

---

## **Effect of Heterospermy on the Quality of Spermatozoa during Preservation of Boar Semen at 18<sup>0</sup>c**

**Lalrintluanga, K.\*, Deka B.C.\*\*, L. Hmar.\*\*\*, Dipak Bhuyan\*\*\*\*and  
Biswas R.K.\*\*\*\*\***

*Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science, Assam  
Agricultural University, Khanapara, Guwahati.*

### **ABSTRACT:**

*A total of 24 ejaculates collected from two Yorkshire boars (Boar –A and Boar –B) were diluted using BTS extender. From each ejaculate, one-third of diluted semen of Boar-A was mixed with one-third of diluted semen of Boar –B and considered as heterospermic semen. The remaining two-third of each ejaculates of Boar-A and Boar-B and heterospermic semen were preserved for 72 hours at 18<sup>0</sup>C in a BOD incubator. The semen were evaluated for sperm motility, live sperm count, percentage of intact acrosome, Hypo-osmotic sperm swelling test (HOSST) at 0, 24, 48 and 72 hours of preservation. The study may be concluded that the heterospermic semen did not have significant effect on the quality of LWY boar semen during preservation.*

**Keywords:** Heterospermy, Preservation, Boar semen, Hypoosmotic sperm swelling test.

Heterospermy is the mixing of semen ejaculates from different boars and is expected to improve the semen quality. However, the correlation between heterospermy admixture and the quality of spermatozoa during preservation remains to be understood. The inherent disadvantages in the use of semen from one boar for the insemination of the sow have been identified (Long *et al.* 1998). Male with unproven sperm motility, concentration and acrosome morphology were found to have serious clinical problems as azoospermia. Severe oligozoospermia, tetrazoospermia and asthenozoospermia are also more difficult to be diagnosed than total or near total infertility (Schafer and Holzmann, 2003). It is important therefore, not to base the verdict about the fertility of boar on a single boar semen sample. It is reasonable to expect that a higher semen quality could be achieved from semen pooled together from different boars. This study was conducted to evaluate the effectiveness of heterospermy on the quality of Large White Yorkshire boar semen during preservation.

### **MATERIALS AND METHODS**

Semen was collected twice a week from Large White Yorkshire (LWY) boars by gloved hand technique (Hancock and Hovell, 1959), using a dummy as mount. Boars were maintained under the same management and used for routine semen collection in Semen Collection Centre, Govt. of Mizoram, Selesih, Aizawl. Semen collections were made in the morning from 6.00 to 7.00 AM. from two boars per collection day. The boar was brought to the semen collection site and was allowed to mount over the dummy. After erection of the penis the corkscrew end was grasped firmly with gloved hand and an intermittent pulsatile pressure was applied on penis for ejaculating semen. The collected semen was strained through filter

gauze into a graduated collecting beaker of 500 ml capacity. A total of 24 ejaculates collected from two Yorkshire boars (Boar –A and Boar –B) were diluted using BTS extender. From each ejaculate, one-third of diluted semen of Boar-A was mixed with one-third of diluted semen of Boar –B and considered as heterospermic semen. The remaining two-third of each ejaculates of Boar-A and Boar-B and heterospermic semen were preserved for 72 hours at 18<sup>0</sup>C in a BOD incubator. The dilution of semen was done in such a way so that each inseminate dose of 100 ml diluted semen contained 4x10<sup>9</sup> sperm. The semen were evaluated for sperm motility, live sperm count (Beatty, 1957), percentage of intact acrosome( Watson, 1975), Hypo-osmotic sperm swelling test (HOSST) (Jeyendran *et al.* 1984) at 0, 24, 48 and 72 hours of preservation.

## RESULTS AND DISCUSSION

The mean values of effect of heterospermy on progressive motility, live sperm count, intact acrosome and Hypo-osmotic sperm swelling test in BTS extender at different hours of preservation at 18<sup>0</sup>C are presented in Tables 1

**Table-1. Mean ± S.E. Of Effect Of Individual And Heterospermic Semen On Progressively Motile Sperm, Live Sperm, Intact Acrosome And Hosst Of Boar Spermatozoa Preserved In Bts Extender At 18<sup>0</sup>c**

Source of Semen.	Preservation period				Overall
	0 hr	24 hr	48 hr	72 hr	
<i>Progressively motile sperm (%)</i>					
Boar A	76.54 ± 1.46	64.50 ± 1.95	56.13 ± 2.31	49.50 ± 2.38	61.66 ± 5.83
Boar B	76.38 ± 1.10	66.63 ± 1.40	60.00 ± 1.43	53.58 ± 1.58	64.14 ± 4.87
Boar A+B	75.83 ± 1.31	66.33 ± 1.84	57.00 ± 1.84	52.29 ± 2.61	62.86 ± 5.21
Overall	76.25 <sup>a</sup> ± 0.74	65.82 <sup>b</sup> ± 1.00	57.71 <sup>c</sup> ± 1.21	51.79 <sup>d</sup> ± 1.29	
<i>Live sperm (%)</i>					
Boar A	90.75 ± 0.99	84.25 ± 1.08	78.00 ± 1.24	70.92 ± 1.25	80.98 ± 4.24
Boar B	90.88 ± 1.16	85.04 ± 0.78	79.33 ± 1.09	73.42 ± 1.32	82.16 ± 3.74
Boar A+B	90.63 ± 0.97	84.67 ± 0.62	78.79 ± 0.96	72.08 ± 1.18	81.54 ± 3.97
Overall	90.75 <sup>a</sup> ± 0.59	84.65 <sup>b</sup> ± 0.48	78.71 <sup>c</sup> ± 0.63	72.14 <sup>d</sup> ± 0.72	
<i>Intact acrosome (%)</i>					
Boar A	89.08 ± 0.94	81.42 ± 1.12	77.75 ± 1.30	70.08 ± 1.61	79.58 ± 3.95
Boar B	89.38 ± 1.12	82.21 ± 1.12	77.79 ± 1.09	72.25 ± 1.28	80.40 ± 3.61
Boar A+B	89.63 ± 0.98	81.79 ± 1.10	77.58 ± 1.09	71.17 ± 1.36	80.04 ± 3.87
Overall	89.36 <sup>a</sup> ± 0.58	81.81 <sup>b</sup> ± 0.63	77.71 <sup>c</sup> ± 0.66	71.17 <sup>d</sup> ± 0.81	
<i>HOSST reacted sperm (%)</i>					
Boar A	59.96 ± 2.18	51.79 ± 1.81	44.50 ± 1.59	37.63 ± 1.36	44.64 ± 4.08
Boar B	60.13 ± 2.18	52.67 ± 1.92	45.54 ± 1.64	38.38 ± 1.45	49.18 ± 4.67
Boar A+B	59.79 ± 2.12	52.00 ± 1.79	44.96 ± 1.52	37.92 ± 1.32	48.66 ± 4.69
Overall	59.96 <sup>a</sup> ± 1.23	52.15 <sup>b</sup> ± 1.04	45.00 <sup>c</sup> ± 0.90	37.97 <sup>d</sup> ± 0.78	

The mean progressively motile sperm of individual and heterosperm in the present study was found to be comparable with the finding of Ogbuewe *et al.* (2007) who reported the sperm

motility of one boar and two boars' semen mixture as 70 and 75 per cent respectively. The mean progressively motile sperm did not differ significantly between Boar A, Boar B and Boar A + B which was in agreement with the report of Ogbuewe *et al.* (2007). The percentage of progressively motile sperm differed significantly ( $P < 0.01$ ) between preservation periods. On critical difference test it was observed that the percentage of progressively motile sperm decreased significantly ( $P < 0.05$ ) with each increase in preservation period. Ehlers *et al.* (1958) observed that in certain cases of bull semen, fructose utilization and lactic acid formation could be enhanced by mixing semen of different bulls and they suggested that mixing of semen led to increased plasma - fructose supply which was supposed to improve sperm quality. In the present finding, the sperm motility from mixed semen of Boar A and Boar B was higher, though not significantly, than that of Boar – A, but lower than that of Boar B during preservation. The improvement of sperm motility in mixed semen over the inferior semen i.e., semen of Boar A, was not significant because the difference in sperm motility between Boar A and Boar B was also not significant. The mean progressively motile sperm irrespective of the source of semen did not vary significantly due to interaction of source of semen and preservation period which showed that the main effects were independent.

The mean live sperm count in individual and mixed semen in the present study was comparable with the report of Ogbuewe *et al.* (2007). The mean live sperm count did not differ significantly between Boar A, Boar B and Boar A + B. This was in agreement with the report of Ogbuewe *et al.* (2007). However, it differed significantly ( $P < 0.01$ ) between preservation periods. On critical difference test it was observed that the percentage of live sperm decreased significantly ( $P < 0.05$ ) with each increase in preservation period. Though not significant, the overall live sperm count in mixed semen from Boar A and Boar B was higher than that of Boar A and lower than that of Boar B during preservation. This non-significant difference in live sperm count between the semen from one boar and the mixed semen from two boars was in accordance with the report of Ogbuewe *et al.* (2007) who recorded the live sperm count from one boar and the admixtures of semen from two boars as 86 and 88 percent respectively.

The mean percentage of intact acrosome in individual and mixed semen in the present study was comparable with the report of Ogbuewe *et al.* (2007) who reported the incidence of intact acrosome as 70 per cent in one boar and 76 per cent from mixed semen of two boars, 79 per cent from three boars, 67 per cent from four boars and 60 per cent from admixture of semen of five boars. The mean percentage of intact acrosome did not differ significantly between Boar A, Boar B and Boar A + B, which was in agreement with the report of Ogbuewe *et al.*, (2007). The percentage of intact acrosome differed significantly ( $P < 0.01$ ) between preservation periods. On critical difference test, it was observed that the percentage of intact acrosome decreased significantly ( $P < 0.05$ ) with each increase in preservation period.

The percentage of HOSST reacted sperm, irrespective of preservation period, did not differ significantly between the sources of semen. But differed significantly ( $P < 0.01$ ) between preservation periods but not due to interaction of source of semen and preservation period which showed that the main effects were independent.

The study may be concluded that the heterospermic semen did not have significant effect on the quality of LWY boar semen during preservation

---

**REFERENCES**

- i. Beatty, R.A. (1957). Nigrosin-Eosin staining of rabbit spermatozoa and the fertility of semen. *Proc. Rol. Soc. Edin.* **67**:1.
- ii. Ehlers, M.H.; Kuskwaha, N.S. and Erb, R.E. (1958). *J. Dairy Sci.* **36**:1021 In: The Biochemistry of semen and of the male Reproductive Tract. Thaddeus Mann, First Published 1964, Great Britain :Butler and Tanner Ltd, Frome. p. 66
- iii. Hancock, J. L. and Hovell, G. L. K. (1959). The collection of boar semen. *Vet. Rec.* **71**:664.
- iv. Jeyendran, R.S. ; Van der Ven, H.H. ; Perez-Pelaez, M. ; Crabo, B.G. and Zaneveld, L.J.D. (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J. Reprod. Fertil.* **70**: 219-225.
- v. Long, C.R.; Rath, G.R.; Welch, *et al.*(1998). *In vitro* production of porcine embryos from semen stored for sex with a high speed cell sorter: Comparison of two fertilization media. *Theriogenology* **49**:363 abstract).
- vi. Ogbuwu, I. P. ; Umesiobi, D.O. ; Okoli, I. C. and Okoro, V.M. (2007). Validation of the polysemen admixture on viability and acrosomal morphology of boars spermatozoa. *Online Health and Allied Sci.* **6** (1):1-5.
- vii. Schafer, S. and Holzmann, A. (2003). The use of transmigration and Spermac<sup>TM</sup> stain to evaluate epididymal spermatozoa. *Anim. Reprod. Sci.* **59**:201-211).
- viii. Watson, P.F. (1975). Use of a Giemsa stain to detect change in acrosomes of frozen ram spermatozoa. *Vet. Rec.* **97**(1):12.