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Efficiency of artificial insemination compared to natural mating in Aseel breed of chicken

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ABSTRACT

Artificial insemination (AI) and natural mating (NM) are a well-known technique of breeding for poultry in developed countries. AI includes collection, examination, dilution and then deposition of semen into the reproductive tract of female animals. Worldwide AI is widely used in developed countries but no initiative has yet been undertaken in Bangladesh. Therefore the current study was undertaken to investigate the suitability and efficiency of this technique to compare the fertility and hatchability traits, egg weight and day-old chick weight produced by Aseel chickens. For AI the chickens were sexually matured at 18 weeks of age and a dose of 50 microliter of volume semen contained 100-200 million spermatozoa. A total of 54 hens and 18 males were selected for this study i.e. each treatment group contained 6 hens and 2 cocks and each treatment group had 3(three) replications. The age at maturity of birds, egg weight and day-old chicks' weight did not vary significantly with treatment groups. The fertility percent demonstrated by artificial insemination using fresh and diluted semen was significantly better ($P<0.05$) than natural mating but hatchability did not differ significantly. The other hatchability traits did not vary significantly.

INTRODUCTION

Artificial insemination (AI) involves the deposition of semen into the female reproductive tract manually. It starts with the collection of the semen from the male and its evaluation in terms of motility, viability and concentration followed by its deposition into the female reproductive tract. Sexual maturity in both male and female birds occurs at 18 weeks of age. One ejaculate of a male can cover up 20 female birds by using AI. A dose of 50 microliter of volume semen should contain 100–200 million spermatozoa per insemination Kharayat et al. (2016). Artificial insemination was first practiced in America during the 1920s and then used widely in Australia with the introduction of laying cages during the late 1950s. For geneticists, AI is the method of choice for maintaining pedigreed mating. Artificial insemination (AI) is considered a valuable tool for the poultry industry (Benoff et al., 1981) due to the efficient utilization of males, which is not possible under natural mating. This decreases the cost of poultry production directly by reducing the number of cockerels needed for male gamete production (Benoff et al., 1981). AI was the first biotechnological tool applied to increase poultry production, as it allowed wider use of genetically superior cockerels with high productive performance (Benoffet al., 1981). That is, the benefits of artificial insemination in the poultry are as follows: i) artificial insemination increases mating ratio at about fourfold, ii) Older males with outstanding performance can be used for several generations, iii) Valuable male birds with the leg injuries can still be used for artificial insemination, iv) Preferential mating is eliminated, and v) AI aids in successful cross breeding.

The problems of native chickens are slow growing, producing a few eggs, the higher percentage of broodiness, light-weight body and their late maturing age compared with the industrial strains. On the other hand, native chickens have some advantages and these include high quality eggs, thicker eggshells better in taste

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and flavor, a high percentage of meat, resistance to heat and cold and some disease (Makarechian, 2002). To improve local chickens like Aseel and other important genetic resources, artificial insemination may play an important role. There is an opportunity to develop the local chickens using breeding technologies using artificial insemination and the local genetic chickens may be used to develop a modern strain.

Fertility is an important parameter in chickens and reflects the total actual reproductive capacity of females and males expressed by their ability when mated together to produce offspring. An egg is said to be infertile when it fails to show any evidence of developing an embryo (Miaziet al., 2012). Hatchability is a trait of economic importance in the chicken industry because it has a strong effect on chick output (Wolcet al., 2010). It is influenced by several factors such as egg weight, turning of eggs, storage, humidity, shell strength, egg size and genetic factors within the chickens kept. The ability of the embryo to successfully escape from the shell is called hatchability (Tarek 1992). To some extent, good hatchability of eggs is heritable but is determined by a complicated genetic constitution and the environment. Artificial insemination has a positive effect on improving fertility and hatchability traits also.

It is well established that artificial insemination in avian species has an advantage over natural mating (Surai and Wishart, 1996). AI resulted in better fertility than natural mating in poultry. Gee et al. (2004) reported that even when under natural mating 80–85% of eggs are fertile and fertility can be increased by another 5-10% simply by applying AI. Egg production of native genetic resources is lower than that of improved strains normally used at farm levels. So, hatching procedures are very important to increase the number of chicks (Kaygısız, et al., 1994).

The application of AI along with natural mating is widely used in developed countries. But no initiative has yet been undertaken in Bangladesh to investigate the suitability and efficiency of this technique. Fresh semen should be inseminated within 15-30 minutes after collection to achieve better fertility (Brillard, 2003). But within such a short period, it is difficult to inseminate a large number of hens. For this reason, in addition to fresh semen, an attempt was made under this study, to investigate the performance of diluted semen. Therefore, keeping in mind the above points, the study was undertaken to compare the fertility and hatchability traits of eggs laid by Aseel through natural mating and AI using fresh and diluted semen. The comparative performance of Aseel chickens with natural mating and AI on egg weight and day-old chick weight was also observed.

MATERIALS AND METHODS

Study location and duration

The research work was conducted under the project entitled "Conservation and Germplasm cryobanking of native chicken genetic resources of Bangladesh" at Advanced Avian Research Farm, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The duration of the study was 10 months.

Experimental birds

The mature Aseel chickens were selected from the birds available in Advanced Animal Research Farm and reared from the early growing stage to a mature stage. A total of 54 hens and 18 males were selected for this study i.e. each treatment group contained 6 hens and 2 cocks and each treatment group had 3 (three) replications.

Experimental design

Treatment → Replication ↓	Natural mating		AI with Fresh semen		AI with diluted semen	
R1	6♀	2 ♂	6♀	2 ♂	6♀	2 ♂
R2	6♀	2 ♂	6♀	2 ♂	6♀	2 ♂
R3	6♀	2 ♂	6♀	2 ♂	6♀	2 ♂
Breeding system	Cocks and hens were kept together and were allowed to have natural mating		Cocks were kept in various locations; fresh semen was collected and inseminated		Cocks were kept in different places, Semen was collected, diluted and inseminated	

General management

Each treatment group of chickens were reared in a deep litter system, demarcated well and kept in a separate pen. They were maintained in a semi-intensive system under standard management practices following the existing rules and regulations of the Bangladesh Veterinary Council regarding animal care and management. All birds were treated equally in all respects, except the mating system and in case of artificial insemination, fresh and diluted semen were used.

A similar diet was supplied to all birds (Table 1). Feed and water were supplied in plastic feeders and drinkers. Throughout the experimental period *adlibitum* clean drinking water was made available all day by using hanging drinkers. As for litter, rice husk were used as a litter material. Each pair of chickens was marked with permanent markers for proper identification. Both natural and mechanical procedures were followed to maintain proper ventilation and illumination in the chickens' house. The average environmental temperature was between 21−35°C and the light and dark pattern was 16 h light and 8 h dark. During the summer season, when the environmental temperature was too hot, lemon juice, Renalytes (Renata Ltd. products) were used with the drinking water and water was changed frequently with the cool water. A breeder diet was formulated for the experimental groups. The diet was made out of the feed ingredients maize, rice polish, soybean meal, animal protein, vitamin-mineral premix, amino acid, salt, toxin binder, and anti-oxidant. The composition of the diet is presented in

Table 1: Nutrient composition of experimental diet

Nutrients	Amount (%)
DM (%)	88.00
Crude protein (%)	21.00
Calcium (%)	2.00
Phosphorus (%)	0.45
Crude Fiber (%)	5.00
ME (Kcal/kg)	3000−3100

Source: Aftab Bahumukhi Farms Ltd. Bangladesh

Training of cocks for semen collection

At the age of twenty eight weeks, all the cocks were trained for semen collection by using the abdominal massage technique as described by Burrows and Quinn (1937). The training was performed twice a week until the experiment started. By thirty two weeks of age, all the cocks had become almost equally ready for semen collection. Then the semen collection was carried out and just after collection the semen sample was transported to the laboratory.

Semen collection

From the matured Aseel cock semen was collected through the massaging method. A special type of wooden chair was made with keeping facilities for locking two legs of cocks; and comfortable sitting and massaging options for the semen collector. The main goal of the semen collection procedure was to obtain the maximum amount of clean, high quality semen with minimum handling and stress. The testes located at the dorsum were stroked and massaged gently until protrusion of the cloaca. The researcher himself collected semen every week at the same time, and under the same conditions to minimize stress and maximize the quality of semen. The cooler period of the day, i.e. at morning (4.00-5.00 P.M.) the collection was performed and the collection was carried out twice a week. However, the following steps were performed for semen collection:

At first, cocks were taken gently onto lap of the collector and two legs of cocks were locked onto the arm of the chair. To start massaging, the left hand is placed on the back of the tail and the right hand on the ventral part of the tail or rear part of the abdomen.

The tom was stimulated by stroking the abdomen and pushing the tail upward and toward the bird's head with the right hand.

The cocks responded and the copulatory organ enlarged and partially protruded from the vent. If the copulatory organ had not protruded, it seemed that the cock was probably not sexually responsive and needed more time and massage.

When the cock responded and the copulatory organ was exposed, the left hand was placed at the cloaca with the forefingers. At the same time, the

right hand was used to provide inward and upward pressure beneath the cloaca.
When semen was ejaculated, it was then squeezed out by a short, sliding, downward movement of the left hand and an upward pressure of the right hand. Care was taken so that the copulatory organ was not touched and harmed during collection. Individual ejaculates were collected into 1 ml micro tubes.
After each collection, the ejaculates were examined visually as well as microscopically. Special care was taken to avoid contamination of semen with feces, urates, and transparent fluid, which lower semen quality.
Just after the collection of semen, the macroscopic parameters were observed and recorded and transported to the laboratory for next analysis using the CASA analyzer.

Semen examination

Before using the semen, the collected semen was examined first using CASA (Computer Assisted Semen Analyzer). The cocks that produced better semen were used for artificial insemination.

Semen dilution

The collected semen was diluted using previously made diluents, which are also known as modified Ringer's solution. For artificial insemination, one group was inseminated with fresh semen, just after collection and another group was inseminated with diluted semen, which was diluted at a 1:2 ratio. For the control group, natural mating was practiced. For the study, semen diluents were used following the composition used by Akcayet al., (2006). The composition of the diluents is given in Table 2.

Table 2: Composition of the semen diluents

Ingredients	Amount
Sodium chloride(g)	9.50
Potassium chloride(g)	0.20
Calcium chloride(g)	0.26
Sodium bicarbonate(g)	0.20
Distilled water (ltr.)	1.00
Glucose(g)	1.00

Source: Akcayet al., 2006

Artificial insemination (AI)

The females were inseminated by "venting" as described by Hafez (1985). Venting was done by applying pressure to the left side of the abdomen around the vent in such a way that it causes the cloaca to come out and the oviduct to protrude. Then a 1 ml plastic syringe without a needle with an appropriate amount of semen was inserted into the oviduct and semen was delivered at a depth of 1.5 to 2 cm. AI was performed twice in a week between 4-5 P.M. to avoid the presence of a hard shelled egg in the uterus. Timing of hen insemination is very important to achieve a high rate of fertility. Because it is generally recognized that AI should be carried out when no hard shelled egg is likely to be present in the uterus or at least not within 3 hours of an oviposition (Giesen*et al*., 1980). As the semen was expelled into the vagina, pressure around the vent was released and then the vent area was massaged, which assisted the hen in retaining sperm in the vagina or the oviduct. AI was performed within 30 minutes of semen collection.

AI with fresh semen: Freshly collected undiluted pooled semen was drawn with a 1 ml syringe and deposited into the vagina @ 0.02 ml for each hen.

AI with diluted semen: Pooled diluted semen was drawn with a 1 ml syringe and deposited into the vagina @ 0.2 ml for each hen

Rough handling of the hens was avoided carefully before, during and after the insemination process. Hens were released gently after insemination to prevent semen regurgitating from the vagina, which might result in lower fertility. However, the following precautions were taken during the insemination:

To avoid contamination, exteriorized vent of the hen was not touched with hands without hand gloves. Syringes used for insemination were cleaned, washed and disinfected. The vents of cocks and hens were properly cleaned with cotton wool moistened with physiological saline before semen collection and insemination. Contaminated semen was not used for insemination.

Data collection

Collection, cleaning, weighing and storing of eggs

The eggs obtained from the different groups were collected and just after collection the eggs were cleaned with wet cloths and weighed according to the treatment groups and the cracked, broken and off-shaped eggs were culled. The weights of eggs were recorded treatment wise. Then the eggs were stored differently (treatment wise) in the Genetics and Animal Breeding Laboratory where the air conditioning facility was available. The healthy eggs were stored for no more than seven days.

Incubation, candling and hatching of eggs

From the storage, the wellshaped and healthy eggs were selected, kept at a normal temperature and then incubated in an automatic forced-air incubator where temperature, humidity and turning were performed automatically. The candling was done twice during the incubation period, on the seventh and eighteenth days of incubation. The eggs were transferred to the hatching tray after 2nd candling and after the hatching chicks were weighed and recorded treatment wise.

Fertility of eggs

After first candling, infertile and clean eggs were identified and carried out for culling and recorded treatment wise and the rest of the fertile eggs were kept for hatching. From the set eggs, the infertile eggs were deducted and fertility was calculated.

$$\text{Fertility (\%)} = \frac{\text{No. of eggs set} - \text{No. of infertile eggs}}{\text{No. of eggs set}} \times 100$$

$$= \frac{\text{No. of fertile eggs}}{\text{No. of eggs set}} \times 100$$

Hatchability of eggs

After hatching, the chicks were carried out from the incubator. The healthy and abnormal chicks were sorted first and then counted. The abnormal chicks were then counted according to abnormal type, and the eggs which were not hatched were checked for dead in shell, late embryonic mortality or sticky chicks. Then hatchability was calculated.

$$\text{Hatchability (\%)} = \frac{\text{No. of chicks hatched}}{\text{No. of fertile eggs}} \times 100$$

Statistical analysis

The effect of treatment on fertility, hatchability, egg production, egg weight, day-old chick weight, embryonic mortality, normal and abnormal chicks were analyzed using the One-way ANOVA procedure in accordance with a Completely Randomized Design (CRD) following the GLM procedure of SPSS computer software 22.00 (SPSS, 2013). The significance of differences among the means of treatments was compared by using Duncan's Multiple Range Test (DMRT) from the same package. All data were presented as Mean ± standard error of Mean (SEM). Significant differences were considered at the level of $P<0.01$ and $P<0.05$.The following linear model summarizes the statistics employed to analyze the data:

$Y_i = \mu + TR_i + E_i$,

Where; Y_i is the dependent variable,μ is the overall mean,$TR_i$is the treatment effect, and E_i is the error.

RESULT

Age at maturity

Figure 1 represents the age at maturity in different breeding situations. The age at maturity obtained by this study did not vary significantly. The hens which were managed by natural mating laid their first egg at 215±15.05 days of age.The hens which were managed by AI with fresh and diluted semen laid eggs at 213±13.07 and 216±13.98 days of age, respectively.

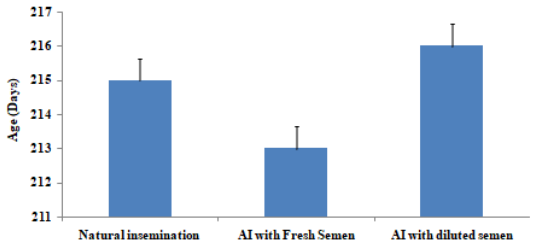

Figure 1: The age at maturity (days) in different breeding situations. Each bar with an error bar represents the Mean±SEM values.

Egg weight

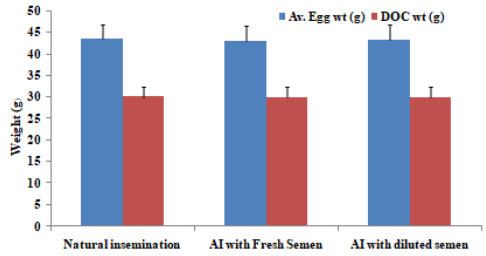

Figure 2: The egg weight (g) and day-old chick weight (g) in different breeding situation. Each bar with an error bar represents the Mean±SEM values.

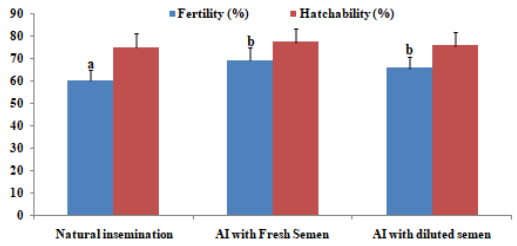

Figure 3: The fertility (%) and Hatchability (%) in different breeding situation. Each bar with an error bar represents the Mean±SEM values; different letters above the error bars of the same feeding period indicate statistically significant differences ($P < 0.05$).

The egg weight produced by the different treatment groups did not differ significantly. The egg weights were 43.39±2.97, 43.03±2.94 and 43.21±2.95 g respectively (Figure 2). The result indicates that the breeding system (Normal mating and Artificial Insemination by fresh and diluted semen) had no effect on egg weight.

Fertility and Hatchability

Fertility is determined on the basis of the number of fertile eggs obtained after first candling compared with set eggs (Figure 3). The breeding system (Normal mating and Artificial Insemination by fresh and diluted semen) had the minimum effect on fertility. The insemination by the fresh and diluted semen (69.25±5.04, 65.68±4.75) showed significantly better ($P<0.05$) fertility than the fertility observed by natural mating (60.00±4.3) and insemination by fresh semen showed significantly better fertility ($P<0.05$) than the diluted semen also.

The hatchability is determined on the basis of fertile eggs. The breeding system (Normal mating and Artificial Insemination by fresh and diluted semen) had no effect on the hatchability (75.00±5.50; 77.21±5.67; 75.68±5.55) of eggs among the different treatment groups, i.e. hatchability did not differ significantly.

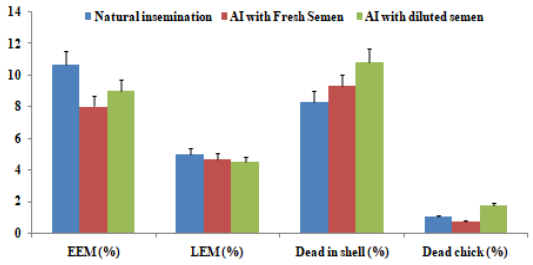

Figure 4 The other hatchability traits (%) in different breeding situation. Each bar with an error bar represents the Mean±SEM values.

Other hatchability traits

Early embryonic mortality (10.67±0.75, 8.02±0.54 and 9.00±0.6 percent); late embryonic mortality (5.00±0.2, 4.70±0.27 and 4.50±0.26 percent); dead in shell (8.33±0.45, 9.32±0.54 and 10.81±0.56 percent) and dead chicks (8.33±0.45, 9.32±0.54 and 10.81±0.56 percent) did not differ significantly between the different breeding

system and insemination with fresh and diluted semen (Figure 4).

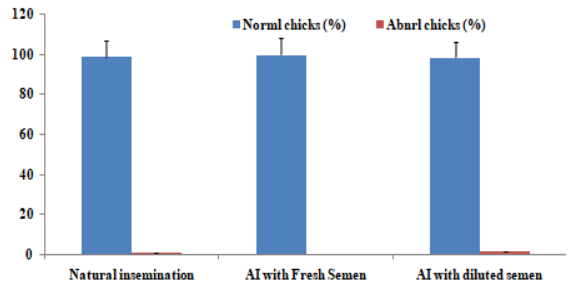

Figure 5: The normal and abnormal chicks production (%) in different breeding situation. Each bar with an error bar represents the Mean±SEM values.

Figure 5 represents the normal and abnormal chick production in different breeding situations. Normal (99.00±6.42, 99.99±6.99 and 98.50±6.88 percent) and abnormal (1.00±0.05, 0.01±0.00 and 1.50±0.09 percent) chicks production did not differ significantly among the treatment groups.

Day-old chick's weight

Figure 2 represents the day-old chicks weight in different breeding situations. The day-old chick's weight did not vary significantly among the treatment groups. The day-old chicks weight were almost similar (30.03±1.91, 29.98±1.93 and 29.90±1.86) within the treatment groups.

DISCUSSION

Age at maturity

The hens which were managed by natural mating laid their first eggs at 215±15.05 days of age. On the other hand, those which were managed by AI with fresh and diluted semen laid eggs at 213±13.07 and 216±13.98 days of age, respectively and did not vary significantly. Li et al. (2018) commented that birds managed with natural mating showed a significantly earlier age at their first egg compared with AI, but in this study the result showed no significant variation among the treatment groups.

Egg weight

The egg weight (43.39±2.97, 43.03±2.94 and 43.21±2.95 g respectively) produced by the different treatment groups did not differ significantly and similar result was observed by Li et al. (2018) when they studied artificial insemination and natural mating.

Fertility and Hatchability

The breeding system (Normal mating and Artificial Insemination by fresh and diluted semen) had the minimum effect on fertility. The insemination by the fresh and diluted semen (69.25±5.04, 65.68±4.75) showed significantly better ($P<0.05$) fertility than the fertility observed by natural mating (60.00±4.3) and insemination by fresh semen showed significantly better fertility ($P<0.05$) than the diluted semen also. Li et al. (2018) observed no differences among the birds managed with AI and natural mating. Though this experiment was not the same as the previous one, the result somehow supported the result of this study. Verma et al. (2018) stated that the fertility (%) were 87.18±2.92a, 80.00±3.00b for Kadaknath and Aseel chickens, respectively.

The hatchability is determined on the basis of fertile eggs. The breeding system (Normal mating and Artificial Insemination by fresh and diluted semen) had no effect on the hatchability (75.00±5.50; 77.21±5.67; 75.68±5.55) of eggs among the different treatment groups,i.e. hatchability did not differ significantly. Habibullah et al. (2015) observed significantly higher hatchability after AI than before AI and this result disagreed with the result of this study. Sayyazadeh et al. (2005) showed that the hatchability percentage increased by artificial insemination. The average hatchability percentage in natural mating for Arian and Ross 308 lines were 82.7 and 83.1 respectively. But when artificial insemination was done, these values were increased and that was 87.2% and 89.4% respectively. A similar result was also observed by Hocking and Bernared (1997), Christensen (2001) and Suraiand and Wishart (1996). Robinson (1996) reported that when artificial insemination is practiced, hatchability and production percentages are increased compared to natural mating.

Other hatchability traits

The comparison in relation to Early Embryo mortality (10.67±0.75, 8.02±0.54 and 9.00±0.6 percent); the Late Embryonic mortality (5.00±0.2, 4.70±0.27 and 4.50±0.26 percent); Dead in shell (8.33±0.45, 9.32±0.54 and 10.81±0.56 percent) and Dead chick (1.00±0.06, 0.75±0.05 and 1.75±0.11 percent) did not differed significantly among the different treatment groups of breeding system and insemination with fresh and diluted semen. Similar result were reported by (Keith, 2008) and (Bramwell et al., 1996). The results of this study were lower than Mroz et al. (2010)who reported 13.0¬23.0% and also lower than Khan et al. (2013) who reported 7.5, 13.2 and 19.3% deaths as early, mid and late embryonic mortality, respectively.

Normal (99.00±6.42, 99.99±6.99 and 98.50±6.88 percent) and abnormal (1.00±0.05, 0.01±0.00 and 1.50±0.09 percent) chick production did not differ significantly among the treatment groups.

Day-old chick's weight

The day-old chick's weight weredid not vary significantly among the treatment groups. The day-old chicks weight was almost similar (30.03±1.91, 29.98±1.93 and 29.90±1.86) within the treatment groups. Habibullah et al. (2015) observed the similar result in the case of broiler parent stock.

CONCLUSION

The age at maturity for natural (215±15.05 days) and artificial insemination with fresh and diluted semen (213±13.07 and 216±13.98 days) did not vary significantly. The egg weight (43.39±2.97, 43.03±2.94 and 43.21±2.95 g respectively) and day-old chicks' weight (30.03±1.91, 29.98±1.93 and 29.90±1.86) also did not vary significantly. The fertility percent (69.25±5.04, 65.68±4.75) demonstrated by artificial insemination using fresh and diluted semen was significantly better ($P<0.05$) than natural mating (60.00±4.3) but hatchability (75.00±5.50; 77.21±5.67; 75.68±5.55) did not differ significantly. The other hatchability traits did not vary significantly.

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