



Egg Production, Fertility, Hatchability and Luteinizing Hormone Profile of Progesterone Hormone Injected to Arabic Gold Chicken (*Gallus turcicus*)

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ABSTRACT

The production and reproduction performance of chicken depends on their hormonal status, especially progesterone hormone, which has been known to correlate with egg production. The present study aimed to analyze the effect of progesterone hormone injection on Arabic Gold chickens (*Gallus turcicus*) regarding egg production and luteinizing hormone concentration in blood plasma. A total number of 60 Arabic Gold chickens aged 26-weeks were divided into three groups based on injected hormone concentration (P₀: control; P₁: 1 mg/chicken; P₂: 2 mg/chicken). The study was conducted using a completely randomized design and the obtained data were analyzed with a descriptive analysis for qualitative data and one-way analysis of variance followed with Duncan's Multiple Range Test as a post hoc test for the quantitative data. The results presented that progesterone hormone injection had a significant effect on hen day production two and six weeks after injection. The P₁ group was able to reach its peak production (82.9%) at week 29, while the P₂ group reached its peak at week 26 (78.9%). In addition, it was found that the P₂ group produced a soft-shelled egg and double egg yolk. Progesterone injection led to no significant effect on the egg weight, shape index, fertility, embryo viability, hatchability, and chick weight at hatch. The luteinizing hormone concentration was higher in P₂ (1.52 ng/ml), compared to P₀ (1.36 ng/ml) and P₁ (1.34 ng/ml) groups. It was concluded that progesterone hormone injection during the production phase of Arabic Gold chicken had a significant effect on egg production and caused varying egg production peak and luteinizing hormone concentration.

Keywords: Arabic Gold chicken, Egg quality, Hen day production, Luteinizing hormone, Progesterone

INTRODUCTION

Arabic gold chicken (*Gallus turcicus*) is a chicken breed widely reared in Indonesia for egg production. The breed is preferred by the local farmers due to its better Feed Conversion Ratio (FCR), which is around 3 to 4 (Rizal et al., 2015), compared to the native Kampung chicken for which the ratio is 6.73 on average (Pagala et al., 2019). The Arabic Gold hens begin to lay eggs by the age of five months and its peak production would be achieved at the age of 8-9 months (Indra et al., 2013). Some factors that have been known to determine egg production of laying hens include the genetic characteristics, age, nutrition intake, and rearing system (Sarica et al., 2012). Pirsaraei et al. (2008) added that hormonal status also plays a significant role in egg production.

Egg production is a complex process that involves hormonal interaction within chickens. Reproductive hormones, such as progesterone, play an important role in reproductional functions in fowls (Scanes, 2015; Han et al., 2017), including the development of reproductive organs, ovulation, albumen synthesis, eggshell's formation, and egg oviposition (Mishra et al., 2019). The progesterone hormone is one of the important steroid hormones that affects cattle and fowl (turkeys and laying egg hens) reproduction (Scanes, 2015). The progesterone hormone has been acknowledged to have a positive correlation with egg production. The progesterone hormone would promote the pre-ovulation release of luteinizing hormone (LH) that impacts egg production (Zaghari et al., 2009).

A previous study indicated that the secretion of progesterone hormone correlates with egg production on

Guinea Fowl by providing an exogenous progesterone hormone to the feed and drinking water (Adeyinka *et al.*, 2007). Aside from feed and drinking water, the exogenous progesterone can also be given by injection. Zaghari *et al.* (2009) conducted a study on the effect of progesterone injection on broiler chickens. In a study performed by Ito *et al.* (2011), it was found that progesterone hormone injection 20 hours before ovulation affects the release of sperm storage tubules in the ureterovaginal junction.

Although effort has been made to increase egg production (Samadi *et al.*, 2020), there is still no report on the enhancement of egg production in Arabic Gold chicken regarding hormone application. Therefore, this study aimed to analyze the effect of progesterone hormone injection on Arabic gold chicken during the production phase towards the performance of egg production, fertility, and LH hormone profile.

MATERIALS AND METHODS

Ethical approval

This study has been approved by the Animal Ethics Committee of Universitas Brawijaya with number 001-KEP-UB-2020 and has been declared to have fulfilled the ethical feasibility of the research. All procedures and care for chickens were in accordance with institutional guidelines for the use of animals in the study.

Experimental design

The study was conducted as experimental research using a completely randomized design in the poultry installation unit of the Politeknik Pembangunan Pertanian Malang, Indonesia, from August to December 2019. A total of 60 Arabic Gold chickens were divided into three treatment groups with 10 replications each and 2 chickens for each replication. The current study had three roosters at approximately 12 months of age to produce semen for the artificial insemination process twice a week. The data of this study were collected from a week of first chicken insemination or at the age of 26 weeks old.

Research sample

The Arabic Gold chickens used in the current study were obtained from “Mitra Ternak Juara Group” farm in Probolinggo, Indonesia, and has been vaccinated with Marek’s Disease, Infectious Bursal Disease (IBD), Infectious Bronchitis (IB), Infectious Laryngo Tracheitis (ILT), and Newcastle Disease (ND) from the age of 1 day to 4 months. The chickens were firstly reared from the age of 16 weeks for battery cage and feed adaptation by using

commercial laying hens feed containing 14-16% protein, 5-7% crude fiber, 4-7% crude fat, 5-7% ash, and 2500-2700 Kcal/kg metabolizable energy, given *ad libitum* along with the drink. The hens were inseminated at the age of 25 weeks until 33 weeks and kept in individual coops with 14 hours of light and 10 hours of dark system daily.

Research treatments

The research treatment of this study entailed the injection of different concentrations of progesterone hormone to the Arabic gold chickens. The progesterone hormone used for the present study was P0478-5G produced by Tokyo Chemical Industry (TCI) Co, Ltd. The progesterone hormone injection doses for experimental hens included 0% or no injection (control), 1 mg/chicken (P₁), and 2 mg/chicken (P₂). The solvent used in the current study was sesame oil (0.5 ml of oil/ chicken, Correa *et al.*, 2005). The progesterone hormone injection was performed subcutaneously. The hormone was injected five hours prior to the estimated ovulation as calculated from the previous egg-laying time. The injections were administered twice a week for four consecutive weeks (on weeks 28 to 31). Eggs were collected from two weeks before the injection time until two weeks after the injection to observe the egg production performance. The eggs from hormone-injected hens were then hatched at the temperature of 37.5°C with a humidity level of 55-60% (Mohan *et al.*, 2015).

Hen day production

The observed variables in the present study included hen day production (HDP), egg qualities, and LH profile. The HDP was measured by comparing the number of eggs produced in a day with the number of hens; the result was then multiplied by 100% (Pirsaraei *et al.*, 2008). The HDP values were calculated for weekly production.

Egg qualities

The observed egg quality parameters included weight, size (length and width), shape index, and eggshell quality. Egg weight was measured using an analytic scale. The shape index is the comparison of egg width and length diameters multiplied by 100% (Duman *et al.*, 2016). The width and length of the egg were measured using vernier calipers. The exterior egg quality was observed by checking its surface, whether smooth, rough, or soft. The egg fertility percentage is achieved by dividing the number of fertile eggs by the total amount of observed eggs, the result is then multiplied by 100%. The egg fertility was measured by the candling method. The candling was performed on the seventh day of the hatching period.

Furthermore, the egg candling was also performed on the fourteenth and eighteenth days of the hatching period to determine the embryo viability. The embryo viability is the percentage of the live embryo at 14 or 18 hatching days of fertile eggs multiplied by 100% (Reijrink et al., 2010). The hatchability is the percentage of hatched eggs of the embryo of fertile eggs multiplied by 100% (Indrawati et al., 2015).

Luteinizing hormone profile

The concentration of LH in the blood plasma of subjects was measured two hours before and after the injection, the result was analyzed using Enzyme-linked Immunosorbent Assay (ELISA).

Statistical analysis

The qualitative data were analyzed descriptively, while the quantitative data were analyzed using analysis of variance, and followed by Duncan's Multiple Range Test. P-value less than 0.05 was considered statistically significant ($P < 0.05$). The results were expressed as the mean \pm standard deviation (SD).

RESULTS

Hen day production

The Arabic Gold chickens began to lay eggs at the age of 20 weeks and the initial egg production was 5.2%. The HDP of Arabic Gold chicken injected with different progesterone levels in this study is presented in Figure 1. Figure 1 demonstrates that egg production gradually decreases as the chickens matured indicating varying peak production time frames. The control group receiving no treatment (P_0) experienced a decrease in production and its peak was on week 27 with HDP at $75.7 \pm 22.6\%$ when other groups did not receive any progesterone hormone injection. The P_1 group reached its peak production on week 29 with HDP at $82.9 \pm 9.64\%$ while the P_2 group reached its peak production on week 26 with the HDP at $77.9 \pm 12.3\%$. The HDP data during the progesterone hormone injection treatment until week 33 is presented in Table 1.

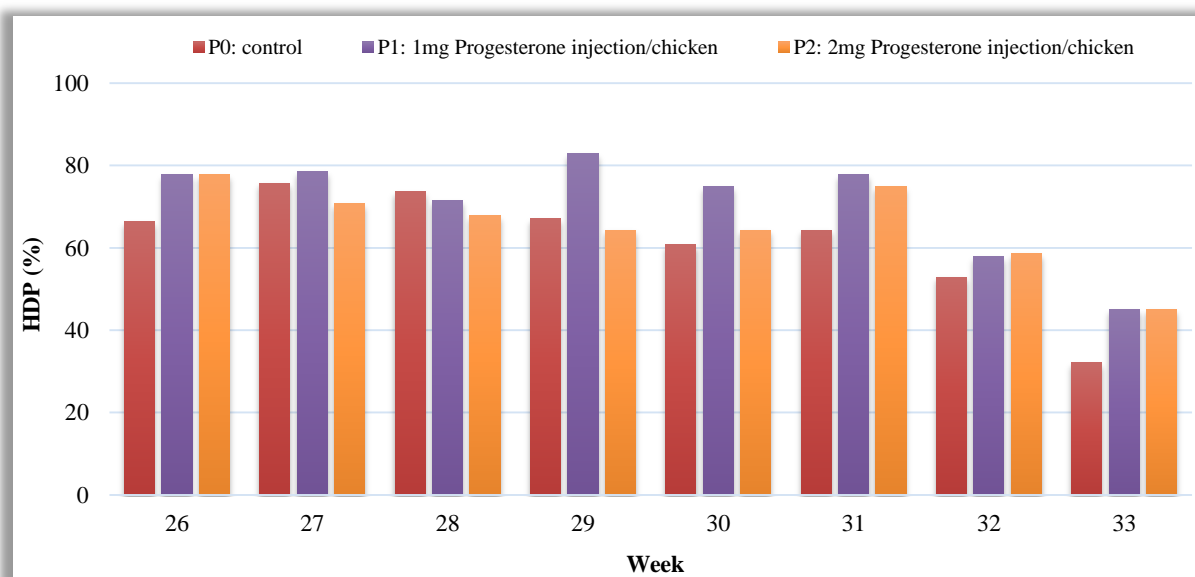


Figure 1. Hen day production of Arabic Gold chickens from week 26 until week 33 with different injected progesterone levels.

Table 1. Average hen day production of Arabic Gold chickens injected with different progesterone levels.

Treatment \ HDP (%)	26	27	28	29	30	31	32	33
P_0	66.4 \pm 25.4 ^a	75.7 \pm 22.6 ^a	73.6 \pm 14.7 ^a	67.1 \pm 19.4 ^a	60.71 \pm 23.6 ^a	64.3 \pm 11.7 ^a	52.9 \pm 11.8 ^a	32.1 \pm 13.6 ^a
P_1	77.9 \pm 18.9 ^a	78.5 \pm 16.5 ^a	71.4 \pm 4.8 ^a	82.9 \pm 9.64 ^b	75.00 \pm 15.52 ^a	77.8 \pm 12.8 ^a	57.9 \pm 12.8 ^a	45.0 \pm 15.1 ^b
P_2	77.9 \pm 12.3 ^a	70.7 \pm 13.2 ^a	67.9 \pm 8.4 ^a	64.3 \pm 14.67 ^a	64.29 \pm 16.1 ^a	75.0 \pm 13.6 ^a	58.6 \pm 11.6 ^a	45.0 \pm 10.7 ^b
SEM	3.81	2.30	1.67	5.77	4.29	4.13	1.80	4.29
p value	0.35	0.61	0.45	0.02	0.22	0.11	0.52	0.05

Different superscripts on each column indicate significant differences ($P < 0.05$). The progesterone hormone was injected at 1 mg/chicken for P_1 and 2 mg/chicken for P_2 from week-28 to 31.

Egg qualities

The egg quality parameters observed in the current study included egg weight, size (width and length), shape index (%), and the eggshell. The exterior egg quality measurement in the present study is presented in Table 2, while the eggshell quality is indicated in Table 3.

Egg fertility and hatchability are two essential parameters in egg reproduction to produce the day-old chick. The egg fertility percentage was measured by conducting the candling test on the seventh day of the incubator. Table 4 indicates the ANOVA test of fertility among treatments. The obtained results revealed no significant difference between the treatment groups and the control group ($P > 0.05$). The embryo viability during the hatchery period was observed through the candling test. In the present study, the embryo observed on day 14 and day 18 based on grouping indicated that the death occurred in the mid-phase. The embryo viability showed the number of living ones from the fertile eggs that fit the hatchery stage. The results of this study showed that the embryo viability due to hormone injection was not different ($P > 0.05$) among all treatments. Therefore, it could be concluded that the progesterone hormone

injection did not affect the chicken embryo viability which was supported by a relatively high result of all groups. The hatchability found in this study did not significantly differ among treatment groups ($P > 0.05$), the hatched eggs were derived from layer hens with similar age (28 to 32 weeks).

Luteneizing hormone profile

Progesterone stimulates the LH hormone release prior to ovulation. The ELISA analysis results on the LH hormone concentration in the current study are presented in Figure 2, and the results of ANOVA analysis of LH concentrations are presented in Table 5. Figure 2 presents the progesterone hormone injection five hours before the ovulation could stimulate the LH hormone release. The ANOVA analysis (Table 5) indicated that the LH concentration was not significantly different ($P > 0.05$) among treatment groups although P_2 had a higher LH concentration than other treatments. A high dose of progesterone treatment could increase the LH concentration in the plasma to 1.52 ng/ml three hours before the predicted ovulation, or around 28 hours before the next oviposition.

Table 2. Exterior egg quality of Arabic Gold chickens injected with different progesterone levels.

Parameter	Treatment group			SEM	P-value
	P_0	P_1	P_2		
Egg weight (g)	38.9±1.58	39.6±1.09	39.7±1.06	0.27	0.27 ^{ns}
Egg width (cm)	35.7±0.65	36.1±0.67	36.2±0.57	0.15	0.18 ^{ns}
Egg length (cm)	46.4±1.16	46.8±0.63	46.4±0.54	0.13	0.49 ^{ns}
Shape index (%)	77.1±1.10	77.3±3.62	78.2±10.8	0.34	0.19 ^{ns}

ns: no significant difference between each treatment group ($P > 0.05$)

Table 3. Eggshell quality of Arabic Gold chickens injected with different progesterone levels.

Parameter	Treatment group		
	P_0	P_1	P_2
Smooth eggshell (%)	93.2	88.1	87.7
Speckled smooth eggshell (%)	4.44	5.03	8.10
Rough eggshell (%)	2.37	6.85	4.25
Soft shell (%)	0	1.96	3.49
Double egg yolk	0	0	1.62
White colour (%)	82.9	63.9	72.8
Brown colour (%)	17.1	36.1	27.2

Table 4. Egg fertility evaluation of Arabic Gold chickens injected with different progesterone levels.

Parameter	Treatment group	P ₀ N = 123	P ₁ N = 129	P ₂ N = 121	SEM	P-value
Egg fertility (%)		88.2±8.74	83.6±3.62	88.1±10.8	1.51	0.54 ^{ns}
Embryo viability to day 14 (%)		97.2±5.91	96.1±12.2	97.4±4.16	0.40	0.93 ^{ns}
Embryo viability to day 18 (%)		94.9±8.86	91.6±14.6	94.1±6.07	1.08	0.73 ^{ns}
Hatchability (%)		89±13.7	84.4±14.8	83.9±10.4	1.62	0.64 ^{ns}
DOC weight (g)		25.9±1.05	26.8±1.43	26.8±0.95	0.32	0.13 ^{ns}

N: Number of hatched eggs, ns: No significant difference between each treatment group (P > 0.05).

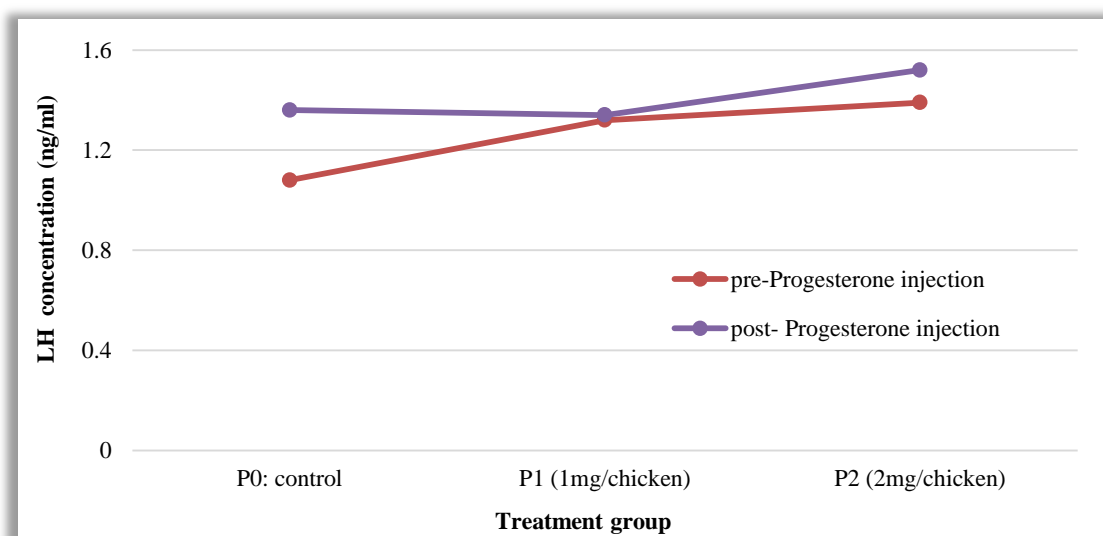


Figure 2. Luteinizing hormone concentration on each treatment group of Arabic Gold chickens from week 26 until week 33 with different injected progesterone levels.

Table 5. Luteinizing hormone concentration of Arabic Gold chickens injected with different progesterone levels.

Treatment	P ₀	P ₁	P ₂	P-value
Pre-progesterone injection	1.08±0.21	1.32±0.61	1.39±0.52	0.32 ^{ns}
Post-progesterone injection	1.36±0.35	1.34±0.44	1.52±0.36	0.52 ^{ns}

ns: No significant difference between each treatment group (P > 0.05).

DISCUSSION

The performance of egg production can be analyzed from the number of eggs produced by each chicken (Javed et al., 2003). The production parameter is achieved from the number of daily egg production, which is then converted to HDP, after that, the HDP collection is calculated to determine the weekly average. The HDP provides

essential information to realize the daily egg production (Farooq et al., 2002; Pirsaraei et al., 2008).

The Progesterone hormone injection was administered during the production phase (28-31 weeks), the egg production data was collected from two weeks before the treatment (week-36) until the egg production reached the percentage of 74.2%. Production data was calculated for 8 weeks started from week 26 until 33 (2

weeks before the treatment, 4 weeks during the treatment, and 2 weeks after the treatment).

Table 1 indicates that progesterone hormone injection affects egg production ($P < 0.05$) in week two of the treatment process (week-29) and two weeks after the treatment (week 33). The present study applied a low-dose progesterone hormone injection (1 mg/chicken), which could improve egg production only until week 29 or similar to the second week of the treatment, followed by a decrease in egg production. The low-dose progesterone treatment is proven to achieve the highest production compared to the control group. Moreover, the high dose of progesterone injection (2 mg/chicken) represented that the egg production dropped for three weeks after the initiation of treatment, but started to increase again from the last week of the treatment (week 31) before the egg production decreased again. This finding was in line with a study by Zaghari *et al.* (2009) that indicated that progesterone injection could significantly affect egg production.

The chicken that gets progesterone injection produces fewer stable eggs than those who receive no treatment. A similar finding by the current study presented that after the progesterone injection was administered, egg production of turkeys (Bacon and Liu, 2004), broilers (Liu and Bacon, 2005), and Japanese quail (Liu and Bacon, 2004) decreased. On the last observation of week 33, all groups experienced a decrease in egg production, but treatment groups had a higher decrease (45%), compared to the control group (32%).

The characteristics of egg quality depend on several factors, namely the age and genus of the chicken, the nutrient of the feed, and chicken weight (Sarica *et al.*, 2012). Besides, other factors that affect egg production include the maintenance system, the oviposition time, molting induction, general stress, heat stress, diseases, addition of an exclusive product to the feed, and the hormonal status of the chicken (Pirsaraei *et al.*, 2008; Ahmadi and Rahimi, 2011). A stressful situation and other physiological conditions can generally affect oviposition, which eventually affects egg quality (Ahmadi and Rahimi, 2011). The treatment of progesterone hormone injection in this study for four weeks during the egg production had no significant effect on the exterior egg quality ($P > 0.05$) due to the similar treatment on feed, maintenance method, and careful treatments provided to all chicken to avoid stress.

Factors that affect the egg weight are the chicken age, strain and breed, feed nutrients, chicken weight, egg-laying time, environmental temperature, and diseases (Bell and Weaver, 2002). The egg weight of Arabic Gold chicken breed in the present study was in the normal

range. The range was in agreement with a study conducted by Bakar *et al.* (2005), which indicated that the weight of Arabic chicken breed could range from around 31 to 52 grams per egg. Table 3 presents that the three treatments of the Arabic chicken breed do not differ significantly regarding the egg weight ($P > 0.05$). The result of the study was lower in value than the results from a study conducted by Yumna *et al.* (2011) in which the weight of eggs was 46.81 ± 2.22 grams during the age of 32 to 36 weeks. This difference could be due to the age difference of the subjects used in these two studies. The progesterone hormone affects the eggshell formation in the reproductive tract (Zhang *et al.*, 2019) which is assumed to affect the egg weight; nevertheless, the finding showed different results. This means that the hormone injection does not have any impact on the egg weight.

Egg shape index (SI) is the width and length ratio of an egg, it is an essential criterion in determining the quality of an egg. An egg that does not have a standard shape, such as misshapen, too round, or a flat surface on one side, is not included in grade AA (almost perfect) or grade A (slightly under AA) since it does not comply with the standard oval egg shape (Duman *et al.*, 2016). The shape index correlates with the egg shape. The variation of the egg index is between 0.65-0.82 (Yuwanta, 2010). The higher the egg shape index (0.82), the more round the egg shape. On the contrary, the lower the egg shape index, the more oval and tapering the egg. Yuwanta (2010) also explains that the egg shape index will progressively decrease as laying time increases; right after the egg is laid, the egg shape index ranges around 0.77, but at the end of laying time, the index is around 0.74. The current study used eggs derived from chickens during the mid-production time, eventually, their shape index was about 77-78%.

The variable of eggshell quality underwent a descriptive analysis. On treatment P₂, there was a double egg yolk occurrence with a percentage of 1.62%. The double egg yolk occurred due to the progesterone hormone injection that stimulated the LH hormone release before the ovulation which resulted in the occurrence of double ovulation from the mature follicle. After the double egg yolk started the hatching stage, on day seven, it is found infertile as a result of the candling test. This finding was in line with Buchanan *et al.* (2002) who reported two or more follicles on each position of the hierarchy that could produce two or more follicles in a day, an egg with multiple ovulation cannot be suitable to undergo the incubation.

Several studies have indicated that progesterone correlated with eggshell quality. The Progesterone hormone affects the ovary and hypothalamus leading to stimulating the surge of LH used for the ovulation process. The progesterone injection can have an impact on the quality of the eggshell during the calcification initiation (Zhang et al., 2019). The findings of the current study indicated that the progesterone hormone injection resulted in the soft-shell egg whose percentage was higher for the high-dose treatment P₂ (4.25%), compared to the P₁ treatment that reached 1.96%. The soft eggshell occurrence was similar to a study performed by Zaghari et al. (2009) concluding that progesterone injection caused an increase in eggshell qualities with a softshell during the first week of the hormone injection initiation. The double egg yolk and soft shell are considered to increase ovulation although these eggs are not suitable for hatching and not to be counted as good eggs (Zaghari et al., 2009). Liu and Bacon (2005) also observed a higher occurrence of the softshell eggs after the injection of progesterone in the broiler chicken farm.

The shell color is one of the parameters of eggshell quality (Liu et al., 2010). Eggshell color is a result of a stack of pigment during the egg formation process in the oviduct. The type of pigment depends significantly on breed type and its genetic characteristics (Liu and Cheng, 2010). The Arabic chicken breed is known to produce eggs with white to the light brown shell which resembles the eggs of free-range chicken (Yumna et al., 2011). In the present study, the color of the shell was still within the normal range. The shell color depends on the pigment produced by shell gland during the shell formation. The color of the egg can be determined when it is inside the uterus and the end of the oviduct. During the shell formation, the epithelial cells on the surface of the shell glands (uterus) start to synthesis the color pigments (Baylan et al., 2017).

Egg fertility and hatchability are two of the essential parameters in egg reproduction to produce the day-old chick (King'ori, 2011). These parameters depend on genetic and environmental factors. Both egg fertility and hatchability measurement in the current study was estimated 83-89%. King'ori (2011), explained the factors affecting fertility and hatchability included nutrient, chicken in the flock, egg factors, hatchery (natural and artificial incubation), and environmental factors.

The spermatozoa are inseminated to the cloaca, and consequently stored for several days in the sperm storage tubule (SST) located between the uterovaginal junction (UVJ) and infundibulum. The site has a receptor for

progesterone and estrogen (Yoshimura et al., 2000) that is shown to determine the fertility rate. The correlation between the progesterone hormone and egg fertility was supported by the finding of Ito et al. (2011) that reported progesterone as one of the factors causing the spermatozoa release towards the fertilization site, which could be active for 20 hours after the oviposition. The highest egg fertility of Arabic gold chickens in this study was found at 2 mg progesterone hormone injection (P₂ treatment) which was at 88.1±10.8%. This finding was higher compared to the finding by Astomo et al. (2016), showing that the egg fertility of the Arabic chicken breed was around 62.5-79.2%.

Hatchery process, the death of embryo detected through the candling test and is classified into three phases, including the death of the initial embryo on day 10 (5 to 10 days), death of the embryo in the middle phase, observed on day 18, and death in the final phase of hatchery process which means day 21, and the embryo would not hatch (Larivière et al., 2009). This study observed the embryo death on days 14 and 18 indicating that the death occurred in the mid-phase. The embryo viability indicated the number of living ones from the fertile eggs that fit the hatchery stage. The results showed that the progesterone hormone injection did not give significant differences (P>0.05) on embryo viability between all treatments. Therefore, it can be concluded that the progesterone hormone injection did not affect the chicken embryo viability proven by a relatively high result of all treatment groups.

The hatchability is a critical factor of hatching in the breeding farm, it depends on many factors, such as hens in the flocks, egg fertility, and egg handling (Liu and Ngadi, 2012). There was no significant difference among treatment groups in terms of hatchability (P>0.05) for the hatched eggs derived from layers aged 28-22 weeks. The obtained result was higher in value, compared to a study conducted by Astomo et al. (2016) that reported the hatchability of the Arabic chicken breed for natural breeding to get the highest percentage of 76.7%. It was also stated that the affecting factors included egg quality, nutrition of hens, duration of egg storing, cage quality (temperature and humidity), and the hens quality as the egg producer. In the current study, the layer hens were selected according to specific parameters, qualified feed, good egg quality, the eggs were stored at 18°C temperature before entering the incubation machine, therefore, the hatchability rate was relatively high.

The hatching weight of Arabic chicken breed due to progesterone hormone injection in this study did not

significantly differ among treatment groups ($P > 0.05$). The result from hatching weight in this study reported a relatively lower one than a study performed by Astomo *et al.* (2016) that stated the hatching weight of Arabic chicken breed ranges around 29.4 g, and was significantly affected by initial egg weight before entering the incubation machine. A bigger egg has more nutrition than a smaller one. The embryo that is lacking nutrients would not grow properly and affect the hatched chicks. The current study indicated that the progesterone hormone injection did not have any significant effect ($P > 0.05$) on the egg weight, it does not have a substantial impact on the hatching weight. The hatched weight of this study was lower than the one performed by Rohmad and Fitri (2016) that presented the hatched weight range of Arabic chicken breed as 31.2-32.53 g and this difference was due to the egg weight and different age of layer hens.

A high-dose of progesterone injection could increase the LH concentration in the plasma up to 1.52 ng/ml at three hours before the time that predicted ovulation, or around 28 hours prior to the next oviposition. This finding was in accordance with a study by Bronneberg *et al.* (2007) that the plasma LH concentration increases by 1-2 ng/ml and starts from 11 hours before the oviposition. The obtained results of a study by Lewis *et al.* (2005) also reported that the LH concentration in chicken was within the range of 1.1-1.2 ng/ml during different photoperiods.

The increase of LH concentration in P_2 was still within normal levels despite the multiple ovulation caused by the double egg yolk. The high LH in P_2 treatment was then expected as the cause of double-yolked egg, as both factors known to be correlated (Bédécarrats *et al.*, 2016). Another research found that multiple ovulation occurs due to the defect in the follicle hierarchy than the distraction on the LH concentration profile (Buchanan *et al.*, 2002).

CONCLUSION

The progesterone hormone injection in Arabic gold chicken during the production phase (28-31 weeks) had a significant effect on egg production two and six weeks after the injection. The progesterone hormone injection caused a varying production peak, and reduce the overall egg production. The progesterone hormone injection did not significantly affect the egg weight, shape index, fertility, embryo viability, hatchability, and hatched weight. Nevertheless, a high-dose of progesterone injection (2 mg/chicken) increased the occurrence of softshell and double egg yolk, as well as LH concentration in plasma blood.

DECLARATIONS

Competing interests

The authors declare that there are no competing interests.

Author's contributions

Iswati and Muhammad Halim Natsir designed the research. Iswati and Muhammad Halim Natsir performed the research and analyzed the data. Iswati wrote the manuscript. Gatot Ciptadi, Muhammad Halim Natsir, and Trinil Susilawati participated in the revision of the manuscript. All authors have read and approved the final version of the manuscript.

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