



## **Reproductive Indices of Naturally Mated and Artificially Inseminated Quail Hens (*Coturnix japonica*): Is Artificial Insemination of Japanese Quail Hens Feasible in a Local Setting?**

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### **ABSTRACT**

Naturally mated and artificially inseminated Japanese quail (*Coturnix japonica*) hens' eggs reproductive indices were investigated to evaluate natural mating or artificial insemination techniques for breeding quails in local farms. A total of 141 (108 hens and 33 cocks) matured quails of about 11 weeks of age were used for the experiment, of which 54 hens were inseminated with Citric-quail egg albumen extended semen collected from 15 fertile cocks. Another 54 hens were naturally mated by another 18 fertile cocks in a ratio of one cock to three hens (1:3). Eggs collected from both naturally mated and artificially inseminated birds were incubated for 18 days. The percentage of incubated eggs that were fertile when candled on the 7<sup>th</sup> and 12<sup>th</sup> day and hatch-out analysis after the 18<sup>th</sup> day of incubation tested fertility. Hatchability was tested by the percentage number of fertile eggs that hatched. Naturally mated and artificially inseminated hens' eggs had a fertility of 84.0 % and 73.3 %, and a hatchability of 82.5 % and 85.5 %, respectively. The study revealed no significant difference in the frequency of fertile or hatched Japanese quail eggs ( $\chi^2$  cal <  $\chi$  critical at 5% or 1% level of probability). Naturally mated and artificially inseminated birds had relative fertile egg rates of 53.4 % and 46.6 %, respectively, and relative egg hatching rates of 52.5% and 47.5 %, respectively. Artificial insemination had the advantage of introducing the same volume of semen to more quail hens than a single cock in a natural mating process. However, natural mating was recommended over artificial insemination in the study area because of the technical constraints that required more workforce and amenities in the insemination process. The authors recommended research on developing restraint instruments and non-electrical semen storage methods for quail birds. The authors also proposed a restraint model for quail birds.

### **Keywords:**

Japanese quail, Artificial insemination, Citric-quail egg albumen extender, Egg fertility and hatchability, Quail restraint instrument.

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## INTRODUCTION

*Coturnix japonica* is also called the Japanese quail. Japanese quails have been domesticated since the 12th century for their meat and eggs [1]. Japanese quail hens start laying eggs within their sixth to the seventh week of age and continuously lay one egg daily. In Nigeria, the commercial quail farming business is highly profitable, and the demand for quail eggs and meat often exceeds the supply. Quail farming requires little investment of resources and space management than chicken farming. As a result, more people are becoming interested in this business. As of 2019, Nigeria produced above 640,000 tons of eggs with an estimated annual increase of 4.10 % and 239,947 tons of poultry meat [2]. Despite this volume, Nigeria is far from meeting its domestic demands in poultry production. The rapid population growth estimated at 2.54 % in 2020 [3] further increases the gap between demand and supply. There have been several efforts by the Federal Government of Nigeria to increase poultry production within the last few decades; this expectation is yet to be realized since poultry products and by-products lag behind other livestock products. The decline of commercial poultry production may be attributed to the shortage of day-old chicks, poor quality feeds, lack of adequate veterinary services and availability of drugs and vaccines, inadequate financial resources and technical skills in managing the birds. FAO [4] reported that inferior production methods and poor management skills are major factors responsible for poultry industry failure. The poultry industry needs to improve its production methods to produce optimal meat and eggs.

Artificial insemination (AI) is the manual placement of semen in the female animal's reproductive tract by a method other than natural mating. In AI, offsprings are produced by facilitating gametes' union (spermatozoa

and oocytes) in the female reproductive tract. AI of farm animals is a common animal husbandry practice in livestock and poultry industries of developed countries. Only very few government-established and private chicken poultry farms in Nigeria practice AI. More so, the use of AI in quail production in Nigeria is not known. Artificial insemination (AI) in avian species increased the number of settable eggs, overall fertility and hatchability of eggs, and reduced production cost per unit of day-old chicks [5][6][7]. A rational AI technique with certified semen in quail farming can revolutionize quail farming in Nigeria. There is a need to develop AI technology in quail production by innovating new quail species-specific semen diluents and performing extensive research on quail hen inseminating techniques that our local farmers can adopt.

This study evaluated the reproductive performance of Japanese quail (*Coturnix japonica*) hens naturally mated and artificially inseminated intra-uterine with semen diluted in citric-quail egg albumen. This study's primary objectives were: first, to evaluate the fertility and hatchability of eggs from Japanese quail birds bred by natural mating and artificial insemination, and to determine if the frequency of fertilized and hatched eggs differed between naturally mated and artificially inseminated Japanese quail hens; and secondly, to identify challenges in using AI in a local setting and proffer solutions to challenges encountered in AI of quail hens.

## MATERIALS AND METHODS

### Study Area / Experimental Site

The experiment was conducted at the University of Abuja Teaching and Research Farm, Animal Science Section, Main Campus, Gwagwalada, Nigeria. Gwagwalada is located between latitude 8°57' and 8°55'N and

longitude 7°05<sup>1</sup> and 7°06<sup>1</sup>E. Gwagwalada has an ambient temperature that ranges from 28 °C to 36 °C in the daytime and 22 °C to 27 °C at night. Gwagwalada has two main seasons; the wet season, which starts from April to October and the dry season, which starts from November to March.

### Management of Experimental Animals

One hundred and fifty (150), five-week-old birds (110 hens, 40 cocks from different pedigree) were obtained from the National Veterinary Research Institute, a reputable research institute located in Vom, Jos, Plateau State, Nigeria. On arrival, feed and water containing anti-stress were supplied. The cocks and hens were isolated in cages for two weeks, where they were closely observed and allowed to acclimatize to the environment. Animals were fed daily on a commercial feed (Vital Feed Egg Max ® by UAC Feed Milling Company) containing crude protein (16.50 %), fat (5.00 %), crude fibre (10.00 %), calcium (3.50 %), phosphorus (0.40 %), and metabolizable energy (2700 kcal/kg). Clean, fresh water was provided *ad libitum*. Vitamins and medications were also supplied when needed.

### Experimental Procedure

When the birds were seven weeks old, 141 birds (108 hens, 33 cocks) were selected for the experiment. The hens were randomly selected while the cocks that had erections and ejaculated when exposed to hens, and produced foam from well-developed proctodeal (foam) glands when pressure was applied on the gland, were selected. This was a test of mating capacity and fertility of quail cocks as described by Sefton and Siegel [8] Adkin-Regan [9] and Mohan *et al.* [10]. Fifteen (15) fertile cocks were randomly selected and caged separately for semen collection, while 54 hens were inseminated randomly and caged separately. The remaining 72 birds, which

comprised 18 cocks, and 54 hens, were randomly distributed into 18 cages in a ratio of one cock to three hens.

### Preparation of Semen Extender

One hundred milliliters (100 ml) of thin quail egg albumen (egg white separated from yolk) was collected in a beaker as described by Ubah *et al* [11] but with a modification of collecting the albumen and discarding the yolk. The thin egg albumen's pH level was first reduced to 7.5 by adding phosphate buffer and then further reduced to 6.8 by adding citric acid, as Wentworth and Mellen [12] described.

Two hundred (200) units of penicillin (Crystalline Penicillin G injection BP 1 MEGA) and 250.0 µg of streptomycin (Streptomycin sulphate injection BP 5 g) were added to 1.0 ml of quail egg albumen as recommended by Wentworth and Mellen [8]. In brief, a Crystalline penicillin injection containing 1,000,000 units was diluted in 1000 ml of water to obtain a solution of 1000 units of penicillin per millilitre. The streptomycin sulphate injection (5 g) was diluted in 2000 ml of water to obtain a solution of 2500 µg of streptomycin per millilitre. Then, 20 ml of penicillin solution and 10 ml of streptomycin solution were mixed and made up to 100 ml with thin egg albumen.

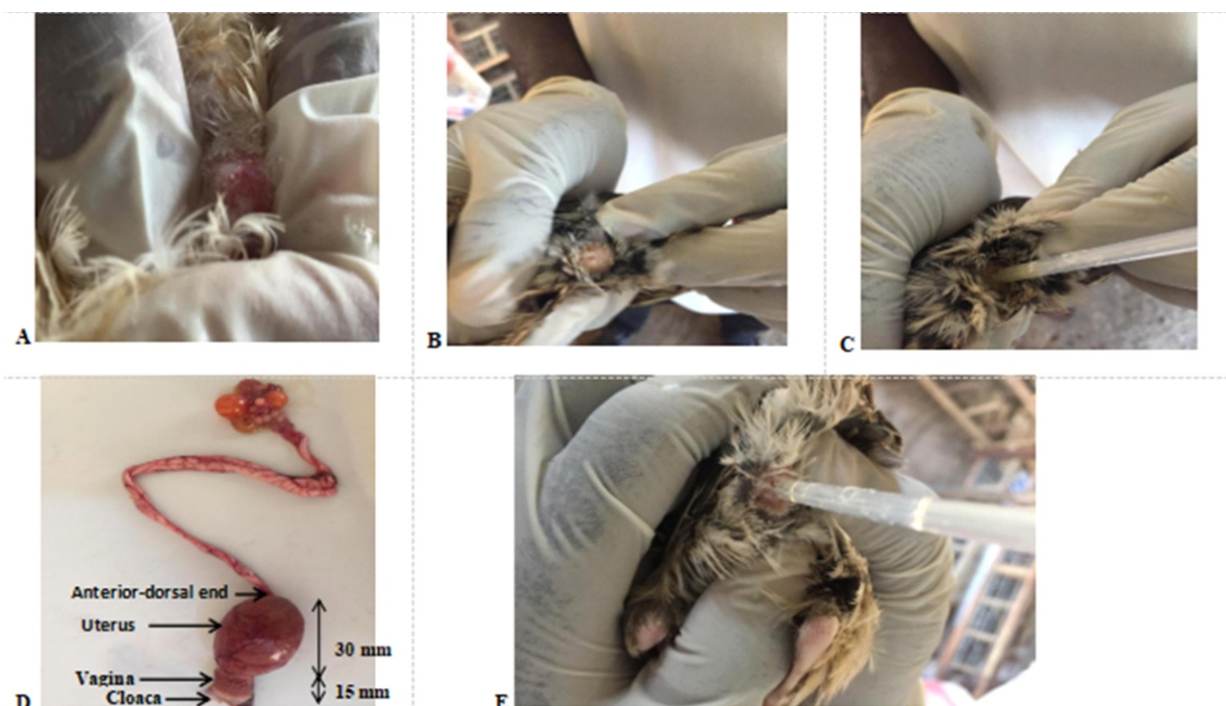
### Semen Collection and Dilution

The 15 cocks earlier selected were subjected to semen collection training massages. The males were starved of feed and water at least 12 hours before semen collection to minimize contamination of the semen with faeces and urine. The collection was timed so that not more than 30 minutes elapsed between collection, dilution and insemination. For proper semen collection, two operators were necessary; one pair of hands held the bird in place while both hands of the other handler were for

manipulation. The cloacal gland's frothy secretion was forced from the gland by a motion with the left hand, while the secretion deposited at the vent was cleared with the right hand, with a clean towel, as described by Wentworth and Mellen [8]. The index finger of the right hand was placed below the pubic bones, and slight pressure was applied upward. The pressure was applied laterally to the cloacal region with the left hand's thumb and index finger. Expressed semen was aspirated from the everted copulatory organ using a miniature graded pipette (Fig. 1 A-C). Semen volume collected from each male was observed and recorded. The semen sample was diluted with the semen extender in a ratio of 1:50.

### Insemination

Before the insemination process, a mature female quail with an egg in the uterus (shell gland) was dissected to observe the oviduct's structure and length (Fig. 1D). Two operators were necessary for the insemination process. As one pair of hands held the birds in place, the other collected the required 0.1ml of diluted semen (inseminate) in the tiny pipette and guided the insertion of the pipette (Fig. 1E). The pipette was inserted about 45 mm deep from the cloaca into the oviduct to the target site (the anterior-dorsal end of the uterus). The inseminate was deposited at the anterior portion of the uterus near the junction of the uterus and isthmus. Insemination of females was done thrice at three days apart.



**Fig. 1: Semen collection from a quail cock and insemination of quail hen.**

A) Pressure application on cloaca. B) Eversion of cloaca. C) Aspiration of semen into graduated pipette. D) Quail oviduct: estimation of distance to the anterior-dorsal end of uterus. E) insemination of quail hen.

### Procedure for Natural Mating

The natural mating process was left to the birds to occur by nature. In each of the 18 cages, one cock from the pool of selected cocks was present at all times to service three hens. Mating occurred when foam was observed on the cloaca of the hen consequent to the cock's erection, mounting and contact of the cock and hen's cloaca.

## Outcome Measures

### *Egg Collection and Incubation*

One hundred and fifty un-cracked eggs were randomly collected from the naturally mated and artificially inseminated quail hens. Only eggs laid after 24 hours of the last artificial insemination were collected for incubation. From each group, 75 eggs were collected, disinfected, identified and set within 24 to 72 hours in the same incubator (HHO Automatic YZ8-48 egg incubator, made in China) at 37 °C – 38 °C and humidity of 70 - 75 g/m. The eggs were incubated for 18 days and were automatically turned in every three hours by the incubator.

### *Fertility and Hatchability Test*

Fertility was tested first by candling on the 7<sup>th</sup> and 12<sup>th</sup> day of incubation. Candling was repeated on the 12<sup>th</sup> day because fertility test of quail eggs by candling on day 7 of incubation is not very reliable in detecting early embryonic development due to the mottled nature of the quail egg's shells. In a dark room, candling was done with an improvised candler (made from a 3-watt LED Ellington rechargeable touch light -made in China) by applying light on the eggs to check for embryonic development. Fertility was further tested by hatch-out analysis after 18 days of incubation. The hatch out analysis involved cracking open the unhatched eggs to check if there was ever an embryonic development. Cracking open of eggs was done to verify the number of fertile eggs incubated as it was assumed that candling of quail eggs was not 100 % reliable due to the mottled nature of their shells. Hatch out analysis also identified dead-in-shell eggs and fertile contaminated eggs.

Fertility was recorded as the sum of fertile eggs detected by candling and hatch out analysis relative to the total eggs incubated.

Hatchability was recorded as the number of hatched eggs relative to the fertile eggs.

Relative fertile egg frequency was calculated as the number of fertile eggs from a group to the total number of fertile eggs in the incubator

Relative hatched egg frequency was calculated as the number of hatched eggs from a group to the total number of hatched eggs in the incubator.

The health status (weight, deformity and mortality) of the chicks from hatched eggs was also observed.

### **Statistical Analysis**

Chi-Square analyzed the data generated for the frequency of fertilized and hatched eggs from naturally mated and artificially inseminated quail hens. The results were presented as relative frequencies (in percentages) to the total fertilized or hatched eggs.

### **Hypothesis:**

H<sub>0</sub>1: There is no significant difference between the frequency of fertilized eggs from naturally mated and artificially inseminated Japanese quail hens.

H<sub>0</sub>2: There is no significant difference between the frequency of hatched eggs from naturally mated and artificially inseminated Japanese quail hens

## **RESULTS**

### **Reproductive Parameters of Eggs from Birds Naturally Mated or Artificially Inseminated.**

Qualitative results of eggs and chicks from naturally mated and artificially inseminated quail hens are presented in Figure 2.

In the naturally mated group, out of 75 eggs incubated, 63 eggs (84 %) were fertile but only 52 eggs (82.5 %) hatched. Three eggs (27.2 %) were contaminated among the unhatched eggs, while eight were dead-in-shell eggs. In the

artificially inseminated hens 55 (73.3 %) out of 75 incubated eggs were fertile while 47 (85.5 %) of fertile eggs hatched; one (12.5 %) of the unhatched eggs was contaminated, while the remaining seven were dead-in-shell eggs.

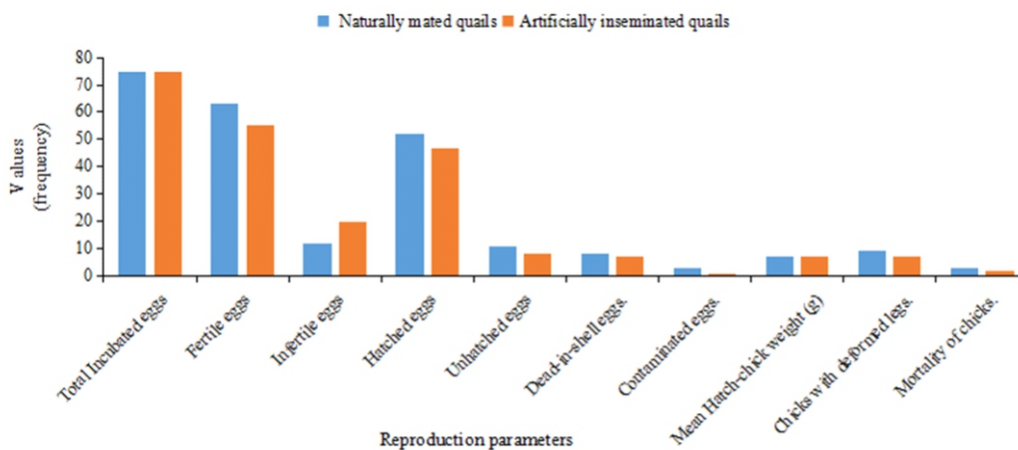


Fig. 2: Qualitative presentation of eggs and chicks from naturally mated and artificially inseminated Japanese quail hens

**Evaluation of the Frequency of Fertilized Eggs from Naturally Mated and Artificially Inseminated Japanese Quail Hens**

There was no statistically significant difference between the frequency of fertilized eggs from naturally mated and artificially inseminated Japanese quail hens. The 'chi-square' test of hypothesis ( $H_0$ ) showed a calculated chi-square ( $\chi^2_{cal} = 0.54$ ), and a critical chi-square

( $\chi^2_{crit} = 3.84$  or  $6.63$ ): d.f. = 1,  $\alpha = 0.05$  or  $0.010$ ). Since  $\chi^2_{cal} < \chi^2_{crit}$  at 5% or 1% level of probability, the study accepted the null hypothesis ( $H_0$ ) which stated that there is no significant difference between the frequency of fertilized eggs from naturally mated and artificially inseminated Japanese quail hens. The result is presented as relative frequencies to the total fertilized eggs in Figure 3.

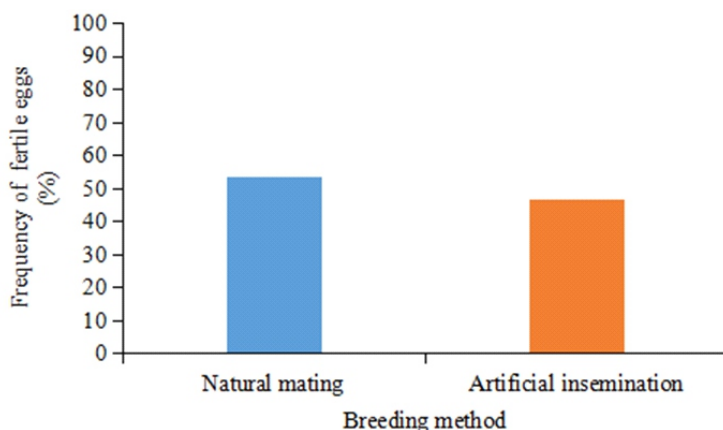


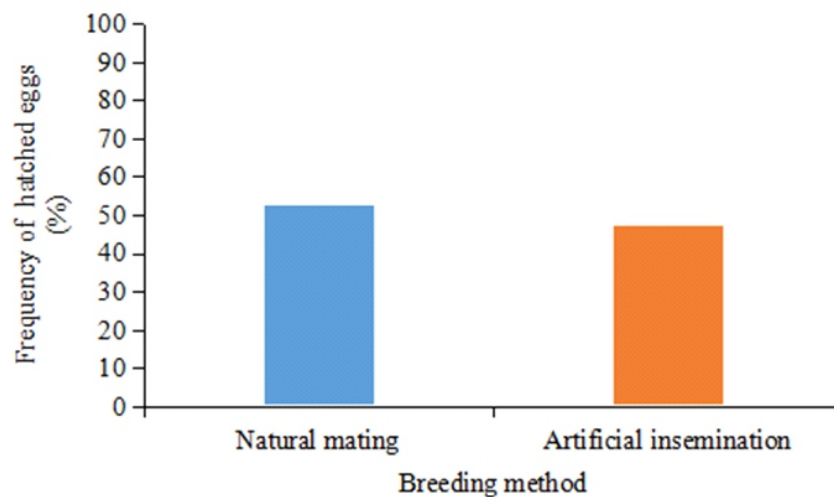
Fig. 3: Relative frequencies of fertile eggs from naturally mated and artificially inseminated Japanese quail hens

There was no significant difference in the relative frequency of fertile eggs between quail hens mated naturally and artificially inseminated. [ $\chi^2$  calculated = 0.54,  $\chi^2$  critical = 3.84 or 6.63 (d.f. = 1,  $\alpha$  = 0.05 or 0.010)]

#### Evaluation of the Frequency of Hatched Eggs from Naturally Mated and Artificially Inseminated Japanese Quail Hens

For the hatched eggs, there was no statistically significant difference between the frequency of hatched eggs from naturally mated and artificially inseminated Japanese quail hens.

The 'chi-square' test of hypothesis ( $H_0$ ) showed a calculated chi-square ( $\chi^2$  cal = 0.25), and a critical chi-square ( $\chi^2$  crit = 3.84 or 6.63): d.f. = 1,  $\alpha$  = 0.05 or 0.010). Since  $\chi^2$  cal <  $\chi^2$  crit at 5% or 1% level of probability, the study accepted the null hypothesis ( $H_0$ ) which stated that there is no significant difference between the frequency of hatched eggs from naturally mated and artificially inseminated Japanese quail hens. The result is presented as relative frequencies of hatched eggs to the total hatched eggs in Figure 4.



**Fig. 4: Relative frequency of hatched eggs from naturally mated and artificially inseminated Japanese quail hens**

There was no significant difference in the relative frequency of hatched eggs between quail hens mated naturally and artificially inseminated. [ $\chi^2$  calculated = 0.25,  $\chi^2$  critical = 3.84 or 6.63 (d.f. = 1,  $\alpha$  = 0.05 or 0.010)]

#### DISCUSSION

The results presented demonstrated that the reproductive indices of naturally mated and artificially inseminated Japanese quail hens were comparable. The similarity may indicate that the fertilization capacity of diluted semen introduced by artificial means into the reproductive tract of the hen was as good as that of raw semen introduced by copulation.

The viability of the spermatozoa that fertilizes

an egg contributes to the embryo's survival throughout the incubation period and the chick's hatching. Cock semen extenders are developed to mimic the natural components of a cock's seminal plasma. The current study used a citric-quail egg albumen semen extender maintained at PH 6.8. Spermatozoa in semen with a PH range of 6.8 - 7.1 have a high fertilizing capacity [13]. Citric-quail egg albumen semen extender is easy to prepare, and the nutritional components of the albumen are

indigenous to the species. Quail egg albumen is rich in antioxidants that remove free radicals [14] that are known to cause a damaging effect on spermatozoa function [15]. Perhaps, the antioxidants in quail egg albumen curtailed excess free radical production during spermatozoa metabolism and preserved its viability.

Similarly, Wentworth and Mellen [12] reported 77.5 % egg fertility for quail hens inseminated intrauterine with semen diluted with quail egg albumen and 54.3 % for quail hens naturally mated. The higher fertility in the naturally mated quail hens in the current study than that recorded by Wentworth and Mellen [12] may be due to differences in climatic and seasonal effects [16]. Chelmonska *et al.* [17] recorded a higher fertility percentage (82.7 %) for quail hens artificially inseminated, against 73.3 % in the current study. Chelmonska *et al.* [17] used proctodeal gland foam from a quail cock as semen diluent. They inseminated intravaginally, while in the current study, citric-quail egg albumen was used as semen diluent for intrauterine insemination. Proctodeal foam contains PGF2 alpha which facilitates long-term fertilization by inducing vaginal contraction and suction of spermatozoa into the sperm storage tubules of the oviduct [18]. Conceivably, a process that encourages long term fertilization would result in a higher fertility percentage. Perhaps, the fertility of artificially inseminated birds could have been better if the proctodeal gland foam was used as a semen diluent rather than quail egg albumen with antibiotics. Even so, the rate of picking a fertilized and hatched egg from naturally mated or artificially inseminated Japanese quail hens was comparable in the current study. Natural mating and artificial insemination of Japanese quail hens gave rise to similar reproductive indices.

In the current study, all other observed reproductive parameters, namely, dead in-shell eggs, contaminated eggs, average chick weight, chick deformity, and chick mortality, were observed in the naturally mated and artificially inseminated quail hens (the data was not subjected to statistical analysis due to the small sample size results). Perhaps factors other than the breeding method were responsible for the parameters observed. Some common causes of embryo death (dead-in-shell) as listed by FAO [19] include; diseased parent flock, improper nutrition of breeders, cracked, dirty or wet eggs, improper egg cleaning and fumigation, and eggs stored improperly or for too long before incubation. Improper incubation parameters such as too high or too low temperature or humidity or faulty egg turning during the incubation period. Inbreeding can also cause early embryonic mortality. However, this factor may not be considered in the current study, because the hens in both treatment groups were of the same pedigree and were bred by cocks of the same pedigree. Eggs can become contaminated/rotten or explode due to; trans-ovarian infectious diseases like salmonellosis from the parent flock, wet and dirty bedding, or nests contaminated with bacteria like *Pseudomonas sp* and *E.coli*. Wet eggs prior to setting, improper egg cleaning procedures, high air humidity during egg storage, contamination during storage, and failure to clean incubators following an egg explosion leaves bacteria in the chamber that can contaminate other eggs [19]. Notwithstanding all eggs laid by naturally mated and artificially inseminated hens were subjected to the same environmental conditions and hygienic measures. A study carried out by Traldi *et al.* [20] indicated that hatchling weight is influenced by egg weight as eggs with similar weight result in hatchlings with similar weight. A deformed leg, also known as spraddle or splay leg, is characterized by the abduction of



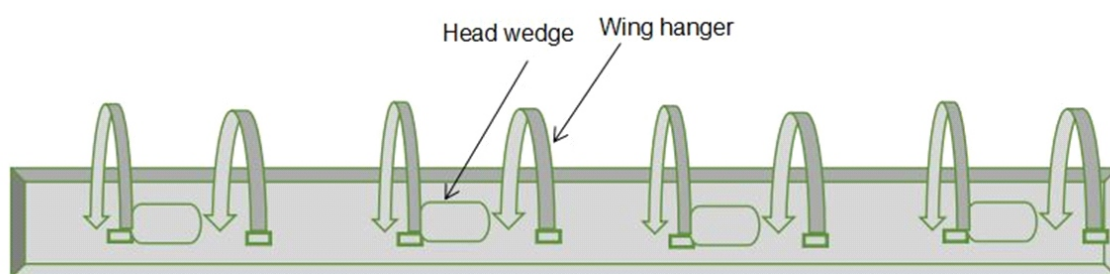
the chick's legs. Mormino [21] reported causes of spraddle leg to include slick floors that result in chicks losing their footing with a twist of the femur of the hip socket. Others include temperature fluctuation during incubation, a laborious hatch that makes legs weak, leg or foot injury, brooder overcrowding and vitamin deficiency.

A post-mortem was not carried out on the mortalities recorded, and the cause of chick death was not verified. Yerpes *et al.* [22] and Olsen *et al.* [23] reported that the primary cause of chick mortality within the first week of hatching is environmental stress and infectious diseases. The current study was limited in monitoring semen characteristics that could affect embryonic and chick survival. However, the results showed that the reproductive indices of the hens bred by natural and artificial insemination were comparable and the semen characteristics could have been similar.

An equal volume of raw semen served more quail hens when extended than deposited directly in the cloaca during natural mating. However, several constraints were encountered in the artificial insemination process. The current study demonstrated that skill and experience are required to perform successful artificial insemination in quails, and intending inseminators must be trained. Artificial insemination in quail is a new venture at the location of the present study, and there are very few knowledgeable and skilled personnel in

artificial insemination in quails in Nigeria. There was a lack of facility to sustain the storage and preservation of extended semen, so semen had to be collected several times from selected quail cocks. This constraint was due to the unstable nature of electricity supply peculiar to the environment, similar to underdeveloped societies. Semen collection was laborious and required more than one person to operate since there were no quail restraint instruments. The authors suggest a quail restraint instrument (Fig.5) that can accommodate many birds, anchor the birds' wings, and deflect the head ventral-wise but still allow the bird's feet to be supported by the floor. This paper does not consider the biomechanical and aesthetic design considerations and calculations.

Thus, considering the constraints mentioned and the similar fertile and hatching rate observed in natural mating and artificial insemination, the practical constraints override artificial insemination's advantages in quail production in the study area. Due to the practical constraints, natural mating may remain the breeding method in small to medium scale Japanese quail production in the study area and like places. However, the artificial insemination breeding technique may be used in genetic research when progenies from a particular breed stock are desired. The significant constraints of artificial insemination can be overcome if semen storage and quail restraint facilities are available.



**Fig. 5: Proposed quail restraint instrument for artificial insemination**

## Conclusion

The study revealed that the frequency of fertile and hatched eggs collected from birds naturally mated and birds artificially inseminated intra-uterine, with semen extended in quail egg

albumen extender were not different. AI had an advantage of semen conservation, but the constraints observed during the study overrides the advantages of artificial insemination in quail production in the study area.

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