

**ASSESSMENT OF CROSSBRED HAMPSHIRE BOAR SEMEN ON LIQUID PRESERVATION AT 15° C**

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**ABSTRACT**

In the present study, a total of forty (40) ejaculates, ten ejaculates from each of four crossbred Hampshire boars were collected. The semen was diluted in Beltsville Thawing Solution (BTS) depending upon the concentration and preserved at 15 °C to evaluate the effects of different boars' semen at 24 hours of preservation. The total 120 gilts/sows were artificially inseminated 60 gilts/sows with single dose semen (90 ml) after 24 hours in one group and other group 60 gilts/sows were inseminated with dose (90 ml) after 24 and 48 hours (double insemination) from the beginning of oestrus. The gestation period and litter size at birth were calculated and reported. The mean volume, sperm concentration, per cent initial motility ( $P < 0.01$ ) and intact acrosome significantly ( $P < 0.05$ ) differed among the boars immediately after the collection of semen. After 24 hours of preservation except sperm motility, the parameters such as live sperm, intact plasma membrane, intact acrosome differ significant ( $P < 0.05$ ) among the boars. The average litter size at birth in boar 1 and boar 4 differed significantly. The highest litter size at birth has been reported in the double insemination group.

**Keywords:** Artificial Insemination, Crossbred Hampshire boar, Litter size, Semen

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In India's North Eastern area, diversified agriculture includes pig farming as a key component (Talukdar *et al.*, 2019). The demand for pig consumption has expanded globally, which has forced the pork sector to produce in a competitive, efficient, and sustainable manner. This includes implementing genetic modifications that will result in higher production and better-quality animal protein (Roca *et al.*, 2015). For the success of pig industry, the information on the reproductive traits of the animal is important (Haque *et al.*, 2019). Semen volume, sperm concentration and gross sperm morphology are semen traits that affect the better fertility (Roca *et al.*, 2015). Therefore, the study was carried out to know the assessment of crossbred Hampshire boar semen on liquid preservation at 15° C. Meghalaya had a total pig population of 7,06,364 in 2019 and produced 14.93 thousand MT of pork (GoM, 2019). Crossbred Hampshire, Saddle Black, White Yorkshire, and native breeds like Niang Megha are the main pig breeds raised in Meghalaya. About 63.85% of Meghalaya households are active in pig farming (GoM, 2019). In Meghalaya, 11.25 percent of the meat consumed is pork (GoM, 2020). To achieve this, reproductive biotechnologies have been used extensively in boar for decades, with artificial insemination (AI) using liquid semen preserved at 17 °C being the most popular (Johnson *et al.*, 2000).

**MATERIALS AND METHODS**

The present study was conducted at the Division of

Animal and Fisheries Sciences, ICAR Research Complex for NEH Region, Umiam, Meghalaya, India. A total of forty (40) ejaculates, ten ejaculates from each of four crossbred Hampshire boars were collected by gloved hand technique with the help of a dummy sow as standard method. Shortly after collection, the semen was filtered through gauze using a Buchner funnel to remove the gel fraction. After collection, semen characteristics were microscopically evaluated, using standard laboratory techniques and the ejaculates having mass activity of 3+ or more and individual sperm progressive motility of 70% or more were chosen for the experiment (Murasing *et al.*, 2020). The semen was diluted in Beltsville Thawing Solution (BTS) and preserved at 15 °C to evaluate the effects of preservation. After that the semen was processed as per the method described by Kommisrud *et al.* (2002). The semen volume, sperm concentration (Salisbury *et al.*, 1978), mass motility (Saxena, 2000), initial individual progressive motility, per cent live sperm (Blom, 1950), Plasma membrane integrity (Arjun *et al.*, 2021; Arjun *et al.*, 2022) and acrosomal integrity (Watson, 1975) was evaluated.

The total of 120 gilts/sows were artificially inseminated with liquid-preserved semen having total sperm concentration of  $2.5 \times 10^9$ , 60 number of gilts/sows as a single dose (90 ml) after 24 hours of oestrus and the rest 60 gilts/sows were inseminated with double dose (180 ml) as dose rate of 90 ml semen after 24 and 48 hours from the beginning of oestrus. The average litter size at birth was

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calculated and reported. The data collected from the study were subjected to statistical analysis as per Snedecor and Cochran (1994).

## RESULTS AND DISCUSSION

In the present study, evaluation of fresh Hampshire crossbred boar semen i.e., immediately after the collection is depicted in Table 1, while evaluation after 24 hours of preservation with BTS extender at 15 °C is presented in Table 2.

The mean semen volume among the four different Hampshire crossbred boars significantly ( $P<0.01$ ) differed. The overall volume was found to be  $255.75 \pm 5.91$  ml. The present finding was lower with the results reported by Sangma *et al.* (2020). The mean mass motility of four different Hampshire crossbred boars' semen was not statistically significant. The overall mass motility of all ejaculates was found to be  $4.35 \pm 0.05$ . Bhoite *et al.* (2005) reported that mass motility of sperms has been an important attribute for acceptance or rejection of the ejaculate for further processing and use in AI, and it has been positively correlated with keeping quality and fertility of that sample. The mean sperm concentration of fresh semen of four different Hampshire crossbred boars differed significantly ( $P<0.01$ ). The overall sperm concentration was found to be  $177.75 \pm 5.77 \times 10^6$ /ml. The present finding was lower than the results reported by Shylesh *et al.* (2019), Sangma *et al.* (2020). The mean per cent initial motility and intact acrosome of fresh semen of four different Hampshire crossbred boars differed significantly ( $P<0.01$ ). The mean per cent live sperm, intact plasma membrane of fresh semen among four different Hampshire crossbred boars were not statistically

significant. The overall mean per cent initial motility, live sperm, intact plasma membrane and intact acrosome of all ejaculates was found to be  $89.15 \pm 0.28$ ,  $85.77 \pm 0.28$ ,  $65.55 \pm 0.41$  and  $93.95 \pm 0.24$ , respectively. The present finding for initial progressive motility, live sperm and intact acrosome of fresh semen was in close agreement with those reported by Sangma *et al.* (2020). The per cent intact plasma membrane of fresh semen was lower than the finding of Shylesh *et al.* (2019). The differences in initial motility, live sperm, intact plasma membrane and intact acrosome percentage of fresh semen among the boars might be due to genetic predisposition, body weight, and individual physiological status of the boars (Haque *et al.*, 2018).

The overall per cent of sperm motility, live sperm, intact plasma membrane and intact acrosome of all ejaculates after 24 hours of preservation was found to be  $77.22 \pm 0.36$ ,  $79.55 \pm 0.33$ ,  $53.77 \pm 0.54$  and  $84.42 \pm 0.38$ , respectively. The mean per cent sperm motility after 24 hours of preservation of semen among the four different Hampshire crossbred boars was not statistically significant. The present finding of sperm motility after 24 hours of preservation was in close agreement with the findings reported by Kanthraj and Athman (2007). The mean per cent live sperm, intact plasma membrane and intact acrosome after 24 hours of preservation of semen among four different Hampshire crossbred boars were differed significantly ( $P<0.05$ ). The present finding of intact plasma membrane after 24 hours of preservation was higher than the findings reported by Kumaresan *et al.* (2009). Boar body weight, individual physiological status, environmental stress and genetic predisposition may all play a role in the variations in sperm motility, live sperm, intact plasma membrane, and intact acrosome percentage

**Table 1. Characteristics of Hampshire crossbred boar semen immediately after collection (mean  $\pm$  SE), (n=10)**

| Parameter                          | Boar 1                | Boar 2                | Boar 3              | Boar 4              | F value             |
|------------------------------------|-----------------------|-----------------------|---------------------|---------------------|---------------------|
| Volume (ml)                        | $302.00 \pm 8.40^c$   | $218.00 \pm 5.12^a$   | $269.00 \pm 5.25^b$ | $234.00 \pm 3.71^a$ | 40.605**            |
| Concentration ( $\times 10^6$ /ml) | $230.0 \pm 3.94^d$    | $154.00 \pm 3.71^b$   | $141.00 \pm 4.33^a$ | $186.00 \pm 2.66^c$ | 113.789**           |
| Mass motility (0-5 grade)          | $4.01 \pm 0.08$       | $4.24 \pm 0.08$       | $4.22 \pm 0.08$     | $4.55 \pm 0.07$     | 1.876 <sup>NS</sup> |
| Initial motility (%)               | $89.40 \pm 0.54^b$    | $87.70 \pm 0.53^a$    | $90.30 \pm 0.44^b$  | $89.20 \pm 0.51^b$  | 4.446**             |
| Live sperm (%)                     | $86.40 \pm 0.49$      | $84.80 \pm 0.57$      | $85.60 \pm 0.52$    | $86.30 \pm 0.57$    | 1.856 <sup>NS</sup> |
| Intact plasma membrane (%)         | $65.70 \pm 0.61$      | $64.40 \pm 1.20$      | $66.40 \pm 0.63$    | $65.70 \pm 0.76$    | 0.991 <sup>NS</sup> |
| Intact acrosome (%)                | $93.50 \pm 0.54^{ab}$ | $94.20 \pm 0.32^{ab}$ | $94.80 \pm 0.44^b$  | $93.30 \pm 0.51^a$  | 2.175*              |

\* $P<0.05$ ; \*\* $P<0.01$ ; NS Non-Significant; Means bearing different superscripts in each row differed significantly

**Table 2. Characteristics of Hampshire crossbred boar semen after 24 hours of preservation with BTS extender (mean  $\pm$  SE), (n=10)**

| Parameter                  | Boar 1             | Boar 2             | Boar 3                | Boar 4                | F value             |
|----------------------------|--------------------|--------------------|-----------------------|-----------------------|---------------------|
| Sperm motility (%)         | $77.20 \pm 0.80$   | $77.60 \pm 0.71$   | $76.80 \pm 0.74$      | $77.30 \pm 0.76$      | 0.191 <sup>NS</sup> |
| Live sperm (%)             | $78.50 \pm 0.63^a$ | $80.90 \pm 0.62^b$ | $80.20 \pm 0.59^{ab}$ | $78.60 \pm 0.60^a$    | 3.767*              |
| Intact plasma membrane (%) | $56.20 \pm 1.05^b$ | $52.10 \pm 1.12^a$ | $52.40 \pm 0.80^a$    | $54.40 \pm 0.93^{ab}$ | 3.768*              |
| Intact acrosome (%)        | $85.80 \pm 0.87^b$ | $83.10 \pm 0.50^a$ | $84.30 \pm 0.65^{ab}$ | $84.50 \pm 0.85^{ab}$ | 2.233*              |

\* $P<0.05$ ; NS Non-Significant; Means bearing different superscripts in each row differed significantly

**Table 3. Litter size at birth (mean  $\pm$  SE) (n=15)**

| Boar number | Litter size (Single dose) | Litter size (double dose) | t- value            |
|-------------|---------------------------|---------------------------|---------------------|
| 1.          | 8.72 $\pm$ 0.23           | 9.36 $\pm$ 0.36           | 1.466*              |
| 2.          | 8.81 $\pm$ 0.35           | 8.90 $\pm$ 0.36           | 0.178 <sup>NS</sup> |
| 3.          | 8.72 $\pm$ 0.35           | 8.72 $\pm$ 0.33           | 0.0 <sup>NS</sup>   |
| 4.          | 8.81 $\pm$ 0.42           | 9.45 $\pm$ 0.28           | 1.253*              |
| F- value    | 1.270 <sup>NS</sup>       | 0.574 <sup>NS</sup>       |                     |

\*P<0.05; NS Non-Significant

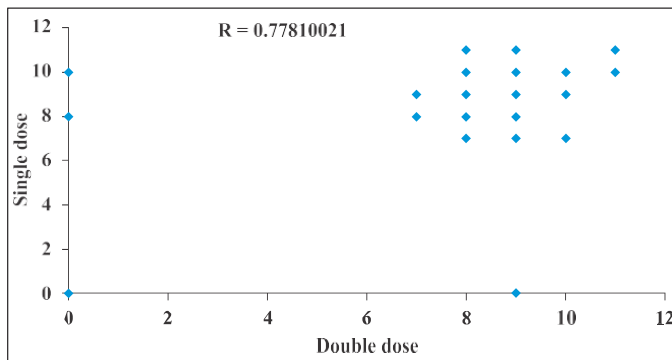


Fig. 1. Correlation between single and double dose insemination of gilts/sows

of semen among the boars.

The average litter size at birth is depicted in Table 3. The average litter size at birth for boar 1 and boar 4 semen differed significantly ( $P<0.05$ ) and the values were positively correlated ( $R=0.77810021$ ) between single and double insemination (Fig. 1); whereas boar 3 and boar 4 were not statistically significant. The present finding was in close agreement with the results reported by Chutia *et al.* (2014). The highest litter size at birth has been reported in the double insemination group which might be due to presence of more numbers of spermatozoa in the female reproductive tract of gilt/sow towards ovulation.

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