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# Effect of genotype on semen quality traits of main and reciprocal crossbred chickens

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**Abstract**

*Semen quality of cocks is of utmost importance in reproduction. Breeding method, breed, strain and genetic constitution are the major components of the genetic factors capable of affecting the semen quality of cocks. This research seeks to determine the effect of genotype on the semen quality traits of 272 $F_1$ crossbred cocks produced at day-old by main and reciprocal crossbreeding of 69 Isa Brown and local frizzle feathered, naked neck and normal feathered chickens. The genotypes of the cocks were Isa Brow x frizzle feathered main cross (IBxF), Isa Brown x naked neck main cross (IBxNa), Isa Brown x normal feathered main cross (IBxN), frizzle feathered x Isa Brown reciprocal cross (FxIB), naked neck x Isa Brown reciprocal cross (NaxIB) and normal feathered x Isa Brown reciprocal cross (NxIB). At 36-40 weeks of age, semen was extracted from the cocks by abdominal massage technique and used to evaluate semen volume (SV), sperm motility (SM), sperm concentration (SC) and live sperm (LS) at weekly intervals. Data obtained from the evaluation were subjected to analysis of variance and tested at 5% level of probability. Genotypes differed significantly ($P < 0.05$) in SC at 37-40 weeks and in SM and LS at all ages. There was no significant difference ($P > 0.05$) in SV. Regardless of genotype and age, the mean SV, SM, SC and LS ranged from 0.31±0.02 to 0.24±0.01ml, 71.84±1.33 to 58.75±2.16 %, 3.22±0.02 to2.92±0.02 x$10^9$/ml and 71.91±1.37 %, respectively. The NxIB and IBxNa genotypes produced largest numerical semen volume at 37 and 39 weeks of age respectively. Whereas SC and LS were significantly highest for IBxF and IBxN respectively at week 37, SM was so for IBxN at all ages. Further, the LS of IBxN genotype was significantly highest at 36-38 and 40 weeks of age. The exotic Isa Brown and normal feathered main cross cocks exhibited the best semen quality traits among all genotypes studied and should be selected for improvement of the reproductive characteristics of cocks.*

**Keywords:** Chicken, crossbreeding, crosses, reproductive traits.

## Introduction

The assessment of semen quality characteristics of poultry is an indicator of their reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs (Peters *et al*., 2004). Variation in semen quality characteristics of the chicken is caused by both genetic and environmental factors. Semen volume and sperm concentration of the domestic fowl depends largely on the relative contribution of the various reproductive glands, the number of spermatozoa that could be obtained from a breed/strain and the extent to which the genetic potential of the animal can be exploited (Hafez, 1978). Genotype or strain of chickens is an important factor that influences semen quality traits of chickens. Omeje and Marire (1990) observed significant genotype effect on body size and semen characteristics of cocks, except the

pH value whereas Ezekwe and Machebe (2004) reported differences in strains with respect to semen volume, concentration, motility, active and sluggish spermatozoa. Peters*et al*. (2008) reported significant sire strain effect on a number of semen quality traits including semen volume and sperm concentration with highest semen volume obtained from exotic White Leghorn followed by Giriraja and semen concentration from Naked neck strain. The objective of this study was therefore, to determine the effect of genotype on the semen quality traits of the hybrids of Isa Brown and local chicken parents generated by main and reciprocal crossing.

## Materials and methods

### *Experimental location*

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike. The University is located on the latitude $05^0 29'$ North and longitude $07^0$ 33' East. It is approximately 122 m above sea level. Umudike has maximum and minimum daily temperatures of 27 – 36°C and 20 – 26° C, respectively and relative humidity of 57 – 91%. It is located within the tropical rainforest zone and the environment is characterized by an annual rainfall of 2177 mm (Nwachukwu *et al*., 2006).

### *Management of parent stock and production of $F_1$ progeny*

A total of 69 parents of three genotypes (frizzle feathered, naked neck and normal feathered) of local and exotic (Isa Brown) chickens were used in the experiment. The chickens consisted of three cocks each of the local genotypes, eight frizzle feathered, seven naked neck and nine normal feathered hens. The Isa Brown chickens consisted of nine cocks and twenty-seven hens. These were fed layer mash containing 2650 metabolisable energy per kilogram weight (MEkg) and 16.5 % crude protein (CP) *ad libitum*. They were reared in small cages of 78 x 74 x 66 $cm^3$ dimension constructed with wire gauze on deep litter pens measuring 2.65 x 1.67 $m^2$. Each cock mated three or two hens in main and reciprocal pattern. The Isa Brown cocks were used in the main crossing while the local cocks were used in the reciprocal crossing. Eggs produced from the mating were collected, set according to their pedigree and hatched in an incubator at weekly intervals. A total of 531 first filial generation ($F_1$) day-old chickens were produced in 12 consecutive hatches. The birds belonged to six genotypes namely, Isa Brown x frizzle feathered main cross (IBxF), Isa Brown x naked neck main cross (IBxNa), Isa Brown x normal feathered main cross (IBxN), frizzle feathered x Isa Brown reciprocal cross (FxIB), naked neck x Isa Brown reciprocal cross (NaxIB), normal feathered x Isa Brown reciprocal cross (NxIB). There were 123 (59 males and 64 females), 49 (28 males and 21 females), 116 (62 males and 54 females), 137 (70 males and 67 females), 42 (19 males and 23 females) and 64 (34 males and 30 females) birds of IBxF, IBxNa, IBxN, FxIB, NaxIB and NxIB genotypes. The males were used for evaluation of semen quality traits. The chicks were vaccinated at day-old against Newcastle Disease and brooded together in cages of 79 x 67 x 61 $cm^3$ dimension each constructed on deep litter pens measuring 2.65 x 1.67 $m^2$ in dimension. They were fed and provided cool, clean water *ad libitum*. Feeding was carried out using round plastic feeders during brooding and conical and rectangular iron feeders hung approximately 25 cm above the liter level afterwards. Four-liter plastic drinkers were used to provide water for the birds. The birds were fed starter mash containing 2800 kcal ME/kg and 20% CP from 1-6 weeks, grower mash containing 2550 kcal ME/kg

and 15% CP from 6-20 weeks and breeder mash containing 2650 kcal ME/kg and 16.5% CP from 20-40 weeks of age.

***Semen collection and determination of semen quality traits***

Semen was collected on farm on individual cocks and evaluated for different parameters starting from 36 to 40 weeks of age. The method of collection was by abdominal massage technique (Lake, 1962). This was achieved by two persons. The assistant gently put his hand on the ventral surface of a cock, placing his palm on the abdomen, while clipping the two shanks of the cock with his first and second fingers. The cock was then massaged at the back by the collector, who stroked close to its tail and quickly applied a slight finger pressure around the base of the tail. The phallus then became erect within the cloaca. Pressure was applied around the cloaca and the tail flattened towards the back of the cock, causing the phallus to protrude from the cloaca. The collector's thumb was then pressed on the cock's abdomen directly beneath its vent. This caused semen to be released from the ductus deferens and the collector gently squeezed the semen from the swollen papillae at the base of the phallus into a graduated test tube. The collected semen was used to determine the following semen quality parameters.

***Semen volume***

The volume of the semen was read in cubic milliliter ($mm^3$) directly from the graduated test tube and converted to milliliter (mL).

***Sperm motility***

This was assessed on farm immediately after collection of semen**.** A drop of semen with the aid of a micro-pipette was placed on a clean microscope slide and covered with a glass cover slip to spread the semen in other to have a uniform thickness and to prevent drying. The semen was viewed under the microscope using a magnification of x 40 objective lens. Two or three fields were examined and the estimated motility was by subjective judgment of the motile sperm. Semen motility was expressed as the percentage of cells that are motile.

***Sperm concentration***

Semen concentration was measured using the direct count method. The Neubaeur haemocytometer which is used for counting blood cells was used. It consists of two counting chambers and two dilution pipettes. The counting chambers were 0.1 mm in depth and had a ruled area on the bottom of the chambers that was 1.0 $mm^2$; the square was sub-divided into 25 smaller squares. Normal saline was mixed with 1 mL of semen at the dilution rate of 1:1000. The diluted semen was then picked up using a micropipette. One drop of the diluted semen was then placed on one end of the haemocytometer and also on the other end and allowed to settle. The loaded Neubaeur haemocytometer was then placed on the microscope at a magnification of x 40. The spermatozoa's head that falls within the sub-divided smaller squares at the four edges and center of the haemocytometer was counted and the average per stain of a cock read. The concentration of the sperm per volume was determined using the formula below.

$$\text{Concentration } (\times 10^9/\text{mL}) = \frac{\text{Number of spermatozoa counted x Dilution rate}}{\text{Volume of fluid (ml)}}$$

***Live/Dead ratio of sperm***

This was determined by mixing freshly collected semen with Eosin/Nigrosin staining dye on the glass slide in order to make the sperm cells visible under the microscope. This is known as supervital staining (Marini and Goodman, 1969) whereby dead spermatozoa will absorb the staining dye while the live ones will not. The stained semen was smeared on the slide and examined under the microscope using a magnification of x 40 objective lens. Unit of expression was percentage live sperm, and was estimated based on the rate of movement of the cells.

***Experimental design and statistical analysis***

The experiment was carried out in nested design with a mixed model involving fixed (hatch and genotype) and random (sire and dam) effects. There were 12 hatches, 6 genotypes, 18 sires and 51 dams. The model of the design was of the form:

$Y_{ijklm} = \mu + H_i + G_j + S_k + D_{kl} + E_{ijklm}$

Where:

$Y_{ijklm}$= Record of the $m^{th}$ progeny of $l^{th}$ dam mated to $k^{th}$ sire belonging to $j^{th}$genotype in $i^{th}$ hatch

$\mu$ = population mean

$H_i$= fixed effect of hatch (i = 1,…, 12)

$G_j$= fixed effect of genotype (j = 1,…, 6)

$S_k$= Random effect of sire (k = 1,…, 18)

$D_{kl}$= Random effect of dam mated to sire (l = 1,…, 51)

$E_{ijklm}$ =Random error, assumed to be independently and identically normally distributed with zero mean and constant variance [iind $(0,\sigma^2$ ) Analysis of variance was performed on the data collected using SAS (1999) statistical software. Genotype effect was tested at 5 % level of probability and significant means separated using Duncan (1955) New Multiple Range Test as provided by the SAS (1999) software.

**Results**

The mean semen volume of main and reciprocal crosses of Isa Brown and local chickens at 36-40 weeks of age are shown in Table 1. Non- significant (P>0.05) difference was observed in semen volume among the genotypes at all ages studied. The mean semen volume for all the genotypes at all ages ranged from 0.31±0.02 to 0.24±0.01.

**Table 1: Mean (± se) semen volume (ml) of main and reciprocal crosses of Isa Brown and local chickens at 36-40 weeks of age**

| Age | Main cross | | | Reciprocal cross | | |
|---|---|---|---|---|---|---|
| (weeks) | IBxF | IBxNa | IBxN | FxIB | NaxIB | NxIB |
| 36 | 0.25±0.02 | 0.28±0.02 | 0.24±0.01 | 0.28±0.01 | 0.26±0.02 | 0.27±0.02 |
| 37 | 0.26±0.02 | 0.30±0.02 | 0.26±0.02 | 0.28±0.01 | 0.27±0.02 | 0.31±0.02 |
| 38 | 0.24±0.01 | 0.29±0.02 | 0.28±0.02 | 0.27±0.01 | 0.25±0.02 | 0.27±0.12 |
| 39 | 0.26±0.02 | 0.31±0.02 | 0.27±0.02 | 0.25±0.02 | 0.28±0.02 | 0.29±0.02 |
| 40 | 0.25±0.01 | 0.24±0.02 | 0.27±0.02 | 0.25±0.01 | 0.27±0.02 | 0.26±0.02 |

IBxF= Isa Brown x frizzle feathered main cross, IBxNa = Isa Brown x Naked neck main cross; IBxN = Isa Brown x normal feathered main cross, FxIB= Frizzle feathered x Isa Brown reciprocal cross, NaxIB = Naked neck x Isa Brown reciprocal cross, NxIB= Normal feathered x Isa Brown reciprocal cross, se = standard error

The mean sperm motility of main and reciprocal crosses of Isa Brown and local chickens at 36-40 weeks of age are presented in Table 2. There was a significant (P<0.05) genotype effect on sperm motility of the cocks at all ages studied. Isa Brown x normal feathered main cross (IBxN) genotype was consistently superior in sperm motility to the other genotypes. The observed mean values of sperm motility for all genotypes ranged from 58.75±2.16 to 71.84±1.33 %. These were slightly lower than 62.55±10.26 to 87.35±10.12% range of values reported by Peters *et al*. (2008) but fell within the range reported for motility in normal cocks (Hafez, 1978). Highest mean values for IBxF and IBN genotypes were obtained at week 38 and for the rest of the genotypes, they were at different weeks.

**Table 2: Mean (± se) sperm motility (%) of main and reciprocal crosses of Isa Brown and local chickens at 36-40 weeks of age**

| Age | Main cross | | | Reciprocal cross | | |
|---|---|---|---|---|---|---|
| (weeks) | IBxF | IBxNa | IBxN | FxIB | NaxIB | NxIB |
| 36 | $62.53^{b}$±2.07 | $62.08^{b}$±1.69 | $71.38^{a}$±1.27 | $67.63^{a}$±1.33 | $69.32^{a}$±2.02 | $62.06^{b}$±1.16 |
| 37 | $63.51^{b}$±2.14 | $63.04^{b}$±1.65 | $70.96^{a}$±1.17 | $63.13^{b}$±1.21 | $70.45^{a}$±2.07 | $68.23^{a}$±1.41 |
| 38 | $63.89^{b}$±1.90 | $63.46^{b}$±1.66 | $71.84^{a}$±1.33 | $63.33^{b}$±1.45 | $68.00^{ab}$±2.22 | $66.58^{b}$±1.39 |
| 39 | $58.75^{c}$±2.16 | $63.69^{b}$±1.67 | $70.46^{a}$±1.24 | $63.93^{b}$±1.40 | $70.68^{a}$±2.22 | $65.87^{ab}$±1.40 |
| 40 | $59.25^{c}$±2.42 | $65.00^{b}$±1.86 | $71.02^{a}$±1.40 | $64.30^{b}$±1.29 | $68.32^{ab}$±2.18 | $67.24^{ab}$±1.36 |

[a-c]Means in the same row with different superscripts are significantly different (P<0.05)
IBxF= Isa Brown x Frizzle feathered main cross, IBxNa = Isa Brown x Naked neck main cross,
IBxN = Isa Brown x Normal feathered main cross, FxIB = Frizzle feathered x Isa Brown reciprocal cross, NaxIB = Naked neck x Isa Brown reciprocal cross, NxIB= Normal feathered x Isa Brown reciprocal cross, se = standard error

The mean sperm concentration of main and reciprocal crosses of Isa Brown and local chickens at 36-40 weeks of age is presented in Table 3. Sperm concentration differed significantly (P<0.05) among the genotypes at all ages except 36 weeks where no significant difference (P>0.05) occurred. Where significant differences occurred, Isa Brown x frizzle and normal feathered main crosses (IBxF and IBxN) ranked highest in sperm concentration among the genotypes. The mean sperm concentration of the main crosses was also generally higher than the reciprocal crosses.

**Table 3: Mean (± se) sperm concentration ($x10^{9}$/ml) of main and reciprocal crosses of Isa Brown and local chickens at 36-40 weeks of age**

| Age | Main cross | | | Reciprocal cross | | |
|---|---|---|---|---|---|---|
| (weeks) | IBxF | IBxNa | IBxN | FxB | NaxIB | NxIB |
| 36 | 3.10±0.02 | 3.04±0.02 | 3.08±0.01 | 3.07±0.01 | 3.04±1.02 | 3.07±0.01 |
| 37 | $3.22^{a}$±0.02 | $3.15^{b}$±0.02 | $3.20^{a}$±0.01 | $3.18^{ab}$±0.01 | $3.15^{b}$±0.02 | $3.19^{ab}$±0.01 |
| 38 | $3.01^{a}$±0.02 | $2.93^{b}$±0.01 | $3.02^{a}$±0.02 | $2.96^{b}$±0.01 | $2.92^{b}$±0.12 | $2.96^{b}$±0.02 |
| 39 | $3.01^{a}$±0.02 | $2.95^{bc}$±0.01 | $3.00^{a}$±0.02 | $2.95^{bc}$±0.01 | $2.92^{c}$±0.02 | $2.98^{ab}$±0.01 |
| 40 | $3.06^{a}$±0.01 | $3.02^{ab}$±0.04 | $3.05^{a}$±0.02 | $2.95^{bc}$±0.01 | $2.94^{c}$±0.02 | $3.03^{ab}$±0.02 |

[a-c]Means in the same row with different superscripts are significantly different (P<0.05)
IBxF= Isa Brown x Frizzle feathered main cross, IBxNa = Isa Brown x Naked neck main cross, IBxN = Isa Brown x Normal feathered main cross, FxIB= Frizzle feathered x Isa, Brown reciprocal cross, NaxIB = Naked neck x Isa Brown reciprocal cross, NxIB= Normal feathered x Isa Brown reciprocal cross, se = standard error

Mean live sperm of main and reciprocal crosses of Isa Brown and local chickens at 36-40 weeks of age are presented in Table 4. Live sperm of the cocks differed significantly (P<0.05) among the genotypes in all ages studied. At 37, 38 and 40 weeks, IBxN genotype recorded highest significant live sperm means of71.20±1.19 %,71.91±1.37 % and 69.25±1.23 %; at 39 weeks, IBxNa recorded 70.44±1.68 %while at 36 weeks IBxN and IBxNa recorded 71.20±1.19 % and 71.19±1.74 %, respectively. The means of live sperm were above 50 percent and ranged from 60.78±1.72 to 71.91±1.37% at all ages. Although these means were not as high as the upper value in a six-week range of 54.70±0.10 to 80.00±0.10% live spermatozoa reported by Rwuaan *et al*. (2010) for normal White Shika brown cocks, values for each genotype were generally high.

**Table 4: Mean (± se) live sperm (%) of main and reciprocal crosses of Isa Brown and local chickens at 36-40 weeks of age**

| Age | Main cross | | | Reciprocal cross | | |
|---|---|---|---|---|---|---|
| (weeks) | IBxF | IBxNa | IBxN | FxIB | NaxIB | NxIB |
| 36 | $62.20^{c}\pm1.73$ | $71.19^{a}\pm1.74$ | $71.20^{a}\pm1.19$ | $67.23^{ab}\pm1.09$ | $66.82^{ab}\pm1.91$ | $65.79^{bc}\pm1.54$ |
| 37 | $60.98^{c}\pm1.72$ | $69.92^{ab}\pm1.74$ | $71.91^{a}\pm1.37$ | $66.68^{b}\pm1.58$ | $67.89^{ab}\pm1.83$ | $65.81^{b}\pm1.67$ |
| 38 | $63.64^{c}\pm1.57$ | $70.36^{ab}\pm1.70$ | $71.14^{a}\pm1.14$ | $63.62^{c}\pm1.40$ | $66.30^{bc}\pm1.85$ | $65.03^{c}\pm1.63$ |
| 39 | $60.78^{c}\pm1.76$ | $70.44^{a}\pm1.68$ | $70.16^{ab}\pm1.17$ | $65.62^{b}\pm1.22$ | $65.66^{b}\pm1.73$ | $66.00^{ab}\pm1.85$ |
| 40 | $62.78^{bc}\pm1.76$ | $67.42^{ab}\pm2.05$ | $69.25^{a}\pm1.23$ | $60.18^{c}\pm1.47$ | $66.73^{ab}\pm2.04$ | $65.45^{ab}\pm1.70$ |

[a-c]Means in the same row with different superscripts are significantly different (P<0.05)
IBxF = Isa Brown x Frizzle feathered main cross, IBxNa = Isa Brown x Naked neck maincross, IBxN = Isa Brown x Normal feathered main cross, FxIB= Frizzle feathered x Isa Brown reciprocal cross, NaxIB = Naked neck x Isa Brown reciprocal cross, NxIB= Normal Feathered x Isa Brown reciprocal cross, se = standard error

## Discussion

The mean semen volume obtained (Table 1) were similar to that of 0.27 ml reported for White Leghorn cocks (Saeid and Al-Soudi, 1975) and a six-week range of 0.3±0.10 to 0.3±0.30 ml reported for Red Shika brown cocks (Rwuaan *et al*., 2010) but were slightly lower than the average of 0.35 ml in Cobb broilers (Cerolini *et al*., 2006) and range of 0.37±0.02 to 0.73 ± 0.01 ml reported by Peters *et al*. (2008) for normal healthy cocks. These differences indicate that the breeding method, breed, strain or genotype is capable of influencing the semen quality of a cock. Manipulation of the genetic constitution of an animal through breeding to improve the semen quality of cocks is therefore important. Hence, these factors should be considered germane to breeding and improvement of the reproductive traits of cocks. The means of semen volume which generally decreased with age suggests that all the genotypes can be engaged in selective breeding experiment for improvement of semen quality traits of chickens at the ages studied. The observed decreased in semen volume with age also implies that senescence reduces semen volume in cocks. The implication of this result is that cocks should be selected at earlier age where largest semen volume can be obtained for breeding efficiency and improvement of semen traits. The genotypic differences in sperm motility observed in Table 2 indicate that genotype of a cock can influence its sperm motility. Isa Brown combined with frizzle and normal genes will result in highest sperm motility among the genotypes studied. Sperm motility is an important semen quality because the fertilization ability of semen depends on the speed with which the spermatozoa can reach the ovum. McGary *et al*. (2002) reported that semen quality is an important factor that determines the breeding value of males, because it influences the fertilization rate of the eggs for hatching as well as the reproductive efficiency of their progeny. Cocks with high motility therefore have greater potential to fertilize an egg. The genotypic differences in sperm concentration (Table 3) is in consonant with the findings of Ezekwe and Machebe, (2004), who observed differences in strains with respect to semen volume, concentration, motility, active and sluggish spermatozoa. The generally higher sperm concentration observed in the main crosses than the reciprocal ones is an indication that the exotic Isa Brown sires should be used in crossbreeding with local hens, especially the frizzle and normal feathered genotypes for improved sperm concentration. The decreased sperm concentration observed with age suggests that fertility and hatchability of eggs will reduce when old cocks are used to cross hens. Hence, to avoid such decline in practical animal breeding, cocks should be used at younger age, especially as yearly, in mating. The observed means of sperm

concentration of the current study were slightly lower than the reported means of 7.0 $x10^9$/ml (Hafez, 1978), 4.30 $x10^9$/ml (Moya *et al*., 1996) and range of means of 3.11±0.04$x10^9$/ml to 4.21±1.45$x10^9$/ml (Peters *et al*., 2008) but higher than 2.0 $x10^9$/ml (Keskin *et al*., 1995) and 1.2 $x10^9$/ml (Nwagu *et al*., 1996). These differences may be attributed to different managements and genetic backgrounds of the strains of the cocks used which are capable of influencing semen quality traits of cocks. Good management and genotypic combination such as IBxF and IBxN will results in improved sperm concentration. The significant differences in the live sperm of the genotypes studied (Table 4) indicate that strains and different forms of crossbreeding in chickens will result in significant semen outputs. This is in agreement with the findings of Peters *et al*. (2008). The means of live sperm were above 50 percent. The proportion of the living spermatozoa which recorded above 50 % in this study indicates that live sperm were more in number than the dead ones. This implies that the semen quality of these cocks was good and could be used in reproduction both by natural mating and artificial insemination without any adverse effect on their progeny.

## Conclusion

Genotype was observed to impart on all the semen quality traits studied except the semen volume. Different breeds and methods of breeding may be employed to effect positive changes in semen volume. Semen quality traits of the main cross were generally better than the reciprocal cross, especially at younger than at older age. Isa Brown and normal feathered cocks should be developed to improve the semen quality traits in chickens.

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