"Revised"

Parental sex effect of parthenogenesis on hatchability and sperm-egg penetration in mated
Chinese Painted quail (*Coturnix chinensis*)¹

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Abstract

Selecting quail for an increased incidence of parthenogenesis also impacts egg weight and albumen pH as well as reduces hatchability and fertility due to decreased sperm-egg penetration (SEP). However, it is unknown which parental sex is responsible for these changes in quail selected for parthenogenesis. Therefore, the objective of this study was to determine which sex influences egg weight, albumen pH, hatchability, and SEP in birds selected for parthenogenesis. In this study, 2 lines of birds were used: 1 line that was selected for parthenogenesis and 1 line not selected for parthenogenesis (control). Treatments were as follows: control females w/control males, control females w/parthenogenetic line males, parthenogenetic line females w/control males, and parthenogenetic line females w/parthenogenetic line males. Fresh eggs were collected daily, labeled and analyzed for albumen pH and SEP or incubated at 37.5°C for 20 d of incubation. Eggs were candled at 10 days of incubation (DOI) and eggs exhibiting little or no embryonic development were removed and broken open to determine hatching failure. This was repeated at 20 DOI for eggs that did not hatch. A dam main effect for egg set weight existed with parthenogenetic line dams exhibiting heavier eggs than control dams. The parthenogenetic line dams and sires exhibited lower albumen pH and hatch but a higher incidence of parthenogenesis than control line dams or sires. However, only a sire main effect existed for fertility and SEP. Sires from the parthenogenetic line yielded the highest infertility due to lower SEP. In conclusion, both the parthenogenetic line dams and sires contribute to reduced reproductive performance. However, it appears that the sire from the parthenogenetic line is responsible for lower fertility due to a reduction in SEP. Because the sire has a negative impact on overall fertility, it is possible that

males selected for parthenogenesis have poorer semen quality resulting in fewer sperm traversing the oviduct or penetrating the perivitelline layer.

Keywords: Parthenogenesis, egg weight, hatchability, albumen pH, embryonic mortality

1. Introduction

Parthenogenesis is a spontaneous, abortive form of embryonic development which can occur in an unfertilized avian egg [1]. During fertilization and the final steps of oogenesis in birds, the second polar body is extruded from the ovum. However, parthenogenesis is thought to occur due to either the second polar body not being extruded from the ovum or by re-entry of the second polar body thereby restoring diploid chromosomes [1]. In fact, in young hens with erratic clutch sequences, Mong et al. [2] reported an increase in triploid chromosomes due to errors that occurred during oogenesis, such as the lack of formation of the second polar body.

Parthenogenesis has been reported in both virgin [3] and mated Chinese Painted quail [4, 5]. However, parthenogenesis negatively impacts hatchability in mated quail [4, 5]. Interestingly, Wells et al. [6] reported that eggs from virgin hens containing parthenogens were larger at set and lost less weight over incubation as opposed to eggs with no parthenogenetic development. These larger eggs that exhibit a lack of egg weight loss during incubation could contribute to the reduction in hatchability for mated quail selected for the parthenogenesis trait [4, 5]. For example, in broiler breeders it has been reported that the first egg in the clutch sequence is larger and loses less weight over incubation versus subsequent eggs in a sequence [7]; however, these first eggs also exhibit decreased embryo viability [8]. In fact, quail selected for parthenogenesis also have decreased clutch lengths and therefore more first eggs in a clutch [9] that potentially contribute to their decrease in hatchability when mated.

Using virgin quail, Santa Rosa et al. [10] revealed that parthenogens alter their environment within the egg by altering albumen pH, ion concentrations, and gas exchange. Interestingly, these same alterations in albumen, due to parthenogenesis, also occur in mated quail [11]. In fact, when comparing albumen from parthenogens to fertilized embryos, albumen pH, O₂, Ca²⁺, and protein concentrations were similar. However, albumen from unfertilized eggs without embryonic development had a higher pH and O₂ concentration and a lower CO₂ concentration as compared to parthenogens. Due to the similarities in albumen pH, O₂, Ca²⁺, and protein concentration between fertilized eggs and eggs containing parthenogens, Santa Rosa et al. [11] concluded that parthenogens are apparently utilizing O₂ and creating CO₂ like embryos in fertilized eggs. Additionally, it is also possible that the parthenogenetic trait reduces pH and alters ionic composition of albumen as it is secreted in the magnum, which could contribute to the reduction of hatchability in fertilized eggs reported in mated Chinese Painted quail that have been selected for parthenogenesis [4, 5]. Because these ionic alterations in albumen would occur after ovulation [10, 11], normal embryonic differentiation in fertilized eggs, and therefore hatchability, may be hindered in the parthenogenetic line of birds due to these changes in secreted albumen.

On the other hand, low fertility is at least partially responsible for the reduction in hatchability exhibited by Chinese Painted quail selected for the parthenogenetic trait [4, 5]. Using sperm-egg penetration (SEP), Santa Rosa et al. [11] determined that there were significantly less sperm holes in the perivitelline layer of eggs from quail selected for parthenogenesis versus a control, unselected line of quail. Perhaps hatchability is negatively impacted in Chinese Painted quail selected for parthenogenesis [4, 5] by altering egg set weight, albumen pH, SEP, and fertility. It is possible that these alterations due to selection for

parthenogenesis could be influenced by the female, male or a cumulative effect of both sexes. Therefore, the objective of this study was to determine which parental sex (male or female) or combination of both impacts egg set weight, albumen pH, fertility, and hatchability in birds selected for the parthenogenetic trait.

2. Materials and Methods

2.1.1. Housing and care

Chinese Painted quail eggs were initially obtained from a random breeding stock and chicks were then divided into 2 lines of birds. One line of birds was selected for the parthenogenetic trait over 10 generations [5, 9]. The other quail were randomly bred to maintain a line that was not selected for the parthenogenetic trait. For both lines of quail, males and females were brooded together until 4 wk of age. Quail chicks were fed a commercial starter diet until 4 wk of age when males were separated from females. After males were separated from females, both sexes were placed on a commercial breeder diet. Quail were fed ad libitum and were exposed to 17 h of light. Prior to sexual maturity, at 4 wk of age when male plumage is visible, males from both the unselected control and parthenogenetic line were separated from females and placed in colony cages. At 6 wk of age, females from the parthenogenetic line were individually caged to monitor their incidence of parthenogenesis as virgins. Birds were treated in accordance with the Guide for Care and Use of Laboratory Animals [12].

2.1.2. Egg collection for parthenogenetic virgin quail

For the parthenogenetic line of birds, to determine each virgin hen's incidence of parthenogenesis, eggs were collected daily and labeled prior to incubation then incubated for 10 d at 37.5°C and 50% relative humidity. After 10 d of incubation (DOI), eggs were broken open

to examine the germinal disc for the presence of parthenogenesis using a 2 x magnifying lamp. If parthenogenesis was detected, the germinal disc was measured across the widest part [3]. Due to the fact that most parthenogens appeared as unorganized, undifferentiated cells, embryos were not staged using Hamburger and Hamilton [13]. Each virgin's incidence of parthenogenesis was determined when 20 eggs were laid [3].

2.1.3. Virgin quail selection for mating

After each virgin hen from the parthenogenetic line had laid 20 eggs containing 20 to 40% parthenogenesis, they were selected for breeding in this trial. For the unselected, or control birds, the quail were randomly selected for mating. There were 4 parental breeding pairs used in this study: control line dams + control line sires (CC), control line dams + parthenogenetic line sires (CP), parthenogenetic line dams + control line sires (PC), and parthenogenetic line dams + parthenogenetic line sires (PP). In this study, parthenogenetic line males were selected for mating based on their sister's incidence of parthenogenesis. The sister's incidence of parthenogenesis also ranged between 20-40%.

2.1.4. Sperm-egg penetration in fresh eggs

After parental pairings were made and birds were placed into individual cages, fresh eggs were collected daily, labeled, and weighed then SEP was performed on eggs from these breeder pairings (n=768 eggs from 48 breeding pairs). As fresh eggs were broken open for SEP, albumen pH was determined using pH strips [10, 11]. Also, eggs that were broken open were classified as either fertilized, unfertilized, or eggs exhibiting parthenogenesis using a 2 x magnifying lamp [3]. Eggs that were classified as fertilized exhibited an area opaca, area pellucida, and periblastic ring. The germinal disc of unfertilized eggs contained numerous

vacuoles, while the germinal disc from eggs exhibiting parthenogenesis appeared as densely layered sheets of epithelial cells. For pictures of each classification see Santa Rosa et al. [11].

2.1.5. Candling at 10 days of incubation (DOI)

Eggs from each parental pairing were also collected daily, labeled, weighed, and incubated at 37.5°C and 50% relative humidity to examine embryonic development. All incubated eggs were weighed to determine egg weight loss at 10 DOI. Also, eggs were candled at 10 DOI, and eggs that were clear or showing little to no embryonic development (n=1,580 eggs from 92 breeding pairs) were broken open to determine at what stage the embryonic failure occurred. Albumen pH was determined using pH strips [10, 11]. Eggs candled at 10 DOI without viable embryos were classified as unfertilized or fertilized eggs with early dead embryos as classified according to Ainsworth et al. [14]. Unfertilized eggs classified as exhibiting parthenogenesis appeared as freshly laid eggs after 10 DOI, with a germinal disc of ≤ 7 mm [4, 5]. Eggs with viable embryos at candling were returned to the incubator.

2.1.6. Hatch Residue Analysis

Incubated eggs that did not hatch were broken open at 20 DOI to determine at what stage the hatching failure occurred [12]. Hatching failures were measured as follows: unfertilized eggs with no development, unfertilized eggs classified as containing parthenogens (≤ 7 mm; 3, 4), early dead (1-6 d), middle dead (6-12 d), and late dead (12-18 d). During the hatch residue analysis 644 eggs were classified as unfertilized, parthenogens or early dead embryos because they were missed when eggs were candled at 10 DOI due to dark shell color. For hatch residue analysis, eggs broken out at 10 DOI were combined with eggs broken open for hatch residue, resulting in 4,577 total eggs examined from 92 breeding pairs for hatching failures. In this study,

because parthenogens are from unfertilized eggs, the percentage of total unfertilized eggs was calculated as a summation of the percentage of unfertilized eggs with and without parthenogens.

2.2. Statistics

A 2 dam (C and P) x 2 sire (C and P) factorial arrangement of treatments was utilized. Data were analyzed as a completely randomized design with each individual parental pair serving as the experimental unit. After making each parental pairing, breeding pair means were determined for egg set weight, incubational egg weight loss, albumen pH, each hatchability parameter, and SEP. When global $P < 0.10$, means were separated using Fisher's protected LSD with α set at 0.05 [15].

3. Results

Fresh egg albumen pH and SEP for eggs classified as fertilized, unfertilized without embryonic development, or eggs exhibiting parthenogenesis are presented in Table 1. The albumen pH from fresh eggs was lower if they contained parthenogenetic development as opposed to eggs classified as fertilized or unfertilized (Table 1; $P < 0.0001$). However, the pH for fertilized and unfertilized eggs were similar. Fertilized eggs had the highest number of sperm entering the germinal disc area as opposed to unfertilized eggs and eggs exhibiting parthenogenesis, yet the number of sperm entering the germinal disc were similar between unfertilized eggs and eggs exhibiting parthenogenesis (Table 1; $P < 0.0001$). However, eggs classified as unfertilized or exhibiting parthenogenesis had the highest percentage without any sperm holes as opposed to fertilized eggs (Table 1; $P < 0.0001$). There were no differences in the percentage of eggs that did not contain sperm penetration between unfertilized eggs and eggs exhibiting parthenogenesis.

Dam and sire effects for fresh egg albumen pH and eggs classified as fertilized, unfertilized without embryonic development, or eggs exhibiting parthenogenesis are presented in Table 2. For both the parthenogenetic line dams and sires, albumen pH from every type of fresh egg (fertilized eggs, unfertilized eggs, and eggs containing parthenogenetic development) was lower as opposed to eggs from the control line dams and sires. Also, there was a dam by sire interaction for fresh egg pH in unfertilized eggs. The fresh egg albumen pH for unfertilized eggs was lower for PP as compared to PC, CP, and CC eggs. However, there were no differences in fresh egg albumen pH of unfertilized eggs for PC, CP, and CC.

Fresh egg breakout revealed a sire main effect for the percentage of fertilized eggs, the mean number of sperm holes in a 1.35 mm^2 area surrounding the germinal disc, and the percentage of eggs that contained no sperm penetration (Figures 1A, 1B, and 1C). The parthenogenetic line males had lower fertility and less sperm penetrating the germinal disc as well as more eggs that did not contain any sperm penetration as opposed to the control line sires ($P=0.10$, 0.008 , and 0.02 , respectively). In contrast, there were no effects due to the dam line for percentage of fertilized eggs, the mean number of sperm holes, and the percentage of eggs that contained no sperm penetration (Figures 1A, 1B, and 1C, respectively).

For eggs broken open at 10 DOI, there were dam main effects for initial egg set weight ($P=0.002$), egg weight loss ($P=.10$), and albumen pH ($P=0.011$; Table 3). The parthenogenetic line dams had a higher initial set weight yet a lower percentage of egg weight loss at 10 DOI than did the control line dams. Also, the parthenogenetic line dams had a lower albumen pH as opposed to the control line dams. However, there was no effect due to sire line for initial egg set weight, egg weight loss, and albumen pH (Table 3). Unfertilized eggs without embryonic development after 10 DOI yielded the highest albumen pH when compared to eggs exhibiting

parthenogenesis, early, or middle embryonic mortality (Table 4; $P < 0.0001$). However, there were no differences in albumen pH among eggs exhibiting parthenogenesis, early, or middle embryonic mortality.

For eggs broken open after 20 DOI, there was a dam main effect for initial egg set weight of each hatching failure (Table 5). For eggs that hatched ($P < 0.0001$), unfertilized eggs ($P = 0.0002$), eggs exhibiting parthenogenesis ($P = 0.0002$), middle dead ($P = 0.03$) and late dead embryonic mortality ($P = 0.0007$), dams from the parthenogenetic line consistently yielded heavier eggs as compared to the control line dams. However, there were no egg weight effects due to sire line for eggs that hatched, unfertilized eggs, eggs exhibiting parthenogenesis, middle dead, or late dead (Table 5).

The dams and sires of the control line had the greatest percentage of eggs that hatched as opposed to the parthenogenetically selected line of dams and sires (Figure 2A; $P = 0.004$ and 0.006 , respectively). However, the parthenogenetic line of dams and sires exhibited the highest percentage of eggs containing parthenogenetic development when compared to the control line dams and sires (Figure 2B; $P < 0.0001$ and 0.025 , respectively). There was also a dam by sire interaction for the percentage of eggs exhibiting parthenogenesis (Figure 3; $P = 0.09$). Parthenogenetic line dams mated to males from the parthenogenetic line had the highest percentage of eggs exhibiting parthenogenesis as opposed to all other breeding pair combinations (PC, CP, CC). However, there were no differences in the incidence of parthenogenesis between PC, CP, or CC.

The parthenogenetic line sires had the highest percentage of unfertilized eggs with no development (Figure 4A; $P = 0.09$) and total unfertilized eggs (unfertilized eggs with and without parthenogenetic development; Figure 4B; $P = 0.0009$). Additionally, the parthenogenetic line

dams yielded a higher percentage of early embryonic mortality as opposed to the control line dams (23.2 ± 2.92 and $14.83 \pm 2.79\%$, respectively; $P=0.043$).

4. Discussion

It appears that parental sex affects egg-set weight, incubational egg weight loss, albumen pH, fertility and hatchability in Chinese Painted quail selected for the parthenogenetic trait. In fact, the parthenogenetic line dams had the greatest impact on initial egg set weight for each of the individual hatch parameters. Also, in virgin quail, Parker and McDaniel [3] reported that the first egg in a clutch sequence was most likely to exhibit parthenogenesis as opposed to subsequent eggs in a clutch sequence. As a result of a shortened clutch sequence due to selection for the parthenogenetic trait, more first eggs in a clutch sequence are produced in the parthenogenetic line [9]. In chickens, Robinson et al. [7] reported that initial egg weight was greater for the first egg in a clutch and that the first egg in the clutch sequence lost less weight over 7 DOI as opposed to subsequent eggs in the clutch sequence. Incubational egg weight loss was also reduced in the current study for the dams from the parthenogenetic line. Using virgin Chinese Painted quail, Wells et al. [6] reported that eggs exhibiting parthenogenesis had a lower percentage of incubational egg weight loss as compared to eggs exhibiting no embryonic development. It appears that this difference in egg weight loss also occurs in mated Chinese Painted quail and that this reduction in egg weight loss is due mostly to the dams of the parthenogenetic line. Because the parthenogenetic line of birds have been intensively selected for the parthenogenetic trait thus resulting in more first eggs in a clutch, this could explain why the parthenogenetic line dams lay heavier eggs with less egg incubational weight loss when compared to the control line dams. Interestingly, the first egg in the clutch sequence is also larger and loses less weight over incubation versus subsequent eggs in a sequence in broiler

breeders [7]; but these first in sequence eggs have poor embryo viability [8]. Therefore in the current study, because the dams of parthenogenetic line of birds lay heavier eggs that lose less egg weight over incubation, it is possible that these alterations lead to lower embryo viability and hence decreased hatchability.

The parthenogenetic line dams not only exhibited altered egg weight and egg weight loss over incubation, but the parthenogenetic trait in dams as well as sires also impacted albumen pH and the incidence of parthenogenesis. In virgin quail, Santa Rosa et al. [10] reported that albumen pH declined over incubation in eggs that exhibited parthenogenesis as compared to eggs exhibiting no embryonic development. In the current study, pH from fresh eggs was lower yet the incidence of parthenogenesis was greater for the parthenogenetic line dams and sires as compared to the control line. Because eggs exhibiting parthenogenesis had a lower pH than eggs with no embryonic development or fertilized eggs (Table 2), it is apparent that the incidence of parthenogenesis in the current study had an overall impact on albumen pH. In fact, in the current study, the presence of the parthenogenetic trait in the dams or sires reduced the albumen pH of all classifications of fresh eggs including parthenogenetic embryos, unfertilized eggs, and fertilized eggs. However, the reduction in pH seen in parthenogenetic embryos was twice as much as seen in both unfertilized and fertilized eggs (Table 1).

However, the albumen pH of every type of fresh egg (fertilized, unfertilized, or parthenogen) was reduced for both dams and sires from the parthenogenetic line. For the dams, it is possible that albumen pH, at the moment of secretion in the magnum, may be reduced in the parthenogenetic line. This reduced albumen pH during early embryonic development in the magnum could impact normal embryonic differentiation in fertilized eggs leading to higher early embryonic mortality and thus lower hatchability that has been reported in the parthenogenetic

line of quail [4, 5]. Also, these alterations in fresh egg albumen for the parthenogenetic line could possibly be due to the first egg of a clutch sequence remaining in the oviduct longer [8], and quail selected for parthenogenesis exhibit more first eggs in a clutch [9]. Because the first egg in a clutch sequence remains in the oviduct longer, it is possible that the egg also remains in the magnum longer. If in fact the egg is in the magnum longer, viable embryos are undergoing cellular division and are taking O₂ from albumen and thus releasing CO₂ [10]. Therefore, viable embryos in the magnum possibly alter albumen pH, because in the current study, albumen pH was lower for eggs exhibiting parthenogenesis and early or middle embryonic mortality as compared to unfertilized eggs.

However, the sire difference in fresh egg albumen pH for the parthenogenetic line versus the control line was less (0.25 pH units for unfertilized eggs, 0.36 pH units for fertilized eggs, and 0.46 pH units for parthenogens) than that seen between the 2 dam lines (0.52 pH units for unfertilized eggs, 0.47 pH units for fertilized eggs, and 0.91 pH units for parthenogens). Additionally no differences were detected in pH by 10 DOI due to sire line. It is unknown how the parthenogenetic line sire could alter albumen pH of unfertilized or fertilized fresh eggs. However, the reduced fertility seen in the current study for the parthenogenetic line sires would yield more unfertilized eggs that could then yield parthenogens and hence a greater chance of lower pH values for these eggs.

Additionally, the inferior fertility for the parthenogenetic line sires, appears to be due to the decrease in the mean number of sperm holes and the increased percentage of eggs without sperm holes. On the other hand, it appears that for the parthenogenetic line dams the incidence of parthenogenetic eggs is reduced due to the control line males high fertility and semen quality thus resulting in fertilized eggs (PC see Figure 3). However, the parthenogenetic line males

appear to have poor semen quality and fertility, therefore allowing for more unfertilized eggs and hence more parthenogenetic eggs. Olsen [1] also reported that hatched turkey parthenogens have poor semen quality. Because the parthenogenetic line sires had a significant reduction in SEP it is possible that the parthenogenetic trait itself leads to poor semen quality thus resulting in less SEP. Conversely, perhaps sperm from the parthenogenetic line males are unable to bind to receptors on the egg surface and penetrate the egg [11]. In chickens, to insure that an egg is fertilized, the ova must be penetrated by a minimum of 30 sperm even though only the DNA from 1 sperm combines with the DNA of the ova [16]. However, in Chinese Painted quail it is unknown how many sperm must penetrate the ovum to insure a fertilized egg. In the present study, fertilized eggs yielded more than 3 times as many sperm penetration holes as unfertilized eggs and eggs exhibiting parthenogens indicating that most eggs were correctly classified as fertilized, unfertilized or parthenogen (Table 1). However, the ranges in sperm-egg penetration were wide for each category of eggs (fertilized: 0-923 holes, unfertilized: 0-475 holes, and parthenogen: 0-817 holes). These data indicate that perhaps more than 30 sperm are required to insure maximum fertilization of the Chinese Painted quail egg and that even unfertilized eggs and parthenogens can exhibit sperm-egg penetration. Therefore, it is possible that even though sperm penetrate the avian egg, syngamy of the male and female pronucleus may not occur allowing for parthenogenetic development. This may especially be true for the parthenogenetic line sires that exhibit reduced fertility even though more than 34 of their sperm penetrate the egg. Additionally, it is also possible that at breakout a few fertilized eggs were misclassified as unfertilized or parthenogen due to pre-ovipositional death of the embryo soon after fertilization.

Because sperm-egg penetration and fertility of the dam lines were not different, it does not appear that the parthenogenetic trait within the dams hinders fertilization. Yet for the

parthenogenetic line dams, there was a 20% decline in hatch of chicks when compared to the number of chicks hatched from the control line dams. In the current study, the reduction in hatch of eggs set for the parthenogenetic line dams appears to be due to approximately 8% and 17% increases in early embryonic mortality and eggs exhibiting parthenogenesis, respectively. Parker et al. [4] reported that prior to mating, virgin hens exhibiting a high incidence of parthenogenesis also exhibited a high incidence of parthenogenesis after mating as opposed to the virgins that did not exhibit parthenogenesis prior to mating. The results for the higher incidence of parthenogenesis from the current trial are similar to those reported by Parker et al. [4]. The increase in parthenogenesis in the parthenogenetic line dams could possibly be due to meiotic failures which occur during oogenesis. For example, Mong et al. [2] reported that failures occurring during meiosis II can result in diploid ova that become triploid at fertilization. In that study, 81% of the triploid embryos examined were due to a lack of formation of the second polar body in oogenesis [2]. In fact, Mong et al. [2] reported that triploid embryos were more common in younger hens that had shorter clutches. Parthenogenetically selected quail hens have shorter clutch sequences [9], possibly resulting in more opportunities for the first egg in the clutch sequence to exhibit parthenogenesis due to these meiotic failures that would yield diploid ova without fertilization and triploidy if they were fertilized. Also, the chromosomal abnormalities such as triploidy reported by Mong et al. [2] could explain the increased incidence of early embryonic mortality in fertilized eggs from the parthenogenetic line dams. For example, in the current study the majority of early embryonic mortality consisted of membranous tissue with no differentiation. Therefore it is possible that this early embryonic mortality exhibiting a lack of cellular differentiation is due to polyploidy as result of meiotic failures. Additionally, this early embryonic mortality appears similar to that of eggs stored for an extended period with excessive

proliferation of the extraembryonic membranes [17]. However, in the current study eggs were never stored more than 2 d.

The approximate 20% reduction in the hatch of eggs set for the parthenogenetic line sires is due to an approximate 10% increase in the incidence of parthenogenesis plus a 10% decrease in fertility. Since parthenogens form from unfertilized eggs, when combining eggs exhibiting parthenogenesis with unfertilized eggs, total infertility was approximately 20 % greater for the parthenogenetic line sires versus the control line sires. As mentioned previously, the increased incidence of unfertilized eggs in the parthenogenetic line is most likely due the to the sire's inability to fertilize an egg as opposed to the dam's fertility.

4.1. Conclusion

In conclusion, it appears that both the dam and sire from the parthenogenetic line of birds negatively impact reproductive performance. For example, the parthenogenetic line dams lay heavier eggs and these eggs lose less weight during incubation. However, both the parthenogenetic line dams and sires exhibit a reduction in albumen pH and hatchability as well as an increased incidence of parthenogenesis, yet only the parthenogenetic line sires exhibit reduced fertility. This increase in infertility is due to a lack of sperm from the parthenogenetic line sires penetrating the germinal disc area. Additional research is needed to determine if the reduction in SEP for the parthenogenetic line sires is due to alterations in sperm concentration, viability, motility, or egg receptors on the sperm plasma membrane.

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Table 1. Fresh egg pH and sperm-egg penetration for eggs classified as fertilized, unfertilized without embryonic development, or containing parthenogenetic development (mean±SEM)¹.

	pH	Number of Sperm Holes	Eggs without Sperm Holes (%)
Fertilized Eggs	9.29±0.033 ^a	107±5.47 ^a	13.08±1.96 ^b
Unfertilized eggs without development	9.38±0.058 ^a	34±9.49 ^b	61.27±3.40 ^a
Eggs exhibiting parthenogenesis	8.76±0.049 ^b	29±8.06 ^b	67.51±2.89 ^a
P value	<0.0001	<0.0001	<0.0001

^{a-b} Percentages within a column with different superscripts are significantly different.

¹ 48 breeding pairs, 768 total eggs.

Table 2. Dam and sire effects for fresh egg pH and eggs classified as fertilized, unfertilized without embryonic development, or containing parthenogenetic development (mean±SEM)¹.

	Fertilized Eggs	Unfertilized Eggs	Parthenogenetic Eggs
DAM LINE			
Parthenogenetic	8.91±0.106 ^b	9.06±0.090 ^b	8.35±0.114 ^b
Control	9.38±0.124 ^a	9.58±0.102 ^a	9.26±0.141 ^a
SIRE LINE			
Parthenogenetic	8.95±0.106 ^b	9.17±0.092 ^b	8.53±0.114 ^b
Control	9.31±0.124 ^a	9.42±0.098 ^a	8.99±0.141 ^a
Interaction			
CC	9.51±0.176	9.57±0.156 ^a	9.40±0.206
CP	9.24±0.176	9.59±0.135 ^a	9.13±0.193
PC	9.11±0.176	9.32±0.127 ^a	8.63±0.193
PP	8.79±0.132	8.80±0.127 ^b	8.20±0.141
P Values			
Dam	0.014	0.0008	<0.0001
Sire	0.08	0.08	0.07
Interaction	0.87	0.055	0.67

^{a-b} Percentages within a column with different superscripts are significantly different.

¹ 48 breeding pairs, 768 total eggs.

410 Table 3. Dam and sire effects for initial egg set weight, egg weight loss, and pH at 10 d of
 411 incubation (mean±SEM)¹.

	Initial set weight (g)	Egg weight loss (%)	pH
DAM LINE			
Parthenogenetic	5.82±0.083 ^a	8.05±0.66 ^b	8.43±0.117 ^b
Control	5.45±0.081 ^b	9.69±0.65 ^a	8.87±0.115 ^a
P value	0.002	0.10	0.011
SIRE LINE			
Parthenogenetic	5.70±0.078	8.23±0.62	8.64±0.108
Control	5.53±0.086	9.74±0.69	8.67±0.125
P value	0.21	0.17	0.91

412 ^{a-b} Percentages within a column with different superscripts are significantly different.

413 ¹ 92 breeding pairs, 1,580 total eggs.

414
 415 Table 4. Albumen pH of eggs classified as unfertilized with no development, unfertilized
 416 exhibiting parthenogenesis, and embryonic mortality after d 10 of incubation (mean±SEM)¹.

	pH
Unfertilized Eggs	9.91±0.058 ^a
Parthenogenetic Eggs	7.89±0.062 ^b
Early embryonic mortality	7.99±0.058 ^b
Middle embryonic mortality	8.06±0.168 ^b
P value	<0.0001

417 ^{a-b} Percentages within a column with different superscripts are significantly different.

418 ¹ 92 breeding pairs, 1,580 total eggs.

419 Table 5. Dam and sire main effects for egg set weight of each hatching failure classification from
420 hatch residue analysis (mean±SEM)¹.

	Hatched eggs	Unfertilized eggs	Parthenogens	Early dead embryos	Middle dead embryos	Late dead embryos
DAM LINE	----- g -----					
Parthenogenetic	5.82±0.071 ^a	5.83±0.087 ^a	5.82±0.083 ^a	5.80±0.100 ^a	5.61±0.118 ^a	5.80±0.100 ^a
Control	5.37±0.067 ^b	5.32±0.086 ^b	5.33±0.089 ^b	5.35±0.099 ^b	5.23±0.105 ^b	5.31±0.090 ^b
P value	<0.0001	0.0002	0.0002	0.0018	0.0277	0.0007
SIRE LINE						
Parthenogenetic	5.63±0.072	5.66±0.082	5.69±0.081	5.67±0.094	5.46±0.109	5.53±0.095
Control	5.54±0.066	5.46±0.091	5.47±0.092	5.46±0.106	5.34±0.113	5.52±0.095
P value	0.302	0.221	0.127	0.205	0.548	0.942

421 ^{a-b} Percentages within a column with different superscripts are significantly different.

422 ¹ 92 breeding pairs, 4,577 total eggs.

Figure 1. Sire main effects for the percentage of fertilized eggs (A), mean number of sperm holes in a 1.35 mm² area surrounding the germinal disc (B), and percentage of eggs not containing sperm in fresh eggs (C). ^{a-b} Means for the percentage of fertilized eggs, number of sperm holes and the percentage of eggs not containing sperm holes (P=0.10, 0.008, and 0.02, respectively) are significantly different. However, there were no effects due to dam line (A: P=0.64, B: P=0.49, and C: P=0.57).

Figure 2. Dam and sire main effects for the percentage hatch of eggs set (A) and eggs exhibiting parthenogenesis (B). ^{a-b} Means for the percentage hatch of eggs set for the dam and sire main effects (A: $P=0.004$ and 0.006 , respectively) were significantly different. For eggs exhibiting parthenogenesis, the dam and sire main effects were also significant (B: $P<0.0001$ and 0.025 , respectively).

Figure 3. Dam by sire interaction for the percentage of eggs exhibiting parthenogenesis. Treatments are represented as follows: control line dams mated to control line sires (CC), control line dams mated to parthenogenetic line males (CP), parthenogenetic line dams mated to control line males (PC), and parthenogenetic line dams mated to parthenogenetic line sires (PP). Means for the percentage of eggs exhibiting parthenogenesis are significantly different ($P=0.09$).

Figure 4. Sire effect on the percentage of unfertilized eggs (A) and total unfertilized eggs (eggs exhibiting parthenogenesis and unfertilized eggs; B) after 20 days of incubation. ^{a-b} Means for the percentage of unfertilized (P= 0.09) and total unfertilized eggs (P=0.009) are significantly different. However, there were no effects due to dam line (A: P=0.31 and B: P=0.12).