Performance Appraisal of Artificial Insemination Technique in Pig under Organized Farm and Field Condition in Nagaland

Manas Kumar Patra[1], Yhuntilo Kent[2], Soya Rungsung[3], Lily Ngullie[4], Rüüsalie Nakhro[5] and Bidyut C. Deka[6]

1,2,3,4,5&6. ICAR Research Complex for NEH Region, Nagaland Centre, Jharnapani, Medziphema, Nagaland

ABSTRACT

Both organized and un-organized small holder pig farmers suffer due to non-availability of quality germplasm in their vicinity. Indiscriminate use of limited number of breeding male available in the region leads to decline in reproductive performance and overall productivity of the farm. Artificial insemination (AI) technique bring immense opportunities in overcoming the deficiency of breeding boar and could promote in establishment of small holder breeding unit for meeting the demand of quality pig germplasm. An attempt has been made to introduce and popularize the AI technique in pig in Nagaland under the ongoing Mega Seed Project on Pig. With this perspective, the present study has been carried out with two objectives, first to evaluate the semen quality and sexual behaviour of boars used for AI and second, to appraise the performance of AI techniques being adopted in organized farm and field condition in Nagaland. The sexual behaviour traits like reaction time (RT), ejaculation time (ET), and the semen quality parameters like volume, concentration, total sperm per ejaculate, motility and weight of gel fraction in ejaculates semen were recorded for representative collection in Large Black, Ghungroo, Hampshire and Hampshire – Ghungroo cross breed boars. Our study has revealed that about 63.64 % of boars responded in training for donating semen and the Ghungroo boar is the quickest (4 days) in getting trained and also responded with shortest RT (2.23 min) as compare to other boars. Further, the semen quality of all the boars were appeared within the permissible range and interestingly, the weight of gel fraction was recorded to be directly proportional to the ejaculation time and inversely proportional to the sperm concentrations in ejaculates. The better performances of AI in terms of conception rate (83.93% vs. 71.21%), litter size (10.09 vs. 6.12) and service per conception (1.19 vs. 1.40) were recorded in organized farm than at field condition. The knowledge of inseminator in the AI technique and use of single insemination was seemed to be the bottleneck in overall performance at field condition. From the present study, it can be concluded that the AI technique could be an effective tool in propagation of quality germplasm in farm as well as field condition provided the correct insemination protocols followed. The proper heat detection, use of double insemination protocol with properly preserved semen should be adopted in farm and field condition for better conception rate and litter size.

Key words: *Artificial insemination, semen quality, sexual behaviour, reproductive efficiency, and pig*

Small scale pig farming predominates throughout the North East Hill region including Nagaland. Pig alone accounts for 55.38 per cent of the total livestock population in Nagaland, but still a wide gap exists between the demand and availability of pork mainly due to traditional production system. The distribution of total pig population mostly located at rural areas (86.05%). Among the rural pig population 73.02 per cent are crossbred whereas, in urban areas the crossbred population is about 91.38 per cent (Livestock census data, 2012, Department of animal husbandry, fisheries and dairying, GOI). Farmers prefer to raise 1 to 2 crossbred pigs for fattening with zero to minimum inputs in terms of family labour and feeding for early return than going for breeding. Non-availability of good numbers of superior quality breeding male, high cost of transportation in hilly terrain, and lack of awareness about artificial insemination (AI) techniques forced the small holder pig breeder to choose natural service by one or two boar maintained in their village (*Kumaresan et al., 2009*). It leads to inbreeding and faster decline in growth and reproductive performance of pig population maintained in the region. In remote districts of Nagaland, people use the Naga local male pig at an

early age to breed their dam and practically do not maintain any breeding boar thereafter. The Naga Local male has been shown to attain sexual maturity at less than three months of age at minimum of three kg body weight and often appeared as cryptorchid (*Karunakaran et al., 2009*).

Artificial insemination (AI) technique could be beneficial for meeting the demand of improved pig germplasm at farmers' door step and might help in faster propagation of elite germplasm. Wide spread use of quality pig germplasm of Large Black, Ghungroo, Hampshire breed and their crosses has been carried out in Nagaland under Mega Seed Project on Pig in recent past. Artificial insemination technique was introduced for the first time in Nagaland by ICAR Research Complex for NEH Region, Nagaland Centre (Nagaland Post, July 29, 2013). The technique has shown tremendous potential in upgrading of the existing pig population. Establishment of AI unit at each district is the earnest need for meeting the demand of quality pig germplasm.

The selection of male with excellent genetic potential, training to donate semen, and evaluation of ejaculates while processing and finally insemination by trained personal are major steps involved in AI for effective utilization and propagation extensively. The different breeds of boar exhibits varied sexual activity and produce ejaculates that differ in quantitative and qualitative traits (*Szostak and Sarzynska, 2011; Wysokinska and Kondracki, 2013; Kondracki et al., 2013*). Similarly, time and number of insemination, knowledge level in heat detection and insemination techniques also influence overall performance of AI techniques adopted in Pig (*Kadirvel et al., 2013*). Adoption studies of any new technique after its introduction for the first time is essential for refinement and further improvement. Considering the fact, the present study has been carried out with two objectives, first to evaluate the semen quality and sexual behaviour of boars used for artificial insemination and second, to appraise the performance of artificial insemination techniques being adopted in organized farm and field condition in Nagaland.

METHODOLOGY

The present study was carried out in breeding farm under Mega Seed Project on Pig, at ICAR Research Complex for NEH Region, Nagaland Centre, Jharnapani. The centre is located at latitude of 25°45'24" N, longitude of 93°50'26" E and altitude of 295 m AMSL. The region falls under monsoon zone with annual rainfall varies from 2000 to 2500 mm with an annual humidity of 70 to 80% and average temperature range of 10 to 25°C. The field trail was carried out in farmers' field, organized farm, and small holder pig breeding unit in different villages of Dimapur district in Nagaland. The intact male animals of Large Black, Ghungroo, Hampshire and Hampshire – Ghungroo cross (50%) breed at eight months of age were selected from the stock available under Mega Seed Project on Pig and maintained in individual pen adjacent to the collection yard following standard feeding and management practices.

The boars were trained to mount on dummy sow at ten months of age. During the four weeks training period, the boars were exposed to the dummy sow placed at collection room daily in the morning hour for at least 30 minutes. Urine collected from estrus sow was sprinkled over the dummy early in the morning while allowing the male into the collection room. The exposure to dummy sow was continued until the majority of male started mounting and ejaculate semen. Once, the boars got trained to mount and ejaculate on dummy, collection of semen was initiated in two to three successive days to make the boars acquainted with the collector and dummy sow.

Semen was collected from each trained male twice in week following strict hygienic measure by glove hand technique using powder free vinyl collection gloves (IMV technologies, France). Sterilized thermo-flask maintained at body temperature was used to collect the gel free fraction of semen filtered through Buchner funnel fitted with a filter paper (ø230mm, IMV technologies, France). The weight of gel portion separated in filter paper was recorded for each collection. During semen collection, the sexual behaviour trait like time for sniffing the dummy (TDS), time for mounting not exposing penis (TMNP), time taken for mounting with exposed penis (TMWP), time elapse to first mounting (ETFM), reaction time (RT) and ejaculation time (ET) were recorded using a stop watch following the procedure as cited (Levis and Reicks, 2005). The macroscopic semen quality parameters like volume, colour, and consistency were recorded imme-

diately after collection. The microscopic parameters like motility, live percent and abnormalities were recorded following standard procedures in representative samples from each boar (Salisbury et al. 1985). The sperm concentration was measured in spectrophotometer at 650 nm. The semen samples having more than 70% motile spermatozoa and less than 20% abnormalities were considered for further processing. The semen fit for use in AI was diluted in BTS QSF extender (IMV Technologies, France) at 1:3 to 1:4 ratio based on sperm concentration. The diluted semen was loaded in golden tip bag (GTB, IMV Technologies, France) at 80 ml per dose and sealed manually using electrical sealing machine. The packaged semen doses were transferred to a B.O.D incubator maintained at 18 °C. The semen was mixed gently at twice daily to make even distribution of spermatozoa in the medium and to avoid settling down of spermatozoa in the GTB. The maximum storage time was allowed up to 72 hours for use in AI.

Insemination and pregnancy determination: Insemination was done at institute farm of Mega Seed project on Pig, Jharnapani, at AICRP farm of SASRD, Medziphema and nearby villages in an around Dimapur district in Nagaland. Inseminators engaged in AI program were either trained local youth/farmers or practicing veterinarians. At institute farm, double insemination protocol was adopted at 24 hour after detection of estrus in sows and after 12 hour of heat detection in gilts and repeat insemination after 24 hours using golden pig/gilt catheter (IMV technologies, France). While insemination, proper care was taken to maintain hygiene. The tip of the catheter was lubricated with B-gel and the other end was fitted in factory seal point of the GTB. The catheter was slowly and gently inserted in anti-clockwise direction into the vagina until it was passed through and locked in the cervix. Once the catheter fixed at cervix, the semen pouch was held up so as to deposit semen by gravitational force. In addition, mild pressure was applied on the semen pouch to facilitate complete passage of semen. Once insemination was done, the catheter was removed slowly and gently in clock-wise direction. The successful service was confirmed by both non-returns to estrus at 21 days post insemination and on abdomen palpation at two and half months post AI. The data generated in present study were analysed using Vassar Stats: Website for statistical computation (*http://vassarstats.net*).

RESULTS AND DISCUSSION

Success rate in training of boar: All together eleven boars comprised of different breeds namely, Large Black, Ghungroo, Hampshire and Hampshire – Ghungroo cross (50%) were used for training to donate semen on indigenously made dummy sow. However, only 63.64 per cent of boar could be trained in the span of four weeks training scheduled (Table 1). The Ghungroo boar was the fastest in getting trained (4 days), followed by Large Black (10 days). The Hampshire and Hampshire - Ghungroo cross breed took longer time but, successfully got trained on an average of 21 and 25 days period, respectively.

Table 1. Success rate in training of boar for use in AI

Breed	No. training trained	No. of for training	Av. days
Large Black	3	1 (33.33%)	10
Ghungroo	1	1 (100%)	4
Hampshire	4	3 (75.0%)	21
Hampshire – Ghungroo cross	3	2 (66.67%)	25
Total / average	11	7 (63.64 %)	15

Sexual behaviour and semen attributes: The sexual behaviour traits namely, time for sniffing the dummy (TDS), time for mounting not exposing penis (TMNP), time taken for mounting with exposed penis (TMWP), time elapse to first mounting (ETFM), reaction time (RT) and ejaculation time (ET) were recorded in all boars

Table 2: Sexual behaviour traits of different breeds of boar used for AI

Breed (Sec)	TSD (sec)	TMNP (sec)	TMWP (sec)	ETFM (min)	RT (min)	ET (min)	DOE
Large Black	45	109	83	143	6.33	10.80	17.13
Ghungroo	28	12	48	46	2.23	7.38	9.61
Hampshire	43.5	50.5	31	66.5	3.19	6.01	9.20
Hampshire–Ghungroo cross	56.50	24.50	6	80.50	2.79	12.35	15.14
Overall average	43.25	49	42	84	3.37	9.14	12.77

TDS: Time for sniffing the dummy, *TMNP:* Time for mounting not exposing penis; *TMWP:* Time taken for mounting with exposed penis; *ETFM:* Time elapse to first mounting; *RT:* Reaction time; *ET:* ejaculation time; *DOE:* total duration of evaluation

for six successive collections (Table 2).

Immediately after allowing the male into collection room, all the boars were seen to do sniffing the dummy as its first response. The Ghungroo male spent the shortest time (28 sec) before started to mount. The libido as manifested by RT which was varied from 2.23 min in Ghungroo, followed by 2.79 min in Hampshire – Ghungroo cross, 3.19 min in Hampshire and the longest in the Large Black (6.33 min). The Ghungroo boar spent less time in TMNP and ETFM as compare to Hampshire and Large Black cross pig. Similarly, the ejaculation time was highest in Hampshire - Ghungroo cross (12.35 min) followed by Large Black (10.80 min) and Ghungroo (7.38 min) boars. The whole collection process completed at shorter time in Hampshire (9.20 min) and maximum time spent in collection room by Large Black boar (17.13 min). Thus, the sexual behaviour in our study is seemed to be influenced by genetic make-up of the male and is well supported by previous reports of Szostak and Sarzynska (2011), Wysokinska and Kondracki (2013) and Kondracki et al. (2013) who have also observed varied sexual activity in terms of quantitative and qualitative traits which are genetically determined.

The semen quality parameters was studied in all together 75 ejaculates collected from boar of all four breeds (Table 3). Overall, the semen volume was lower in Hampshire (93.69 ml), and maximum volume was recorded in Ghungroo pig (188.82 ml). However, the concentration and total sperm per ejaculates were highest in Hampshire, followed by Ghungroo and Large Black. The Hampshire - Ghungroo crosses pig produced semen with the lowest concentration (141.32 million / ml) and also possessed lowest total sperm per ejaculates. The colour and consistency of ejaculates were correlated with concentration of spermatozoa and it was varied from thin creamy white in Large Black and Hampshire – Ghungroo crosses to thick creamy white in Hampshire boar. The initial motility was also recorded at the highest (85.4%) in Hampshire – Ghungroo cross and lowest in the Large Black (66.3%) boar.

Similarly, the live percent of total spermatozoa was recorded in the range of 70% to 83.24% in all the ejaculates collected for this study and abnormalities in total spermatozoa was also within the normal range of less than 20%. The average weight of gel fraction was recorded in the range of 27.71 g to 47.5 g and the weight was appeared to be directly proportional to the ejaculation time and inversely proportional to the sperm concentration in ejaculates. Variability exhibited by different boars in respect of ejaculatory efficiency are genetically determined and most likely to be dependent on a breed, cross-breeding variant or genetic line, as well as environmental factors as also observed in bull (*Galina et al. 2007*). However, those boars which produce large amounts of high-quality semen and, simultaneously, can develop sexual reflexes quickly and easily are particularly useful for the insemination service purposes (*Najjar et al. 2010*). Hence, careful corroboration of sexual behaviour and semen parameters offers the necessary criteria for selection of high fertilization capacity ejaculate.

Performance of artificial insemination in farm and field level: The performance of different breeds of boar used in artificial insemination techniques under organized farm condition revealed that the conception rate was highest in Ghungroo (91%) followed by Hampshire – Ghungroo cross (87.5%) and Hampshire (83.3%, Table 4). The litter size obtained through AI was very good in all the four breeds and it was ranges from 8.80 in Ghungroo to 11.86 in Hampshire - Ghungroo crosses. Following double insemination at farm condition, the highest litter size was recorded as 19 in Large Black and 16 in Ghungroo and Hampshire – Ghungroo cross. Similarly, the service per conception was less than 1.5 in all the four breeds and it

Table 3. Semen quality parameters in ejaculates obtained from different breeds of boar

Semen attributes	Large Black	Ghungroo	Hampshire	Hampshire - Ghungroo cross
No. of collection	(24)	(12)	(14)	(25)
Volume (ml)	164.84 ± 7.37	188.82 ± 6.71	93.69 ± 6.19	174.40±13.76
Colour	Thin creamy white	Creamy white	Thick creamy white	Thin creamy white
Motility (%)	66.3	80.7	76.5	85.4
Concentration (millions/ml)	197.09 ± 39.44	259.28 ± 21.88	535.63±61.36	141.32±14.77
Total sperm per ejaculates (billion)	32.49	48.96	50.18	24.65
Gel weight (g)	42.38 ± 3.61	35.39 ± 6.09	27.71 ± 2.66	47.5±1.44

Table 4. Performance of artificial insemination conducted at Mega Seed Project farm

Breeds	No. of insemination	No. of animals conceived	Conception rate	Service per conception	Litter size
Large Black	20	15	75.0	1.33	10.67 (6-19)
Ghungroo	22	20	91.0	1.11	8.80 (3-16)
Hampshire	6	5	83.3	1.20	11.75 (10-13)
Hampshire –Ghungroo cross	8	7	87.5	1.14	11.86 (9-16)
Overall	56	47	83.93	1.19	10.09 (3-19)

Values in parenthesis indicate the range of litter size

Table 5. Performance of artificial insemination conducted in field condition in Nagaland

Location of AI adopted farm	No. of insemination	No. of animals conceived	Conception rate	Service per conception	Litter size
Dimapur town	21	15	71.43	1.40	6.4 (1-12)
AICRP on Pig farm, SASRD Medziphema	15	10	66.67	1.50	5.75 (2-11)
Jharnapani village	10	9	90.0	1.11	6.0 (3 -9)
Medziphema Village	8	5	62.50	1.60	4.60 (2-7)
Pungluwa village	7	4	57.14	1.75	5.0 (4-6)
NEPED piggery model farm	5	4	80.0	1.25	9.25 (8-11)
Sub Total	66	47	71.21	1.40	6.17 (1-12)

Values in parenthesis indicate the range of litter size

was ranged between 1.11 to 1.33. Overall, the performance of artificial insemination was found to be better or as per the natural service and is well supported by the previous reports (*Lamberson and Safranski, 2000; Kadirvel et al., 2013; Am-in et al. 2010*).

The feedback data on conception rate, litter size and service per conception were acquired from the inseminators who had collected the semen from the institute and were engaged in AI in field condition. Overall, it was revealed that the performance of artificial insemination was comparatively poor relative to the organized farm condition in all respects (Table 5). The conception rate in AI under field condition was significantly lower (71.21%, Z=1.664, p=0.04) than in farm condition (83.93%). The service per conception rate was also significantly higher in field condition (1.40, vs. 1.19, $p<0.05$) than in the organized farm. Similarly, the litter size was also observed as higher (10.09 vs. 6.17, $p<0.01$) in organized farm than those in field condition.

The lower performance of AI in field condition most likely to be due to the handling error of semen dosses, failure in maintaining of correct temperature while storage and transportation and finally single insemination at very early or late stage of estrus. Among the field trials, the insemination conducted at Jharnapani area showed better conception rate (90%) due to its closeness to the institute where semen was stored at optimum temperature. In present study, the artificial insemination technique in field condition was implemented by either veterinary practitioners or non-veterinary personnel. The knowledge level about the reproductive physiology and artificial insemination techniques influenced the performance in terms of conception rate, service per conception and litter size (Table 6).

Both veterinary practitioner and non veterinary personnel frequently used single AI protocol in field condition, but the conception rate was much higher in the cases where AI was done by veterinary practitioner (63% vs. 40%). However, inseminators' knowledge level has no influence on conception rate while double insemination was adopted. Overall, the performance of double AI was better in terms of conception rate (77% vs. 54%, $p<0.05$) and litter size (8.34 vs. 3.20) as compare to single AI. Thus, besides the quality of boar semen, several factors influenced the performance of AI. Apart from the male factors, the genetic make-up of the female, body condition, nutrition and management factors also affect the fertility as a whole and the conception and litter size in particular.

CONCLUSION

From the present study, it is concluded that the AI technique could be an effective tool in propagation of quality germplasm in farm as well as field condition

Table 6. Performance of artificial insemination carried out by veterinary practitioners and non-veterinary personnel field condition in Nagaland

Attributes	Veterinary professional	Trained non-veterinary personal	Total
No. of semen dose used	72	34	106
No. of animals covered	44	21	65
No. of Single AI	16	10	26
No. of double AI	28	11	39
Conception rate at Single AI (%)	63.0	40.0	54.0
Conception rate at double AI (%)	75.0	81.82	77.0
Overall conception rate (%)	70.0	71.43	71.0
Litter size at single AI	2.50 (2-4)	3.33 (1-7)	3.20 (1-7)
Litter size at double AI	6.67 (2-11)	10 (8-12)	8.34 (2-12)

Values in parenthesis indicate the range of litter size

provided the correct insemination protocols followed. The proper heat detection, use of double insemination protocol with properly preserved semen should be adopted in farm and field condition for better conception rate and litter size. Hence, for effective use of AI technique in Nagaland, emphasis should be given on capacity building to produce quality semen and skilled manpower for dissemination at farmers' door steps.

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