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Effect of holding time and removal of seminal plasma on preservation of boar semen at liquid state

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ABSTRACT

Effects of 3 holding times and 3 levels of removal of seminal plasma on the quality of Hampshire boar semen during preservation in BTS extender for different periods were studied. The semen samples were held at 24°C prior to extension and preservation. The effect of removal of 0, 50, 75 and 100% of seminal plasma was studied after holding for 5 h by subsequently replacing and extending it with BTS before preservation. The mean sperm motility, live sperm count and intact acrosome were significantly higher at 5 h than at 6 and 7 h of holding. The corresponding parameters recorded at zero hour of preservation were 83.89±1.25, 89.22±1.29 and 88.78±1.35%, which decreased significantly with increase in hour of preservation and recorded to be 66.39±2.17, 70.78±1.83 and 75.83±1.73% at 96 h of preservation in semen held for 5 h. All the 3 sperm parameters were significantly higher in semen without removal of seminal plasma, i.e., non-removal than in 50, 75 and 100% removal of seminal plasma. The mean sperm motility, live sperm count and intact acrosome recorded were 66.39±2.17, 71.39±1.36 and 70.61±1.22% at 96 h of preservation without removal of seminal plasma.

Key words: Boar semen quality, Holding, Preservation, Seminal plasma removal

Success of artificial insemination depends on the quality of semen during preservation. Susceptibility of boar spermatozoa to cooler temperature poses a hindrance in preservation of semen. Efforts were made earlier for circumventing the drawback and to improve the quality of boar semen during preservation by adopting processing techniques like holding of semen before processing (Galli *et al*. 1991) and replacement of seminal plasma with extender (Shimatsu *et al*. 2002). However, there is diversity of opinion with regard to the efficacy of processing techniques. Therefore, the present investigation was designed to study the effect of holding time and removal of seminal plasma on preservation of boar semen at 18°C.

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MATERIALS AND METHODS

Ejaculates (18) collecting 6 from each of 3 adult healthy Hampshire boars by simple fist method were used to study the effect of holding semen on its preservation for different periods in BTS extender that was found suitable for preserving boar semen. Immediately after collection semen was taken in a 100 ml conical flask and placed in a beaker containing water (30°C) and kept in a BOD incubator maintained at 24°C. Semen was taken out of the incubator in aliquots after allowing 5, 6 and 7 h of holding time and extended in BTS extender @ 1: 3 and preserved in 5 ml glass vials in a BOD incubator at 18°C for 96 h and evaluated for sperm motility, live sperm and intact acrosome following standard methods at 0 h (i.e., immediately after extension), 24, 48, 72 and 96 h of preservation.

Additional 18 ejaculates collected as before were subjected to study the effect of removal of seminal plasma on preservation of semen in BTS extender allowing the holding time that was found superior in the first experiment. After holding, the semen was split into 4 parts and 3 split samples were centrifuged at 3,000 rpm separately for 20 min and 50, 75 and 100% seminal plasma was pipetted out from 3 parts respectively. The removed portion of seminal plasma

was replaced with equal volume of BTS extender to bring it to the original volume. The fourth part was not centrifuged and kept as such considering it as 0% seminal plasma removal. All the 4 split samples were extended (1 : 3) in BTS extender and preserved and evaluated for the same criteria as before.

The data generated were subjected to statistical analysis as per Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The mean values of sperm motility, live sperm and incidence of intact acrosome in Hampshire boar semen at different hours of preservation in BTS extender at 18°C for different holding times prior to extension are furnished in Table 1. The highest and the lowest percentage before extension and after 96 h of preservation of semen in the extender in respect of sperm motility, live sperm and intact acrosome were obtained at 5 and 7 h of holding respectively. The mean sperm motility, live sperm and intact acrosome differed significantly (P<0.01) between holding times and between preservation periods. The interaction between holding time and preservation period was not significant. The mean% motile, live sperm and intact acrosome were significantly (P<0.01) higher in 5 h than in 6 and 7 h and in 6 h than in 7 h of holding time irrespective of preservation period. The highest percentage of motile and live spermatozoa recorded at 0 h of preservation with 5 h of holding was in agreement with that reported by Lalrintluanga (1994) and Tallilepzuk (1998). The significantly higher overall mean sperm motility and live sperm obtained on preservation after 5 h of holding time as compared to that after 6 and 7 h and after 6 h than after 7 h of holding irrespective of hour of preservation recorded in the present study could be due to higher metabolic rate for longer hour of holding that could lead to depletion of available nutrients on prolonged preservation and increase in pH and subsequent cell senescence (Tamuli 1993). Lalrintluanga (1994) also observed the highest sperm motility and live sperm on preservation at 15°C following holding for 5 h at 24°C. Mann and Lutwak-Mann (1981) maintained that the decrease in sperm motility and live sperm with increase in holding time might be due to lack of sufficient substrate in undiluted semen resulting in death of proportion of spermatozoa during holding at higher temperature. However, Pursel *et al*. (1973) found that optimum holding time was 6 h before preservation when boar semen was preserved subsequently at 5°C.

The highest percentage of intact acrosome obtained with 5 h of holding at 0 h of preservation found credence from the observation of Tamuli (1993), Lalrintluanga (1994) and Tallilepzuk (1998). The significantly higher overall mean intact acrosome after 5 h of holding time than after 6 and 7 h of holding irrespective of preservation period in the present study could be due to damage to the sperm plasma membrane and acrosome due to longer holding (Robertson *et al*.1989) or due to increase in pH along with increase in holding time. Lalrintluanga (1994) also observed the highest incidence of intact acrosome after 5 h of holding at 24°C after preservation at 15°C.

The overall mean percentages of motile, live sperm and sperm with intact acrosome declined significantly (P<0.01) with the increase in the preservation period irrespective of holding time. This could be due to progressive weakness of sperm cells and peroxidation effect (Jones and Mann 1977) on acrosome with increase in the period of preservation. Reduction in sperm motility and live sperm with the increase in preservation period was also recorded by Lalrintluanga (1994), Tallilepzuk (1998) and Kommisrud *et al*. (2002).

Table 1. Mean sperm motility, live sperm count and intact acrosome in Hampshire boar semen at different hours of preservation in BTS extender at 18ºC for different holding times

Hour of preservation	Sperm motility (%) holding time (h)				Live sperm count (%) holding time (h)				Intact acrosome (%) holding time (h)			
	5	6	7	Overall	5	6	7	Overall	5	6	7	Overall
0	83.89	81.11	74.16	79.72[a]	89.22	85.67	80.33	85.07[a]	88.78	86.72	81.33	85.61[a]
	±1.25	±1.25	±1.58	±0.96	±1.29	±1.19	±1.23	±0.86	±1.35	±1.01	±1.14	±0.79
24	78.61	74.17	70.28	74.35[b]	82.22	78.78	75.67	78.89[b]	84.17	77.5	73.5	78.39[b]
	±1.5	±1.42	±1.87	±1.02	±1.57	±1.25	±1.42	±0.87	±1.13	±0.96	±1.42	±0.9
48	76.11	67.78	62.78	68.89[c]	81.11	73.06	68.61	74.26[c]	81.33	73.61	69.5	74.81[c]
	±1.49	±1.63	±2.26	±1.28	±1.5	±1.74	±1.34	±1.12	±0.98	±1.56	±1.24	±0.99
72	70.00	61.94	56.67	62.87[d]	74.56	67.44	62.83	68.58[d]	77.06	71.06	61.94	70.02[d]
	±2.29	±1.35	±2.25	(1.37	±1.99	±1.49	±2.48	±1.33	±1.42	±1.68	±1.69	±1.24
96	66.39	57.22	49.44	57.69[e]	70.78	62.94	58.67	64.13[e]	75.83	66	58.72	66.85[e]
	±2.17	±1.63	±2.25	±1.5	±1.83	±1.46	±2.3	±1.28	±1.73	±1.87	±2.16	±1.46
Overall	75.00[a]	68.44[b]	62.67[c]		79.58[a]	73.58[b]	69.22[c]		81.43[a]	74.98[b]	69.00[c]	
	±1.02	±1.11	±1.31		±1	±1.06	±1.16		±0.77	±0.98	±1.1	

Within parentheses are standard errors (SE).Overall means bearing different superscripts in column and row differ significantly (P<0.01).

Table 2. Mean sperm motility, live sperm count and intact acrosome in Hampshire boar semen at different hours of preservation in BTS extender at 18ºC in different levels of seminal plasma removal

Hour of preservation	Sperm motility (%) level of seminal plasma removal (%)					Live sperm count (%) level of seminal plasma removal (%)					Intact acrosome (%) level of seminal plasma removal (%)				
	0	50	75	100	Overall	0	50	75	100	Overall	0	50	75	100	Overall
0	83.33	74.72	66.11	63.06	71.81[a]	85.94	80.5	70.89	68.38	76.43[a]	89	80	71.72	66.22	76.74[a]
	±0.99	±1.11	±2.51	±2.56	±1.34	±0.6	±1.09	±2.33	±2.14	±1.19	±0.94	±1.65	±2.56	±2.03	±1.38
24	78.89	65.83	59.17	54.72	64.65[b]	80	73.94	63.5	61.61	69.76[b]	81.72	70.16	63.5	58.44	68.46[b]
	±0.95	±2.98	±2.98	±2.76	±1.66	±0.77	±2.15	±2.37	±2.78	±1.38	±1.09	±1.82	±3.07	±2.24	±1.48
48	76.11	59.44	49.72	45	56.60[c]	75.28	62.94	56.94	50.78	61.49[c]	77.56	63.22	56.33	52	62.28[c]
	±1.49	±2.35	±1.87	±2.21	±1.55	±0.6	±2.23	±2.23	±1.66	±1.39	±1.28	±1.78	±2.78	±2.33	±1.55
72	70	39.72	35.28	28.23	42.50[d]	73.94	46.61	41.33	35.56	49.36[d]	72.5	52.5	40.17	33.72	49.72[d]
	±2.29	±1.59	±1.37	±0.99	±4.83	±0.9	±1.3	±1.26	±1.35	±1.85	±1.39	±1	±1.18	±1.09	±1.85
96	66.39	26.67	25.28	21.67	33.82[e]	71.39	33.06	34.11	29.4	42.01[e]	70.61	40.44	33.44	26.78	42.82[e]
	±2.17	±1.37	±1.37	±1.14	±2.04	±1.36	±0.87	±1.22	±1.09	±2.1	±1.22	±1.3	±1.22	±0.92	±2.07
Overall	71.80[a]	53.20[b]	47.11[c]	43.20[d]	77.30[a]	59.40[b]	53.40[c]	49.20[d]	78.28[a]	61.27[b]	53.03[c]	47.43[d]			
	±1.25	±2.04	±1.85	(1.97	±0.68	±1.98	±1.68	±1.78	±0.88	±1.61	±1.82	±1.77			

Within parentheses are standard errors (SE). Overall means bearing different superscripts in column and row differ significantly (P<0.01).

Lalrintluanga (1994) also observed decline in intact acrosome with progression of preservation period. It emerged from the study that holding of Hampshire boar semen for 5 h before processing resulted in better semen quality on preservation as compared to 6 and 7 h of holding.

The mean values of sperm motility, live sperm and incidence of intact acrosome in Hampshire boar semen at different hours of preservation in BTS extender at 18°C for different levels of seminal plasma removal after holding at 24°C for 5 h that was found most suitable in the previous experiment are presented in Table 2. The mean sperm motility, live sperm and intact acrosome differed significantly (P<0.01) between the levels of seminal plasma removal and also between preservation periods. The interaction between levels of seminal plasma removal and preservation period was also highly significant (P<0.01). It was revealed that the percentages of sperm motility, live sperm and intact acrosome were significantly (P<0.01) higher in semen without removal of seminal plasma i.e., 0% removal as compared to that with 50, 75 and 100% removal of seminal plasma irrespective of hour of preservation. The percentages of sperm motility, live sperm and intact acrosome were significantly (P<0.01) higher for removal of lower percentage of seminal plasma over the successive higher percentage of removal of seminal plasma. The lowering of sperm motility and live sperm due to higher removal of seminal plasma might be attributed to washing of semen for removal of the plasma that resulted in pellet formation at the bottom of the centrifuge tube and possible stickiness of the cells which could damage cell membranes (Islam 1998) and thus lower the percentage of sperm motility and viability. Pursel *et al*. (1973) also observed that the percentage of sperm motility and live sperm dropped markedly on replacing seminal plasma. However, Shimatsu *et al*. (2002) observed that Modena extender could be effective in storing miniature pig semen for 5 days at 15°C on complete substitution of seminal plasma. The discrepancy might be due to the difference in the extender used and preservation temperature.

The highest percentage of sperm motility, live sperm and intact acrosome obtained during preservation of sperm held and extended without removal of seminal plasma (0% removal) might be due to availability of sufficient substrate and buffer for supporting sperm metabolism and neutralizing lactic acid during holding. The lowest sperm motility and live sperm recorded in 100% replacement of seminal plasma during preservation might be due to sperm loss because of removal of supernatant (Katkov and Mazur 1998) and physical damage caused to the sperm population during centrifugation (Alvarez *et al*. 1993). Rozeboom (2000) reported that inclusion of seminal plasma in the insemination medium of pigs increased the chances of conception at subsequent insemination by attenuating the inflammatory response of the uterus to the previous insemination. Reduction in percentage of motile, live sperm and intact acrosome with ascending level of removal of seminal plasma could be due to increase in injury on the sperm membrane (Alvarez *et al*. 1993) resulting from progressive reduction of biological protection that was rendered by the seminal plasma. Detrimental effect of washing or removal of seminal plasma causing damage to the plasma membrane and acrosome has been documented (Robertson *et al*. 1989). Incidence of intact acrosome was reported to be markedly dropped on replacement of seminal plasma (Pursel *et al*. 1973).

The significant (P<0.01) decrease in sperm motility, live sperm and intact acrosome with increase in hour of

preservation could be due to deterioration in sperm quality with increasing depletion of available sperm nutrient substrate. Kato *et al*. (1990) also observed that incidence of intact acrosome was higher after 10 days than after 15 days of preservation when centrifuged boar spermatozoa were resuspended with seminal plasma in Tris – citrate glucose extender with or without egg yolk and catalase.

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