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An Imminence Towards Novel Approach of Biotechnology with the Possibility Artificial Insemination in Turkey Birds in Nepal

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ABSTRACT

The experiment was carried out on Broad Breasted Bronze and medium white turkey at National Agriculture Research Center, Khumaltar, Lalitpur from May 20 to July 22. The effect of treatment was analyzed using one-way ANOVA to study the Breed and season effect on turkey production. Three toms including 2 Bronze and 2 White along with 9 hens including 4 Broad Brested Bronze and 5 Medium White were used in the experiment. Completely randomized Design (CRD) was employed to investigate and compare the Artificial insemination and Natural breeding condition in Turkey birds. The total number of birds (n =28) used for the study were randomly allotted to 3 treatments (3x3) factors replicated 3 times in each unit. The experimental research was factorial with different level of semen volume: 0.3 ml, 0.4 ml and 0.5 ml made available from the Black Tom, weight and semen count. Data were analyzed for descriptive statistics between the variables were analyzed using R programming. The statistically significant means were then compared using Duncan's Multiple Range Test (DMRT). The majority of the data shows non-significant results in terms of semen volume, weight of Tom, semen count, egg-weight, and egg number (p>0.05). Therefore, thorough further research and the publication of research articles corroborating the aforementioned findings can open many doors to new breeding methods for Turkey bird species that are both advised and efficient.

Key words- Artificial Insemination, Turkey, Broad Brested Bronze, Medium White

INTRODUCTION

Historically, artificial insemination (AI) is the first generation of modern reproductive biotechnologies (Thibier, 1990) and has become one of the most important techniques in livestock to achieve genetic improvement. It has widely been used as the most available management practice for cattle breeders, in addition to making high genetic-merit bulls available to all (Webb, 2003). Besides genetic improvement, prevention of reproductive diseases, and inbreeding control, another advantage of AI is the provision of accurate breeding records; i.e., insemination dates, pregnancy rates, interestrus intervals, and days to first service (Ferguson & Skidmore, 2013). Artificial insemination practices started in the 1930s in Turkey. After the private sector was authorized to perform AI in 1985, it gained momentum (Suraj *et. al.*, 2001) and is widely used today (Burrows & Quinn, 1939). Subedi et al(2018) reported an overwhelming success at artificial insemination in Turkey farming which has been seen successful. As, commercial farmers of Nepal have been rearing large number of Turkey flock, artificial insemination could be flourished and large number of poultry can be produced through artificial insemination (Subedi et. al. , 2018). Since the development of Broad Breasted Bronze turkeys, fertility and hatchability have been major problems in the turkey industry. The loss in turkey eggs from these two factors amounts to approximately 40% of all eggs set. Infertility or apparent infertility makes up about two-thirds of this loss.Since the early work of Burrows and Byerly & Marsden (1938) indicates that fertility could be improved in turkeys by using artificial insemination, considerable work has been carried out using this technique as a tool for studying various phases of the fertility problem. Little experimental work is available to indicate the effect of artificial insemination upon fertility in large mass-mated Bronze turkey breeding flocks.Low fertility is one of the most common and costly problems in turkey breeder flocks. As a partial solution to this problem, there has been a growing interest in the artificial insemination of turkeys. Artificial insemination is a practical solution to infertility due to inactive toms, insufficient toms, and non-receptive hens, but is not a solution to infertility due to poor management. Despite the exploitation of AI chickens (Etches, 1996), a well-developed pectoral muscle in turkeys, has prevented turkey toms to mate naturally (Etches, 1996), making AI a necessity.Additionally, since; frame of the bird with active feeding habits, feed restriction was found to have significant effect on the growth of the body weight performances in the turkey (Jha et.al., 2018) and expelling a limited amount of semen, rearing a large number of toms can be highly expensive to farmers of Nepal. Therefore, the study was done with the objective to increase the efficiency of artificial insemination for future application in Nepal.

Materials and Methods

Experimental Area and design

The experiment was carried out on Bronze and medium white turkey at National Agriculture Research Center, Khumaltar, Lalitpur from May 20 to July 22. The effect of treatment was analyzed using one-way ANOVA to study the Breed and season effect on turkey production.

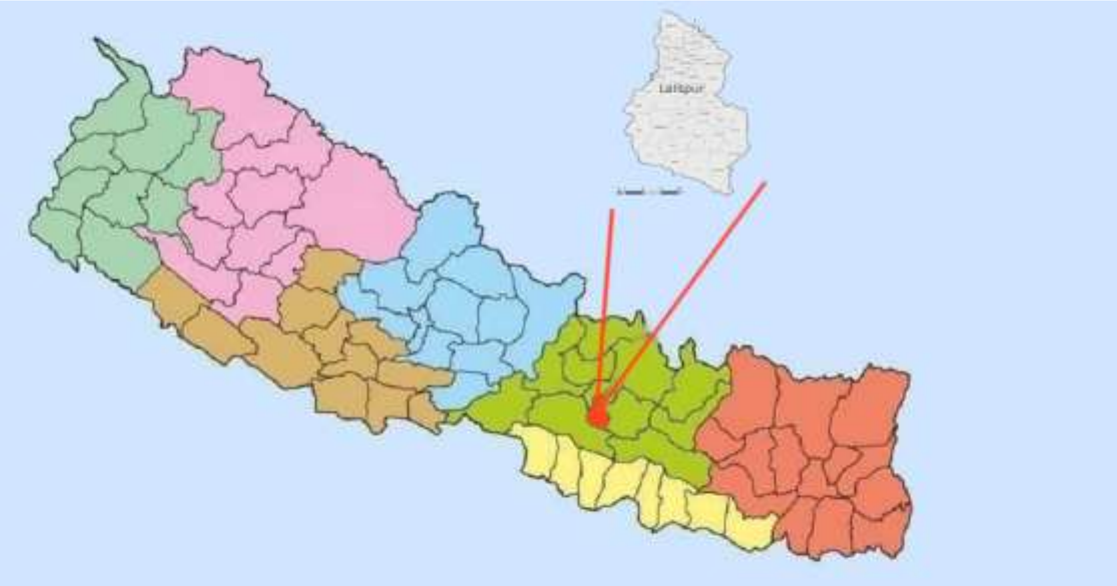

Fig 1: Research Area

3 toms including 2 Black and 2 White along with 9 hens including 4 BroadbrestedBronze and 5 Medium White were used in the experiment.

Treatment design

Completely randomized Design (CRD) was employed to investigate and compare the Artificial insemination and Natural breeding condition in Turkey birds. The total number of birds (n =28) used for the study were randomly allotted to 9 treatments (3x3) factors replicated 3 times in each unit. The experimental research was factorial with different level of semen volume: 0.3 ml, 0.4 ml and 0.5 ml made available from the Black Tom, weight and semen count .

Layout of experimental design

The experiment was carried out in Completely Randomized Design. The overall layout of experiment is shown in figure. A, B and C represent the three Tom used for experiment.

Broad Brested Black	1A	2B	3C
	♀	♀	♀
	♀	♀	♀
	♀	♀	♀
Medium White	♀	♀	♀
	♀	♀	♀
	♀	♀	♀

Fig: Layout of the experiment

Data collection

For the study of different parameters, all 28 birds were used to minimize the error. The following observations related to the objectives of the study were recorded for the treatment.

a. Weight of the Turkey

The weight of the individual Tom was recorded to see the response of Tom's weight on semen volume during the research period. Average weight of the experimental bird was recorded with the help of digital balance.

b. Semen volume

The semen volume of individual Tom was recorded to see the response of semen volume on the hatchability of egg and egg count.

c. Semen count

The collected semen from the experimental Tom was sent in the lab to record the sperm count. Semen count was taken as a reference to observe its impact on the hatchability of egg.

d. Egg number and weight

The egg number and its weight were recorded of both artificial inseminated and Natural breeding Hens. Record was made to analyze the effect of both treatment on these parameters.

Statistical Analysis

All the collected data were then entered in MS Excel and converted into text files (MS-DOS). Effect of treatment were analyzed using one-way ANOVA procedure in accordance with the Completely Randomized Design (CRD).

Data were analyzed for descriptive statistics between the variables were analyzed using R programming. The statistically significant means were then compared using Duncan's Multiple Range Test (DMRT) (Duncan, 1995) computer software as modified by Kramer (1957). Meat quality was evaluated using a t-test at $P \leq 0.05$ level of significance.

Statistical models

The linear model : $Yi = \mu + TRi + Rep + Ei$, was used to summarize the statistics employed to analyze the data;

where; Yi is the dependent variable,

μ is the overall mean,

TRi is the treatment effect (the effect due to natural mating and artificial breeding techniques)

Rep is the replication and

Ei is the error.

Semen Collection

The tom was stimulated by stroking the abdomen by right hand and pushing the tail upward and toward the bird's head with left hand. Individual ejaculates were collected into 1 ml microtubes. 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 the semen quality. Semen was used within 30 minutes of collection. Collected semen was pooled in an equal amount according to required semen volume to eliminate the effect of individual variability of gamete donors.

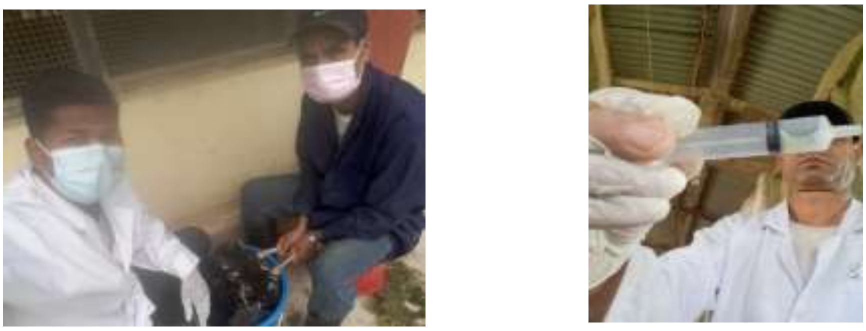

Fig 2: At a glance sequential activity for semen collection Here, a) Massaging of Tom b) Collection of semen

Method of separating Spermatozoa from semen

Spermatozoa were washed by diluting the semen 1:1 with Millonig's phosphate buffer (Millonig, 1962) and centrifuged to remove the buffer and seminal plasma. The spermatozoa were then resuspended with 2% phosphate-buffered glutaraldehyde and fixed. The sperm were further fixed with a 0.5% phosphate-buffered solution of osmium tetroxide for 1 hour.

After dehydration with a graded ethanol solution, a drop of ethanol with suspended sperm was placed on glass coverslips and the sperms were allowed to settle. The sperm was then scanned with a JEL 848 SEM.

Semen analysis

To ensure quality of semen for AI, semen analysis was done. First, observation was done on an electronic microscope to check the viability of the collected semen. The percent of live-dead and abnormal spermatozoa were counted after preparing smears and staining with eosin and nigrosine according to the methods described by Lake and Stewart (1978). Stained spermatozoa were counted as dead.

The percent of abnormal spermatozoa was evaluated by observing morphology of a total count of 100 spermatozoa. Sperm motility was assessed by examining a drop of semen (4–5 μl) under the microscope at ×10 magnification and sperm concentrations were determined using a Neubauerhaemo-cytometer.

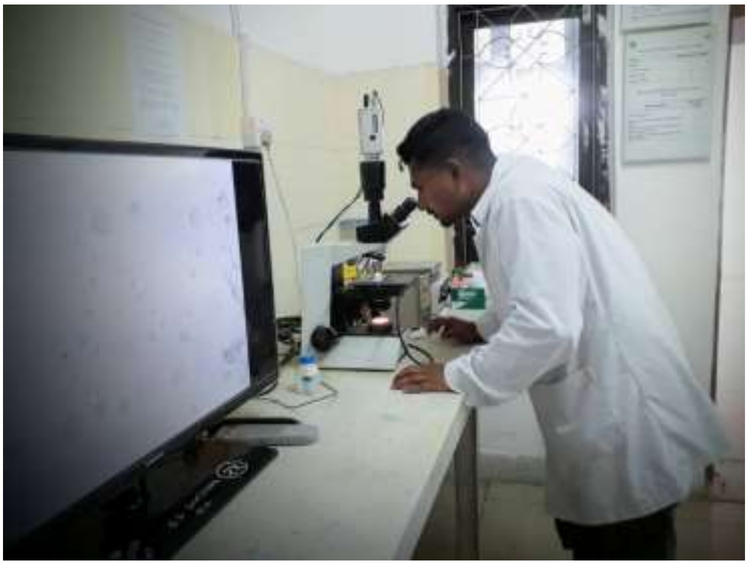

Figure 3: Observation of collected semen under the electronic microscope in Lab of National Animal Breeding Research Centre, Khumaltar, Lalitpur.

AI in turkey hen

The females were inseminated following the "Venting" method as described by Hafez (2013). The venting was done by applying pressure to the left side of the abdomen around the vent in such a way that it caused the cloaca to come out and the oviduct to protrude. Then 1 ml plastic syringe without a needle with the appropriate amount of semen was inserted into the oviduct and semen was delivered at the depth of 1.5 to 2 cm inside the vent.

AI was performed once a week between 4–5 p.m. to avoid the presence of a hard-shelled egg in the uterus. 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 oviposition (Giesen et al., 1980). AI was completed within 30 minutes of semen collection.

Only the ejaculates with a milky appearance, free of fecal material with >70% mass motility were used for AI. Freshly collected undiluted pooled semen was drawn with a 1 ml syringe and 0.02 ml was deposited into the vagina.

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. Rough handling against hen 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.

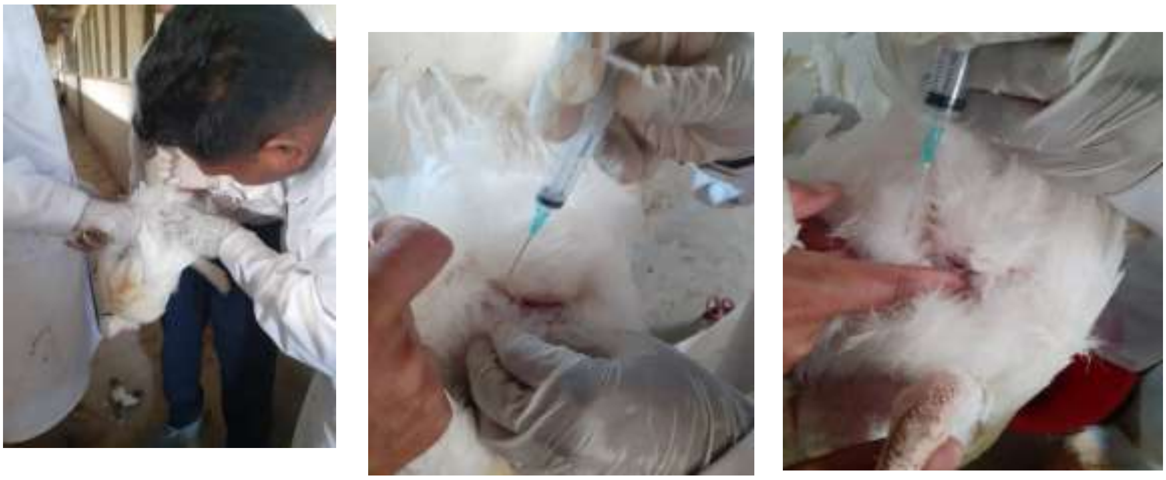

Figure 4 : Sequence of steps for Inseminating female turkey bird after collecting sperm from male turkey bird.

Result and Discussion

Hatchability of eggs

Volume of semen

Result of the present study showed that the hatchability of the eggs has increased after the application of Artificial insemination in Hens. While after natural breeding, the hatchability percentage was only about 81.3% , artificial inseminated hens have higher percentage of hatchability i.e. 91.7% and 95.7 % respectively. However, below data shows that the Volume of semen of Tom has no significant difference on a number of chicks hatched. Similar result was obtained by Subedi *et. al*. (2018) during their study in Rampur, Chitwan.

Table 1: Response of different volumes of semen on no. of chicks hatched.

Factor	Volume of semen(ml)	No of chicks born(no±SE)	P value	Level of sig.
Volume of semen	0.3	3±0.00	>0.05	NS
	0.4	4±0.00		
	0.5	1±0.00		

The findings deciphers that weight of Tom has no significant difference in the number of chicks hatched. The finding is parallel to that of Subedi *et. al.* (2018) during their study at Rampur, Chitwan, Nepal. Thus, it can be asserted that the three different group of volume of semen used were ample for insemination and no. of chicks hatched. However, few compelling factors like sample size, nutrition, environment and other non- genetic factors could be additionally responsible and might lead to variation. McCartney (1956) and Shaffner and Andrews (1948), also didn't find any relation between different semen volume on no. of chicks hatched. However, Sampson and Warren (1939), Raimo (1943) and Allen and Champion (1955) found that adequate semen volume are required to ensure fertility in eggs. Therefore, further research is recommended.

Weight of Tom

Table 2: Response of different weight of Tom on No. of chicks hatched.

Factor	Weight of tom	No of chicks hatched(no±SE)	P value	Level of sig.
Weight of Tom	7	3±0.00	>0.05	NS
	8.5	4±0.00		
	9	1±0.00		

This data shows that weight of Tom has no significant difference in the number of chicks hatched. A parallel findings was obtained by Subedi *et. al.* (2018) during their study in Rampur, Chitwan, Nepal. Thus, it can be asserted that the three different group of weight upto (7 kg, 8.5 kg and 9 kg) of Tom used were standard and attained maturity for and no. of chicks hatched. However, few compelling factors like sample size, nutrition, environment and other non- genetic factors could be additionally responsible and might lead to variation. Previous study reported that differences in size of tom (large white strains approximately 33 kg) and hen (approximately 9 kg at the onset of laying) resulted in unsuccessful mating and consequently low fertility (Zambelli & Levy 2010) that compelled eventually to adopt AI for commercial turkey production. In addition, (Juliet and Bakst, 2008) also reported that turkey is the only commercial species, which is completely dependent upon AI for fertile egg production. Surai and Wishart (1996) emphasized that AI has relative advantages over natural mating in case of avian species resulting in better fertility than natural mating in poultry. Therefore, before reaching to a concrete conclusion,further research is suggested.

Weight of eggs

Table 3: Response of Egg weight on no. of chicks hatched.

Factor	**Egg weight (in gms)**	**No of chicks born(no±SE)**	**P value**	**Level of sig.**
Weight of eggs	70	3±0.00	>0.05	NS
	75	4±0.00		
	65	1±0.00		

The above table shows that there is a non-significant difference between the weight of the egg and the number of chicks born. Contradicting results were found by Anandh & Jagatheesan. (2015) where it was reported that the fertility rate and hatchability of all eggs set or rich eggs were advanced in heavyweight eggs compared to light eggs. Similarly, a counter results were also evoked by Lerner and Gunns(1952). Lerner and Gunns (1952) stated that in flocks in which there is selection for large egg size, the best hatchability, at a given age, is obtained from eggs that are below the mean egg weight. However a greater incidence of late mortality has been observed in embryos of large-egg lines of chickens and in large eggs of turkeys (Reinhart and Moran, 1979), although hatchability does not differ significantly between egg weight groups. Few persuasive factors like sample size, nutrition, environment and other non- genetic factors could be additionally responsible and might lead to variation. Therefore further research is prioritized for a discrete conclusion.

Response of number of chicks on no. of chicks hatched.

Factor	Egg no.	No. of chicks born (no ± SE)	P value	Level of significance
No. of eggs	6	4 ± 0.00	>0.05	NS
	5	3 ± 0.00	>0.05	NS
	7	1 ± 0.00	>0.05	NS

The table insinuates that the no. of eggs has no significant difference on a number of chicks hatched. The persuasive factors like sample size, nutrition, environment and other non- genetic factors could be responsible and might lead to variation. Therefore further research is prioritized for a discrete conclusion. As, controverting results were obtained by C. A. Onbasilar and E. Kocakaya (2020). They found that increasing the number of eggs led to a decrease in hatchability and chick quality, likely due to reduced ventilation and increased carbon dioxide levels. However, the study did find that hatchability increased when the number of eggs per unit area was decreased.Therefore further research is prioritized for a discrete conclusion.

Conclusion

The artificial insemination research process in Turkey produced hopeful and soon-to-be results. As this is the first successful attempt at breeding in Turkey birds using AI under government farms in Nepal, the research results are still encouraging even though the majority of the data shows non-significant results in terms of semen volume, weight of Tom, semen count, egg-weight, and egg number. Therefore, thorough further research and the publication of research articles corroborating the aforementioned findings can open many doors to new breeding methods for Turkey bird species that are both advised and efficient.

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Authors' Contributions:

Niraj Banskato Designed and implemented the experiment, data recording, and data analysis and prepared the manuscript. Ajeet Kumar Jha, M Dahal, Rahul Senchuri, D adhikari and S Upreti were ebulliently involved in experiment and writting the manuscript.

Conflicts of Interest

The authors have no relevant financial or non-financial interests to disclose.

References

Allen, C. J., & Champion, L. R. (1955). Competitive fertilization in the fowl. *Poultry Science*, *34*(6), 1332-1342.

Anandh, M. A., & Jagatheesan, P. N. (2015). Reproductive performance of Beltsville small White and Broad Breasted Bronze Turkeys (Meleagris gallopavo) under hot humid climatic condition. *Indian Journal of Animal Research*, *49*(6), 847-850.

Burrows, W. H., & Quinn, J. P. (1939). *Artificial insemination of chickens and turkeys* (No. 525). US Department of Agriculture.

Byerly, T. C., & Marsden, S. J. (1938). Weight and hatchability of turkey eggs. *Poultry Science*, *17*(4), 298-300.

Duncan, A.B (1995) Multiple Range Test and Multiple F. Test. Brometric, 11: 1 – 43

Etches, R. J. (1996). *Reproduction in poultry*. CAB international.

Ferguson, J. D., & Skidmore, A. (2013). Reproductive performance in a select sample of dairy herds. *Journal of dairy science*, *96*(2), 1269-1289.

GIESEN III, A. F., McDaniel, G. R., & Sexton, T. J. (1980). Effect of time of day of artificial insemination and oviposition-insemination interval on the fertility of broiler breeder hens. *Poultry science*, *59*(11), 2544-2549.

Hafez, H. M. (2013). Salmonella infections in turkeys. In *Salmonella in domestic animals* (pp. 193-220). Wallingford UK: CABI.

Jha, A. K., Sah, A. K., Tiwari, M. R., & Sudi, M. S. (2018). Effect of feed management schemes on productive performance of white turkeys. *Nepalese Journal of Agricultural Sciences 2018, volume 17*, 40.

Juliet, A. L., & Bakst, M. R. (2008). The current state of semen storage and AI technology. Biotechnology and Germplasm Laboratory, Beltsville Agricultural Research Center. *Agricultural Research Service, USDA, Beltsville, MD, USA*.

Kramer, G. (1957). Experiments on bird orientation and their interpretation. *Ibis*, *99*(2), 196-227.

Lake, P. E., & Stewart, J. M. (1978). *Artificial insemination in poultry*. Her Majesty's Stationery Office..

Lerner, I. M., & Gunns, C. A. (1952). Egg size and reproductive fitness. *Poultry Science*, *31*(3), 537-544.

Mccartney, M. G. (1956). Relation between semen quality and fertilizing ability of White Holland turkeys. *Poultry Science*, *35*(1), 137-141.

Millonig, R. C., Amrein, B. J., & Borman, A. (1962). Antigenicity of bovine cortical bone. *Proceedings of the Society for Experimental Biology and Medicine*, *109*(3), 562- 564.

Onbaşılar, E. E., Kahraman, M., Güngör, Ö. F., Kocakaya, A., Karakan, T., Pirpanahi, M., ... & Yalçın, S. (2020). Effects of cage type on performance, welfare, and microbiological properties of laying hens during the molting period and the second production cycle. *Tropical Animal Health and Production*, *52*, 3713-3724.

Raimo, M. A. (1943). Revista de Educação, 1943, v. 29, n. 30/39, SP.

Reinhart, B. S., & Moran Jr, E. T. (1979). Incubation characteristics of eggs from older small white turkeys with emphasis on the effects due to egg weight. *Poultry Science*, *58*(6), 1599-1605.

Sampson, F. R., & Warren, D. C. (1939). Density of suspension and morphology of sperm in relation to fertility in the fowl. *Poultry Science*, *18*(4), 301-307.

Shaffner, C. S., & Andrews, F. N. (1948). The influence of thiouracil on semen quality in the fowl. *Poultry Science*, *27*(1), 91-102.

Subedi, M., Luitel, H., Devkota, B., Bhattarai, R. K., Phuyal, S., Panthi, P., ... & Chaudhary, D. K. (2018). Antibiotic resistance pattern and virulence genes content in avian pathogenic Escherichia coli (APEC) from broiler chickens in Chitwan, Nepal. *BMC veterinary research*, *14*(1), 1-6.

Surai, P. F., & Wishart, G. J. (1996). Poultry artificial insemination technology in the countries of the former USSR. *World's Poultry Science Journal*, *52*(1), 27-43.

Surai, P. F., Fujihara, N., Speake, B. K., BrilIard, J. P., Wishart, G. J., & Sparks, N. H. C. (2001). Polyunsaturated fatty acids, lipid peroxidation and antioxidant protection in avian semen-Review. *Asian-Australasian Journal of Animal Sciences*, *14*(7), 1024-1050.

Thibier, M. (1990). New biotechnologies in cattle reproduction. In *7th Congress of the Federation of Asian Veterinary Associations (FAVA), Chonburi (Thailand), 4-7 Nov 1990*.

Webb, A. D., Dickens, G. R., & Oliver, N. H. (2003). From banded iron-formation to iron ore: geochemical and mineralogical constraints from across the Hamersley Province, Western Australia. *Chemical Geology*, *197*(1-4), 215-251.

Zambelli, D., & Levy, X. (2010). Clinical approach to the infertile male. In *BSAVA manual of canine and feline reproduction and neonatology* (pp. 70-79). BSAVA Library.