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Reproductive performance in cervical and postcervical artificial insemination (PCAI) with liquid boar semen in Gunghroo × Hampshire crossbreed pig in Nagaland

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ABSTRACT

Genetic advancement for the modern swine industry is primarily accomplished through the use of reproductive biotechnology, mainly artificial insemination (AI). The objective of the present study was to compare the fertility outcome by cervical and post-cervical artificial insemination (PCAI) using normal (three billion) and reduced (one billion) number of spermatozoa in pig. Pluriparous weaned sows were grouped into 4 groups, i.e. Group- 1, AI with three billion spermatozoa by intra-cervical insemination; Group-2, AI with one and half billion spermatozoa by intra-cervical insemination; Group-3, AI with three billion spermatozoa by PCAI and Group-4, AI with one and half billion spermatozoa by PCAI. Non-significantly higher farrowing rate was recorded in Group-3 compared to Group-1. Post-cervical AI with lower number of spermatozoa (Group-4) resulted into farrowing rate which was similar to cervical insemination with higher number of spermatozoa (Group-1). There was significant difference in litter size at birth and litter size at weaning between Group-1 and 2. Litter size at birth and litter size at weaning was significantly higher in Group-4 compared to Group-2. Also, litter size at birth and litter size at weaning was significantly higher in PCAI animals (Group-2 and 4) compared to cervical inseminated animals (Group-1 and 2). In conclusion, PCAI with liquid boar semen was found to have improved farrowing rate, litter size at birth and litter size at weaning compared to cervical insemination.

Keywords: Artificial insemination, Cervical, PCAI, Pig, Reproductive performance

Artificial insemination (AI) is one of the most successful reproductive biotechnologies adopted across all the major livestock species. The fact that male germplasm can be collected, evaluated more precisely, processed, stored and deposited in female reproductive tract more efficiently makes AI programme successful throughout the world. AI in pig has been adopted over 50 years ago and is being used extensively worldwide (Knox 2016). Traditional cervical AI requires around three billion spermatozoa to be deposited in female genital tract per insemination. However, only a very small proportion of the spermatozoa reach the sperm reservoir (Cassar *et al.* 2005). Traditional AI method leads to wastage of male germplasm and only a handful of sow can be inseminated per ejaculate. The number of spermatozoa required to be inseminated is directly related to the place of deposition of these spermatozoa.

Backyard pig rearing is an integral part of Naga tradition. Pig husbandry has a significant role in the livelihoods and socio-cultural practices of the tribal farmers in Nagaland (Kumaresan *et al.* 2007, Singh and Mollier 2016, Singh *et al.* 2019). AI with liquid boar semen in pig was introduced in Nagaland to improve the non-descript pig population (Singh *et al.* 2018). There is dearth of information regarding study of different AI dose and route of insemination and its effect on reproductive performance particularly in Indian condition. Therefore, to further improve upon the traditional AI technology, the present study was done to compare the cervical and PCAI with normal and reduced number of spermatozoa per insemination in Gunghroo × Hampshire crossbreed pig.

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MATERIALS AND METHODS

The experiment was done at pig research farm of ICAR Research Complex for NEH Region, Nagaland Centre, Medziphema, Nagaland. Semen was collected from four boars (Gunghroo ×Hampshire) by using glove hand method. Semen was transported in thermous flask to laboratory within 15 min of collection. After examining the semen (macroscopically as well as microscopically), it was diluted in Primxcell (IMV, France) extender. The dilution was done in such a way that each insemination dose (80 mL) contained either three billion or one and half billion motile spermatozoa. Processed liquid boar semen was stored at 17ºC for three days. AI was done on second day of estrus following AM-PM schedule in pluriparous Gunghroo × Hampshire sows. Postcervical CAI was done using Safe Blue catheter (Minitube, Germany) and cervical insemination was done using Golden Gilt (IMV, France) catheter. Adult pluriparous sow were grouped into four groups, Group-1 (40 sows inseminated with three billion

spermatozoa by cervical insemination); Group-2 (40 sows inseminated with one and half billion spermatozoa by cervical insemination); Group-3 (30 sows inseminated with three billion spermatozoa by PCAI) and Group-4 (30 sows inseminated with one and half billion spermatozoa by PCAI). Farrowing rate, litter size at birth, and litter size at weaning were compared among different groups. Farrowing per cent was calculated by dividing the number of pigs farrowed out of total pigs inseminated in each group. The data obtained were analysed using statistical package R version 3.6.1. One way ANOVA was used to see the significant difference between the sample variances between groups. Farrowing rate was compared between different groups by Chi square test. Results are presented as mean±SD and differences were considered significant when $P<0.05$.

RESULTS AND DISCUSSION

The present study tested the fertility effects of normal versus reduced numbers of spermatozoa per insemination and cervical versus PCAI in weaned pluriparous sows. There was non-significant difference ($P>0.05$) in farrowing rate between different groups owing to the number of spermatozoa and site of semen deposition (Table 1). Although, lower number of spermatozoa in cervical insemination, i.e. 1.5 billion per dose led to lower farrowing rate compared to 3.0 billion spermatozoa nonetheless it was not significant ($P=0.5923$). Also, farrowing rate did not differ significantly between Group-1 and Group-2. Non-significantly higher ($P>0.05$) farrowing rate was recorded in Group-3 compared to Group-1. Postcervical AI with lower number of spermatozoa (Group-4) resulted into farrowing rate which was similar to cervical insemination with higher number of spermatozoa (Group-1). This may be due to the fact that in cervical insemination, there is great loss of spermatozoa in backflow of semen and also due to cervical barrier to the spermatozoa. This reduces the effective number of spermatozoa reaching to the uterus and further to utero-tubal junction for fertilization of ova. Billions of spermatozoa are deposited intra-cervically during mating or AI. However, only a very small proportion of the spermatozoa reach the sperm reservoir (Mburu *et al.* 1996). Optimal fertility can be achieved with only 1×10^7 spermatozoa (Krueger and Rath 2000) after surgical insemination near the utero-tubal junction, instead of the 3 billion spermatozoa normally inseminated in the cervix. Knox *et al.* (2017) reported higher farrowing rate with use of 2.5 than 1.5 billion spermatozoa. Non-significantly ($P>0.05$) higher farrowing rate was recorded in PCAI (Group-3 and 4) compared to cervical insemination with lower number of spermatozoa (Group-2). Lower number of spermatozoa in cervical insemination leads to poor sperm reservoir formation at utero-tubal junction compared to PCAI. Intrauterine deposition of spermatozoa avoided loss of spermatozoa at cervical barrier and subsequently more number of spermatozoa reached to the site of fertilization. If insemination close to the utero-tubal junction can be undertaken, a theoretical 100-fold reduction in insemination dose can be obtained (Hunter 2001). Steverink *et al.* (1998) observed a close relationship between fertility, number of spermatozoa inseminated and the process of backflow. There was non-significant difference in farrowing rate between Group-3 and Group-4. Reduction in number of spermatozoa among PCAI groups does not affect farrowing rate. The finding of present study indicate that low dose of spermatozoa deposited intrauterine resulted in to similar farrowing rate as was observed in cervical insemination with higher number of spermatozoa. There is wide variability in estrus exhibition and time of ovulation, particularly in weaned sows; therefore, two times insemination is done, to improve the likelihood that at least one AI will occur within most fertile period. Postcervical AI with lower number of spermatozoa resulted into better utilization of semen of highly productive boar. This could be utilized for enhancing the AI efficiency in pig as more number of semen doses will be produced per ejaculate of boar compared to traditional cervical insemination which has a very low efficiency. With lower sperm used with intrauterine AI, there appears to be a minimum number of sperm needed to achieve similar fertility to controls, (Watson and Behan 2002). The impact of lower sperm used for mated females has been modeled for increased economic value as a result of greater genetic gains through access to higher indexing sires (Fontana *et al.* 2014).

Table 1. Farrowing rate, litter size at birth and litter size at weaning

Group	No. of animals	No. of animals farrowed	Farrowing rate (%)	Litter size at birth	Litter size at weaning
G-I	40	32	80[a]	11.09±1.44[a]	8.93±0.84[a]
G-II	40	30	75[a]	9.36±1.65[b]	8.23±0.93[b]
G-III	30	26	86.67[a]	14.34±1.59[c]	11.69±1.08[c]
G-IV	30	25	83.33[a]	13.28±1.13[c]	11.24±1.01[c]

Means with different superscript differ significantly ($P<0.05$).

However, Braken *et al.* (2003) observed that cervical insemination with a low number of spermatozoa may not result in acceptable fertility even if precisely timed relative to ovulation. While, Watson and Behan (2002) recorded comparable reproductive performance between one billion spermatozoa deposited in the uterine body versus 3 billion spermatozoa deposited intracervically (86.9% versus 91.1% farrowing rate). Pregnancy rate of 77.3% and 12.6 viable fetuses were recorded when 0.5 billion spermatozoa were deposited into the distal uterine horn (Wolken *et al.* 2002).

Reduction in number of spermatozoa in intracervical insemination from 3 billion to 1.5 billion did not lead to significant difference in farrowing rate, however, the same resulted in to significant ($P<0.05$) reduction in litter size at birth and litter size at weaning (Table 1). Lower number of spermatozoa may have led to pregnancy but litter size was

significantly lower which may be due to loss of spermatozoa and fertilization of fewer ova. Litter size at birth and litter size at weaning was significantly (P<0.05) higher in PCAI animals (Group-2 and 4) compared to cervical inseminated animals (Group-1 and 2). No significant difference in litter size at birth or weaning was observed among two groups of PCAI. As optimal numbers of spermatozoa are available at utero-tubal junction in intrauterine inseminated animals, the further litter size will be affected by the genetic potential of mother. Litter size at birth and litter size at weaning was significantly (P<0.05) higher in Group-4 compared to Group-2.

The result of present study revealed that PCAI with lower number of spermatozoa resulted into bigger litter size at birth and also at weaning. Effect of greater number of sperm on litter size and trends on conception and farrowing rate has been demonstrated in earlier study (Knox *et al.* 2017). The higher fertility with use of intrauterine AI occurs due to deposition of semen closer to the site of fertilization (Fontana *et al.* 2014). Watson and Behan (2002) recorded litter size of 12.1 versus 12.5 between one billion spermatozoa deposited in the uterine body versus 3 billion spermatozoa deposited intracervically. The high fertility at a lower sperm dose is achieved by reducing the time and distance sperm must travel to the site of fertilization (Rath 2002) and reducing sperm loss through leakage during (Steverink *et al.* 1998) and back-flow following intrauterine AI (Hernandez-Caravaca *et al.* 2012). However, it was observed that as the number of sperm falls below 2.0 to 1.5 billion, farrowing rate or litter size start to decline (Hernandez-Caravaca *et al.* 2012). Single conventional insemination with 0.5 billion sperm results in lower numbers of embryos compared to 3 billion sperm (Bracken *et al.* 2003). At this time, data is still limiting on the lower limit for numbers of sperm required with use of intrauterine insemination. However, with low sperm numbers and volumes, backflow of sperm still has the potential to negatively affect fertility (Bortolozzo *et al*. 2015) particularly in cervical insemination.

In conclusion, PCAI with lower number of spermatozoa resulted in to comparable reproductive efficiency in term of farrowing rate with that of cervical insemination with three billion spermatozoa. The result of present study also revealed that intrauterine insemination with lower number of spermatozoa resulted into bigger litter size at birth and also at weaning. The PCAI with liquid boar semen at low number of spermatozoa per doses should be of great benefit in cases of semen from superior boars. This will allow wide and effective use of the selected males. Also, it may be of particular significance in case of insemination with cryopreserved semen. However, before this insemination technique is used routinely, more investigations are needed to understand the mechanisms related to sperm capacitation, sperm colonization of the oviducts and identify the minimal sperm numbers needed to obtain maximal fertility results for processed and unprocessed boar spermatozoa.

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