USE OF PHENOTYPIC TRAITS TO PREDICT COCKS FERTILITY

1- THE SECONDARY SEXUAL TRAITS AND THE SKELETAL CONFORMATION

By

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Abstract: *Genetic selection procedures applied to improve breeder performance may modify phenotypic traits which depress male fertility. Identification of traits that reliably indicate individual male fertility would facilitate selection for reproduction.*

It is hypothesized that male fertility might correlate with comb and wattle measurements, skeletal conformation, and degree of fluctuating asymmetry (FA) of bilateral traits.

A total of thirty six Bandarah strain cockerels were classified into two groups according to the secondary sexual traits. Males with large comb and wattle (GL), and males with small comb and wattle (GS). Eighteen males per group, individually housed with 10 females per male, fertility was evaluated at five periods of cock's age (31-34 wk), (35-38 wk), (39-42wk), (43-46 wk), and (47-50wk). At 50 wk, comb area, length and width (CA, CL, CW), wattle area, length and width (WA, WL, WW), keel length (KL), posterior pelvic width and length (PPW, PPL), dorsal pelvic width and length (DPW, DPL), and tarsometatarsal length and width (TL, TW) were measured.

Results of the overall means showed that GL males revealed a higher fertility ($p < 0.01$) than GS, however, the differences were significant ($P<0.05$) only at the 4^{th} and 5^{th} periods.

GL males had a larger ($P<0.01$) comb and wattle measurements compared to GS males, also, there was a significant, strong positive correlation between comb area and fertility.

Although no significant differences in skeletal structure were found, there was a significant positive correlation between fertility and TW within either groups.

Further analysis revealed that within GS, males with greater WW FA and DPW FA tended to have lower fertility (P<0.05). This research provide evidence that phenotypic traits is a useful indicator to predict males fertility in Bandarah local strain.

INTRODUCTION

The recent decline of fertility of naturally mated fowl (McGary *et al*., 2003) is related, in part, to the differential reproduction among males, as some males have high fertility whereas others have low fertility and, hence, contribute to a reduction in overall flock fertility.

Physiological studies found that fertility under natural mating conditions does not correlate with semen characteristics (McCartney and Brown, 1976). Jones and Mench, (1991) suggested that differential male fertility in natural mating systems is likely due to behavioral differences rather than semen quality, as reproductively successful males must satisfy additional physiological and behavioral requirements.

Genetic selection for traits such as growth rate and yield have been negatively associated with the expression of morphometric traits related to reproduction (Soller *et al.*, 1965; and Siegel and Dunnington, 1985). An additional consequence of selection for growth is that skeletal conformation and leg dimensions have likely been modified to physically support birds' bodies. These physical modifications may impede semen transfer (Soller *et al.*, 1965; Wilson *et al.*, 1979; Hocking and Duff, 1989; and Siegel and Dunnington, 1985), for example, through altered compatibility for the male and female cloacal positioning during copulation, which could reduce concomitant fertility.

Along with the potential for physical modifications to impact fertility, the degree of development of the secondary sexual characters could also affect the reproductive potential of an individual. For example, comb and wattle growth are androgen dependent (Zeller, 1971) and have been shown to correlate with a male's health status in red jungle fowl (Hamilton and Zuk, 1982). Sexual selection theory states that this differential expression (individual variation in the degree of phenotypic expression) of secondary sexual characters may reliably indicate individual male quality (Hamilton and Zuk, 1982; and Kodric-Brown and Brown, 1984). Evidence provided by Zuk *et al.*, (1995) supports this theory, as when female red jungle fowl were given a choice of two males during a preference test, they more frequently mated with males possessing large combs.

Asymmetry refers to the random deviations from symmetry in the development of bilaterally symmetrical traits (Johnstone, 1994; and Møller, 1994), Fluctuating asymmetry (FA), a specific type of asymmetry, occurs without any directionality, deviating in either direction with a normal distribution and a mean of zero (Møller, 1994). FA has been demonstrated to convey an individual's ability to cope with environmental and social stress (Leary and Allendof, 1989; and Møller *et al*, 1995) and reflects male reproductive quality in some avian species, as poor quality males tend to be more asymmetric (Møller, 1990). In addition, females have been shown to prefer to mate with males that are more symmetric (Møller, 1992).

These previous findings led us to investigate the morphological characterize as sexual traits potentially indicating male fertility levels. Once identified, these traits could be incorporated into the genetic selection regimen with the intent of improving fertility.

MATERIALS AND METHODS

1-Experimental Design

This experiment was designed to study the potential relationship between physical traits and fertility of individual males. Experiment was carried out at El-Sabahia Poultry Research Station, Animal Production Research Institute, Ministry of Agriculture. In January 2005, Bandarah strain was used in this study. These birds were a part of the routine procedure according to the breeding program.

All birds were raised on floor pens, mixed sex in open sided house. Food and water were allowed *ad libitum*. A photoperiod of 16L-8D was provided. Males at 21 wk of age were kept isolated from the females before the study to ensure sexual rest and the replenishment of their sperm reserves.

Thirty six males (31 wk of age) were classified according to their physical traits into two equal groups: males with well-developed secondary sexual characteristics, such as large comb and wattle (GL), and males with alter-developed secondary sexual characteristics, like small comb and wattle (GS). Each male of each group was randomly housed in individual pedigree pens containing an average of 10 females.

Data on fertility collected for each male at the following ages: period 1 (31-34 wk), period 2 (35 to 38 wk), period 3 (39 to 42 wk), period 4 (43 to 46 wk) and period 5 (47 to 50 wk). Comb and wattle dimensions were measured at early and late age periods, whereas leg and pelvic measurements were taken at age of the period 5.

2-Fertility

Eggs were collected from each pen at each period. Incubated in automatic digital incubator. Fertility was determined at 7th day of incubation.

3-Physical Measurements

Comb area (CA), length (CL), width (CW), wattle area (WA) length (WL), and width (WW) were measured for each male by image analysis[1] of digital pictures of the left and right sides of the head. Each picture includes a metric ruler for calibration. The computer mouse was used to trace CA, CL, CW, WA, WL and WW, and the distance (cm) or area (cm^2) was calculated by the Scion Image Analysis Software of irregular shapes. The WL and CL were measured as the maximum horizontal distance between the front and the rear of the comb/wattle. The CW was measured as the maximum vertical distance from the highest peak of the comb to the base and WW as the maximum vertical distance from base of the wattle to the distal end. Tracing the perimeter of the comb or the wattle with the computer mouse allowed Scion software to calculate CA or WA. (McGary *et al.*, 2003). Combs and wattles for each male were measured at early and late ages. Trait size did not change with age (P>0.05) thus mean trait size was calculated and used for statistical analysis.

At 50 wk of age males were weighed and were slaughtered to complete bleeding. Calipers[2] were used to measure tarsometatarsal length (TL; from the tibio-tarsal joint to the joint of the hallux) and tarsometatarsal width (TW. The width of leg above the spur). A cloth measuring tab was used to measure keel length (KL), defined as the maximum distance from the anterior end of the sternum to the posterior end of the xyphoid process.

The thoracic/pelvic regions were then skinned, and the muscle mass was removed from the bones according to (Chambers, 1990). Posterior and dorsal pelvic dimensions were measured according to (McGary *et al.*, 2003) with calipers from the outermost processes located on the skeleton. The digital photos of the posterior (Figure 1a) and dorsal (Figure 1b) skeletal views include letters to denote the points at which the measurements were taken. Mean posterior pelvic length (PPL; Figure 1a) was calculated as (EA+FB)/2, and mean posterior pelvic width (PPW; Figure 1a) was calculated as (EF+CD+AB)/3. The PPW estimate was based on the average of measurements at three separate points, as this pelvic area varied greatly

[1] Scion Corporation, Frederick, MD.
[2] Instrument, company, Gasrovo, Sulgaria.

depending on where the measurement was taken. Mean dorsal pelvic length (DPL; Figure 1b) was calculated as (GI+HJ)/2, and mean dorsal pelvic width (DPW; Figure 1b) was (GH+IJ)/2. The degree of relative FA between the left (L) and right (R) bilateral traits (WL, WW, TL, TW, PPL, PPW, DPL, DPW) was calculated using the formula [L-R]/[L+R] (Møller, *et al.*, 1995).

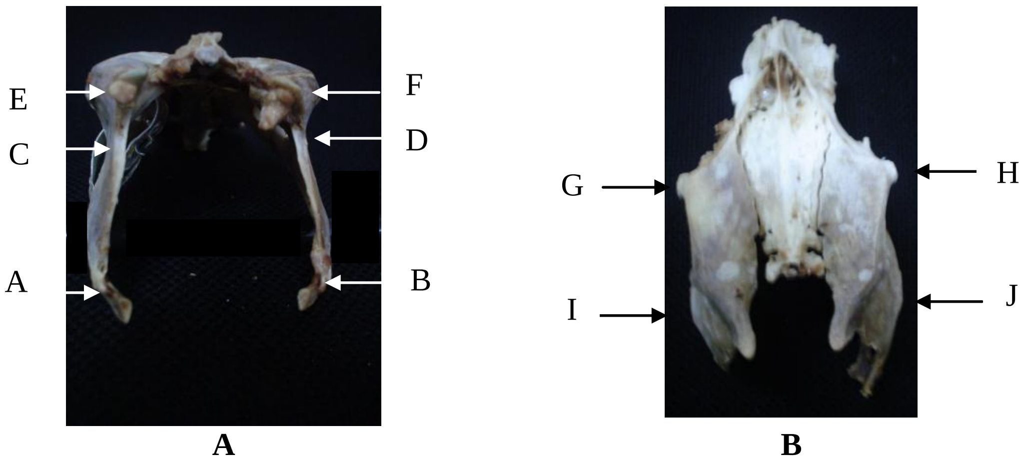

Figure (1): Digital photographs of the a) posterior and b) dorsal views of the thoraco-pelvic region of the Bandarah male. Length and width measurements were taken using digital calipers at the outermost points of the processes, as denoted by the capital letters on each photograph. The posterior pelvic length (PPL) was calculated as (EA + FB)/2, posterior pelvic width (PPW) was (EF + CD + AB)/3. Dorsal pelvic length (DPL) was calculated as (GI + HJ)/2, and dorsal pelvic width (DPW) was (GH+ IJ)/2.

4- Statistical Analysis

Mean fertility across age was directly compared between the two groups by mixed-model repeated-measures ANOVA, with age as the repeated measure. Factors in the model were group, age and the interaction between them. Physical characteristics differences were analyzed by the t-test. Pearson correlations were used to determine relationships between fertility and physical traits. Concerning the correlation analyses, means of fertility percentages for periods 4 and 5 for each individual male/group were used. Data were analyzed using SAS package (1990).

RESULTS AND DISCUSSION

Table (1) showed that there was a significant (P<0.01) difference between GL and GS in percentage of fertility (98.79 vs, 95.22%). The different periods differed significantly (P <0.05). Both the 4th and 5th periods showed significantly lower fertility than the rest period. A group × age interaction on fertility was observed (P<0.05). GL showed a similarity of fertility between the different age periods. Age negatively impacted fertility in GS. Furthermore, GS fertility values decreased sharply than GL within age periods 4th and 5th. (Figure 2)

Fertility in this study decline with age for GS, as reported by McGary *et al.,* (2002). In addition, previous research showed that the fertility decline may relate to altered male reproductive endocrinology and behavior (Rosenstrauch *et al.,* 1998; and Weil *et al.,* 1999); physical impairment upon copulation (Soller *et al.,* 1965; and Wilson *et al.,* 1979), reduction in sperm concentration and semen volume (Sexton, 1987) or a combination of the above.

Because body weight did not differed significantly between the two groups, (GL) and (GS) were (2075 and 2050gm) respectively, also body weight did not correlate with fertility within either group (P>0.05), it seems unlikely that body weight it self did not impacted male mating ability at least in this local strain. These findings are agreement with (McGary *et al.,* 2003) who found that body weight did not correlate with broiler fertility.

Table (2) showed the secondary sexual characters (CA, CL, CW, WA, WL and WW) were larger for GL as compared to GB (P<0.01). A strong positive relationship was found between GL comb area and fertility (r=0.56, P<0.05); however, this correlation was not significant for GS (Figure 3). Furthermore, no correlation were found between other secondary sexual characters and fertility in either groups (P>0.05).

The relationship between secondary sexual characters and fertility was previously confirmed in domestic fowl by (McGary *et al.,* 2002), who found that male broiler breeders with larger combs within specific strains were likely to have higher fertility, as a significant positive correlation was found between male and their individual fertility level, also, suggested that, if female broiler breeders more frequently crouch for and subsequently mate with males having large, symmetrical combs and wattles, differential fertility may be related to fact that high quality males secure a higher mating frequency, which should in turn, improve reproduction. Hence, the next logical step in this experiment was to determine if fertility levels were related to keel length, leg dimensions and pelvic conformation.

Table (3) showed that the leg dimensions and pelvic conformation did not differ between groups (P>0.05). Figure (4) showed the leg dimensions, there was a strong positive correlation between TW and fertility in either GL (r=0.583, P<0.01) or GS (r=0.544, P<0.05). Interestingly, this was the only skeletal measurement that significantly correlated with fertility.

McGary *et al.,* (2003) anticipated that variation in leg dimensions may alter the intersexual cloacal distance upon mating. Although TW was not differed significantly between groups, TW correlated with fertility within them. These results suggest, therefore, that leg size in Bandarah local strain does not seem to have a direct role on male fertility.

Contrary to the previous results that larger musculoskeletal dimensions (Wilson *et al.,* 1979; and Duncan *et al.,* 1990) would impede semen transfer and reduce fertility, it was found that no significant differences between groups in KL, DPW, DPL, PPL and DPW. Also, all these traits did not significantly correlate with fertility within either group (P>0.05).

Table (4) demonstrated that fluctuating asymmetry of wattle and skeletal measurements did not show any differences between groups (P>0.05) except for, WW FA and DPW FA which were significantly greater in GS males than GL (p<0.01). Figure (5) showed that GS males with greater WW FA tended to have lower fertility (r= -0.536, P<0.05). The same relationship was found for DPW FA as well, which correlated with GS fertility (r= -0.553, P<0.05), no relationship existed between GL fertility and WW FA or DPW FA (P>0.05). The other FA measurements did not show any correlation with fertility within either group (P>0.05).

Local strains may face several nonspecific stressors; such as heat stress, feed restriction, management, and social stress that may have altered the degree of FA of bilateral traits as indicated previously (Bilcik and Estevez, 2005). In support of previous results that differential FA among males could be used as a tool to reliably selected males of high reproductive quality (Møller, 1990). This results showed that although GS males had shorter WW as compared to GL, they had a higher degree of FA, as stressors have been shown to influence the degree of FA in domestic fowl (Møller *et al.,* 1995). Furthermore, FA of WW negatively correlated with fertility within GS, these results seem to be supported by (Morris and Casey, 1998; and Cadee 2000) which they found a relationship between FA of secondary sexual characters and reproductive success in wild species.

Similar to the degree of FA of secondary sexual characters, it is found that males within GS with a greater degree of FA of DPW had lower fertility. This findings agreement with (McGary *et al.*, 2003) who found that DPW FA negatively correlated with broiler breeder fertility, they suggested a direct impact of pelvic conformation on male's physical ability to mount and successfully inseminate upon cloacal content.

In conclusion, the present study provides further evidence suggesting the potential for physical traits to predict male fertility. Males with large comb area are positively correlated with fertility levels. In addition, it was found a negative relationship between fertility and the degree of FA for both of wattle width and dorsal pelvic width within males which had alter-developed secondary sexual characters in Bandarah local strain. It is suggest that the relationship between bone measurements and fertility should be further explored in local breeders.

Table (1): Fertility percentage (mean±S.E) across the five age periods for GL and GS

Period / Group	1	2	3	4	5	Overall mean
GL	99.07±0.66[x]	98.47±0.73[x]	98.90±0.96[x]	98.78±0.84[x]	98.73±0.99[x]	98.79±0.80[A]
GS	98.23±0.48[x]	97.51±0.31[x]	96.96±0.40[x]	93.86±0.38[y]	89.52±0.53[y]	95.22±0.45[B]
Overall mean	98.65±0.56[a]	97.99±0.58[a]	97.93±0.80[a]	96.32±0.58[b]	94.13±0.76[b]	

GL: males with large comb and wattle.
GS: males with small comb and wattle.
Means with different superscripts within column (capital) or within rows are significantly different at ($P < 0.05$).
Means of interaction between the main factors with different superscripts (x,y) are significantly different at ($P < 0.05$).

Table (2): Mean± S.E of both GL and GS for comb area (CA), comb length (CL) comb width (CW), wattle area (WA), wattle length (WL), and wattle width (WW)

Traits / Group	CA (cm^2)	CL (cm)	CW (cm)	WA (cm^2)	WL (cm)	WW (cm)
GL	22.59±0.87[a]	7.29±0.16[a]	3.79±0.17[a]	10.23±0.79[a]	4.075±0.24[a]	2.85±0.15[a]
GS	8.62±0.75[b]	4.95±0.30[b]	2.28±0.18[b]	4.21±0.49[b]	2.84±0.13[b]	1.47±0.14[b]

GL: males with large comb and wattle.
GS: males with small comb and wattle.
Means with different superscripts within column are significantly different at ($P < 0.05$).

Table (3): Mean±S.E of both GL and GS for tarsometatarsal length (TL), tarsometatarsal width (TW), keel length (KL), posterior pelvic length (PPL), posterior pelvic width (PPW), dorsal pelvic length (DPL), and dorsal pelvic width (DPW)

Traits / Group	TL (mm)	TW (mm)	Kl (mm)	PPL (mm)	PPW (mm)	DPL (mm)	DPW (mm)
GL	116.29±1.89	15.09±0.37	187.1±2.4	59.46±0.44	48.6±0.41	48.03±0.76	55.14±0.52
GS	111.84±1.92	14.23±0.39	179.4±3.62	56.59±0.75	47.3±0.61	45.92±0.27	54.11±0.56

GL: males with large comb and wattle.
GS: males with small comb and wattle.

Table (4): Mean±S.E of the degree of fluctuating asymmetry (FA) between the left and right wattle length (WL), wattle width (WW), tarsometatarsal length (TL), tarsometatarsal width (TW) posterior pelvic length (PPL), posterior pelvic width (PPW), dorsal pelvic length (DPL) and dorsal pelvic width (DPW) for groups

Traits / Group	WL FA	WW FA	TL FA	TW FA	PPL FA	PPW FA	DPL FA	DPW FA
GL	0.013±0.002	$0.021±0.002^{b}$	0.019±0.006	0.022±0.003	0.030±0.008	0.021±0.009	0.021±0.006	$0.018±0.003^{b}$
GS	0.022±0.004	$0.033±0.003^{a}$	0.021±0.005	0.026±0.004	0.025±0.005	0.031±0.006	0.025±0.007	$0.039±0.004^{a}$

GL: males with large comb and wattle.
GS: males with small comb and wattle.
Means with different superscripts within column are significantly different at ($P < 0.05$).

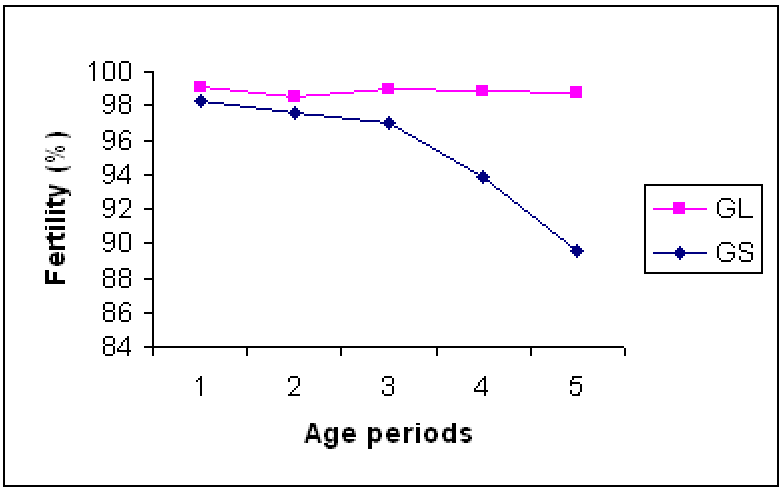

Figure (2): Fertility percentage across the five periods for males with large comb and wattle (GL) and males with small comb and wattle (GS).

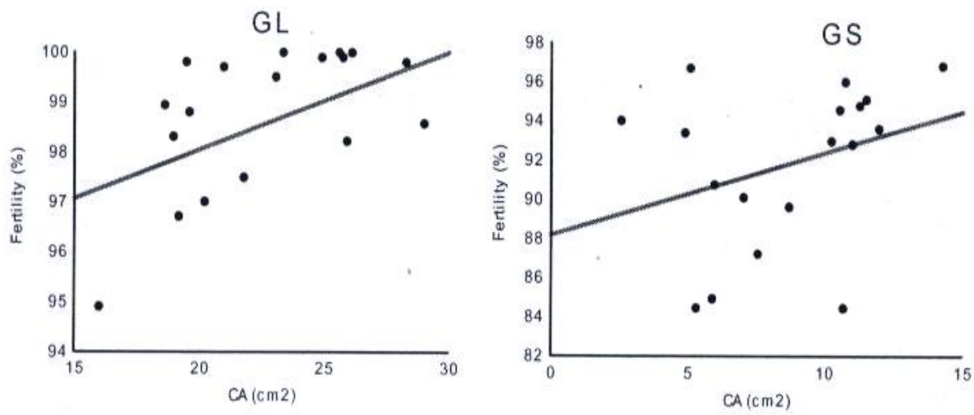

Figure (3): Correlation between fertility and comb area (CA)

GL: males with large comb and wattle.
GS: males with small comb and wattle.

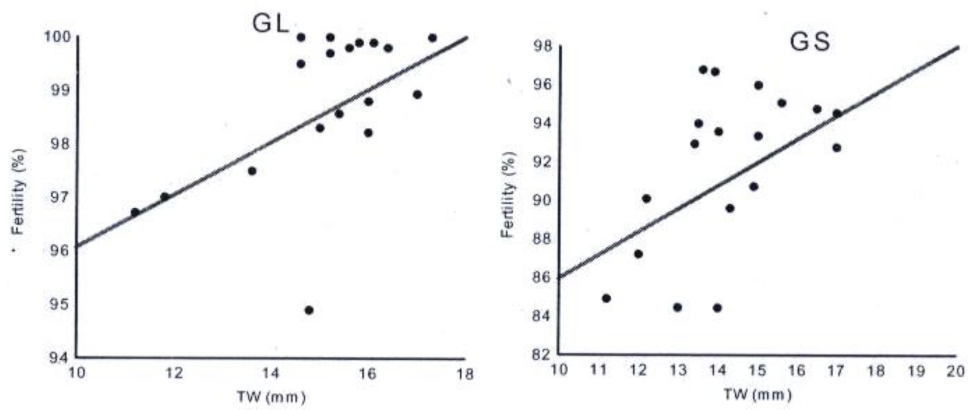

Figure (4): Correlation between fertility and tarsometatarsal width (TW)

GL: males with large comb and wattle.
GS: males with small comb and wattle.

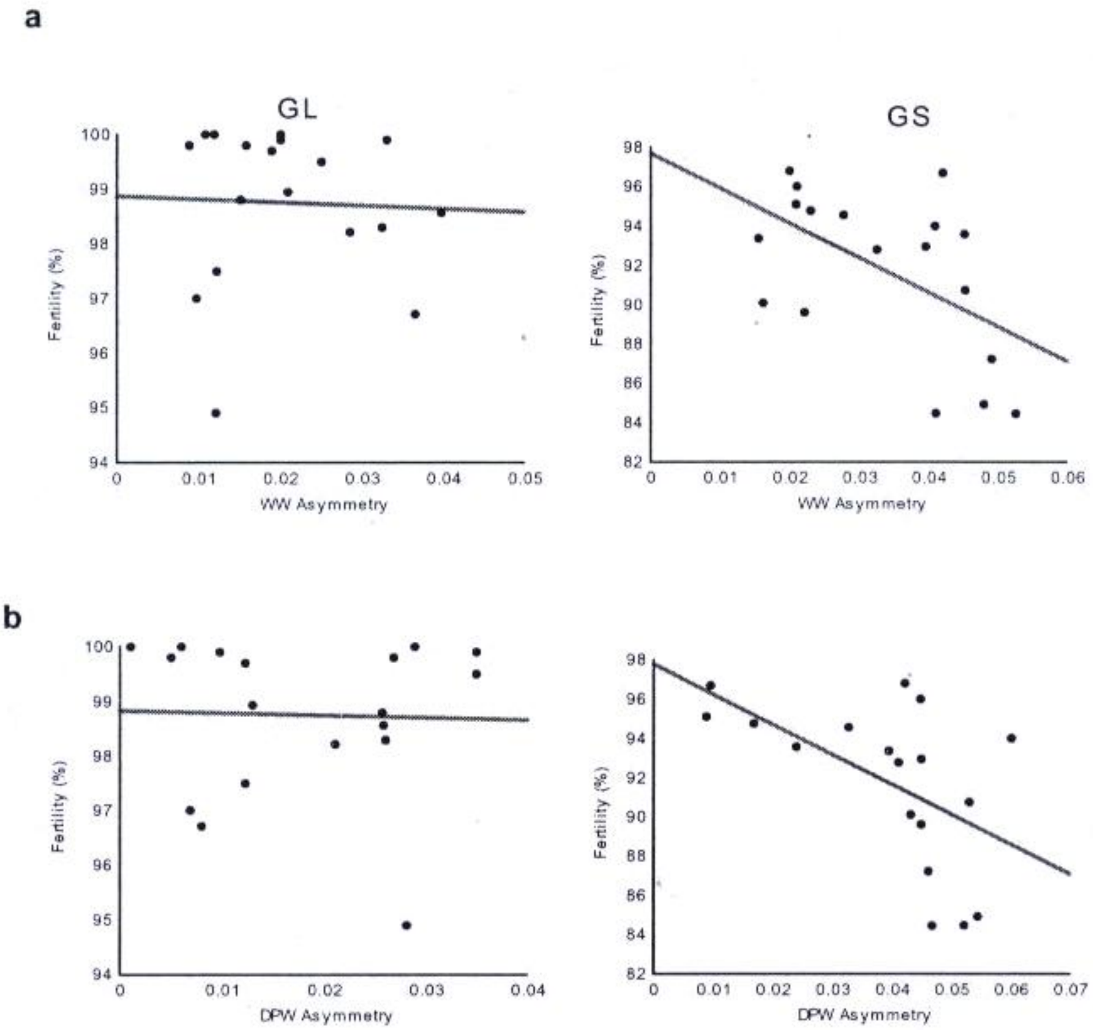

Figure (5): Correlation between fertility and (a) wattle width (WW) asymmetry, (b) dorsal pelvie width (DPW) asymmetry

GL: males with large comb and wattle.
GS: males with small comb and wattle.

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الملخص العربي

استخدام الصفات المظهرية للتنبؤ بخصوبة الديوك

1- صفات الجنس الثانوية والتكوين الهيكلى

أماني عادل الصحن

معهد بحوث الإنتاج الحيوانى-مركز البحوث الزراعية- مصر

1. إجراء الانتخاب الوراثي من أجل تحسين أداء قطيع التربية ربما يغير من الصفات المظهرية للذكور وبالتالى تقل قدرته الإخصابية. التعرف على الصفات المحددة لخصوبة الذكور فردياً سيحسن الإنتخاب من أجل التناسل.

2. من المفترض أن خصوبة الذكر ربما ترتبط بقياسات العرف والدلايات والتكوين الهيكلي وعدم التناسق (FA) للصفات الثنائية الشكل.

3. إستخدم فى هذه الدراسة عدد 36 ديك من سلالة البندرة، تم توزيعهم بالتساوى إلى مجموعتين على أساس صفات الجنس الثانوية: ديوك ذات عرف و دلايات كبيرة (GL) وديوك ذات عرف و دلايات صغيرة (GS) 18 ذكر/ لكل مجموعة تم تسكينهم فردياً (ديك/ 10 إناث) تم تقييم الخصوبة خلال 5 فترات من عمر الديوك (31: 34 أسبوع)، (35: 38أسبوع)، (39 : 42 أسبوع)، (43 : 46 أسبوع)، (47 : 50 أسبوع). عند 50 أسبوع، تم قياس مساحة العرف وطوله وعرضه (CA, CL, CW)، مساحة الدلاية وطولها وعرضها (WA, WL, WW)، طول عظمة القص (KL)، عرض وطول عظام الحوض الأمامية (PPW, PPL)، عرض وطول عظام الحوض الظهرية (DPW, DPL) وطول وعرض عظمة الرسغ مشطية (TL, TW).

4. خلال الخمس فترات العمرية كانت خصوبة ذكور GL أعلى من ذكور GS وكانت هناك إختلافات معنوية عند الفترة الرابعة والخامسة.

5. ذكور GL كان تمتلك قياسات عرف ودلايات أكبر (P<0.01) من ذكور GS وكان هناك ارتباط معنوي قوي موجب بين مساحة العرف والخصوبة للديوك GL.

6. على الرغم من عدم وجود اختلافات معنوية في البناء الهيكلي بين المجموعتين إلا أنه كان هناك ارتباط معنوي موجب بين الخصوبة وTW داخل كل مجموعة.

7. أظهرت ديوك GS المنخفضة الخصوبة زيادة فى صفة عدم تناسق عرض الدلايات و كذلك زيادة فى صفة عدم تناسق عرض عظام الحوض الظهرية.

من هذه النتائج يمكن أستخدام الصفات المظهرية كأحدى الدلالات للتنبؤ بخصوبة الديوك في سلالة البندرة المحلية.