



## RESEARCH PAPER

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## Reproductive performance of floored and cage housed broiler breeder roosters

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### Abstract

Male fertility is one of the most factor that affect the profitability of broiler breeder flock. The aim of the present study was to compare the effect of natural and artificial insemination on the reproductive parameter of male broiler breeders. Reproductive parameters of meat-type Hubbard roosters (n=1,800) were studied that were housed in cages (n=600) to inseminate cage housed hens or floored (n=1,200) to mate floored hens naturally (n=600) or artificially (n=600) under controlled environment where the average house temperature and relatively humidity were 21-24° C and 50-65% respectively. Body weight, semen quality, sperm penetration rate over days post insemination, egg lay, and hatchability were recorded for a complete production cycle and analyzed using SPSS software to assess the roosters' reproductive performance with varying body weight, housing system and post insemination duration. Housing type did not affect (P>0.05) seminal volume, sperm concentration, viability and morphology. However, variation was significant in sperm penetration and hatch (P<0.01). Cage housed flock exhibited better sperm penetration and hatch (90.81±21.29; 84.25±14.89) than floored flocks (65.85±20.82; 77.49%). Fewer (P<0.01) sperms penetrated the perivitelline membrane overlying the germinal disc with lengthening post insemination duration over 3,4 and 5 days but remained sound enough (≥42) to cause fertility decline till next insemination. Sperm penetration decreased with increasing body weight of rooster. Semen from roosters weighing 4-4.9 kg exhibited highest (91±11) sperm penetration followed by roosters having 4.5-5 kg (60±22) and above 5 kg (42±19). Sperm penetration was negatively associated with rooster body weight (r<sup>2</sup>=0.52) and age (r<sup>2</sup>=0.45); the regression equation predicts that current roosters will be unfit for floor mated reproduction once weighed ≥6.3 kg or age ≥92 weeks. We thus conclude that better housing, careful application of AI and regular monitoring of flock reproductive efficiency for in time corrective measures ensures smooth profitable business operation. Broiler Breeders may regularly monitor reproductive efficiency of their flocks through adoption of sperm penetration based monitoring plan that guides us for minor management changes in advance that can avoid a costly reduction in the number of quality chicks hatched.

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## Introduction

Reproductive efficiency of roosters and the hens they mate determines the breeding flock fertility which is as important to the entrepreneur as the egg lay is (Akhlaghi *et al.*, 2014). Fertility is essential to produce the maximum number of quality chicks per hen housed. Without producing fertile eggs, the best incubators and hatchery management procedures cannot produce chicks (Bramwell *et al.*, 1996). Through intense genetic selection and improved nutritional management, there has been a steady increase in the growth rate of broiler breeders and their progeny. Intensive selection based on growth traits in meat-type breeder flocks has resulted in their moderate but constant decline in fertilizing potential. A negative correlation between reproductive and growth traits may be responsible for such the decline in the fertilizing potential of birds selected for rapid growth (Brillard, 2004).

The consequent belief of the poultry men in artificial insemination to find its place in the broiler industry as one of the tool for fertility maintenance at acceptable fertility levels is gradually turning to reality, much like it has been in turkey breeding programs. Apart from hen's contribution to egg production and provision of optimal microenvironment in the oviductal sperm storage, for the subsistence of residing spermatozoa (Bramwell *et al.*, 1996), the rooster role is also critical in flock fertility (Saemi *et al.*, 2012). Subfertility is thought to be largely a problem in males because the ratio of males to females in a flock is very low (Lin *et al.*, 2005). The deterioration in fertilizing ability has been attributed to many factors like age, weight and decline in semen quality (Khan, 2011).

A better approach (Wishart and Staines, 1999) to quantify breeding efficiency is to estimate the numbers of sperm that interact with the egg in the infundibulum. These can be identified in laid eggs, as sperm in the outer perivitelline layer (OPVL sperm), or holes produced by sperm in the inner perivitelline layer (IPVL holes). Eggs can contain up to 250,000 OPVL sperm, so the scale improves on binary estimation of fertilization status.

This *in vivo* sperm penetration assay is a relatively new technique that is used to determine the number of sperm that bind, acrosome react, and penetrate the outer layer of the ovum prior to fertilization. A linear relationship ( $r^2 = 0.81$ ) exists between parameters of sperm quality and IPVL holes from eggs laid by inseminated hens (Robertson *et al.*, 1998). Thus, enumeration of IPVL holes provided a clearer validation of sperm quality tests as a basis for selection of breeding males. The quantitative advantage of using sperm penetration measurements for differentiating between fertility of semen samples from different males, from groups of males subjected to different dietary or environmental regimens, or after liquid or frozen storage, seems convincing (Wishart and Staines, 1999). The sperm penetration assay was developed in 1990 at the University of Georgia primarily as a research tool and has been used with great success. Since that time, the assay has been modified as a research tool to include its use as an *in vivo* assay using laid eggs. However, possibly more important is its use to evaluate commercial flocks to predict their future fertility.

The technique excludes the female variation in the form of hen's mating efficiency, sperm storage, sperm transport and sperm binding etc, assesses rooster reproductive efficiency in the post insemination phase and was thus opted for the current trails. The aim of the present study was to compare the effect of natural mating and artificial insemination on the reproductive efficiency of male broiler breeders.

## Materials and methods

### Study location

The field study was carried out in commercial poultry farm located in Lahore, Pakistan. The city is characterized by a long (December-January) and short (August) raining season. The experiment was conducted from November 2015 through April, 2106 under controlled environment where the ambient temperature averaged between 40 and 45° C with Relative Humidity (RH) ranged from 65-90% in hot humid July to August months. The average house temperature and RH during the experimental trials were 21-24° C and 50-65% respectively.

### *Birds and experimental conditions*

Hubbard Boiler Breeder cockerels (n=1,800) were first brooded and raised on littered floor till they aged 18 weeks and were then randomly divided into three groups. Group-1 (n=600) was housed in cages (n=600) to artificially inseminate cage housed flock, Group-2 was separately housed to inseminate the floored hens and Group-3 (n=600) mated the floored hens naturally. Each of the roosters' group had to serve 10,000 hens. Insemination on floor and cages continued from 23<sup>rd</sup> through 65<sup>th</sup> week of flock age comprising roosters and hens of the same age. The flocks were inseminated at 7<sup>th</sup> day.

Each of the four sheds had a floor space of 18800 square feet. In battery cages manufactured by Guangzhou Guangxing Poultry Equipment Company Limited (<http://www.cnguangxing.com>), hens were housed in Hot Dip Galvanized 3 tier cages, measuring, 658 cm<sup>2</sup> areas per female bird and 3 birds per cage, 1645 cm<sup>2</sup> area per male bird and one male per cage.

The floor houses were equipped with semi-automated feeders being picked up 15 minutes after feeding thereby reducing hen house floor space requirement to 1.88 square feet per bird. It is 13% less than the usually required for layers in tropical climates (Maba, 2008 and Banga-Mboko *et al.*, 2007). The cage housed hens occupied 0.89 square feet floor area per hen.

The floored flocks were provided with nests. Wheat straw served the purpose of substrate in the floored pens. Both the cage housed, and floored flocks were watered through automatic drinkers and fed manually.

The cage housing had the facility of automatic manure removal daily. Both type of flocks observed similar lighting schedule of 16 light hours with 60 LUX and 8 dark hours with zero light intensity during the entire production period.

### *Feeding and vaccination*

Feed for hens and roosters was formulated as per management guidelines for Hubbard Classic breed that contained 2750 Kcal kg<sup>-1</sup>, 16% crude proteins,

3.5% calcium (Ca) and 0.06% methionine (M) as per formulation by Banga-Mboko *et al.* (2007).

The floor housed hens and cockerels were daily offered 118 and 110 g respectively on 25<sup>th</sup> week that peaked at 175 for hens on 29<sup>th</sup> week and at 142 per day/bird for cockerels on 52<sup>nd</sup> week.

The caged house hens and cockerels were daily offered 105 and 97 g a bird on 25<sup>th</sup> week that peaked at 157 on 29<sup>th</sup> week for hens and at 100 on 54<sup>th</sup> week for cockerels. The flocks were vaccinated against Newcastle, IB, Coccidiosis, Mareks, IB Variant, IBD (Live & Attenuated), ILT, AI H9, *E. coli*, IBH and EDS.

### *Semen Collection and insemination*

Floored roosters and cage housed roosters served their respective floored and cage housed hens. Cage housed hens were inseminated artificially on each 7<sup>th</sup> day throughout the flock production period.

The schedules followed matched the five-day industrial work-week (Van Krey and Siegel, 1976). Semen was collected from cockerels on alternate day. Semen taken from each 3-4 cockerels used to be pooled together, mixed with 0.4 cc diluent (30% Beltsville Poultry Semen Extender; Hudson *et al.*, 2016) that approximately made final volume of 2cc and gently stirred to inseminate 28-32 hens. Each injector contained 2 million sperms with average penetration value of >60.

Semen was collected and inseminated using the abdominal massage procedure described by Burrows & Quinn (1937) and adopted by Yahaya *et al.* (2013).

The testes located at the dorsum were stroked and massaged until there was protrusion of the cloacae. The semen was then milked and collected directly using a glass funnel.

Three persons were involved in the insemination process. The first person carefully caught and restrained one hen at a time with his hands.

The second person carried out abdominal massage of the breeder hens according to the method of Lake and Stewart (1978) followed by Yahaya *et al.* (2013) and as soon as there was partial eversion of the cloaca, he exerted a controlled pressure on the lower abdomen for eversion of the vagina. Thereafter, the tuberculin syringe with pooled semen was inserted into the hen's vagina by the third person, who released the semen intravaginally as soon as the vagina started to relax.

#### *Measurements*

Five variables; feed intake, body weight, semen quality, sperm penetration rate and hatchability were recorded for a complete production phase from 25<sup>th</sup> to 65<sup>th</sup> week.

#### *Semen evaluation*

Fresh semen collections were evaluated to determine semen characteristics. Seminal volume was measured in graduated collecting tubes. Sperm forward motility was assessed by placing a portion of ejaculate diluted with 2.9% sodium citrate solution (1:100) on a slide, using a Zeiss (Jena, Germany) compound light microscope (400 × magnification), equipped with a warm stage (37°C). Sperm viability and abnormality were determined, using a portion of ejaculate stained with eosin-nigrosin solution, by observing 100 spermatozoa per slide. Spermatozoa with detached heads, abaxial heads, malformed heads, bent tails, coiled tails, double tails, and protoplasmic droplets were considered as abnormal (Pursel *et al.*, 1972). Sperm concentration was determined using a Neubauer hemocytometer.

#### *Perivitelline layer (PVL) sperm penetration assay*

Determination of PVL sperm penetration assay was carried through the slightly modified version (Namdari *et al.*, 2016) of the procedure developed by Bramwell *et al.* (1995).

The egg was broken, and the albumen was separated from the egg yolk which was then put on a glass container with the germinal disc placed on the top.

The surplus albumen was separated from the PVL through tissue paper.

The germinal disc (Blastodermic area) was cut with scissors and immediately rinsed in 1% NaCl solution to detach excess yolk from the egg membrane. Then the PVL was placed on a microscopic slide followed by the addition of some egg albumin, fixed by adding 3-4 drops of 10% formalin for 1 hour, and rinsed with 1% NaCl solution.

The fixed PVL was then replaced with periodic acid-Schiff and allowed to dry at room temperature. The inner PVL holes were counted using a light microscope (OSK856861, Japan) at 400 ×.

The blastodermic area was positioned on each slide and centered in the field of vision (area = 40 mm<sup>2</sup>) and the number of holes within this area counted.

#### *Hatchability*

Eggs were set in forced-air incubator for 18 days at 99-99.5°F and 60-65% relative humidity (83-88°F wet bulb) and transferred to hatcher where temp and humidity were 98.2°F to 98.5°F and 90% respectively.

#### *Statistical analysis*

Data were processed using version 22 of the SPSS software. ANOVA was performed and mean values for input and output variable obtained from the cage housed and floored flocks were compared among treatments using the LSD at 5% significance level. Regression analysis was performed with the help of Pearson's correlation and multiple regression analysis.

## **Results**

#### *Reproductive parameters*

Housing type did not affect ( $P > 0.05$ ) seminal volume, sperm concentration, viability and morphology. However, variation (Table 5.1) was significant in sperm penetration and hatch ( $P < 0.01$ ).

**Table 1.** Reproductive Parameters of Floored and Cage Housed Broiler Breeder Roosters (Means\* ± SE).

Parameter	Floor	Cage	P Value
	Mean*± SE	Mean± SE	
Sperm Volume (ml)	0.5±0.02 <sub>a</sub>	0.5±0.03 <sub>a</sub>	0.32
Sperm concentration (10 <sup>6</sup> /mL)	1110±75 <sub>a</sub>	1160±89 <sub>a</sub>	0.57
%Live Sperm	88.7±1.3 <sub>a</sub>	88.2±1.2 <sub>a</sub>	0.69
%Abnormal Sperm	3.2±0.3 <sub>a</sub>	2.8±0.2 <sub>a</sub>	0.43
%Motile Sperm (%)	79.7±2.4 <sub>a</sub>	68.3±2.9 <sub>b</sub>	0.01
Sperm Penetration Rate mm <sup>-2</sup>	66±21 <sub>a</sub>	90±21 <sub>b</sub>	<0.01
%Hatchability	77.49±15.72 <sub>a</sub>	84.25±14.89 <sub>b</sub>	<0.01

#### *Effect of Rooster Housing on Sperm Penetration and Consequent Hatch*

Average sperm penetration for floored and cage housed cockerels is given in Table 1. Rooster housing affected sperm penetration ( $P<0.01$ ) and consequent

hatch ( $P<0.05$ ). Cage housed roosters exhibited better sperm penetration and consequent hatch (90±21; 84.25±14.89) than floored roosters (66±21; 77.49±15.72). Postinsemination duration and housing did not interacted significantly ( $P>0.05$ ).

**Table 2.** Sperm Penetration (SP mm<sup>-2</sup> GDIPVL) Over Days Postinsemination in Artificially Inseminated Floored and Cage Housed Flocks.

Housing System		Days' Post Insemination				P values		
Floor	Cage	Day-2	Day-3	Day-4	Day-5	Housing	Days	Interaction
54.6±27 <sub>a</sub>	65.5±23 <sub>b</sub>	79±14 <sub>a</sub>	59±13 <sub>b</sub>	45±11 <sub>c</sub>	42±12 <sub>d</sub>	0.001	0.001	0.87

#### *Postinsemination Fertility*

Table 2 presents the sperm penetration over days post insemination. Sound number of sperms (42-87) penetrated the perivitelline membrane overlying the germinal, to ensure optimum fertility level. Sperm penetration was the highest ( $P<0.01$ ) in the eggs laid the next day after insemination in both cage housed or floored flocks. Fewer ( $P<0.01$ ) sperms penetrated the perivitelline membrane overlying the germinal disc with lengthening post insemination duration over 3, 4 and 5 days. Sperm penetration till next insemination was sound enough ( $\geq 42$ ) to cause fertility decline in either flock.

#### *Age Effect on Sperm Penetration*

Sperm penetration was negatively ( $P<0.05$ ) associated with rooster age (Table 4). Weekly age increment reduced one sperm to penetrate the perivitelline layer overlying the germinal disc and the regression equation predicts no penetrating sperm at 92<sup>nd</sup> week onwards.

#### *Body Weight Effect on Sperm Penetration*

Sperm penetration decreased with increasing body weight of rooster (Table 3). Semen from roosters weighing 4-4.9 kg exhibited highest (91±11) sperm penetration followed by roosters having 4.5-5 kg (60±22) and above 5 kg (42±19).

Sperm penetration was negative ( $P<0.05$ ) when regressed on rooster body weight (Table 4). Each 100 grams body weight gain by cockerel reduced 3 SP mm<sup>-2</sup> GDIPVL.

It is thus predicted that the current rooster will be totally unfit for floor mated reproduction at a heavy body weight ( $\geq 6.3$  kg) because they will supply no sperm that succeed to penetrate the perivitelline layer overlying the germinal disc.

Body weight related decrease in sperm penetration did not harm rooster reproductive performance in artificially inseminated flocks for all the age groups

(Table 3) but dropped too low (<27) to cause subfertility in naturally mated flock after the rooster weighed above 5 kg.

### Discussion

In the present study, sperm quality parameters such as semen volume, sperm concentration, live sperm, abnormal sperm, motile sperm, sperm penetration rate and hatchability did not differ in the two housing system. In line with the values reported for broiler breeder semen evaluated by Namdari *et al.*, 2016 variation in sperm penetration and hatch were significant but seminal volume, sperm concentration and viability remained unchanged. However sperm morphology did not match with reference trials due to

type of treatment. In the present study, the numerically better sperm penetration and consequent hatch for cage housed cockerels is attributed to insemination type applied in the two housing systems whereby artificial insemination ensured optimal supply of semen to the fertilization site. Older roosters performed better than younger (Bramwell *et al.*, 1996) with respect to sperm penetration and fertility when flock is inseminated artificially Semen extender was used in inseminating the cage housed roosters' semen that might have favored the transit of sperms through the oviduct in their attempt to reach the infundibulum area, limiting their access to the ovum perivitelline layer (Abouelezz *et al.*, 2015).

**Table 3.** Roosters' Body Weight Effect on Sperm Penetration.

Body Weight (g)		Mean* ± SE	P Value
Minimum	Maximum		
4000	4499	81± 11 <sub>a</sub>	0.0001
4500	5000	60± 22 <sub>b</sub>	
>5000		42± 19 <sub>c</sub>	

There have been conflicting reports of the relationship of concentration and motility to fertility. The majority of reports have found no relationship between the two (Cooper and Rowell, 1957), while other researchers have seen positive correlations (Boone and Huston, 1963). Sperm motility has been correlated to the fertilizing ability of males (McDaniel *et al.*, 1997) and motility based roosters' selection at early age improves flock fertility (Parker *et al.*, 2000).

In the current study, sperm penetration was better ( $P < 0.01$ ) was better in cage housed than floored flocks. Sperm-egg binding has exhibited a high correlation ( $r = 0.83$ ) to fertility (Barbato *et al.*, 1998). The better sperm penetration rate by roosters housed in the modern cage systems may be attributed to several management factors that favored reproductive performance. Besides increased spatial density of birds, an easier control of microclimate, a simplified waste disposal and an easier supervision of individual birds for production level and health status reported by Azeroul (2005) and Pistikova *et al.* (2006), cage

housing enhanced roosters' reproductive efficiency through provision of clean environment and uniform feed allowance per bird. Fertility and hatchability are most sensitive to environmental and genetic influences (Stromberg, 1975).

Heritability estimates for fertility and hatchability in chickens range from 0.06-0.13 (Sapp *et al.*, 2004), this indicates that the non-genetic factors have a higher influence on these traits. Both are interrelated heritable traits that vary among breed, variety and individuals in a breed or variety. System of husbandry and rearing technology (Weis, 1991) are among other factors including egg age (Tarongoy *et al.*, 1990), storage condition (Brah and Sandhu, 1989), flock age (Rogue and Soares, 1994), mating system (Gebhardt-Henrich and Mark, 1991) that influence the hatchability. Variation in reproductive efficiency may be attributed to difference in management practice and environment (Kalita 1984). Considering the overall hatchability traits: breed has little effect on hatchability in poultry (Islam *et al.*, 2002).

When a hen is inseminated, she will lay fertilized eggs for a period of time without need for further artificial insemination or copulation (Lake, 1975). In the current trails, egg fertility in artificially inseminated flocks declined over days postinsemination till next insemination mainly with decreasing number of sperms in the sperm storage tubules in the hen. However, it did not change in the naturally mated flock due to ample daily supply of sperms. The length of the fertile period is positively correlated with the OPVL sperm in the first fertile egg (Wishart, 1987; Brillard and Antoine, 1990) and in cases of severe subfertility, the problem appears to be one of differing sperm transport among hens, rather than insufficient sperms/AI. Gumułka and Kapkowska 2005 lengthened fertility duration by 2 days postinsemination and concluded that high values for effective fertility can be obtained from broiler breeders in adequate environmental and

technological conditions of AI. Subfertility in the floor mated flocks is a problem of sperm allocation among hens rather than total number of sperm transferred by the roosters. The striking feature of the distribution of IPVL holes in eggs from broiler flocks was the enormous variation. Although majority of eggs contained sufficient sperm to ensure fertilization but considerable number of eggs from the same flock were unfertilized.

The standard deviation for number of OPVL sperm in eggs from naturally mated broilers was twice that with AI (Wishart *et al.*, 1992). Thus, it seems that the main cause of subfertility in floor-mated broiler flocks is that many hens rarely, if ever, mate. In floor-mated broiler flocks, dose and quality of sperm deposited are variable and, as with AI, sperm transfer and a hen's oviducal storage capacity affect outcome.

**Table 4.** Regression of Sperm Penetration on Roosters' Body Weight and Age.

Parameter	b <sub>0</sub> ± SE	b <sub>1</sub> ± SE	P for b <sub>1</sub>
Body Weight	188.583±17.359	-0.030±0.004	<0.001
Age	108.7±7.8	-1.172±0.674	<0.001

\* Means with same subscripts didn't differ for the same parameter.

In the current study, roosters were less reproductively efficient with increasing body weight. Heavy body weight lowers mating frequency that may have declined sperm penetration. Rooster' body weight is ineffective on sperm penetration (Bramwell *et al.*, 1996) when flock is artificially inseminated and rather older males had high sperm penetration and fertility. Heavy breed flocks and the extensive systems require more cocks (Islam *et al.*, 2002).

Higher fertility has been recorded for light (White Leghorn) when compared with heavy breeds (Barred Plymouth Rock: Rhode Island Red, White Rock and New Hampshire) (Islam *et al.*, 2002). Both increased body weight and age reduce fertility in broiler breeders (Bramwell *et al.*, 1996). However, Swan (1977) reported higher hatchability percentage with meat strains as compared to egg strains which is in disagreement with the current findings and aforementioned studies.

## Conclusion

Reproductive efficiency of roosters and the hens they mate determines the breeding flock fertility that varies with housing system, mating type, age and body weight. Better housing, careful application of AI and regular monitoring of flock reproductive efficiency for in time corrective measures ensures smooth profitable business operation. Adoption of sperm penetration based reproduction monitoring plan guides us for minor management changes that can avoid a costly reduction in the number of quality chicks hatched.

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