

CANADIAN BROILER HATCHING EGG PRODUCERS ASSOCIATION

**Breeder Parent Age Effects on Fertility, Embryonic Mortality
and Broiler Chick Quality**

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ABSTRACT

Past research has focused on the breeder hen's reproductive ability as a flock ages, but little attention has been given to the rooster's role in age related fertility declines or to the interaction between the hen and rooster. The objectives of this study were to examine the effect of male age on sperm mobility; female age on egg components; and the combined age effect on fertility, embryonic survival, and broiler chick quality. Two flocks, young (Y) and old (O) of Cobb 500 hens, (n=62 hens/flock) were artificially inseminated. Pooled semen from roosters of the same flock ages (n=15/flock) was used. The age groups examined were: 1) Young hens x Young roosters (YY), 2) Old hens x Young roosters (OY), 3) Young hens x Old roosters (YO), and 4) Old hens x Old roosters (OO). Sperm quality and fertilizing ability were examined using sperm mobility and sperm penetration assays. Egg production and egg components were examined. Two broiler trials were conducted when the breeder flocks were 28 (Y) and 51wk of age (O) (Trial 1), and 37 (Y) and 60 wk of age (O) (Trial 2). The parent group OY had fewer sperm holes observed around the germinal disk. There were differences in sperm concentration and mobility between individual roosters, regardless of age. However, each flock had roosters with low, average and high sperm mobility. The young hens had higher egg production than the old hens. Differences were observed in egg weight and egg components between the young and old hens. During Trial 1 the young hens had the greatest fertility however, during Trial 2 there was a parent interaction. The YY parent group had the highest fertility and the OY parent group had the lowest fertility. Hen age had a greater impact on offspring performance than rooster age. Semen from the old roosters had the same fertilizing capacity as that of the young roosters indicating that it is not poor sperm quality that contributes to an age related decline in fertility. Selecting roosters based on sperm mobility may be one way to increase fertility in commercial broiler breeder production systems.

INTRODUCTION

As broiler breeder flocks age, fertility and embryonic viability decrease, resulting in fewer saleable chicks. This is a concern for the Canadian poultry industry as Canadian hatching egg producers are paid based on the number of saleable chicks produced. Most research on age related declines in fertility has focused on hens. There is a lack of research investigating whether aging of the male (impact of sperm quality, not mating frequency) plays a significant role in the decline. Research has shown that as male broiler breeders age they tend to mate less frequently (Casanovas, 2002). This decrease in mating leads to "old sperm" stored in the hen's reproductive tract being used to fertilize eggs; this has negative consequences for fertility and embryonic mortality (Lodge et al., 1971; Pierson et al., 1988).

There have been recent developments in objective methods to measure sperm quality and fertilizing ability: 1) sperm penetration assay and 2) sperm mobility assay. Both assays provide an excellent opportunity to improve the knowledge base in this area of poultry reproduction. The first method examines the number of holes in the yolk membrane overlying the embryo (Bramwell et al., 1996). Each hole represents the penetration of one spermatozoa and the number of holes has been shown to be highly correlated with fertility. The second method (sperm mobility assay (Froman and McLean, 1996)), has been established as a predictor of semen quality (Froman et al., 2003).

Limitations in research design and lack of access to newer techniques have not allowed previous research to make the distinction between the effects of male and female aging on fertility and embryonic mortality as a broiler breeder flock ages. Therefore the main objectives of this research were to determine the effects of age on 1) male sperm quality and fertilizing ability; 2) female egg production and egg components; and 3) how broiler performance is affected by the age of each parent. To accomplish this, the effects of rooster age on sperm quality and fertility, hen age on egg components, and the age of both parents on fertility, embryonic mortality and broiler chick performance were examined. It was hypothesized that older roosters would have lower sperm mobility and fewer sperm

penetrations into the egg and that increasing hen age would result in a change in the egg component ratios. It was also hypothesized that older parents would have lower fertility, higher embryonic mortality and inferior broiler chick quality and performance.

MATERIALS AND METHODS

All experimental procedures were approved by the Animal Care and Use Committee (Livestock) at the University of Alberta, in accordance with the guidelines set forth by the Canadian Council on Animal Care (1993).

Broiler Breeder Management. One hundred and sixty-nine 1-day-old Cobb 500 broiler breeder chicks (136 females and 33 males) were obtained from a commercial hatchery (Maple Leaf Hatchery, Wetaskiwin, Alberta, Canada) and reared in floor pens. These birds comprised the old flock (**OF**). The birds were weighed each week and feed was restricted as recommended by the breeder management guide (Cobb-Vantress Inc., 2005) to maintain target breeder body weights (**BW**). At 21 wk of age the birds were individually weighed, wingbanded and placed in individual cages. When the OF reached 23 wks of age, 133 1-day-old Cobb 500 broiler breeder chicks (104 females and 29 males) were obtained from the same commercial hatchery and reared in the same manner as the OF birds. These birds comprised the young flock (**YF**). All broiler breeders were photostimulated at 21 wk of age. Fifteen roosters and 62 hens from each of the OF and YF were selected for the trial. The birds selected were those closest to the target breeder BW at 21 wk of age (Cobb-Vantress Inc., 2005). All broiler breeders were individually weighed weekly and feed restricted to maintain recommended breeder target BW (Cobb-Vantress Inc., 2005) throughout the production cycle. In order to examine the effect of age, the trial began when the YF was nearing peak production (27 wk of age) and the OF was nearing the end of their production cycle (50 wk of age).

Semen Collection, Sperm Concentration and Sperm Mobility. Semen was collected from each rooster twice per week (with 3 d between the 1st and 2nd weekly collections). Each week, one collection was used to examine the sperm mobility of individual roosters using a sperm mobility analyzer (596a Chicken Mobility Analyzer, Animal Reproduction Systems, Chino, California, USA) following the procedure described by Froman (2006). The second weekly collection was used for insemination of the hens. Semen from old roosters was pooled as was the semen from young roosters. Each hen received a suboptimal dose (70,000,000 sperm cells) in order to exacerbate any problems that might be observed due to poor semen quality. Half the hens (n=31) from the YF were inseminated with pooled semen from the young roosters while the other half of the hens (n=31) from the YF were inseminated with pooled semen from the old roosters. Hens from the OF were divided into two groups and inseminated with semen from the young and old roosters in the same manner as the hens from the YF. Thus the artificial insemination (**AI**) produced the following parent age groups:

- Young hens x Young roosters
- Old hens x Young roosters
- Young hens x Old roosters
- Old hens x Old roosters

Egg Collection. Daily egg production from both the young and old hens was recorded beginning when the OF was 50 wk and the YF was 27 wk and continuing until the end of the trial. Egg production was calculated on a weekly basis throughout the trial.

Egg Components and Sperm Penetration Assay. Eggs were collected 3 times (collection = all the eggs produced in 1 wk) during the fertility trial to examine egg components and perform a sperm penetration assay (Bramwell et al., 1995). The first collection occurred when the OF was 50 wk and the YF 27 wk of age; the 2nd when the OF was 55 wk and the YF 32 wk of age; and the 3rd when the OF was 59 wk and YF 36 wk of age. At each collection, egg components were examined in 30 eggs. Each egg was weighed, broken open and the yolk and shell weighed. Albumen weight was determined by

subtracting the wet yolk and eggshell weight from the total egg weight. The yolk and the shell were then dried (Despatch V Series Model VRC2-26-1E, Minneapolis, Minnesota, USA) at 65°C for 5 days and reweighed. All egg component weight data were expressed as percentage of the total fresh egg weight. Shell thickness was measured using a micrometer screw gauge (SR44 battery model, Mitutoyo, Aurora, Illinois, USA).

All additional eggs collected at these times were used to assess fertility and the sperm penetration assay (Bramwell et al., 1995). Sperm penetration determined by examining the yolk membrane overlaying the embryo for the number of sperm holes surrounding the germinal disc (Bramwell et al., 1995).

Reproductive Fitness and Carcass Characteristics. At the end of the trial all broiler breeders (OF - 63 wk, YF - 40 wk) were weighed and euthanized via cervical dislocation. The liver, heart, *Pectoralis major* and *minor* were removed and weighed. The reproductive organs of the hens (left ovary and oviduct) were weighed and the number of large yellow follicles (follicles that were more than 1 cm in diameter) were counted. The abdominal fat pad of the females was also weighed. The testes of the roosters were weighed. All data were expressed as a percentage of BW.

Broiler Hatchability and Performance Trials

Hatchability, Embryonic Mortality and Chick Quality. When the OF was 51 wk of age and the YF 28 wk of age the hens were inseminated. From 2 to 6 d after insemination, all eggs collected were incubated. On d 18 of incubation, eggs were individually weighed and placed in a hatcher (Jamesway Incubator Company, Inc. Cambridge, Ontario, Canada).

After 21.5 d of incubation, equal numbers of chicks per each hen x rooster parent group (broiler Trial 1 - 52 chicks/parent group, broiler Trial 2 - 38 chick/parent group) were weighed, neck tagged, had their shank and chick length measured and were visually assessed for quality based on the Tona Score (Tona et al., 2003). Chicks were placed in floor pens and individual BW, mortality and feed

consumption were recorded weekly. At 6 wk of age, the broilers were processed at the Alberta Chicken Producers Poultry Technology Centre. The hatchability and broiler performance trial was repeated when the OF was 60 wks of age and the YF was 37 wk of age.

Statistical analysis. All data were subjected to analysis of variance using the Mixed procedure of SAS (SAS Institute Inc., 2002-2003). Where significance ($P < 0.05$) for the main effects or interactions were found, the LSmeans were separated using pdiff of SAS (SAS Institute Inc., 2002-2003).

RESULTS & DISCUSSION

Broiler Breeder Fertility Trial

Broiler Breeder Body Weights. The OF hens and roosters were consistently heavier than YF hens and roosters (Table 1) which was expected as the OF birds were 23 wk older than the YF birds. The hens and roosters from the OF were heavier than the recommended target BW for the duration of the trial.

Previous research has shown that reproductive efficiency in broiler breeders is negatively impacted by obesity (Robinson and Wilson, 1996; Wolanski et al., 2004). Obese female broiler breeders can experience decreased duration of fertility, higher embryonic mortality due to thinner eggshells and shorter lay sequences (Robinson et al., 1983). When males become heavier towards the end of the production cycle they tend to mate less frequently. This may be a consequence of their as leg problems may limit their ability to mate or their size may interfere with successful mating. Any mating inefficiencies due to behavior were removed in this study because the hens were artificially inseminated.

Egg Production. The YF had higher average weekly egg production than the OF during the entire trial (Table 2). However, the YF was nearing peak production and the OF was reaching the end of their lay cycle when the fertility trial began. This confirms previous reports (Robinson et al., 1990) that egg production decreases with age and the results of this research agree.

Egg Components. At all 3 collections the OF hens had heavier eggs with a higher percentage of wet yolk, compared to eggs from the YF hens (Table 3). As the YF hens aged their eggs became heavier and had a higher percentage of wet yolk. The percent wet shell was higher in eggs from YF hens during the first collection but higher in the eggs from OF hens in both later collections; there was no difference in percent dry shell between flock ages or collections. The albumen fraction of the egg was greater in eggs laid by the YF hens than those laid by the OF hens. The percentage of dry yolk was higher in eggs laid by OF hens during the first collection with no difference between the two flocks at the second collection and then higher in eggs laid by the YF hens at the third collection. The percentage of dry yolk decreased in both flocks as they aged. During the first collection, eggs from the OF hens had thicker shells than those eggs collected from YF hens. There was no difference between flock ages at the following 2 collection times.

The results of this study agree with previous research which has shown that as hens age they produce larger and heavier eggs and the proportions of egg components change (Fletcher et al., 1981, 1983; Reinhart and Hurnik, 1984; Suarez et al., 1997).

Sperm Penetration. There was a significant hen age by rooster age interaction effect on the average number of holes seen surrounding the germinal disk. However, this effect was only seen during the 2nd collection when the OF was 55 wk old and the YF was 32 wk old. The parent combination of OY resulted in significantly lower numbers of sperm holes seen around the germinal disc, with the average being 5.27 ± 1.23 holes. There was no difference between the other three parent groups OO, YO, YY with each having an average number of sperm holes of 7.62 ± 1.15 , 8.93 ± 0.86 , 8.91 ± 0.87 , respectively. During collection 1 (OF - 50, YF - 27 wk) and 3 (OF - 60, YF - 37 wk) there was no difference between the parent groups. The highest numbers of sperm holes were seen during the first 2 d post-insemination with the number of holes decreasing as the days post-insemination increased (Table 4).

Previous research (Bramwell et al., 1996) had tested the effect of age (male and female) on the number of spermatozoa that penetrated the perivitelline layer. That research used a similar experimental design, inseminating young and old hens with semen from young and old roosters, and the results similar to the findings of the current study. However, Bramwell et al. (1996) examined a young flock that was 39 wk of age and an old flock that was 69 wk of age (much older than would normally be seen in the Canadian Industry). It is interesting that old hens mated with young roosters had the lowest number of sperm holes since this simulates what would occur in a spiking situation. These results indicate that the increase in fertility observed in spiked flocks might be due to increased mating frequency by older males and not due to the fertilizing ability of the young roosters.

Bramwell et al. (1996) also examined how the number of sperm holes was affected as the number of days post insemination increased. They reported that the number of sperm holes decreased as the number of days post insemination increased. These results were similar to the results from this study, showing that hens need to be exposed to fresh sperm cells at regular intervals in order maintain higher fertility.

Sperm Mobility. There were significant differences seen in sperm concentration and sperm mobility between individual roosters (Table 5). Significant differences were also observed in the average sperm mobility of all roosters combined from week to week (Table 6).

It is known that sperm concentration and sperm mobility can vary from rooster to rooster (Froman et al., 1999). Previous research has shown sperm mobility is a phenotypic trait that does not change with age and can be selected for genetically (Froman and McLean, 1996; Froman et al., 1997). In the current study the volume of the semen samples collected from individual roosters was small and due to this only one sperm mobility measurement could be performed on an individual rooster each week. Therefore it is statistically impossible for the authors to examine the interaction between roosters and week of collection. It is recommended that future research examine duplicate samples individual

males if semen volume permits. The sperm concentration was also variable between roosters. However it is interesting to note that the males with high sperm concentration did not necessarily have high sperm mobility. This finding agrees with Froman's (2006) report that sperm concentration was not highly correlated to sperm mobility.

When examining the pooled semen samples for sperm concentration and mobility there were no differences between the YF and OF roosters from wk to wk. As there were high mobility and low mobility males in each flock pooling of masked any individual differences, resulting in no difference between the flocks. In the current study, by pooling the semen samples a flock's entire sperm population was combined. However in a breeder barn individual birds are still responsible for mating individual hens. Pooling situations could occur if males with low mobility sperm mate with the same hens as roosters with high sperm mobility. If males underwent a selection process that included measuring sperm mobility, the need for spiking could be reduced and producers would have the chance to remove "dud" males from a flock. Froman et al. (1997) have shown that when males are selected based on sperm mobility, fecundity can be increased.

Reproductive Fitness and Carcass Characteristics of Broiler Breeders. When examining the different carcass characteristics of the roosters from the OF and YF, few differences were found (Table 7). However the YF roosters had heavier *Pectoralis minor* muscles than those from the OF, when expressed as a percentage of BW. This may have been due to a large number of old roosters having necrotic myopathy of the *Pectoralis minor* muscle. All other body characteristics were not significantly different between the two flocks. The roosters had similar carcass composition regardless of age. Wolanski, et al. (2004) reported similar findings when they examined end-of-season carcass and reproductive traits in original and replacement males.

When the hens were examined, there was no difference in heart or *Pectoralis major* weights between the YF and OF, when expressed as a percentage of BW (Table 8). Bilgilli and Renden (1985)

reported that body weight was positively correlated with body fat. The hens from the OF had significantly heavier BW and percent abdominal fat than the hens from the YF (Table 8). The percent weight of the left ovaries and oviducts was increased in the YF hens. The YF hens also had more large yellow follicles, a higher percent of *Pectoralis minor* muscles and livers than the old hens (Table 8).

The YF hens were maintaining high egg production at the time of euthanasia so it was logical that they would have higher number of large yellow follicles than the OF hens, who were nearing the end of their reproductive cycle. The OF hens were 61 wk of age, at the end of their production cycle and significantly over their target BW. This could have a direct effect on the number of large yellow follicles the OF hens had. Due to the OF hens being obese the percentage that each carcass measurement contributed to the overall weight would be diminished due to the increased percentage of fat contributing to the OF hens total BW.

Broiler Hatchability and Performance

Incubation. Hen age had a significant effect on egg weight at setting, transfer egg weight, percent weight loss at transfer and chick weight, with the old hens having heavier egg and chick weight and the young hens having great weight loss at transfer in both Trials 1 and 2 (Table 9). The results of this study agree with previous findings that older hens have heavier eggs (Reinhart and Hurnik, 1984; Suarez et al., 1997).

Fertility, Hatchability and Embryonic Mortality. During Trial 1 the young hens had higher fertility than the old hens ($81.51 \pm 2.71\%$ vs. $66.96 \pm 2.71\%$ respectively). During Trial 2 there was a hen age x rooster age interaction with the YY parent group having the highest fertility ($79.5 \pm 3.71\%$) and the OY parent group having the lowest fertility ($57.5 \pm 3.71\%$), and YO and OO having moderate fertility ($70.0 \pm 3.71\%$ and $64.5 \pm 3.71\%$, respectively). There were no differences in total hatchability or hatchability of fertile egg data were not significant during either of the two trials (data not shown). It is interesting that OY parent group had the lowest fertility as older flocks are generally spiked with

younger roosters in order to improve declining fertility levels. The reason for this result is unclear. There was no evidence of reproductive failure on the part of the hens, as observed by examining the reproductive fitness data. It may be that in a barn setting increased mating frequency masks any problems due to low sperm numbers by the sheer numbers of sperm delivered through multiple matings. In this research the suboptimal dose of sperm cells (70,000,000/AI dose) may be revealing quality problems that have not been observed before.

The only difference observed in embryonic mortality occurred during mid incubation (7 to 14 d) with the OO parent group having the highest percent mortality of $1.08 \pm 0.49\%$, while the other parent groups were not different from one another.

Chick Quality. There was a trial by hen age by rooster age interaction for shank length, chick length and Tona Score (Tona et al., 2003) (Table 10). Shanks were shortest in chicks from the YO and YY parent groups in Trial 1. The OO and OY parent groups produced the longest chicks in both trials. There was no clear pattern to the Tona Score and this could be due to the small number of chicks that were used to determine the score in this project or the fact that the score blends a number of quality measures, which may mask individual quality characteristics. As a flock matures, egg size becomes larger and the chicks that hatch from larger eggs tend to be heavier (Yannakopoulos and Tscrveni-Gousi, 1987). Heavier chicks also tend to be longer and have longer shanks (Wilson, 1991).

Broiler Performance. There was no difference in broiler mortality between the parent age groups during Trial 1. However, during Trial 2 the YY parent group had the greatest early mortality (mortalities occurring before 14 d of age, $10.22 \pm 0.21\%$), followed by the YO parent group ($4.97 \pm 0.21\%$). The OO and OY groups had no mortality during this period. During broiler Trial 1 the OF hens had heavier broilers from wk 3 until the end the trial (Table 11). During Trial 2, there was a hen age by rooster age interaction for BW (Table 11) with the YO parent group having the lightest broilers

from 4 weeks of age until the end of the trial while there was no difference among the BW of the broilers from the other 3 parent groups (Table 11).

It was interesting that the hen age had the greatest impact on broiler performance with the broilers from OF hens having the heaviest final BW than the broilers from YF hens. Previous research has shown that chicks from older hens start out heavier approximately 17% heavier than chicks from young hens. However by 42 d the amount of difference in weight is smaller than at hatch decreasing to approximately 5-6% difference in BW (Noy and Sklan, 1997).

In Trial 2 broilers from the YO parent group had significantly lower BW from wk 4 to 6. The reason for this is not clear. This result suggests that the pairing of YO has a negative effect on broiler BW. It cannot be attributed entirely to the hen age as broilers from the YY parent group had similar BW as the other two parent groups (OO, OY).

SUMMARY & CONCLUSIONS

There were no differences in the reproductive capacity of the broiler breeder roosters, regardless of age. It does not appear that suboptimal semen quality plays a role in the reproductive problems seen in broiler breeder flocks as they age. By pooling the semen, the contribution of males with higher quality semen was diluted. In future research it would be interesting to divide the roosters by age and then further classify them as low and high mobility males and examine the differences. This would eliminate the effect of dilution and would provide a clearer picture of the effect male age is having on semen quality. The hens had the greatest impact on many of the production parameters examined in this research.

INDUSTRY IMPLICATIONS

It does not appear to be suboptimal sperm quality that is causing the decline in fertility and the increase in embryonic mortality witnessed in breeder flocks as they age. While there was no apparent difference in semen quality there were differences observed in sperm concentration and sperm mobility between individual males. The difference in sperm mobility showed that a flock could be comprised of males that have low average and high mobility. Examining sperm mobility of broiler breeders could be a very useful tool for the producer as it would provide the producer with valuable information to use in deciding what males to keep and which to replace. If a producer could eliminate males with low sperm mobility the cost of feeding and housing males that have greater likelihood of lower fertility would be eliminated, ensuring they are getting the maximum return on investment. Stocking a barn with high sperm mobility males will increase the likelihood of high fertility as a flock ages and therefore more saleable chicks, increasing profit potential.

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Table 1. Average body weight (BW) for broiler breeder hens and roosters of 2 flocks (1 old and 1 young) for each week of a 14 wk period

Flock Age (wk) ¹		Hens		Roosters	
Old	Young	Old (g)	Young (g)	Old (g)	Young (g)
50	27	4,393±40 ^{2 hi z} (n=62) ³	3,109±43 ^{1 y} (n=62)	5,233±75 ^z (n=15)	4,045±75 ^{h y} (n=15)
51	28	4,281±40 ^{j z} (n=61)	3,189±43 ^{ij y} (n=62)	5,216±75 ^z (n=15)	4,164±75 ^{fg y} (n=15)
52	29	4,417±41 ^{ghi z} (n=61)	3,241±43 ^{gi y} (n=62)	5,235±75 ^z (n=15)	4,260±75 ^{ef y} (n=15)
53	30	4,445±41 ^{fghi z} (n=61)	3,322±43 ^{gh y} (n=62)	5,245±75 ^z (n=15)	4,364±75 ^{def y} (n=15)
54	31	4,338±40 ^{ij z} (n=61)	3,441±43 ^{fg y} (n=62)	5,260±75 ^z (n=15)	4,448±75 ^{cde y} (n=15)
55	32	4,477±41 ^{efgh z} (n=61)	3,525±43 ^{ef y} (n=62)	5,254±77 ^z (n=14)	4,533±75 ^{bcd y} (n=15)
56	33	4,505±41 ^{defgh z} (n=60)	3,579±43 ^{e y} (n=62)	5,241±77 ^z (n=14)	4,595±75 ^{abc y} (n=15)
57	34	4,520±41 ^{cdefg z} (n=60)	3,642±43 ^{de y} (n=62)	5,240±77 ^z (n=14)	4,631±75 ^{abc y} (n=15)
58	35	4,540±41 ^{bcdef z} (n=60)	3,702±43 ^{cd y} (n=62)	5,248±77 ^z (n=14)	4,688±75 ^{ab y} (n=15)
59	36	4,575±41 ^{abcde z} (n=59)	3,751±43 ^{bcd y} (n=62)	5,253±77 ^z (n=14)	4,703±75 ^{ab y} (n=15)
60	37	4,597±42 ^{abcd z} (n=58)	3,786±43 ^{bc y} (n=62)	5,248±77 ^z (n=14)	4,705±75 ^{a y} (n=15)
61	38	4,642±42 ^{ab z} (n=58)	3,827±43 ^{a y} (n=62)	5,248±77 ^z (n=14)	4,749±75 ^{ab y} (n=15)
62	39	4,624±42 ^{abc z} (n=58)	3,869±43 ^{ab y} (n=62)	5,196±77 ^z (n=14)	4,711±75 ^{a y} (n=15)
63	40	4,688±42 ^{a z} (n=58)	3,951±43 ^{a y} (n=62)	5,236±77 ^z (n=14)	4,768±75 ^{a y} (n=15)

^{a-j} Means within the same column with different superscripts differ significantly ($P \leq 0.05$)

^{z-y} Means within the same row (within sex) with different superscripts differ significantly ($P \leq 0.05$)

¹ Flock Age = the age in wk of each flock (Old and Young) for each week of trial

² SEM = standard error of the means

³ n = # of experimental units, each experimental unit = 1 bird

Table 2. Average egg production of the old and young hens during a 13 wk period.

Flock Age (wk) ¹		Average Egg Production (%) ²	
Old	Young	Old	Young
50	27	68.85±1.92 ^{3a y} (n=61) ⁴	85.27±1.91 ^{ab z} (n=62)
51	28	66.74±1.92 ^{ab y} (n=61)	87.12±1.91 ^{a z} (n=62)
52	29	66.27±1.92 ^{ab y} (n=61)	86.89±1.91 ^{a z} (n=62)
53	30	66.51±1.92 ^{ab y} (n=61)	86.66±1.91 ^{a z} (n=62)
54	31	63.00±1.92 ^{bcd y} (n=61)	85.04±1.91 ^{ab z} (n=62)
55	32	64.43±1.94 ^{bc y} (n=60)	85.96±1.91 ^{a z} (n=62)
56	33	58.96±1.94 ^{de y} (n=60)	83.43±1.91 ^{ab z} (n=62)
57	34	59.19±1.94 ^{cde y} (n=60)	82.05±1.91 ^{abc z} (n=62)
58	35	58.26±1.95 ^{de y} (n=59)	80.90±1.91 ^{bc z} (n=62)
59	36	56.67±1.99 ^{ef y} (n=57)	78.13±1.91 ^{cd z} (n=62)
60	37	58.67±1.99 ^{de y} (n=57)	78.82±1.91 ^{c z} (n=62)
61	38	56.42±1.99 ^{ef y} (n=57)	72.83±1.91 ^{e z} (n=62)
62	39	52.66±1.99 ^{f y} (n=57)	73.52±1.91 ^{de z} (n=62)

^{a-f} Means within the same column with different superscripts differ significantly ($P \leq 0.05$)

^{z-y} Means within the same row (within sex) with different superscripts differ significantly ($P \leq 0.05$)

¹ Flock age = the age in wk of each flock (Old and Young) for each week of trial

² Average Egg Production = (egg production for hen 1+ egg production for hen 2+...+ egg production for hen x)/n

³ SEM = standard error of the means

⁴ n = # of experimental units, each experimental unit = 1 bird

Table 3. The effect of hen age (old and young) on egg weight, different egg components and average shell thickness (n=30)¹

	Collection 1 ²	Collection 2 ³	Collection 3 ⁴
Egg Weight (g)			
Old	70.91 ^a	71.01 ^a	70.58 ^a
Young	56.09 ^{bx}	60.45 ^{by}	62.56 ^{bz}
SEM ⁵	0.57	0.57	0.57
% Wet Yolk ⁶			
Old	31.06 ^{ay}	31.21 ^{ay}	32.72 ^{az}
Young	26.02 ^{bx}	28.20 ^{by}	29.82 ^{bz}
SEM	0.22	0.22	0.22
% Wet Shell ⁷			
Old	8.81 ^{by}	16.65 ^{az}	16.73 ^{az}
Young	9.78 ^{ax}	14.76 ^{by}	15.21 ^{bz}
SEM	0.13	0.13	0.13
% Albumen ⁸			
Old	60.13 ^b	52.29 ^b	50.57 ^b
Young	64.23 ^a	57.08 ^a	54.97 ^a
SEM	0.32	0.32	0.32
% Dry Yolk ⁹			
Old	16.32 ^{az}	9.65 ^y	9.10 ^{bx}
Young	13.15 ^{bz}	9.72 ^y	9.70 ^{ay}
SEM	0.12	0.10	0.10
% Dry Shell ¹⁰			
Old	8.49	8.64	8.63
Young	8.65	8.72	8.71
SEM	0.10	0.10	0.10
Average Eggshell Thickness ¹¹ (mm)			
Old	0.34 ^{az}	0.32 ^y	0.32 ^y
Young	0.32 ^b	0.32	0.32
SEM	0.003	0.003	0.003

^{a-b} Means within the same column with different superscripts differ significantly ($P \leq 0.05$)

^{z-x} Means within the same row (within sex) with different superscripts differ significantly ($P \leq 0.05$)

¹ Means based on 30 eggs/hen age/collection time

² Collection 1: Old-55 and Young-27 wk

³ Collection 2: Old-55 and Young-32 wk

⁴ Collection 3: Old-60 and Young-37 wk

⁵ SEM = standard error of the means

⁶ % Wet Yolk = (wet yolk weight/ egg weight)*100

⁷ % Wet Shell = (wet shell weight/ egg weight)*100

⁸ % Albumen = 100% - (% wet yolk + % wet shell)

⁹ % Dry Yolk = (dry yolk weight/ egg weight)*100

¹⁰ % Dry Shell = (dry shell weight/ egg weight)*100

¹¹ Average Eggshell Thickness = (aircell shell thickness + equator shell thickness + sharp-end shell thickness)/3

Table 4. The average number of holes made by sperm cells in the perivitelline membrane covering the germinal disc for each day after insemination during 3 different collections

Days After Insemination	Collection 1 ¹	Collection 2 ²	Collection 3 ³
1	11.5±1.7 ⁴ a (25) ⁵	10.1±1.1 ^a (57)	10.5±1.4 ^{ab} (39)
2	9.6±1.2 ^{a y} (50)	13.4±1.3 ^{a z} (47)	11.8±1.3 ^{a zy} (44)
3	7.9±1.1 ^{ab} (57)	7.4±1.1 ^{abc} (63)	6.9±1.3 ^{bc} (42)
4	4.4±1.2 ^{c y} (52)	7.9±1.1 ^{ab z} (65)	7.7±1.3 ^{c zy} (46)
5	5.0±1.3 ^{bc} (41)	4.3±1.2 ^c (50)	5.4±1.5 ^c (31)
6	3.9±1.4 ^c (40)	4.7±1.3 ^{bc} (41)	4.6±1.5 ^c (32)

^{a-c} Means within the same column with different superscripts differ significantly ($P \leq 0.05$)

^{z-y} Means within the same row (within sex) with different superscripts differ significantly ($P \leq 0.05$)

¹ Collection 1: Old-55 and Young-27 wk

² Collection 2: Old-55 and Young-32 wk

³ Collection 3: Old-60 and Young-37 wk

⁴ SEM = standard error of the means

⁵ n = # of experimental units, each experimental unit = number of germinal discs examined.

Table 5. The average¹ sperm concentration and sperm mobility of individual roosters of 2 different flock ages*

Flock Age	Sperm Concentration ² (billion/ml)	Sperm Mobility ³ (%)
Old	2.55±0.49 ⁴ ijk	.
Old	4.96±0.38 ^{bcd}	13.07±3.83 ^g
Old	3.79±0.47 ^{defghi}	21.57±5.80 ^{fg}
Old	5.18±0.37 ^{bc}	23.04±3.63 ^{fg}
Old	4.77±0.35 ^{bcde}	25.31±3.29 ^f
Old	4.52±0.51 ^{bcdefg}	27.17±4.72 ^{def}
Old	4.84±0.35 ^{bcd}	35.25±3.29 ^{de}
Old	6.31±0.36 ^a	35.62±3.63 ^{cde}
Old	4.01±0.41 ^{defgh}	39.32±4.36 ^{bcd}
Old	4.27±0.35 ^{bcdefgh}	41.35±3.29 ^{bcd}
Old	4.63±0.36 ^{bcdef}	41.45±3.63 ^{bcd}
Old	3.11±0.37 ^{hij}	41.89±4.36 ^{bcd}
Old	3.62±0.36 ^{fghi}	49.41±3.45 ^{ab}
Old	2.31±0.53 ^{jk}	63.20±6.71 ^a
Young	1.20±0.54 ^k	.
Young	4.21±0.35 ^{bcdefgh}	28.00±3.29 ^{ef}
Young	5.20±0.35 ^b	28.17±3.29 ^{ef}
Young	5.03±0.47 ^{bcd}	32.28±5.18 ^{def}
Young	4.75±0.36 ^{bcde}	33.22±3.45 ^{def}
Young	3.75±0.39 ^{efghi}	33.59±4.07 ^{def}
Young	4.31±0.62 ^{bcdefgh}	34.82±5.71 ^{def}
Young	3.28±0.37 ^{ghij}	35.48±3.83 ^{cde}
Young	3.61±0.39 ^{fghij}	41.63±4.36 ^{bcd}
Young	6.20±0.35 ⁵ a	42.11±3.29 ^{bcd}
Young	3.79±0.35 ^{defgh}	45.10±3.63 ^{bc}
Young	4.09±0.35 ^{defgh}	45.48±3.29 ^b
Young	4.16±0.36 ^{cdefgh}	46.57±3.83 ^b
Young	3.98±0.49 ^{defgh}	52.44±5.81 ^{ab}
Young	4.07±0.35 ^{defgh}	63.05±3.63 ^a

^{a-k} Means within the same column with different superscripts differ significantly ($P \leq 0.05$)

* Two roosters (1 from the old flock and 1 from the young flock) do not have a mobility value due to low sperm concentrations and small sample size.

¹ Average based on 1 semen sample/wk for 13 wk for each rooster

² Sperm Concentration = # of sperm cells/ml

³ Sperm Mobility = % of the sperm in a sample that are mobile

⁴ SEM = standard error of the means

Table 6. The average sperm concentration and sperm mobility the roosters of both the old and young flock combined for a 12 wk period¹

Week	Sperm Concentration ² (Billion/ml)	Sperm Mobility ³ (%)
1	3.74±0.23 ^{4 def}	33.98±2.68 ^{de}
2	4.40±0.25 ^f	29.55±2.41 ^{abc}
3	4.46±0.25 ^{cdef}	35.15±2.76 ^{ab}
4	4.82±0.23 ^{ef}	31.81±2.69 ^a
5	4.12±0.24 ^{bcde}	37.89±2.85 ^{def}
6	4.13±0.22 ^{ef}	33.08±3.04 ^{def}
7	4.38±0.21 ^{bc}	41.60±2.36 ^{abc}
8	4.48±0.21 ^f	30.70±2.24 ^{ab}
9	4.30±0.22 ^{bcde}	37.84±2.55 ^{abcd}
10	3.15±0.22 ^a	56.13±3.04 ^e
11	3.77±0.22 ^b	44.27±2.55 ^{cd}
12	4.14±0.23 ^{bcd}	41.27±2.61 ^{bcd}

^{a-f} Means within the same column with different superscripts differ significantly ($P \leq 0.05$)

¹ Means based on two pooled (by flock age, old and young) semen samples per week

² Sperm Concentration = # of sperm cells/ml

³ Sperm Mobility = percentage of the sperm in a sample that are mobile

⁴ SEM = standard error of the means

Table 7. The effect of rooster age on body weight (BW) and relative weight of the left and right testes, breast muscle, liver and heart from roosters of 2 different ages (old and young) when the old roosters were 63 wk of age and the young roosters were 40 wk of age

Age	n ¹	BW ² (g)	Left Testis ³ (%)	Right Testis ⁴ (%)	<i>Pectoralis minor</i> ⁵ (%)	<i>Pectoralis major</i> ⁶ (%)	Liver ⁷ (%)	Heart ⁸ (%)
Old	14	5,236±82 ^{9a}	0.24±0.02	0.24±0.02	3.60±0.27 ^b	14.52±0.47	1.48±0.09	0.50±0.03
Young	15	4,768±79 ^b	0.30±0.02	0.30±0.02	4.37±0.26 ^a	15.31±0.46	1.70±0.09	0.57±0.03

^{a-b} Means within the same column with different superscripts differ significantly ($P \leq 0.05$)

¹ n = # of experimental units, each experimental unit = 1 rooster

² BW = average live body weight of the roosters

³ Left Testis = (left testis weight/BW)*100

⁴ Right Testis = (right testis weight/BW)*100

⁵ *Pectoralis minor* = (*Pectoralis minor* weight/BW)*100

⁶ *Pectoralis major* = (*Pectoralis major* weight/BW)*100

⁷ Liver = (liver weight/BW)*100

⁸ Heart = (heart weight/BW)*100

⁹ SEM = standard error of the means

Table 8. The effect of hen age on body weight (BW), average number of large yellow follicles (LYF) and relative weight of the left ovary and oviduct, breast muscle, abdominal fat, liver and heart from the hens of two different flock ages (old and young) when the old hens were 63 wk of age and the young hens were 40 wk of age

Flock	n ¹	BW ² (g)	Left Ovary ³ (%)	Number of LYF ⁴	Left Oviduct ⁵ (%)	<i>Pectoralis minor</i> ⁶ (%)	<i>Pectoralis major</i> ⁷ (%)	Abdominal Fat ⁸ (%)	Liver ⁹ (%)	Heart ¹⁰ (%)
Old	58	4,688±38 ^{11 a}	1.33±0.04 ^b	5±0.10 ^b	1.58±0.03 ^b	3.87±0.08 ^b	14.23±0.25	4.38±0.18 ^a	1.58±0.03 ^b	0.39±0.01
Young	62	3,951±39 ^b	1.63±0.04 ^a	6±0.10 ^a	1.78±0.03 ^a	4.40±0.08 ^a	13.97±0.25	2.98±0.18 ^b	1.88±0.04 ^a	0.37±0.01

^{a-b} Means within the same column with different superscripts differ significantly ($P \leq 0.05$)

¹ n = # of experimental units, each experimental unit = 1 hen

² BW = average live body weight of the hens

³ Left Ovary = (left ovary weight/BW)*100

⁵ Left Oviduct = (left oviduct weight/BW)*100

⁶ *Pectoralis minor* = (*Pectoralis minor* weight/BW)*100

⁷ *Pectoralis major* = (*Pectoralis major* weight/BW)*100

⁸ Abdominal Fat = (abdominal fat pad weight/BW)*100

⁹ Liver = (liver weight/BW)*100

¹⁰ Heart = (heart weight/BW)*100

¹¹ SEM = standard error of the means

Table 9. Effect of hen age on egg weight at setting and transfer (18 d), relative egg weight loss, chick weight at hatching for two flocks of different ages (old and young) during two different broiler trials conducted 10 wk apart*

	Age ¹ (wk)	Egg Weight at Setting ² (g)	Egg Transfer Weight ³ (g)	Weight Loss at Transfer ⁴ (%)	Chick Weight ⁵ (g)
Trial 1					
Old	50	69.4 ^a	62.7 ^a	9.8 ^b	49.5 ^a
Young	27	56.0 ^b	50.2 ^b	10.4 ^a	39.4 ^b
SEM ⁶		0.42	0.42	0.15	0.34
Trial 2					
Old	60	73.0 ^a	63.5 ^a	12.9 ^b	50.8 ^a
Young	37	65.3 ^b	55.6 ^b	14.6 ^a	44.5 ^b
SEM		0.52	0.38	0.46	0.34

^{a-b} Means within the same column with different superscripts differ significantly ($P \leq 0.05$)

* Means based on 104 eggs /group of hens for trial 1, and 76 eggs/group of hens for trial 2

¹ Age = the age in weeks of the broiler breeder hens during each trial

² Egg Weight at Setting = the weight of the when placed into the incubator

³ Transfer Weight = the weight of the egg when transferred from the incubator to the hatcher at 18 d of incubation

⁴ % Weight Loss at Transfer = (egg wt at transfer/initial egg wt)*100

⁵ Chick Weight = weight of the chick at hatch

⁶ SEM = standard error of the means

Table 10. The effect of combined parent age on chick quality as measured by shank length, chick length and Tona Score*[‡]

Trial	Parent Age ¹	Shank Length ²	Chick Length ³	Tona Score
1	OO	30.1±0.13 ^{4bc}	183±0.67 ^a	94±0.7 ^b
1	OY	30.9±0.13 ^a	183±0.67 ^a	96±0.7 ^b
1	YO	29.3±0.13 ^d	175±0.67 ^c	98±0.7 ^a
1	YY	29.0±0.13 ^d	173±0.67 ^d	98±0.7 ^a
2	OO	30.7±0.15 ^a	184±0.78 ^a	98±0.8 ^a
2	OY	30.4±0.15 ^b	183±0.78 ^a	94±0.8 ^b
2	YO	29.9±0.15 ^c	180±0.78 ^b	94±0.8 ^b
2	YY	30.1±0.15 ^{bc}	181±0.78 ^b	96±0.8 ^b

^{a-d} Means within the same column with different superscripts differ significantly ($P \leq 0.05$)

* Means based on 52 chicks/parent group for trial 1 and 38 chicks/parent group for trial 2

[‡] Two flocks at different ages (old and young) were used for the parent combinations and two trials were conducted. During Trial 1 the old flock was 51wk of age and the young flock 28 wk and during Trial 2 the old flock was 61wk of age and the young flock 38 wk

¹ Parent Age: OO (Old hen x old roosters), OY (old hen x young roosters), YO (young hen x old roosters), YY (young hen x young roosters)

² Shank length – measured from the top of the hock to the pad of the bottom of the foot

³ Chick length – measured from the tip of the beak to the tip of the longest toe

⁴ SEM = standard error of the means

Table 11. Body weight as affected by hen age (old or young) and by combined parent age (OO, OY, YO, YY)

	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6
Hen age ¹						
Old	173 ± 27.8 ⁶ (52) ⁷	486 ± 27.8	943 ± 27.8 ^a (52)	1,520 ± 27.8 ^a (52)	2,179 ± 27.9 ^a (51)	2,586 ± 28.0 ^a (51)
Young	156 ± 28.8 (52)	441 ± 27.8	842 ± 27.8 ^b (52)	1,367 ± 27.8 ^b (52)	2,029 ± 29.0 ^b (52)	2,444 ± 29.0 ^b (52)
Parent age combination						
OO ²	160 ± 2.7 ^b (38)	413 ± 8.5 ^a (38)	816 ± 18.6 ^{ab} (38)	1,358 ± 31.2 ^a (37)	2,028 ± 51.8 ^a (37)	2,465 ± 68.0 ^a (37)
OY ³	173 ± 2.8 ^a (38)	425 ± 8.2 ^a (38)	842 ± 17.8 ^a (38)	1,352 ± 30.0 ^a (37)	1,959 ± 50.0 ^a (37)	2,384 ± 65.8 ^a (36)
YO ⁴	148 ± 2.7 ^c (38)	364 ± 8.3 ^b (37)	727 ± 18.3 ^c (36)	1,169 ± 30.8 ^b (36)	1,704 ± 51.1 ^b (36)	2,082 ± 67.3 ^b (34)
YY ⁵	150 ± 2.8 ^c (38)	386 ± 8.3 ^b (37)	779 ± 18.4 ^b (34)	1,271 ± 31.9 ^a (32)	1,922 ± 53.4 ^a (30)	2,358 ± 71.1 ^a (30)

^{a-c} Means within the same column with different superscripts differ significantly ($P \leq 0.05$)

¹ Hen age = hens from the old flock (51 wk of age) and hens from the young flock (28 wk of age)

² OO = parent age combination of old hens and old roosters (old flock – 61 wk of age)

³ OY = parent age combination of old hens and young roosters (old flock – 61 wk of age and young flock – 38 wk of age)

⁴ YO = parent age combination of young hens and old roosters (old flock – 61 wk of age and young flock – 38 wk of age)

⁵ YY = parent age combination of young hens and young roosters (young flock – 38 wk of age)

⁶ SEM = standard error of the means

⁷ (n) = # of experimental units, each experimental unit = 1 broiler

BUDGET:

Expenditure	Predicted Cost (\$)	Actual Cost (\$)
Airfare	755.30	577.38
Accommodations (Hamilton)	120.00	93.50
Shuttle (Hamilton to Niagara Falls and return)	231.00	241.50
Accommodations (Niagara Falls)	608.49	603.12
Meals (for 6 days)	270.00	126.85
Registration for Conference	120.00	99.01
Total	2,104.79	1,741.36

Already received: \$1,579.79

Still owed: \$161.57