microsatellite markers in poultry. Anim. Genet. 34: 441–443.

Crooijmans, R.P.M.A., A.J.A. van Kampen, J. J. van der Poel, and M.A.M. Groenen, 1994. New microsatellite markers on the linkage map of the chicken genome. J. Hered. 85: 410–413.

Crooijmans, R.P.M.A., J. J. van der Poel, and M.A.M. Groenen, 1995. Functional genes mapped on the chicken genome. Anim. Genet. 26:73–78.

Davies, J. L., Y. Kawaguchi, S. T. Bennett, J. B. Copeman, H. J. Cordell, L. E. Pritchard, P. W. Reed, S.C.L. Gaugh, S. C. Jenkins, S. M. Palmer, K. M. Balfour, B. R. Rowe, M. Farrall, A. F. Barnett, S. C. Bain, and J. A. Todd, 1994. A genome-wide search for human type 1 diabetes susceptibility genes. Nature 371:130–135.

Dietrich, W. F., J. C. Miller, R. G. Steen, M. Merchant, D. Damron, R. Nahf, A. Gross, D. C. Joyce, M. Wessel, R. D. Dredge, A. Marquis, L. D. Stein, N. Goodman, D. C. Page, and E. S. Lander, 1994. A genetic map of the mouse with 4,006 simple sequence length polymorphisms. Nat. Genet. 7:220–245.

Donis-Keller, H., P. Green, C. Helms, S. Cartinhour, B. Weiffenbach, K. Stephens, T. P. Keith, D. W. Bowden, D. R. Smith, E. S. Lander, D. Botstein, G. Akots, K. S. Rediker, T. Gravius, V. A. Brown, M. B. Rising, C. Parker, J. A. Powers, D. E. Watt, E. R. Kauffman, A. Bricker, P. Phipps, H. Muller-Kahle, T. R. Fulton, S. Ng, J. W. Schumm, J. C. Braman, R. G. Knowlton, D. F. Barker, S. M. Crooks, S. E. Lincoln, M. J. Daly, and J. Abrahamson, 1987. A genetic linkage map of the human genome. Cell 51:319–337.

Ellegren, H., B. P. Chowdhary, M. Johansson, L. Marklund, M. Fredhokm, I. Gustavsson, and L. Andersson, 1994. A primary linkage map of the porcine genome reveals a low rate of recombination. Genetics 137:1089–1100.

Gyapay, G., J. Morissette, A. Vignal, C. Dib, C. Fizames, P. Millanseau, S. Marc, G. Bernadi, M. Lathrop, and J. Weissenbach, 1994. The 1993-94 genethon human genetic linkage map. Nature Genet. 7:246–339.

Haldane, J.B.S., 1922. Sex ratio and unisexual sterility in hybrid animals. J. Genet. 12:101–109.

Khatib, H., E. Genislav, L. B. Crittenden, N. Bumstead, and M. Soller, 1993. Sequence tagged microsatellite sites as markers in chicken reference and resource population. Anim. Genet. 24:355–362.

Levin, I., L. B. Crittenden, and J. B. Dodgson, 1993. Genetic map of the chicken Z chromosome using random amplified polymorphic DNA (RAPD) markers. Genomics 16:224–230.

Levin, I., L. Santagelo, H. Cheng, L. B. Crittenden, and J. B. Dodgson, 1994. An autosomal genetic linkage map of the chicken. J. Hered. 85:79–85.

Neumann, P. E., 1990. Two-locus linkage analysis using recombinant inbred strains and Bayes' theorem. Genetics 126:277–284.

Ooijen, van J. W., 1994. A computer program for drawing genetic linkage maps. J. Hered. 85:66.

Radionov, A. V., Y. A. Myakoshina, L. A. Chelysheva, I. V. Solovei, and E. R. Gaginskaya, 1992a. Chiasmata in the lambrush chromosomes of Gallus Gallus Domesticus: The cytogenetic study of recombination frequency and linkage map lengths. Genetika 28:53–63.

Radionov, A. V., L. A. Chelysheva, I. V. Solovei, and Yu. A. Myakoshina, 1992b. Chiasma distribution in the lambrush chromosomes of the chicken Gallus Gallus Domesticus: Hot spots of recombination and their feasible role in proper disjunction of homologous chromosome at the first meiotic division. Genetika 28:151–160.

Rohrer, G. A., L. J. Alexander, J. W. Keele, T. P. Smith, and C. W. Beattie, 1994. A microsatellite map of the porcine genome. Genetics 136:231–245.

Silver, J., 1985. Confidence limits for estimates of gene linkage based on analysis using recombinant inbred strains and backcrosses. J. Hered. 76:436–440.

Ziegle, J. S., Y. Su, K. P. Cororan, L. Nie, P. E. Mayrand, L. B. Hoff, L. J. Mcbride, M. N. Kronick, and S. R. Diehl, 1992. Application of automated DNA sizing technology for genotyping microsatellite loci. Genomics 14:1026–1031.

Age Effect of Male and Female Broiler Breeders on Sperm Penetration of the Perivitelline Layer Overlying the Germinal Disc[1]

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ABSTRACT An experiment was conducted to test the effect of age (male and female) on the number of spermatozoa penetrating the perivitelline layer (PL) overlying the germinal disc (GD) in broiler breeders. Eighty young broiler breeder hens (39 wk old, Y), and 80 old spent broiler breeder hens (69 wk old, O) were randomly divided into eight groups of 20 hens each by age. Hens were inseminated weekly for 4 consecutive wk with 5×10^7 pooled sperm/50 μL from either young or old broiler breeder males. Sperm penetration (SP) of the PL at the GD was assessed in a random sample of 12 oviposited eggs from each hen group for each day postinsemination, with the remainder of the eggs incubated for 10 d to obtain fertility values. For the main effect of sex, and for age within sex, there were differences in mean SP (7.3 vs 4.8; Y vs O hens; $P < 0.02$) and fertility (73.7 vs 54.9%; Y vs O hens; $P < 0.002$) values. Old males had higher mean SP values and fertility (7.2 and 70.6%) than young males (4.8 and 58.0%; $P < 0.03$ and 0.01, respectively). Following artificial insemination of a constant number of sperm, age of hens appears to contribute more to the decrease in SP and fertility than the age of male broiler breeders.

Eggs were obtained from naturally mated broiler breeder flocks from different strains (A and B), lines (male and female), and ages. There was an effect on overall mean SP values due to strain (105.8 vs 78.6 holes per GD area; Strains A and B, respectively; $P < 0.0001$), and line within Strain B (106.4 vs 50.8 holes per GD; male and female line, respectively; $P < 0.0001$). There was a quadratic relationship between SP of the PL and age in Strain A with values ranging from 153.3 to 20.0 holes per GD area ($P < 0.003$). In Strain B, SP holes in the PL decreased in the male line due to age (127.8 to 59.7 per GD; $P < 0.01$), with an effect of age on the female line also (62.1 vs 37.8 holes per GD; $P < 0.05$).

(*Key words*: broiler breeder, age, fertility, sperm penetration)

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INTRODUCTION

Age has an adverse effect on the reproductive success of birds. The age-related decrease in reproduction in the fowl is due, in part, to a decline in egg production (Atwood, 1929; Bahr and Palmer, 1989; Etches, 1990; Robinson *et al.*, 1990). As Lerner *et al.* (1993) stated, this decline begins to occur once the species-specific maximum for egg production has been reached. In addition, eggs laid following the peak in egg production exhibit a continued decrease in fertility and hatch of fertile eggs (Harper and Arscott, 1969; Kirk *et al.*, 1980). It is well documented that with advancing maternal age, fertility declines in mammals (see Adams, 1975; Mikamo and Hamaguchi, 1975 for review) and amphibians (see Mikamo and Hamaguchi, 1975 for review). However, the factors that influence the age-related decline in avian reproduction are poorly understood.

Fasenko and co-workers (1992) suggested that the decrease in fertility and hatchability observed with increasing hen age may be due to a decline in the ability of older hens to retain spermatozoa in the uterovaginal sperm host glands. The decrease in sperm storage in older hens has been previously observed in turkeys (Van Krey *et al.*, 1967; Christensen, 1981) and broiler breeders (Pierson *et al.*, 1988). Brillard (1993), however, indicated that the number of sperm residing in the sperm storage tubules of previously virgin old and young chicken hens was equivalent. Brillard (1993) did note that the release of the spermatozoa from the sperm host glands in old hens was twice that observed in young hens. Using artificial insemination it has been shown that older hens have decreased fertility, but that the decline in fertility can be reduced by the insemination of an increased number of sperm (de Reviers and Brillard, 1986) or by using duplicate inseminations (Brillard and McDaniel, 1986; Brillard *et al.*, 1989).

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Sperm must first cross the perivitelline layer (PL) of the ovum at the germinal disc (GD) region in order to gain access to the female pronucleus for fertilization (Romanoff, 1960). Upon coming in contact with the PL, the acrosome reaction is initiated and the spermatozoon gains entry into the ovum through the digestion of a hole in the PL (Bakst and Howarth, 1977; Okamura and Nishiyama, 1978; Howarth, 1984; Koyanagi *et al.*, 1988). Recently, Bramwell and co-workers (1995) have shown that the number of holes caused by sperm penetration (SP) of the PL *in vivo* was highly correlated with fertility.

The purpose of this study was to ascertain the effects of age of both the male and the female broiler breeder on SP of the PL using artificial insemination in caged birds and natural mating in commercial flocks. The use of artificial insemination would eliminate physical factors that contribute to the decline in fertility in naturally mated birds. These factors include male competition, physical injuries, size of the male, libido, and responsiveness of the female to the male's aggression. The examination of SP in eggs laid by commercial flocks allows us to compare the results obtained under controlled conditions to commercial situations.

MATERIALS AND METHODS

Animals, Housing, Management, and Feeding

Eighty young (Y) Arbor Acres broiler breeder hens (39 wk old) were obtained from a commercial breeder and randomly divided into four groups of 20 hens each. Eighty old (O) Arbor Acres broiler breeder spent hens (64 wk old) and 24 O roosters were selected and removed from a commercial breeder house, the same source as the Y hens, and housed separate from each other for 5 wk prior to their use in the experiment (69 wk old at initiation of study). Old hens were randomly selected from those that were sitting in the nests, thus increasing the chances of obtaining hens that were still in egg production. The O hens were randomly divided into four separate groups of 20 hens each. All hens (total of eight groups) were individually caged and fed the University of Georgia (UGA) Breeder diet (2,915 kcal ME/kg, 15% CP, and 3% Ca) to maintain their recommended weight. Twenty Y Arbor Acres males (same source as all other Y and O birds) and the 24 O males were individually caged and fed 350 kcal ME per bird per d. All birds were maintained on 17 h of light/d for the duration of the experiment.

Semen Collection, Artificial Insemination, and Sperm Penetration Assay

Semen was collected from males using the abdominal massage method as described by Burrows and Quinn (1937). Semen was pooled by age of the male and the sperm cell concentration of the pooled sample determined using the packed cell volume technique described by Maeza and Buss (1976). Each of the two semen aliquots were diluted with minimum essential medium (Howarth, 1981) to obtain the desired sperm cell concentration needed for artificial insemination. Motility was determined in the diluted semen samples prior to insemination by direct observation under a light microscope. Sperm motility in the semen samples used for insemination was ~ 80% or greater for both Y and O males. All hens were inseminated 10 to 12 h following the beginning of the light phase in the daily photoperiod.

Experiment 1. Artificial Insemination Study

Hens were inseminated once weekly, by group, for 4 consecutive wk with 5×10^7 pooled sperm/50 μL from either Y or O broiler breeder males. Inseminations in this manner created four hen treatment groups, which were as follows: 1) Y hens inseminated with sperm from Y males, 2) Y hens inseminated with sperm from O males, 3) O hens inseminated with sperm from Y males, and 4) O hens inseminated with sperm from O males. Eggs were collected and recorded daily by hen and group to determine hen egg production. All eggs laid were identified by group and day postinsemination (DPI) for 8 consecutive d following insemination. Twelve eggs from each hen group for each DPI were used for the determination of *in vivo* SP of the PL, whereas the remainder of the eggs for each DPI were used for the determination of fertility. Sperm penetration was determined in the laid eggs by fixing and staining the intact PL section with Schiff's reagent as described by Bramwell and co-workers (1995). Holes were counted from five 0.269-mm^2 areas (1.35 mm^2 total area) of PL overlying the GD region using a light microscope at 100×. Eggs used to obtain SP values were stored in an egg cooler at ~ 13C for up to 6 wk prior to evaluation (no effects of storage for this duration on results). The percentage of fertile eggs was determined by candling following 10 d of incubation. Eggs that appeared to be infertile or dead at candling were broken open for macroscopic inspection of the blastodisc to identify early dead embryos.

Experiment 2. Naturally Mated Flocks

A total of 30 eggs were obtained from each of 12 different naturally mated commercial broiler breeder flocks (two flocks per age; Strain A) representing six age groups (26, 32, 37, 44, 55, and 63 wk of age). *In vivo* SP of the PL overlying the GD region of the ovum (1.35 mm^2 area) was determined for laid eggs from each of the above-mentioned flocks as previously described. Mean fertility values for each flock were provided by the breeder, with all flocks from 37 to 63 wk of age having been previously spiked with young males.

Thirty eggs were obtained from Strain B male line flocks at 27, 30, 33, 36, 45, and 56 wk of age and from Strain B female line flocks at 28, 34, 35, 36, 52, and 58 wk of age, respectively. *In vivo* SP of the PL from the GD region was

determined in eggs laid by each of the above-mentioned flocks as previously described. Mean fertility values for each flock were provided by the breeder. Flocks from both lines of Strain B had not been spiked.

Statistical Analysis

To analyze SP and fertility in artificially inseminated hens, a 2 × 2 factorial arrangement was used [male (Y and O) and female (Y and O) as main effects] with a split-plot in time (days postinsemination). Each group of 20 hens served as the experimental units of measure. The relationship between SP and fertility was determined by correlating the replicate means of each insemination group of hens.

Eggs from naturally mated flocks were randomly split into two groups of 15 eggs each for the determination of SP to obtain two mean SP values. Naturally mated flock data was subjected to a completely randomized design. All statistical evaluations were made based on analysis of variance by using the General Linear Models procedure of SAS® (SAS Institute, 1985) unless otherwise stated. Student-Newman- Kuhl's multiple range test was used to compare means in both the artificial insemination study and the naturally mated hens. All differences were considered significant at $P < 0.05$ level unless otherwise stated.

RESULTS

Experiment 1. Artificial Insemination

Sperm penetration of the PL overlying the GD and fertility of eggs laid by both Y and O hens inseminated with semen from either Y or O males was determined. In Figure 1, the main effects of sex and age within sex upon SP and fertility are shown. Young hens had significantly higher SP values (7.27, top), and fertility (73.7%, bottom) than O hens (4.79, and 54.9%, respectively). When comparing the males based on age, the O males had significantly higher SP and fertility values (7.24 and 70.6%) than Y males (4.82 and 58.0%), respectively. As expected, mean daily egg production per hen from the O hens was significantly lower over the 4-wk period than that of the Y hens (37.3 vs 79.2%, respectively) but not different between groups of hens of like age.

Young hens inseminated with sperm from O males had significantly more SP holes than O hens inseminated with sperm from Y males (8.60 vs 3.70; Groups 2 and 3, respectively). Mean SP values for groups 1 and 4 (5.94 and 5.88, respectively; SEM = 0.663) were not different from either Group 2 or 3. Mean fertility values for Groups 1 through 4 were 71.3, 76.1, 44.7, and 65.1% (SEM = 2.51), respectively, with only Group 3 significantly different from all others (data not shown).

There was a group by DPI interaction as Groups 1 and 2 (Y hens) had significant linear relationships for the decline in SP over succeeding DPI (Figure 2; top and bottom).

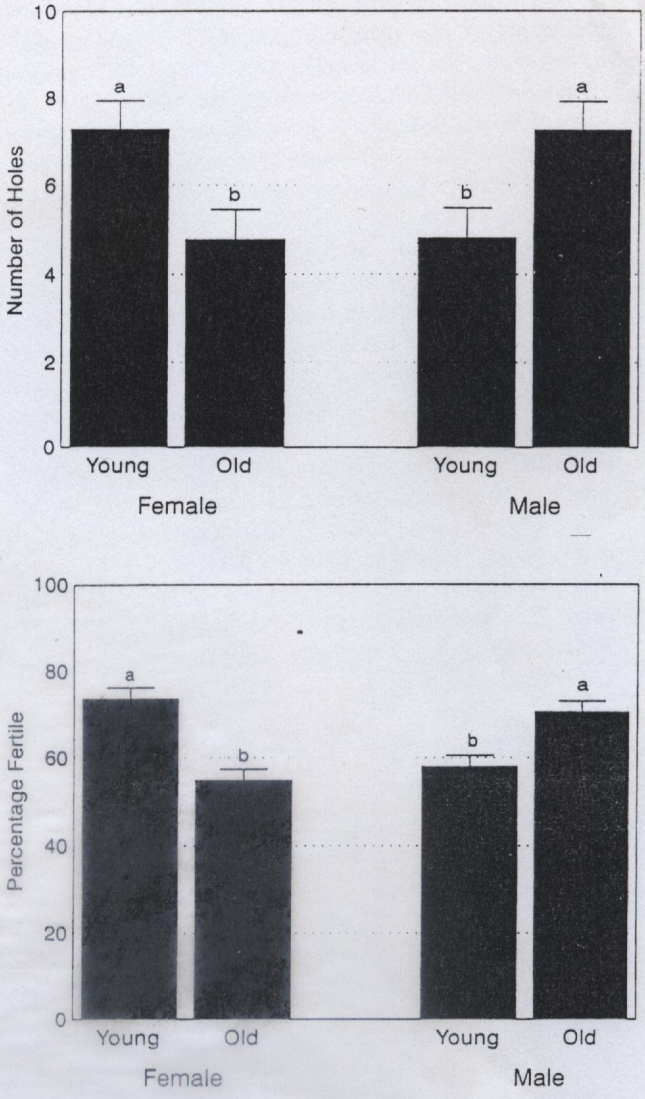

FIGURE 1. Sperm penetration holes in the perivitelline layer overlying the germinal disc (top) and percentage fertile eggs (bottom) by sex and by age. Values are expressed as means ± SEM. Columns with different letters are significantly different ($P < 0.05$). Values represent the replicate means of the 2nd through the 8th d postinsemination over 4 wk from two replicate groups of 20 hens each (n = 14).

Groups 3 and 4 (O hens) had a quadratic relationship for the decline in SP over DPI (Figure 2; top and bottom). The regression coefficients were significantly different between the Y male groups (1 and 3; Figure 2, top) based upon age of the hen. The relationship between SP over DPI from groups inseminated with sperm from O males was also dependent upon age of the hen (Figure 2, bottom). Figure 3 shows the linear effect of DPI on SP and fertility for all four groups combined. This linear relationship was significant between both SP and fertility over DPI.

There was a significant positive correlation between SP values and fertility for Groups 1, 2, and 4 (r = 0.94, 0.81, and 0.85, $P < 0.03$) whereas the correlation value for Group 3 was (r = 0.59, $P > 0.16$). The correlation for all groups combined was significant but not as high as within individual groups (r = 0.73, $P < 0.0001$).

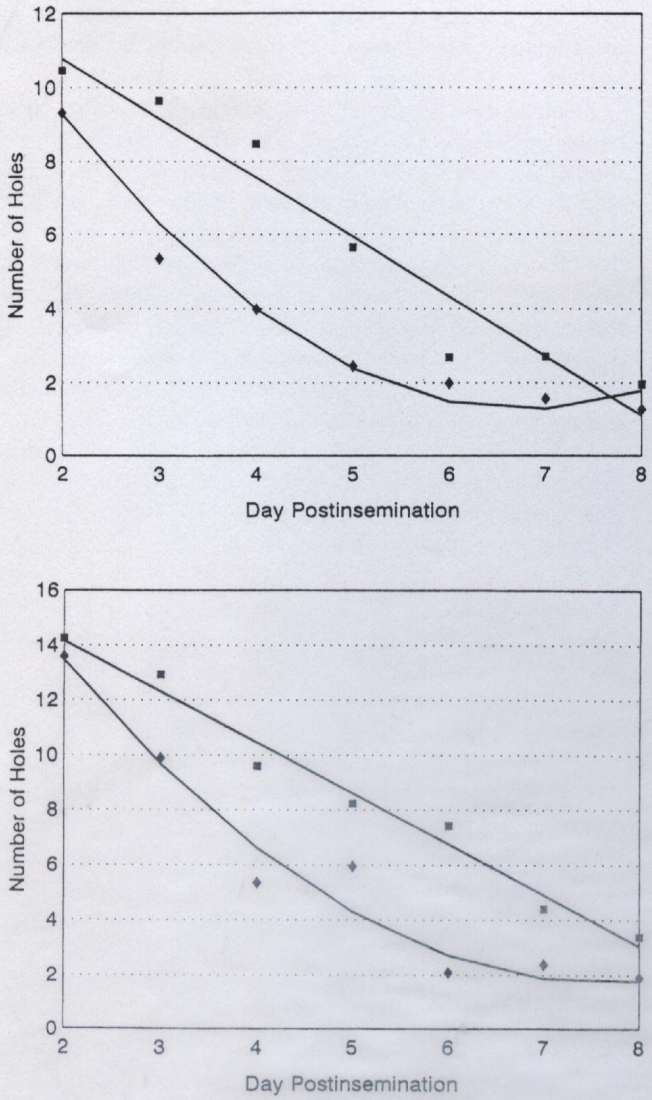

FIGURE 2. Sperm penetration holes in eggs laid for each day postinsemination for the four treatment groups. Top (Groups 1 and 3), sperm from young males inseminated into young hens (■; $y = 13.7 - 1.6x$, $r^2 = 0.94$, $P < 0.0003$), and old hens (♦; $y = 17.4 - 4.8x + .4x^2$, $r^2 = 0.96$, $P < 0.01$). Bottom (Groups 2 and 4), sperm from old males inseminated into young hens (■; $y = 17.6 - 1.8x$, $r^2 = 0.97$, $P < 0.0001$), and old hens (♦; $y = 23.3 - 5.7x + 0.4x^2$, $r^2 = 0.95$, $P < 0.04$). SEM = 0.663. Values represent the means from each day postinsemination for the 4 wk within each of two replicate groups of 20 hens each (n = 2).

Experiment 2. Naturally Mated Flocks

Mean SP of the GD PL in laid eggs from naturally mated flocks of Strain A was significantly affected by the age of the flock (Figure 4). An overall quadratic relationship was detected between SP and flock age for Strain A broiler breeders. Although a significant linear relationship was detected between mean SP and age when comparing values in flocks that were spiked (37 to 63 wk; as shown in Figure 4). There were no significant differences in mean SP between flocks 26 to 44 wk of age. Whereas mean SP from 55-wk-old flocks was significantly lower than that of the 37-wk-old flocks and SP in 63-wk-old flocks was statistically different from flocks of all other ages.

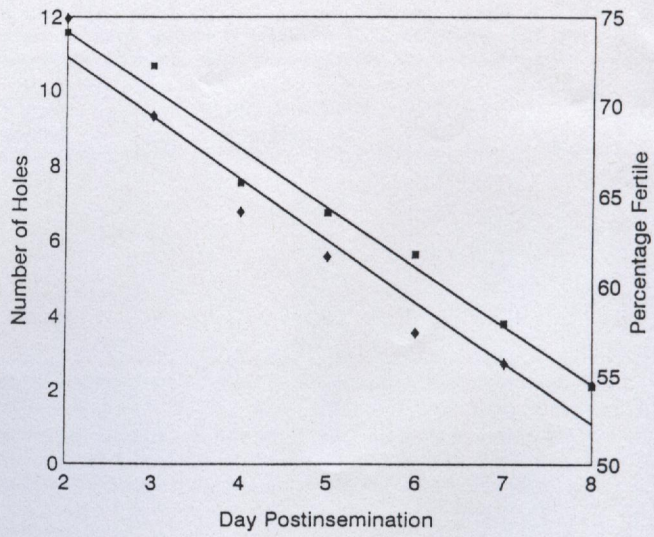

FIGURE 3. Sperm penetration holes (♦) and fertility (■) in eggs laid for each day postinsemination for all four treatment groups combined. A linear relationship was detected between percent fertile ($y = 14.2 - 1.6x$, $r^2 = 0.71$, $P < 0.0001$) and between sperm penetration ($y = 80.7 - 3.3x$, $r^2 = 0.21$, $P < 0.02$) and day postinsemination. Values represent the means of day postinsemination for the 4 wk within each of two replicate groups of 20 hens per group for the four treatment groups (n = 8).

Fertility values were provided by the breeder and were based on flock averages. Mean reported fertility values for flock ages 26, 32, 37, 44, 55, and 63 wk of age were 93.4, 96.7, 98.1, 96.3, 93.2, and 92.3% (SEM = 1.18), respectively. Although these values were all very high, reported fertility for flocks at 37 wk of age was statistically different than flocks at 26, 55, and 63 wk of age, respectively.

Mean SP values in eggs laid by broiler breeders from Strain B were significantly different than those found in Strain A (78.6 vs 105.8). However, this difference was found when comparing the parent stock of Strain A with a combination of the male and female lines (grandparents) of Strain B. A statistical difference in SP due to age was seen for both lines combined in Strain B (data not shown), and there was a significant difference in overall mean SP by line (106.4 vs 50.8; SEM = 1.84) for the male and female lines, respectively. Mean fertility was also statistically different between the male and female lines (90.0 vs 85.4%, respectively).

In the male line (Strain B), a significant quadratic relationship between mean SP of the GD PL and flock age was detected (Table 1). The mean SP value obtained from flocks at 56 wk of age was significantly lower than all other flock ages in the male line. Again, fertility of the eggs was based upon the flock average provided to us by the breeder, with the exception of the flock at 27 wk of age, for which fertility was not furnished. However, because percentage fertile was given for an entire flock and there was only one flock per age, comparisons between fertility by age within a line could not be made.

In the female line of Strain B (Table 2), there was a trend that indicated an effect of age on SP of the GD PL ($P < 0.09$). This relationship between SP and age was of a linear

TABLE 1. Sperm penetration and fertility from eggs laid by a male line from Strain B commercial broiler breeder flocks[1]

Flock age	Mean sperm penetration	Mean flock fertility
(wk)		(%)
27	113.30	...
30	112.15	86.0
33	108.16	92.0
36	127.81	93.0
45	117.05	95.0
56	59.66	84.0
SEM	4.31	...

[1]A quadratic relationship was detected between sperm penetration and age of flock ($y = -106.4 + 12.4x - 0.2x^2$, $r^2 = 0.88$, $P < 0.0005$).

[2]Each sperm penetration value represents the replicate means of 15 eggs per group with two groups per flock per age (n = 2). Fertility values were provided by the breeder and are based upon whole flock averages.

nature in the female line as opposed to that seen in the male line of Strain B and overall in Strain A. Again, comparisons between fertility by age could not be made within a line due to the nature of this data.

DISCUSSION

As expected, there was a considerable affect of age on reproduction as related to the hen. In accord with previous reports, egg production in the O hens was extremely low (37%). This may be partially due to the age of hens at the conclusion of the study (73 wk) and a result of transport and relocation of the hens at 64 wk of age. As expected, along with this decrease in egg production, there was a corresponding decline in fertility in O hens as compared to Y hens.

Prior to this study, it was unknown whether sperm could penetrate the PL at the site of fertilization as readily in ova from O hens as compared to Y hens. Results from this study indicate that, when artificially inseminated with similar numbers of sperm, mean SP of the GD PL was decreased in O hens as compared to Y hens regardless of the age of the sperm donor. However, this method of quantifying SP of the PL *in vivo*, is also dependent upon sperm transport and storage as well as sperm binding and penetration capacities. In this study, a significant difference in the slopes of the lines for SP over DPI was found between the Y and O hen groups inseminated with sperm from males of the same age. The quadratic regression response of SP over DPI in the O hens may indicate the impaired ability of the O hens to store viable sperm as readily as the Y hens, or a decrease in the ability to transport spermatozoa to the site of fertilization. However, we are not able to identify the precise cause from the data reported here.

Results reported here lend some support to the suggestion by previous authors (Pierson *et al.*, 1988; Fasenko *et al.*, 1992; Brillard, 1993) that the decline in fertility in older hens may be due to a sperm storage and transport problem in the female oviduct. Two possibilities exist that affect sperm storage: 1) sperm are released from the sperm storage tubules in O hens more readily or in larger numbers than in Y hens, and 2) sperm stored in O hens do not retain their viability as long as sperm stored in Y hens. If viable sperm were released from the sperm storage tubules in larger numbers in the O hens, this result should be reflected in a subsequent increase in SP values *in vivo*; therefore, the latter explanation seems more likely. It is also possible that the number of sperm receptors on the surface of the ovum may decrease during hen senescence, although this has not been confirmed.

FIGURE 4. Sperm penetration holes in laid eggs (♦) and flock fertility (■) from different age commercial flocks of Strain A. Flocks 37 to 63 wk of age were spiked. For all flocks, a quadratic relationship was detected between sperm penetration and age of flock ($y = -102.4 + 13x - 0.2x^2$, $r^2 = 0.65$, $P < 0.002$; SEM = 15.16) with a linear relationship detected for spiked flocks after production peak ($y = 322.1 - 4.7x$, $r^2 = 0.77$, $P < 0.0001$). Each sperm penetration value represents the replicate means of 15 eggs per group with two groups per flock with two flocks per age (n = 4).

TABLE 2. Sperm penetration and fertility from eggs laid by a female line from Strain B commercial broiler breeder flocks[1,2]

Flock age	Mean sperm penetration	Mean flock fertility
(wk)		(%)
28	52.29	88.5
34	56.00	93.1
35	56.78	93.0
36	62.11	87.5
52	39.82	77.0
58	37.79	73.0
SEM	5.05	...

[1]A linear relationship was detected between sperm penetration and age of flock ($y = 79.7 - 0.7x$, $r^2 = 0.57$, $P < 0.005$).

[2]Each sperm penetration value represents the replicate means of 15 eggs per group with two groups per flock per age (n = 2). Fertility values were provided by the breeder and are based upon whole flock averages.

It has been well documented that as males age the decline in fertility is concomitant with a reduction in the number of spermatozoa in the ejaculate and the volume of semen produced (Lake, 1989; Sexton *et al.*, 1989). In this study, we inseminated a constant number of sperm from both the Y and O males as opposed to a constant volume as used in many other studies relating to age of the male (Ansah *et al.*, 1980; Rosenstrauch *et al.*, 1994). This method allowed us to base our results of SP data on the physiological status of the sperm *in vivo* and not due to a insemination dosage effect as seen by Bramwell *et al.* (1995). When artificially inseminating hens with 50 million total sperm from Y or O males, we did not see a decline in fertility or SP values with increased age of the male. We did, however, detect an increase in SP values in sperm from O males as compared to Y males. The reasons for this result remain unclear, but may indicate that the physiological capabilities of sperm to penetrate and fertilize the ovum remain largely intact upon male senescence. Interestingly, Bramwell *et al.* (unpublished data) reported that the percentage of dead sperm per ejaculate decreased significantly over time in broiler breeder males. In this experiment, hens were inseminated with 50 million total sperm from either the Y or O males. Although the percentage dead sperm per ejaculate was not determined in this study, if the Y males had a higher percentage dead sperm in the 50 million total sperm to be inseminated, then SP and fertility may be reduced due to the insemination of fewer live sperm per hen.

Although individual hens were not used as the experimental unit in the naturally mated broiler breeder flocks, a wide range of SP values was seen from the individual hens. Mean SP values and the subsampling ranges for flocks in peak production from Strain A (153; range = 5 to 718), the male line (128; range = 0 to 564), and the female line (62; range = 6 to 338) of Strain B are given. Although SP in some hens is extremely high, there are still those hens that either are not being serviced or in which sperm are not capable of, or available for, penetration of the PL as readily as others. The minimum and maximum SP values from these flocks may both negatively affect the eventual production of broiler chicks. Sperm penetration that is too low will negatively affect fertility in a dose-dependent manner (Bramwell *et al.*, 1995), whereas SP values that are too high may increase early embryonic mortality (Bekhtina, 1968; Bramwell *et al.*, 1995). Although there is considerable variation in SP between hens, mean values obtained from multiple groups of hens are indicative of the overall status of a given flock.

When assessing SP in eggs laid by naturally mated commercial breeder flocks, physical problems are introduced as contributing factors to the decrease in fertility (Mauldin, 1989) and SP upon aging. In the hen, physical impairments or the lack of response to male aggression contribute to the decrease in fertility; whereas male competition, physical injuries, and decreased libido are contributing factors in the male (Sexton, 1983; Ottinger and Mench, 1989; O'Sullivan *et al.*, 1991). Many producers try to overcome the negative effects of the older males by spiking flocks with young males beginning at ~ 40 wk of age. According to the literature, these young males should have higher concentrations of sperm in ejaculates of greater volume. The young males used to spike flocks should be physically stable and have increased libido as compared to the older males. However, as we reported from Strain A, mean SP values continued to drop dramatically even following spiking of the flocks with young males. Although fertility in the 44-wk-old flocks was not significantly reduced from the 37-wk-old flocks in Strain A, SP values declined an average of 39% (range; 153 to 94 holes per GD). The decrease in mean SP of a flock following the peak in production continued in a linear fashion as the age of the flocks increased (Figure 4). If a standard level of SP was established for each strain and monitored, extreme mean deviances from the standard might indicate a reproductive problem in that flock long before a decline in fertility is detected. If the SP values continue to rapidly decline over time, eventually fertility will be significantly effected. If the SP values were lower than expected for that given flock age and strain, the slope of the linear line (postpeak in production) in Figure 4 would be increased (tail of linear line lowered toward the x axis) and could effect fertility greater than normally expected as the flocks age. Thus, SP values could be used to detect differences in sperm available for fertilization within a flock or strain before they affect flock fertility.

The question remains, however, given these same circumstances from Strain A flocks, would the drop in SP be greater if the flocks had not been spiked? Considering the reports of Bramwell *et al.* (unpublished data) that a higher percentage of the ejaculate from young males contains dead sperm, and according to the results reported here, sperm from the old males appears to have at least the same physiological capacities to fertilize the egg *in vivo* as Y males. Negating the possible indirect benefits of spiking flocks, this capacity would suggest that if the physical impairments males develop upon aging could be sufficiently reduced, spiking may not be necessary. However, it would also depend upon the older males maintaining proper libido.

When comparing the male and female lines of Strain B (Tables 1 and 2), we reported that the male line had a significantly greater number of SP holes. Although there was a less than 5% difference in fertility between the two lines, mean SP in the male line was more than twice that found in the female line (106.4 vs 50.8). The mean difference in fertility between the two lines is not as large as the mean difference in SP values. Because there is often an excess of sperm available for fertilization, small changes in SP values can not be detected by monitoring fertility alone. The fewer sperm active at the site of fertilization in the female line may eventually

lead to a more serious fertility problem if the mean SP numbers continue to be reduced in succeeding generations. Following several generations of selection for specific traits, fertility may not change dramatically but that line may eventually become fixed to have consistently lower SP values. Evidence of this may be seen from the mean SP values in the female line of Strain B. With decreased numbers of sperm active at the site of fertilization, there becomes increasingly less chance of a single sperm cell penetrating the PL at precisely the right location and at the correct time to fertilize the ovum. If SP was monitored consistently, the genetic trend towards fewer sperm active at the GD region could be reversed or maintained before it negatively affects fertility.

In conclusion, it is apparent that age does negatively affect SP values and fertility in broiler breeders. Using artificial insemination of a constant number of sperm, hen senescence significantly reduces SP values. In contrast, physiology of sperm from older males appears to be relatively unaffected in their fertilizing and SP abilities. In a commercial setting using natural mating, SP is greatly reduced dependent upon the age of the flock. A major contributing factor to decreased SP due to hen senescence appears to be the hen's physiological status. In addition, based upon the results of this study, the reduction in SP and fertility due to increased age of the male in naturally mated flocks may be due more to physical problems as opposed to physiological restrictions of the male gamete.

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REFERENCES

Adams, C. E., 1975. Effects of maternal age on ovulation, fertilization and embryonic development. Pages 231–248 *in:* Aging Gametes, Their Biology and Pathology. R. J. Blandau, ed. S. Karger, Basel, Switzerland.

Ansah, G. A., D. C. Crober, R. B. Buckland, A. E. Sefton, and B. W. Kennedy, 1980. Artificial insemination of individually caged broiler breeders 1. Reproductive performance of males in relation to age and strain of females. Poultry Sci. 59:428–437.

Atwood, H., 1929. Observations concerning the time factor in egg production. Poultry Sci. 8:137–140.

Bahr, J. M., and S. S. Palmer, 1989. The influence of aging on ovarian function. Crit. Rev. Poult. Biol. 2:103–110.

Bakst, M. R., and B. Howarth, Jr., 1977. Hydrolysis of the hen's perivitelline layer by cock sperm *in vitro*. Biol. Reprod. 17: 370–379.

Bekhtina, V. G., 1968. Morphological features of polyspermy fecundation in hens. *In:* Summaries from Pushkin Research Laboratory of Livestock Breeding. Leningrad Region, U.S.S.R. Cited by World's Poult. Sci. J. 24:148.

Bramwell, R. K., H. L. Marks, and B. Howarth, 1995. Quantitative determination of spermatozoa penetration of the perivitelline layer of the hen's ovum as assessed on oviposited eggs. Poultry Sci. 74:1875–1883.

Brillard, J. P., 1993. Sperm storage and transport following natural mating and artificial insemination. Poultry Sci. 72: 923–928.

Brillard, J. P., and G. R. McDaniel, 1986. Influence of spermatozoa numbers and insemination frequency on fertility in dwarf broiler breeder hens. Poultry Sci. 65: 2330–2334.

Brillard, J. P., G. R. McDaniel, M. de Reviers, and J. W. Drane, 1989. Expression of several traits of fertility in young and old dwarf broiler breeder hens inseminated with duplicate doses of semen. Poultry Sci. 68:558–563.

Burrows, W. H., and J. P. Quinn, 1937. The collection of spermatozoa from the domestic fowl and turkey. Poultry Sci. 16:19–24.

Christensen, V. L., 1981. Effect of insemination intervals on oviducal sperm storage in turkeys. Poultry Sci. 60: 2150–2156.

de Reviers, M., and J. P. Brillard, 1986. Variations in the sperm production, the sperm output and in the number of sperms to be inseminated in aging broiler breeders. World's Poult. Sci. J. 42:98. (Abstr.)

Etches, R. J., 1990. The ovulatory cycle of the hen. Crit. Rev. Poult. Biol. 2:293–318.

Fasenko, G. M., R. T. Hardin, and F. E. Robinson, 1992. Relationship of hen age and sequence position with fertility, hatchability, viability, and preincubation embryonic development in broiler breeders. Poultry Sci. 71: 1374–1383.

Harper, J. A., and G. H. Arscott, 1969. Seasonal decline in fertility of turkey eggs. Poultry Sci. 48:2109–2133.

Howarth, B., 1981. Preservation of the fertilizing capacity of cock semen incubated *in vitro* at 41 C. Poultry Sci. 60: 1075–1078.

Howarth, B., Jr., 1984. Maturation of spermatozoa and mechanism of fertilization. Pages 61–174 *in:* Reproductive Biology of Poultry. F. J. Cunningham, P. E. Lake, and D. Hewitt, ed. British Poultry Science Ltd., Longham-Group, Harlow, UK.

Kirk, S., G. C. Emmans, R. McDonald, and D. Arnot, 1980. Factors affecting the hatchability of eggs from broiler breeders. Br. Poult. Sci. 21:37–53.

Koyanagi, F., S. Masuda, and H. Nishiyama, 1988. Acrosome reaction of cock spermatozoa incubated with the perivitelline layer of the hen's ovum. Poultry Sci. 67:1770–1774.

Lake, P. E., 1989. Recent progress in poultry reproduction. World's Poult. Sci. J. 45:53–59.

Lerner, S. P., N. French, D. McIntyre, and C. Baxter-Jones, 1993. Age-related changes in egg production, fertility, embryonic mortality, and hatchability in commercial turkey flocks. Poultry Sci. 72:1025–1039.

Maeza, A. S., and E. G. Buss, 1976. Sperm concentration and sperm numbers as related to fertility in chickens. Poultry Sci. 55:2059. (Abstr.)

Mauldin, J. M., 1989. An analysis of reproductive efficiency in Georgia hatcheries. General Report Number 113. Extension Poultry Science Department, The University of Georgia, Athens, GA.

Mikamo, K., and H. Hamaguchi, 1975. Chromosomal disorder caused by preovulatory overripeness of oocytes. Pages 72–97 *in:* Aging Gametes, Their Biology and Pathology. R. J. Blandau, ed. S. Karger, Basel, Switzerland.

Okamura, F., and H. Nishiyama, 1978. The passage through the vitelline membrane in the domestic fowl, *Gallus gallus*. Cell Tissue Res. 188:497–508.

O'Sullivan, N. P., E. A. Dunnington, and P. B. Siegel, 1991. Relationships among age of dam, egg components, embryo lipid transfer, and hatchability of broiler breeder eggs. Poultry Sci. 70:2180–2185.

Ottinger, M. A., and J. A. Mench, 1989. Reproductive behavior in poultry: implications for artificial insemination technology. Br. Poult. Sci. 30:431–442.

Pierson, E. E., L. M. Krista, and G. R. McDaniel, 1988. Effect of age and physiological status on sperm storage 24 hours after artificial insemination in broiler breeder hens. Br. Poult. Sci. 29:193–197.

Robinson, F. E., R. T. Hardin, and A. R. Robblee, 1990. Reproductive senescence in domestic fowl: egg production, sequence length and intersequence pause length. Br. Poult. Sci. 31:871–879.

Romanoff, A. L., 1960. Fertilization and fertility. Pages 75–111 *in:* The Avian Embryo. The MacMillan Co., New York, NY.

Rosenstrauch, A., A. A. Degen, and M. Friedländer, 1994. Spermatozoa retention by sertoli cells during the decline in fertility in aging roosters. Biol. Reprod. 50:129–136.

SAS Institute, 1985. SAS® User's Guide: Statistics. Version 5 Edition. SAS Institute Inc., Cary, NC.

Sexton, T. J., 1983. Maximizing the utilization of the male breeder: a review. Poultry Sci. 62:1700–1710.

Sexton, T. J., J. A. Renden, D. N. Marple, and R. J. Kemppainen, 1989. Effect of dietary energy on semen production, fertility, plasma testosterone, and carcass composition of broiler breeder males in cages. Poultry Sci. 68:1688–1694.

Van Krey, H. P., A. T. Leighton, Jr., and L. M. Potter, 1967. Sperm gland populations and late seasonal declines in fertility. Poultry Sci. 46:1332. (Abstr.)

The Suppressive Effects of Testosterone on Growth in Young Chickens Appears to be Mediated via a Peripheral Androgen Receptor; Studies of the Anti-Androgen ICI 176,334

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ABSTRACT ICI 176,334 is a nonsteroidal anti-androgen that has been shown to selectively block peripheral androgen receptors in rats and is presumed to do so in chickens. In chickens, androgens stimulate secondary sexual characteristics (e.g., comb), but inhibit growth and the immune tissues. The present study examined the effect of dietary ICI 176,334 (5 or 25 mg/kg body weight) on growth in chickens in the presence or absence of testosterone treatment (as 1-cm long silastic implants). Treatments began at 2 wk of age and continued through 6 wk of age. Testosterone alone reduced body growth (average daily gain and shank-toe length, together with weights of the body, skeletal muscle, and the bursa of Fabricius, an immune tissue), and stimulated comb development. At the low dose (5 mg/kg), ICI 176,334 alone had no effect on body growth or organ weight with the exception that comb weight was reduced. At the high dose (25 mg/kg), ICI 176,334 decreased growth (body weight, average daily gain, and shank-toe length) and organ weights (breast muscle, bursa of Fabricius, testis, and comb weights). This effect may represent a toxicity. As might be expected with an anti-androgen, ICI 176,334 (at either 5 or 25 mg/kg) completely suppressed the stimulation of comb growth evoked by testosterone. Similarly, ICI 176,334 (5 mg/kg) overcame, albeit partially, the growth-suppressive effects of testosterone (on body weight, average daily gain, shank-toe length, and breast muscle weight) and also had inhibitory effects on the weights of the testis and bursa of Fabricius. The anti-androgen, ICI 176,334, did not influence the reduction in circulating concentrations of luteinizing hormone occurring after testosterone treatment. The present data are consistent with the growth-suppressive effects of testosterone in chickens being mediated via a peripheral androgen receptor. No effects of either testosterone or ICI 176,334 were observed on circulating concentrations of insulin-like growth factor-I despite the marked changes in growth rate.

(*Key words:* chicken, anti-androgen, growth)

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INTRODUCTION

Gonadal steroids are known to influence growth in both mammals and birds via direct or indirect (either intact steroid or following steroid metabolism) binding to intracellular receptors (androgen or estrogen receptors). In mammals, it is well understood that androgens stimulate growth, particularly muscle growth (Heitzman, 1976). In poultry, however, the picture is less clear. Androgens stimulate growth in turkeys (e.g., Wise and Ranaweera, 1981; Maurice *et al.*, 1985; Fennell and Scanes, 1992a). However, in chickens, androgens inhibit growth (Turner, 1948; Ma, 1954; Visco, 1973; Fennell and Scanes, 1992b). The relative potency of androgens in stimulating turkey growth, in inhibiting chicken growth, and also in inducing comb development are the same (testosterone < 5α-dihydrotestosterone < 19-nortestosterone). It is possible that the effects of androgens on growth are mediated by the androgen receptor or, following aromatization, the estrogen receptor, and are either direct effects of the steroid or indirect effects via modulation of the insulin-like growth factor-I (IGF-I) axis.

The present study examines the effect of an anti-androgen (ICI 176,334)[1] on growth, comb development, and circulating concentrations of IGF-I, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in young chickens, in the presence or absence of testosterone. ICI 176,334 is peripherally selective in blocking the rat androgen receptor without affecting (unlike other anti-androgens) the hypothalamic-pituitary axis and LH secretion (Furr *et al.*, 1987).

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