ± 3.66 [a]

[1] Values were observed after 0.5 and 2h incubation in water bath of 37℃. Mean ± S.E. for 6 ejaculates from Large White boar.

[a,b] Means in the same column with different superscripts at 0.5 and 2h incubation time differ significantly($p<0.05$), respectively.

Table 3-2. Comparison of percentage of NAR on the liquid boar semen diluted with two diluents and preserved at the 15℃

Diluent	Incubation time(h)	Storage length of liquid semen(day)[1]			
		1	3	5	7
BF_5	0.5	86.8±2.40 [a]	70.8±3.48 [b]	24.8±4.79 [b]	4.0±0.63 [b]
Bütschwiler		89.0±1.67 [a]	84.1±2.04 [a]	71.8±4.57 [a]	63.1±1.94 [a]
BF_5	2	83.1±2.04 [a]	56.8±0.98 [b]	16.1±4.02 [b]	2.6±1.36 [b]
Bütschwiler		88.1±1.83 [a]	73.8±2.99 [a]	65.1±4.11 [a]	58.8±1.60 [a]

[1] Values were observed after 0.5 and 2h incubation in water bath of 37℃. Mean ± S,E. for 6 ejaculates from Large White boar.

[a,b] Means in the same column with different superscripts at 0.5 and 2h incubation time differ significantly($p<0.05$), respectively.

BW_{25}, Androhep and Reading(Revell and Glossop, 1989) diluents showed high motility and NAR acrosome as compared with the Bütschwiler diluent. In future, we need to compare the above diluents under the same environment. In this study, the diluted sperm with Bütschwiler diluent in 100 ml plastic bottle will be stored for 7 days at 15℃. However, the sperm with BF_5 diluent was not used after 3 days at 15℃ preservation temperature.

3. Effect of glycerol concentration of liquid semen in BF_5 diluent

The percentages of motility and NAR acrosome on the liquid boar semen diluted different glycerol concentration in BF_5 diluent are presented in Table 4-1 and 4-2.

As shown in Table 4-1, the motility after 0.5 and 2h incubation at 37℃, respectively, was significantly higher for sperm preserved in 0, 1 and 2% final glycerol concentrations than for sperm preserved in 3 and 5% final glycerol concentrations.

As shown in Table 4-2, NAR acrosome after 0.5 and 2h incubation at 37℃, respectively, was significantly higher for sperm preserved 1 and 2% final glycerol concentrations than for sperm preserved in 0, 3 and 5% final glycerol concentrations.

Glycerol concentration affects post-thaw survival of sperm frozen by either the straw or pellet method. The general view is that relatively low concentrations of glycerol(1 to 3%) are required for maximum post-thaw survival(Pursel *et al.*, 1978; Paquignon, 1985; Almlid and Johnson, 1988). Graham and Crabo(1972), comparing post-thaw viablity of boar sperm frozen with 0, 1, 2, or 5% glycerol, found that both motility and NAR acrosome of sperm decreased at 5% level. In the present study, values for motility were maximum at 1 or 2% glycerol and for NAR acrosome were maximum at 1% glycerol

4. Fertilizing capacity of BF_5 and Bütschwiler diluents

Farrowing rate, litter size and average pig

Table 4-1. Comparison of glycerol concentration on the percentage of motility of liquid boar semen diluted with BF_5 diluent

Final glycerol concentration(%)	Incubation time(h)	Storage length of liquid semen at 5℃(day) [1]			
		1	3	5	7
0	0.5	80.0±3.16 ^{a}	75.0±3.16 ^{a}	65.0±3.16 ^{ab}	65.0±3.16 ^{a}
1		81.3±3.66 ^{a}	76.3±3.66 ^{a}	70.0±6.32 ^{a}	68.1±4.83 ^{a}
2		81.3±3.66 ^{a}	73.1±3.65 ^{a}	70.0±3.16 ^{a}	68.1±4.83 ^{a}
3		73.1±4.83 ^{ab}	70.0±6.32 ^{ab}	65.0±6.32 ^{ab}	55.0±3.16 ^{b}
5		65.0±6.32 ^{b}	61.3±7.96 ^{b}	58.1±7.96 ^{b}	50.0±6.32 ^{b}
0	2	78.1±4.83 ^{ab}	70.0±5.47 ^{a}	51.3±4.84 ^{a}	51.3±4.84 ^{a}
1		80.0±5.47 ^{a}	73.1±7.30 ^{a}	55.0±9.48 ^{a}	51.3±6.59 ^{a}
2		80.0±3.16 ^{a}	68.1±7.96 ^{a}	51.3±7.96 ^{a}	51.3±7.96 ^{a}
3		70.3±3.16 ^{bc}	53.1±4.83 ^{b}	38.1±4.83 ^{b}	31.3±7.96 ^{b}
5		63.1±3.65 ^{c}	50.0±5.47 ^{b}	35.0±3.16 ^{b}	30.0±3.16 ^{b}

[1] Values were observed after 0.5 and 2h incubation in water bath of 37℃. Mean ± S.E. for 6 ejaculates from Large White boar.

^{a,b,c} Means in the same column with different superscripts at 0.5 and 2h incubation time differ significantly($p<0.05$), respectively.

Table 4-2. Comparison of glycerol concentration on percentage of NAR of liquid boar semen diluted with BF_5 diluent

Final glycerol concentration(%)	Incubation time(h)	Storage length of liquid semen at 5℃(day) [1]			
		1	3	5	7
0	0.5	82.3±2.39 ^{b}	59.1±4.11 ^{b}	54.0±1.26 ^{bc}	43.3±2.42 ^{b}
1		82.8±3.25 ^{b}	73.1±0.98 ^{a}	64.6±1.31 ^{a}	57.1±0.98 ^{a}
2		90.0±1.67 ^{a}	69.0±1.67 ^{a}	59.1±1.83 ^{ab}	51.3±0.81 ^{ab}
3		82.0±2.89 ^{b}	68.0±1.67 ^{a}	55.3±3.20 ^{bc}	50.3±2.34 ^{ab}
5		78.0±1.67 ^{c}	55.3±1.50 ^{b}	52.1±3.25 ^{c}	51.0±6.32 ^{ab}
0	2	81.1±0.98 ^{a}	51.0±1.16 ^{b}	35.1±5.87 ^{c}	37.0±4.57 ^{c}
1		81.0±1.67 ^{a}	60.3±3.20 ^{a}	53.0±7.12 ^{a}	51.0±0.63 ^{a}
2		82.3±1.03 ^{a}	56.3±6.08 ^{ab}	47.1±2.40 ^{ab}	43.3±2.06 ^{ab}
3		72.3±4.22 ^{b}	52.3±5.71 ^{ab}	40.3±0.81 ^{c}	40.3±1.96 ^{bc}
5		70.1±1.60 ^{b}	48.1±4.11 ^{b}	42.8±2.04 ^{bc}	37.3±1.36 ^{c}

[1] Values were observed after 0.5 and 2h incubation in water bath of 37℃. Mean ± S.E. for 6 ejaculates from Large White boar.

^{a,b,c} Means in the same column with different superscripts at 0.5 and 2h incubation time differ significantly($p<0.05$), respectively.

Table 5. Fertilizing capacity of liquid semen stored in 100ml plastic bottle with BF_5 of 1% glycerol or Bütschwiler diluents

Diluent	No. of gilts inseminated[1]	Farrowed No.	%	No. of pigs born alive per litter	Average pig weight at birth(kg)
BF_5	20	17	85.0	9.8±1.48	1.36±0.29
Bütschwiler	20	16	80.0	10.3±1.69	1.34±0.36

[1] Gilts were inseminated twice with liquid boar semen stored at 5℃ in BF_5 or 15℃ in Bütschwiler diluents for 6~7 days. Sperm concentration was 50×10^8 sperm/80ml in 100ml plastic bottle.

weight at birth for semen diluted in BF_5 or Bütschwiler diluents are presented in Table 5.

Farrowing rate, litter size and average pig weight at birth did not differ significantly between BF_5 and Bütschwiler diluents. In this study, we found out that liquid boar semen can be stored for 6~7 days at 5℃ in BF_5 diluent and 15℃ in Bütschwiler diluent.

SUMMARY

This study was done to find out the methods of long-term use of liquid boar semen in 100 ml plastic bottle for artificial insemination and to investigate differences between BF_5 and Bütschwiler diluents according to storage temperature, and effect of final glycerol concentration in BF_5 diluent. Liquid boar semen diluted with BF_5 diluent showed significantly higher sperm motility($p<0.05$) after 0.5 and 2h incubation at 37℃ than Bütschwiler diluent at all storage length when it was preserved in the 5℃ refrigerator. The NAR acrosome in BF_5 diluent after 0.5 and 2h incubation at 37℃, respectively, during preservation periods was similar to that in Bütschwiler diluent. When liquid boar semen was preserved at 15℃, liquid boar semen in the Bütschwiler diluent showed significantly higher percentages of sperm motility and NAR acrosome from third day to seventh than that in BF_5

diluent. In the effect of final glycerol concentration of liquid boar semen in the BF_5 diluent, the final glycerol concentration of 1% showed higer percentages of sperm motility and NAR acrosome than that of 0, 2, 3, and 5%. Farrowing rate, litter size and average pig weight at birth did not differ significantly between BF_5 and Bütschwiler diluents. As a result of this study, we found out that liquid boar semen can be stored for 6~7 days at 5℃ in BF_5 diluent and at 15℃ in Bütschwiler diluent.

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