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Exploration of age-related changes in reproductive parameters of female Japanese quail (*Coturnix japonica*)

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ABSTRACT

The decline in reproductive efficiency during post-peak period of production in poultry species holds significant economic implications. This study aimed to investigate the productive and reproductive performance of Japanese quails across distinct production stages and the association between these parameters and some genes expression and histometric alterations within the reproductive system. A total of 180 quails from a commercial flock were selected at varying egg production stages, including young, mature, and old, with 45 female and 15 male quails allocated to each group. The quails were maintained for six weeks. During recording period, daily records of egg production and egg weight were recorded. Additionally, oviduct histometric and Follicle biometric measurements, along with mRNA transcript abundance assessments related to follicular selection and yolk accumulation, were conducted on the oviduct, ovary, and small yellow follicles at the end of the experimental period. The results revealed a decrease in egg production in the old group compared to the young and mature groups ($P < 0.05$); meanwhile, the old group had the highest egg weight, and F1 follicle weight ($P < 0.05$). Additionally, the number of prehierarchical follicles was lower in the mature and old groups compared to the young group ($P < 0.05$). The lowest oviduct length, primary and secondary fold height, and thickness of the isthmus and magnum were noted in the old group ($P < 0.05$). Fertility and hatchability were lower in the old group compared to the other groups ($P < 0.05$). The mRNA transcript abundance of anti-Mullerian hormone (*AMH*), was highest in the old group and lowest in the young group ($P < 0.05$), while the mRNA transcript abundance of bone morphogenetic protein 15 (*BMP15*) was higher in the mature group compared to the other groups ($P < 0.05$). Additionally, the young quails had the highest occludin (*OCLN*) mRNA transcript abundance compared to other groups ($P < 0.05$). Overall, the study findings indicate decreased production and reproductive performance, as well as reduced hatchling quality over the production period, attributed to a declining number of follicles, noncooperative gene expression related to follicle selection and yolk accumulation, and diminishing oviduct fold size.

Introduction

Efficient reproduction is vital for poultry productivity and profitability. In quails, factors like age, diet, sex ratio, and environmental conditions significantly influence reproductive efficiency. Aging correlates with decreased egg production, fertility, and chick production, impacting various reproductive parameters (Lillpers and Wilhelmson, 1993; Farooq et al., 2012; Hao et al., 2021., He et al., 2023). Studies have highlighted the detrimental impact of aging on production and reproduction in poultry (Holmes et al., 2003; Liu et al., 2018; Ma et al., 2020).

Aged birds exhibit several significant changes, including reduced reproductive hormones (Buyuk et al., 2010), decreased yolk synthesis and accumulation (Zakaria et al., 1983), a lower number of ovarian follicles (Zakaria et al., 1983; Hao et al., 2021), and decreased oviduct weight and length (Gonzalez-Moran, 2016), all of which contribute to a

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poor fertility and hatchability (Santos et al., 2015; Hameed et al., 2016). It is widely acknowledged that changes in the dynamics and growth of ovarian follicles and yolk accumulation in follicles are the most critical factors associated with lower reproductive performance in post-peak production birds (Zakaria et al., 1983; Johnson et al., 1986; Hao et al., 2021).

The mechanism of follicular selection and yolk accumulation in birds is complex and involves various hormones and factors. Anti-Mullerian hormone (*AMH*), bone morphogenetic protein 15 (*BMP15*), occludin (*OCLN*), and the specific yolk receptor (*LR8*) are the most important genes in process of follicular development and yolk accumulation in birds (Schuster et al., 2004; Schneider, 2009; Stephens and Johnson, 2016; Francoeur et al., 2024).

While some roles of genes related to follicular selection and yolk accumulation have been elucidated, the intricate regulation of this network and its association with aging or the post-peak production period remains incompletely understood in quails. Additionally, it is important to note that oviduct is a site of egg formation and fertilization. Aging is associated with oviduct atrophy in birds (Kimaro et al., 2013; Saemi et al., 2018; Varga et al., 2019) and decreases the length of the oviduct, fold size, the tunica mucosal area, and the tubal glands (Gonzalez-Moran, 2016; Sukhadeve et al., 2021).

Changes in expression of genes related to follicular selection and yolk accumulation between different ages of quails have not been thoroughly investigated, therefore this study aimed to assess the expression of above-mentioned genes, as well as egg production, oviduct histometric parameters, and fertility in Japanese quails across different age groups.

Materials and methods

This study received approval from the Animal Care Committee and Animal Research Ethics Board within the Department of Animal Science at University of Tehran, Iran (Approval Number: 73131587.6.23). All procedures adhered to the guidelines set forth by the Animal Care and Use Committee of the Iranian Council for Animal Care.

Bird management and experimental groups

One hundred and eighty laying Japanese quails (*Coturnix japonica*) were randomly selected from a commercial flock at different age, including young (6 weeks of age), mature (21 weeks of age), and old (40 weeks of age) (n=45 female and 15 male quails/group). All the male quails in this study had a same age (6 weeks of age). The quails were maintained for six weeks (adaptation and recording period). During recording period daily records of egg production and egg weight were recorded (Hansen *et al.*, 2003). Each group was subdivided into five replicates, each, consisting of 12 birds. Within each replicate, there were three male and nine female quails, maintaining a 1:3 male-to-female ratio (Hansen et al., 2003). The birds in each replicate were housed in cages measuring 80×80 cm and were subjected to a lighting schedule of 16 hours of light and 8 hours of darkness, with a brightness of 10 lux. The ambient temperature was maintained at 24±2 ºC, and ventilation was provided at a rate of 540 m^3/h throughout the experimental period. The quails were fed a basal diet (Table 1) formulated to meet their nutritional requirements according to National Research Council guidelines (1994) and had ad libitum access to fresh water.

Table 1
Ingredients and chemical composition of the diet.

Ingredient	Amount (%)
Corn	54.25
Soybean meal, 44% CP	34.80
Dicalcium phosphate	1.45
CaCO3	5.25
Common salt	0.20
NaHCO3	0.17
Vegetable oil	3.23
DL-Met, 99%	0.15
Mineral premix[1]	0.25
Vitamin premix[2]	0.25
Total	100
Calculated nutrient content	
AME (kcal/kg)	2900
CP (%)	20
Calcium (%)	2.5
Available phosphorus (%)	0.35
Sodium (%)	0.15
Lysine (%)	1.59
Methionine (%)	0.45
Met + cys (%)	0.77
Threonine (%)	0.77

[1] mineral premix supplied the following per kg diet: choline, 300 mg; iron, 50 mg; manganese, 120 mg; Zn, 110 mg; copper, 10 mg; selenium, 0 mg; iodine, 2 mg.

[2] Vitamin premix supplied the following per kg of diet: vitamin A, 11,000 IU; vitamin D3, 3500 IU; vitamin E acetate, 150 IU; vitamin K3, 5.0 mg; vitamin B1, 3.0 mg; vitamin B2, 12 mg; vitamin B3, 55 mg; vitamin B5, 15 mg; vitamin B6, 4 mg; vitamin B9, 2 mg; vitamin B8, 0.25 mg; and vitamin B12, 0.03 mg.

Egg weight and production

Throughout the experiment, the daily production of eggs and their respective weights [by a digital balance (0.01 g)] were recorded.

Necropsy

At the end of the experiment, ten females from each group (two birds per replicate) were randomly selected, weighed, and euthanized. The oviduct and ovary were then collected for subsequent histometric and biometric evaluations, respectively (Rafieian-Naeini et al., 2021). Additionally, samples of the walls of small yellow follicles (SYFs) were harvested (sun et al., 2022) and stored at -80 °C for relative gene expression analysis of follicular selection and yolk accumulation.

Ovarian follicle biometry

Following euthanasia, the ovaries were collected, and measurements were recorded for F1 follicle weight and diameter by digital balance (0.01 g) and digital caliper (0.01 mm), respectively, as well as the counts of small (SWF, 1-2 mm), large white (LWF, 2-4 mm), and small yellow follicles (SYF, 4-6 mm) (Hrabia et al., 2004).

Oviduct length and histometry

After oviduct collection, its length was measured, and small segments (approximately 2 cm) of the isthmus and magnum were excised for histometric analysis. These samples were fixed in 10% neutral buffered formalin, embedded in paraffin blocks, and sectioned using a rotary microtome (Rotary microtome, Didsabz company, model DS4055, Urmia, Iran) into 7-micron sections. Following staining with hematoxylin and eosin (H&E), optical microscopy equipped with a camera was employed for evaluation. At a magnification of 40X, images of the isthmus and magnum tissue were captured, and the length and width of the oviduct folds were measured (Rafieian-Naeini et al., 2021) utilizing ImageJ software (version 1.52a).

Reproductive performance and hatchling quality

All eggs collected during the experiment [young (n=201), mature (n=187), old (n=122)] were incubated. After 18 days, hatchability was determined ([number of chicks hatched/number of eggs set] × 100). Unhatched eggs were examined to assess infertility, calculate the fertility rate ([number of fertile eggs/total number of eggs set] × 100), and categorize embryonic mortality as early (1 to 6 days), mid (7 to 12

days), or late (13 to 18 days) embryonic mortality (Ainsworth et al., 2010; Parker et al., 2012). Additionally, hatchling quality and weight were evaluated (Tona et al., 2003).

RNA extraction, cDNA synthesis, and real-time PCR

Small yellow follicles (4-6 mm) were carefully dissected from the ovary, and the yolk was gently expelled into a petri dish. Subsequently, the follicular layers of all SYFs were rinsed with phosphate-buffered saline (PBS) to remove any adhering yolk and then stored at -80 °C until further evaluation of relative gene expression. Total RNA extraction from SYFs was performed using a commercial RNA extraction kit (RNX-Plus, SinaClon Co, Iran) following the manufacturer's instructions. Before adding RNX-Plus, tissues were homogenized with liquid nitrogen and then mixed with this reagent. Samples of RNA were stored at -80 °C. Before stored, RNA integrity was electrophoretically verified using ethidium bromide. The extracted RNA underwent DNase treatment (DNaseI, RNase-free, CinnaGen Co. Iran) to eliminate potential DNA contamination. Next, cDNA synthesis was carried out using a cDNA synthesis kit (AddScript cDNA Synthesis kit, Addbio Co. Korea) as per the manufacturer's protocol. Briefly, a mixture comprising of 3 µL of RNA, 10 µL of reaction buffer, 2 µL of dNTP mixture, 2 µL of oligo dT20, 1 µL of AddScript enzyme solution, and 2 µL of nuclease-free water was prepared to yield a final reaction volume of 20 µL. The cDNA synthesis was performed by incubating the mixture in a Thermal Cycler (Peqlab Biotechnologie GmbH, Primus 25 advanced, Erlangen, Germany) with a thermal program consisting of 10 min of priming at 25 °C, 60 min of reverse transcription at 50 °C, and 10 min of reverse transcriptase inactivation at 80 °C. The primers targeting the *AMH, BMP-15, LR8, OCLN*, and *β-actin* (housekeeping) genes are listed in Table 2.

For the real-time PCR reaction, 1 µL of cDNA was combined with 14 µL of a solution containing 2 µL of forward and reverse primers, 7.5 µL of Green PCR Master Mix (QuantiNovaTM SYBR Green PCR kit), and 4.5 µL of RNase-free water. Real-time PCR was performed using a Rotor-Gene 3000 (Corbett Co. Australia) with a thermal program comprising 10 min of predenaturation at 95 °C, followed by 40 cycles consisting of 15 s of denaturation at 95 °C and 60 s of annealing and extension at 60 °C. The comparative Ct value method was employed to determine the target gene expression concentrations relative to the *β-actin* gene. The relative changes in gene expression derived from real-time qualitative PCR experiments were calculated using the $2^{-\Delta\Delta CT}$ method, as described by Livak and Schmittgen (2001).

Statistical analysis

Continuous data were analyzed using analysis of variance (ANOVA) in a completely randomized design, utilizing the Proc GLM function of SAS 9.4 software (SAS Institute Inc., 2013). Before analysis, the normal distribution of the data was assessed through Shapiro–Wilk and Kolmogorov–Smirnov tests using the UNIVARIATE SAS procedure. Binary distributed data, such as fertility and hatchability, were analyzed using the GENMOD procedure with a logit odds ratio link function. Tukey's range test was employed for multiple comparisons of means. Results are presented as mean ± SEM, with significance levels indicated as $P < 0.05$ for statistically significant differences and $0.05 \leq P \leq 0.10$ for tendencies.

Results

Body weight, egg weight, and egg production

Table 3 presents the body weight (BW), egg weight, and egg production of laying Japanese quails at different ages. Body weight was not affected by production period ($P > 0.05$). However, egg weight was observed to be significantly higher in old group compared to other groups, while egg production was lowest in the old group ($P < 0.05$).

Ovarian follicle biometry

Various follicle biometric measurements of laying Japanese quails at different ages are shown in Table 4. The F1 follicle weight and diameter were higher in old group compared to other groups ($P < 0.05$). Conversely, the number of SWFs was lowest in the old group and highest in the young group ($P < 0.05$). Moreover, the number of LWFs and SYFs was higher in the young group compared to other groups ($P < 0.05$).

Oviduct length and histometry

Histometric evaluation of the isthmus and magnum in laying Japanese quails at different ages is presented in Table 5 and visually depicted in Fig. 1. The primary and secondary fold height and thickness of the isthmus were lower in the old group compared to other groups ($P < 0.05$). Similarly, the primary fold height and thickness in the magnum exhibited a similar trend, with the lowest values observed in old quails ($P < 0.05$). Although the secondary fold height of the magnum was not

Table 3
Body weight, egg weight, and egg production of the laying Japanese quail at the different ages (n=45 female quails per group).

Variable	Experimental group[1]			SEM[2]	*P* Value
	Young	Mature	Old		
Body weight (gr)	260.80	271.00	269.40	3.55	0.46
Egg weight (gr)	11.66[b]	12.11[b]	13.16[a]	0.10	<0.01
Egg production (%)	70. 47[a]	64.44[a]	41.52[b]	2.61	<0.01

a– c: Within each row, means with different superscripts differ significantly ($P < 0.05$).
[1] Quails at the different ages, including young, mature, and old.
[2] Standard error of the mean.

Table 2
Primer sequences used for real-time PCR.

Gene	Direction	Primer sequences (5′-3′)	Product size (bp)	Accession number
AMH	Forward	CCAATCCCTGCGAAACCT	136	XM_015886199.1
	Reverse	CACCTCCCCTGCGAAACAC		
BMP15	Forward	GCTGGAGGGGACAAAAgTGA	107	XM_015860035.2
	Reverse	TAGCGTGGGTTGTAGCGATG		
LR8	Forward	GCCTCCTGTAAAGTGTTCTACCA	189	XM_015848918.2
	Reverse	CACTGCCTAGTCCCATGGAT		
OCLN	Forward	TGAGACCGACTACACCACG	187	NM_205128.1
	Reverse	CTGATTGAGGCGGTCGTTGA		
β-actin	Forward	GACCTTCAACACCCCAGCCAT	118	NM_205518.2
	Reverse	GGGCACAGTGTGGGTAACACC		

Abbreviations: *AMH*, anti-Mullerian hormone; *BMP15*, bone morphogenetic protein 15; *LR8*, specific yolk receptor or very low-density lipoprotein receptor (*VLDLR*); *OCLN*, occludin.

Table 4
Follicle biometric measurements of laying Japanese quail at the different ages (n=45 female quails per group).

Variable	Experimental group [1]			SEM[2]	*P* Value
	Young	Mature	Old		
F1 follicle weight (gr)	3.01[b]	3.36 [b]	3.95[a]	0.01	<0.01
F1 follicle diameter (mm)	17.50[b]	18.43[ab]	18.97[a]	0.17	<0.01
Number of SWF[3]	19.60[a]	13.10[b]	11.10[c]	2.23	<0.01
Number of LWF[4]	18.10[a]	16.40[ab]	13.60[b]	0.65	<0.05
Number of SYF[5]	2.40[a]	1.30[b]	0.70[b]	0.13	<0.01

a– c: Within each row, means with different superscripts differ significantly ($P < 0.05$).
[1] Quails at the different ages, including young, mature, and old.
[2] Standard error of the mean.
[3] SWF: Small white follicle (1-2 mm) (Hrabia et al., 2004).
[4] LWF: Large white follicle (2-4 mm) (Hrabia et al., 2004).
[5] SYF: Small yellow follicle (4-6 mm) (Hrabia et al., 2004).

Table 5
Comparison of the isthmus and magnum folds of the laying Japanese quail at the different ages (n=45 female quails per group).

Variable	Experimental group [1]			SEM[2]	*P* value
	Young	Mature	Old		
Oviduct length (cm)	33.20[a]	32.20[a]	17.50[b]	0.46	<0.01
Isthmus					
Primary fold height (μm)	1280.19[a]	1262.73[a]	1032.52[b]	26.69	<0.01
Secondary fold height (μm)	690.02[a]	729.49[a]	485.58[b]	19.16	<0.01
Primary fold thickness (μm)	330.47[a]	354.38[a]	266.73[b]	7.58	<0.01
Secondary fold thickness (μm)	323.02[a]	343.95[a]	228.73[b]	8.35	<0.01
Magnum					
Primary fold height (μm)	1096.53[a]	1082.66[a]	897.34[b]	29.20	<0.01
Secondary fold height (μm)	571.30	559.64	537.32	23.59	0.82
Primary fold thickness (μm)	510.23[a]	559.20[a]	370.81[b]	14.47	<0.01
Secondary fold thickness (μm)	385.75[a]	353.46[ab]	290.00[b]	12.79	<0.01

a– c: Within each row, means with different superscripts differ significantly ($P < 0.05$).
[1] Quails at the different ages, including young, mature, and old.
[2] Standard error of the mean.

significantly affected by age, the old group showed thinner secondary folds compared to the young group ($P < 0.05$).

Reproductive performance and hatchling quality

Table 6 presents the reproductive performance and hatchling quality of laying Japanese quails at different ages. Fertility and hatchability were lower in the old group compared to other groups, with the highest values observed in the young group ($P < 0.05$). Early and middle embryonic mortality rates were highest in the old group; however, the different age groups did not have a significant effect on late embryonic mortality. Hatchling body weight was found to be higher in the old group, while hatchling quality was highest in the young group ($P < 0.05$).

Relative mRNA abundance

The mRNA transcript abundance of genes related to follicle selection and yolk accumulation is illustrated in Fig. 2. The mRNA transcript abundance of *AMH* was higher in the old group compared to the young group, which exhibited the lowest values ($P < 0.05$). Furthermore, the mRNA transcript abundance of *BMP15* was found to be higher in the mature group compared to other groups ($P < 0.05$). No significant difference was observed between different age groups regarding *LR8* mRNA transcript abundance. However, the mature group demonstrated a lower *OCLN* mRNA transcript abundance compared to the young and old groups ($P > 0.05$).

Discussion

Our results revealed a clear association between aging and reproductive parameters, representing a decline in the number of prehierarchical follicles, leading to reduced fertility and hatchability. Additionally, we observed notable alterations in the expression patterns of genes linked to follicle selection, yolk accumulation, oviduct histometry, and hatchling quality.

The relationship between BW and egg production in birds has been well documented in the literature (Lacin et al., 2008; Chen et al., 2006; Pan et al., 2014). In the current study, no significant variations were recorded among different ages in terms of BW, however egg production was affected by aging showing a lower egg production in the old group compared to other groups. This, aligns with findings from Santos et al. (2015) and Abd El-Azeem et al. (2018), who reported a stable BW in European and Japanese quail breeders throughout the production cycle. In contrast, studies by Lacin et al. (2008), Chen et al. (2006), and Pan et al. (2014) indicated an increase in post-peak production body weight in broiler breeders and a decline in body weight after 68 weeks in laying hens, suggesting that BW changes in laying birds may be influenced by species, production type, and recording duration.

Egg production is known to be significantly impacted by the age of birds (El-Wardany et al., 2016; Liu et al., 2018; Hao et al., 2021; He et al., 2023). The decrease in egg production through the reproductive aging is often linked to the alterations in ovarian follicle dynamics and growth. In the current study, the old group displayed characteristics such as a reduced number of prehierarchical follicles, shorter oviduct length, and diminished fold size, accompanied by disrupted gene expression related to follicular selection and yolk accumulation (*AMH, BMP15*, and *OCLN*). These observations provide insights into the decline in post-peak egg production.

Regarding ovarian follicle biometry, it was observed that the decline in quantity and quality of ovarian follicles was associated with ovarian aging, as supported by previous research (Lillpers and Wilhelmson, 1993; Bala et al., 2015; Yao et al., 2020; Hao et al., 2021). A decrease in number of follicles and mRNA transcript levels of *OCLN* was noted in the mature and old groups, while the mRNA transcript levels of *AMH* were highest in the old group. Elevated *AMH* mRNA levels in human granulosa cells have been linked to follicular aging, leading to decreased proliferation and growth of granulosa cells, as reported by Kedem et al. (2014). On the other hand, decreased *OCLN* mRNA levels in hen granulosa cells have been associated with increased yolk accumulation, as discussed by Stephens and Johnson (2017). Therefore, the alterations in mRNA transcript levels of these genes, along with the decline in follicle numbers in the mature and old groups, and increased ovulation interval is likely contributed to the enlargement of F1 follicles in the old group.

The oviduct plays a crucial role in fertilization and provides the pathway for sperm to reach the oocyte (Varga et al., 2019). In the current study, we observed significant reductions in the length of the oviduct, as well as in the height and thickness of the first and second folds in the magnum and isthmus regions in the old group compared to other groups. These findings are consistent with prior research indicating that oviduct length and fold height decrease in non-laying hens

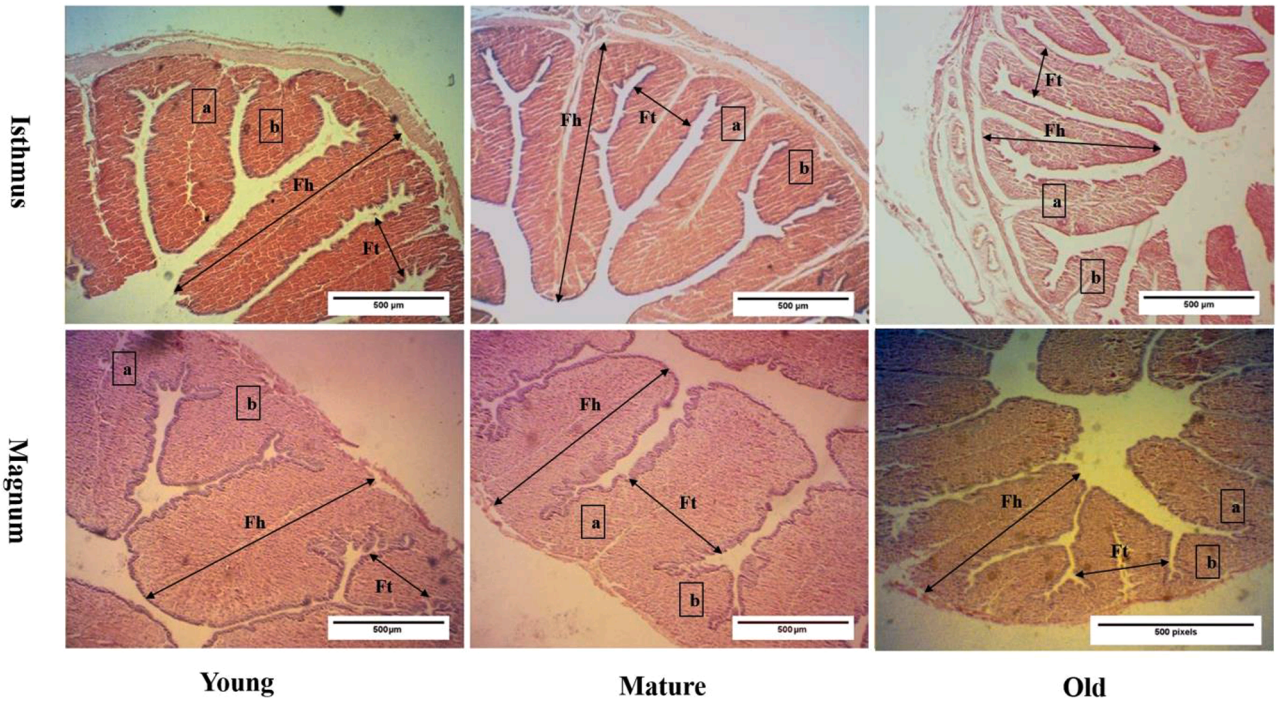

Fig. 1. Histological comparison of the isthmus and magnum folds of Japanese quails in different production period groups. The Isthmus and magnum sections were stained with hematoxylin and eosin. Photos show folds with 40X magnification (Scale bar = 500 mm). (a) Primary fold, (b) Secondary fold, (Fh) Fold height, (Ft) Fold thickness.

Table 6
Reproductive performance and hatchling quality of laying Japanese quail at the different ages (n=45 female quails per group).

Variable	Experimental group [1]			SEM[2]	*P* Value
	Young	Mature	Old		
Numbers of total egg set	201	187	122		
Number of fertile eggs	198	174	94		
Fertility	98.50%[a]	93.04% [b]	77.04%[c]	0.01	<0.01
Number of chicks hatched	147	120	61		
Hatchability	73.13%[a]	64.17%[b]	50.00%[c]	0.02	<0.01
Early embryonic mortality [3]	12.12%[b]	12.64%[ab]	12.76%[a]	0.01	<0.05
Middle embryonic mortality [4]	3.03%[b]	6.32%[ab]	8.51%[a]	0.009	<0.05
Late embryonic mortality[5]	10.60%	12.06%	13.82%	0.01	0.07
Hatchling body weight (gr)	7.89[c]	8.27[b]	8.78[a]	0.37	<0.01
Hatchling quality	97.98[a]	96.21[ab]	95.38[b]	0.05	<0.05

a– c: Within each row, means with different superscripts differ significantly ($P < 0.05$).
[1] Quails at the different ages, including young, mature, and old.
[2] Standard error of the mean.

and quails after a certain duration, as demonstrated by Gonzalez-Moran (2016) and Sukhadeve et al. (2021). Although sex steroid hormones concentrations have not been assessed in the current study, the changes observed in the oviduct morphology could be attributed to alterations in sex steroid synthesis, and variations in progesterone receptor expression (Gonzalez-Moran, 2016). The diminished fertility observed in the old group may be partially linked to the decrease in fold height in the magnum and isthmus regions, potentially affecting the transport of sperm to the site of fertilization.

Fertility, hatchability, and early embryonic development significantly influence the production of one-day-old chicks. The impact of aging on quail fertility and hatchability has been documented by findings of Farooq et al. (2012), Othman et al. (2014), and Santos et al. (2015). In the current study, both fertility and hatchability were lower in the mature and old groups compared to the young group. This decrease in fertility in the mature and old groups may be attributed to the reduced number of follicles and dysregulation of genes involved in follicular selection and yolk accumulation. It has been shown that an increase in the expression of *BMP15* at 20 weeks of age did not affect oocyte quality, ovulation, fertility, and embryo development in mice, but a decrease in the expression of this gene at 40 weeks of age resulted in a reduction in oocyte quality, ovulation, fertility, and embryo development (Park et al., 2020).

The mRNA transcript levels of *BMP15* showed an increase in the mature group and a decrease in the old group, with a corresponding decrease in fertility observed in the latter group. While *BMP15* may not directly impact fertility in quails, the decrease in fertility could be influenced by other factors or potential interactions of *BMP15* regulation with other genes, leading to disruptions in gene regulation and ultimately resulting in reduced fertility. The effects of *BMP15* on fertility may vary depending on factors such as species, age, production period, and other influencing variables.

Hatchability was lower and mortality rates during early and middle stages of incubation were higher in the old group compared to the other groups. Egg characteristics influenced by the age of the bird can impact hatchability, with albumen liquefaction playing a crucial role in embryonic development and mortality (Meuer and Baumann, 1988; Brake et al., 1997). Some studies have reported that liquefaction during the early stage of incubation is essential for the easy transfer of glucose, albumen, and ions to the blastoderm and the reduction of the gas diffusion barrier created by the eggshell (Meuer and Baumann, 1988; Brake et al., 1997). Moreover, the increased albumen height and Haugh unit in eggs of old quails may disrupt protein balance and alter albumen liquefaction, potentially contributing to increased embryonic mortality and decreased hatchability in this group (Taghipour-shahbandi et al., 2023).

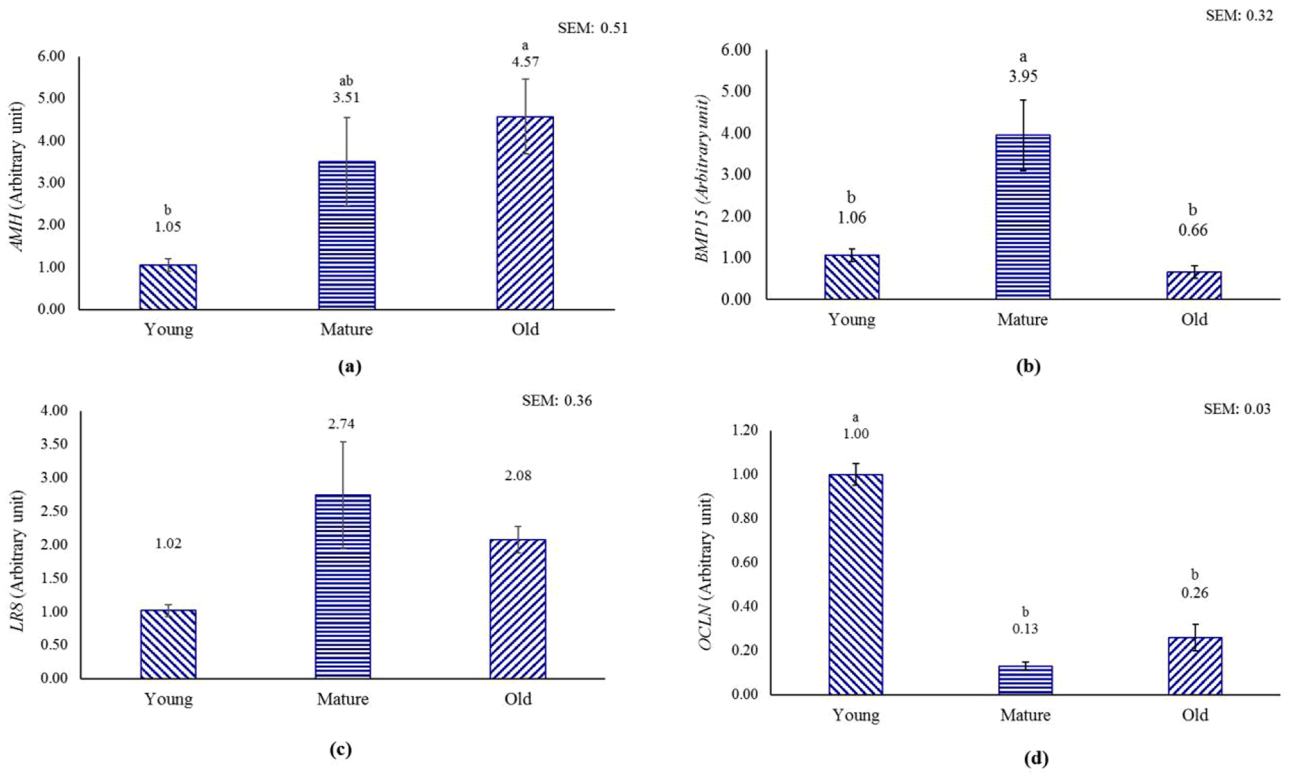

Fig. 2. Comparison of (a) *AMH* (Anti-Mullerian hormone), (b) *BMP15* (Bone morphogenetic protein 15), (C) *LR8* (Specific yolk receptor), and (d) *OCLN* (Occludin) transcript in small yellow follicles of the laying Japanese quail at the different ages. Within each experimental group, values with different superscripts (a, b) are significantly different ($P < 0.05$).

Quantitative methods used to assess hatchling quality, including morphological measurements and hatchling weight, tend to increase with the age of the bird (Ulmer-Franco et al., 2010; Machado et al., 2020). The increase in hatchling weight can be linked to higher yolk and egg weights (Tona et al., 2004a, 2004b). The decrease in follicle numbers and changes in gene expression related to follicular selection and development may lead to alterations in yolk and egg weights. As maternal age increases, the quality of chick production typically declines due to factors such as oocyte aging, reduction in clutch length, and increased ovulation interval (Tona et al., 2004a, 2004b), all of which are associated with decreased egg production (Fasenko et al., 1992; Hao et al., 2021). However, further evaluation is needed to determine the specific impact of these factors on chick quality in the old group.

The current study demonstrated that *AMH* mRNA transcript abundance in the follicular wall of SYFs in old group was significantly higher compared to the young group. The increased *AMH* mRNA transcript abundance in human granulosa cells is a marker of follicular aging. This leads to a reduced response of the follicles to FSH, inhibiting the proliferation and growth of granulosa cells, preventing follicular development to the next stage, inhibiting ovulation, and helping maintain ovarian reserve (Kedem et al., 2014; Kotlyar and Seifer, 2021). In addition, concomitant with the increase in *AMH* mRNA transcript abundance in old group, there was a decrease in *BMP15* mRNA transcript abundance. Previous study has demonstrated the effect of *BMP15* on reducing follicular *AMH* and *OCLN* (Stephens and Johnson, 2017). It has also been reported that *OCLN* plays a role in yolk accumulation and follicular development (Stephens and Johnson, 2017).

No significant changes in LR8 mRNA transcript abundance were observed in different groups in the current study. Consistent with the present research, the expression of this gene did not show any changes in follicles of mature and immature hens (Recheis et al., 2005; Seol et al., 2007). finally, it seems that aging and ovarian senescence disrupt the regulation of genes associated with follicular selection and yolk accumulation, leading to a disturbance in follicular growth and development and consequently a decrease in egg production and fertility.

Conclusion

This study demonstrated that the decrease in follicle numbers and changes in mRNA transcript abundance related to follicular selection and yolk accumulation in the mature group indicate the onset of the aging process. These changes were accompanied by a decline in fertility and hatchability and altered structure of the oviduct. Consequently, there was a remarkable decrease in egg production, fertility, and hatchability in old Japanese quail. The findings provide the basis for future research on avian aging and post-peak production period.

Declaration of competing interest

There are neither conflicts of interest nor conflicts due to professional or financial affiliation for part of any of the authors. Mahdi Zhandi

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