

Original Research Paper

# Exogenous Progesterone for Offspring Sex Ratio Manipulation in Arabic Chickens (*Gallus turcicus*) Leading to Increasing Female Chicks Proportion

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**Abstract:** Female birds of egg-laying hens are more valuable than males, because male birds have no commercial value. The opportunity to manipulate offspring sex ratio in poultry has been observed in female birds; hence, female birds have heterogametic ZW chromosomes. High progesterone concentration 2-5 h before ovulation is thought to affect the segregation of female sex chromosomes as the basis for determining the sex of offspring. This study aimed to investigate the effect of progesterone induction in Arabic hens, which was conducted 5 h before ovulation, along with the time of the first meiosis; therefore, segregation of sex chromosomes leading to an increase in female offspring. The study utilized 60 Arabic hens divided into three treatment groups: Without injection (P<sub>0</sub>), progesterone injection 1 mg/hen (P<sub>1</sub>), progesterone injection 2 mg/hen (P<sub>2</sub>). The results showed that the percentage of female offspring at P<sub>0</sub> and P<sub>1</sub> was 54.74 and 53.33%, respectively, while P<sub>2</sub> hens resulted in 62.92% females, the highest female offspring rate than the other groups. Chi-square analysis of sex ratio showed that P<sub>0</sub> and P<sub>1</sub> hens had no significant effect (P>0.05) while P<sub>2</sub> hens being significant (P<0.05) from the expected sex ratio (50:50). Progesterone concentration of hens produced female offspring in the P<sub>2</sub> group was 7.15 ng/ml being highly significant (P<0.05) than that found in the hens produced male offspring (4.53 ng/ml). Progesterone 5 h preceding ovulation in the Arabic hens with a 2 mg/hen dose can increase the proportion of female offspring; therefore, an opportunity for sex ratio manipulation in Arabic chickens.

**Keywords:** Arabic Chicken, Progesterone, Sex Chromosome, Sex Ratio Manipulation

## Introduction

Arabic chickens are very popular among farmers in Indonesia as one of the local laying hens; they have a high productivity of eggs than other native chickens, with no nestling traits and are more resistant to disease. There are two types of Arabic chicken: Silver Arabic chicken and Gold Arabic chicken. Gold Arabic chickens have better egg production than Silvers, while Gold Arabic chickens are preferred by the local layer chicken industry (Indra *et al.*, 2013).

The chicken farming industry requires the sex of the chicken, according to business, to produce eggs or meat (Susilawati, 2014). The Arabic chicken farm industry certainly needs more females as egg producers.

The Arabic chicken industry's major problem is how to increase female offspring higher than males because males do not give economic benefits. This problem makes farmers look for an avenue to manipulate the sex ratio of hatched chicks, minimizing Day Old Chick (DOC) with unwanted sex. The practical implications are essential for commercial poultry farming as they can find ways to reduce the male chicks in the production of laying hens culled after hatching (Aslam *et al.*, 2013; Krautwald-Junghanns *et al.*, 2018). Manipulation of the sex ratio of chicken breeds aims to increase the number of offspring of one sex in a population and suppress the acquisition of unnecessary sex (Afriani *et al.*, 2011).

Sex ratio manipulation in chickens usually bases on the principle of sex determination and differentiation.

The sex chromosome in male chickens is ZZ, while in hens is ZW; the sex of the offspring is influenced by the hen chromosome (Hens, 2001). The sex ratio manipulation strategy in poultry began to develop with biological evolution that the hens were able to allocate one of its sex chromosomes (Z or W) to determine the sex of the offspring because heterogametic existed in females. (Smith, 2007; Kuroiwa, 2017; Navara, 2018). In chickens and quails, the offspring sex is determined 2-4 h before ovulation during the first meiotic division, when one sex chromosome remains in the oocyte and separates it into the polar body (Pinson *et al.*, 2011a). The factors that influence the allocation of the sex ratio of chicks is maternal hormones. Maternal hormones are expected biased offspring sex ratio (not following the 1:1 proportion), a phenomenon known as sex ratio manipulation (Alonso-Alvarez, 2006; Gam and Navara, 2016).

Maternal steroid hormones are predicted to influence the sex of eggs during oogenesis, especially meiosis I. When Z and W chromosome segregation occurs, one of the chromosomes (Z or W) remains in the oocyte and the other is in the polar body (Pinson *et al.*, 2011b; Navara, 2018). The progesterone is predicted to have significant effect in meiosis I (Correa *et al.*, 2005). Progesterone is the primary steroid produced by birds' pre ovulatory follicles during meiosis I about 2 to 5 h before ovulation, which is a critical time for genetic sex determination. Correa *et al.* (2005) investigated the injection of high doses (2 mg) of progesterone as an exogenous hormone to increase hormone levels. Progesterone in estimated meiosis has produced a primary sex ratio of 75% female offspring in white leghorn layer chickens (*Gallus gallus domesticus*).

This is the first study; until this study was conducted, there had not been any reported attempts to regulate the sex ratio of offspring in Arabic chickens leading to the female sex as egg producers. Therefore, the current study aims to determine whether the administration of progesterone in Arabic hens, at 5 h before expected ovulation, can change offspring sex as an opportunity for manipulation of the sex ratio towards females, which are more profitable for the poultry industry.

## Materials and Methods

### Experimental Animals

This study used 60 Arabic Gold Females (*Gallus turcicus*) obtained from "Mitra Ternak Juara Group" farm in Probolinggo, Indonesia. The chicken is 28 weeks old, placed in individual cages. Mating chickens with Artificial Insemination (AI) twice a week (Habibullah *et al.*, 2015; Pinson *et al.*, 2011a) with semen collected from three male Arabic Gold roosters to keep egg fertility. All chickens were fed on a commercial chicken feed. It contained 2500-2700 Kcal/kg metabolizable energy, 14-16% protein, 4-7% crude fat, 5-7% crude fiber and 5-7% ash; and drinking water *ad-lib*.

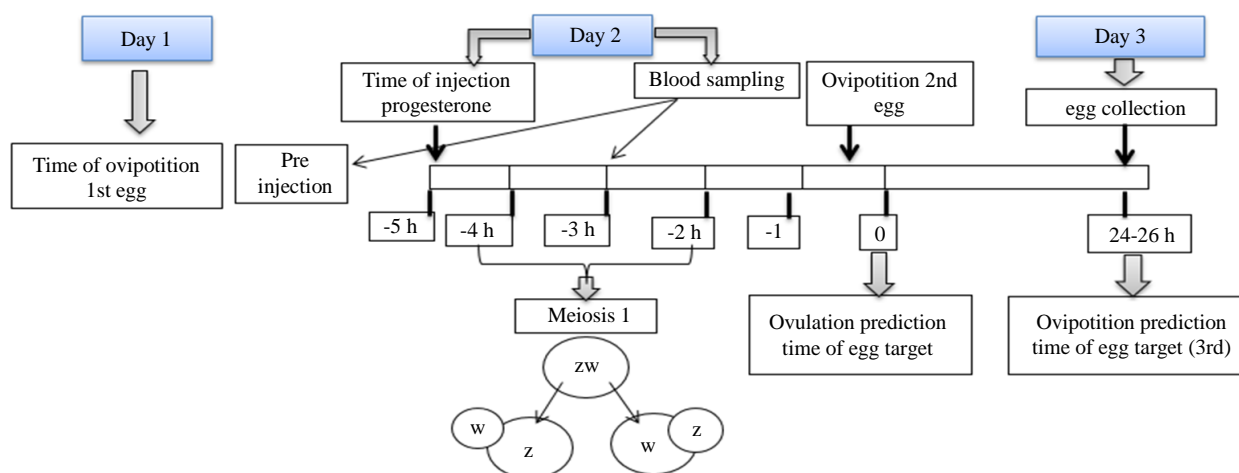
### Injection Time

The study was conducted experimentally with a Completely Randomized Design (CRD); chickens were randomly divided into three treatments and ten replicates, each replicates consisting of two chickens (P<sub>0</sub>: Control without injection; P<sub>1</sub>: Progesterone injection 1 mg/hen, P<sub>2</sub>: Progesterone injection 2 mg/hen). The total number of chickens per group is 20 hens; according to a study simulation by (Wilson and Hardy, 2002). This study utilized Progesterone hormone P0478-5G (Production: Tokyo Chemical Industry (TCI) Co., LTD) is dissolved in 0.5 ml of sesame oil and injected subcutaneously 5 h before ovulation predictions at the next oviposition. The estimated egg-laying time was calculated from observations of the previous day's oviposition. The injection was carried out twice a week for four weeks (28-31 week of chick age) according to each chicken's egg-laying cycle. The schedule of progesterone injection is shown in Fig. 1.

The same schedule was reported by (Pinson *et al.*, 2011b), with different hormonal treatments. The egg-laying point was observed every about 2 h on the first day as the first egg oviposition. On the second day, a blood sample was collected and injection of the progesterone was performed at 5 h preceding the next expected ovulation. The injection time is the prediction time of meiosis I (segregation of Z and W chromosomes) until about 2 h before the target egg ovulation; the second day, the oviposition time was also recorded. The third day or one day after injection was the target egg collection due to the progesterone injection, about 24-26 h from the second egg oviposition. At the oviposition, time was recorded to ensure that the target egg laid according to the predicted time. Hens that were not laying at the expected time, continued for the laying cycle and the next injection period; therefore, each group received eight injections for four weeks. Blood sampling was carried out 2 h after the hormone injection assuming a critical period to increase the progesterone level 2-3 h before the estimated ovulation (Correa *et al.*, 2005).

### Hatching Eggs

The targeted eggs were collected from each hen were stored at 18°C before entering the hatching process. According to the expected point of oviposition time, targeted eggs hatched at a temperature of 99.5°F and a humidity of 55-60% (Mohan *et al.*, 2015). Seven days of hatching, candling was carried out to determine the fertility of the hatching eggs, infertile eggs were removed from the hatching machine and fertile eggs are continued to hatch (Abdellatif, 2001; Al-Maksousi *et al.*, 2019).



**Fig. 1:** Schematic diagram of a one-time Progesterone injection 5 h before estimated ovulation

### Sex Ratio

The newly hatched chicks reared for four weeks of age to confirm sex using secondary sex signs (Tamzil *et al.*, 2018). The sex ratio in this study was the secondary sex ratio which was performed at a particular time after hatching (in this study 4 weeks) until the secondary sex sign appeared (Al-Maksousi *et al.*, 2019).

### Blood Progesterone Determination

Blood samples collected from the brachial vein 2 h after injection to verify the Progesterone surge in the treatment group chickens. Progesterone concentration in the blood was determined using the *Enzyme-Linked Immunosorbent Assay* (ELISA) method. Blood serum was obtained by fresh blood clotting for 10-20 min at room temperature, then pipetting the clear liquid above the sediment (serum) and in a centrifuge at 2000-3000 RPM for 20 min; the ELISA test procedure is following the Chicken Progesterone ELISA Kit, (Bioassay Technology Laboratory; Cat.No E0053Ch). The standard curve range was 0.05 ng/ml-10 ng/ml and the test sensitivity was 0.019 ng/ml. Readings of results on the Bio-rad ELISA Reader with absorbance measured at 450 nm (Mohammadi and Ansari-Pirsaraei, 2014; Al-Maksousi *et al.*, 2019).

### Statistical Analyses

The sex ratio is the ratio of female and male offspring percentage from each treatment. The sex ratio of chicks for each treatment was analyzed by the chi-square ( $X^2$ -test) compared to the sex ratio percentage of 50:50. The concentration of progesterone among treatment groups were analyzed by Analysis Of Variance (ANOVA) with a confidence level of 95% ( $P < 0.05$ ). If there is a significant difference, Duncan's Multiple Range Test

(DMRT) was applied. The t-test comparison of progesterone concentrations between the hen-producing male and female chicks was analyzed (Correa *et al.*, 2005).

## Results

### The Target Eggs

Treatment of progesterone injection in Arabic hen resulted in varying target egg numbers. The average target eggs of the three treatment groups were 77.71%, there were 13.13% hens with oviposition but not at the expected time and 9.17% of the hens not laying or had no oviposition. The data of the target eggs in each treatment group is shown in Table 1.

The target eggs based on the expected egg-laying time, were entered the hatching machine. In the evaluating of hatching, there were infertile eggs, die during hatching and not hatching. A newly hatched one day-old chick reared for up to 4 weeks in order to sex identification and sex ratio determination. The data of the hatching egg and the sex ratio of Arabic chicks is shown in Table 2.

### Secondary Sex Signs in Arabic Chicken

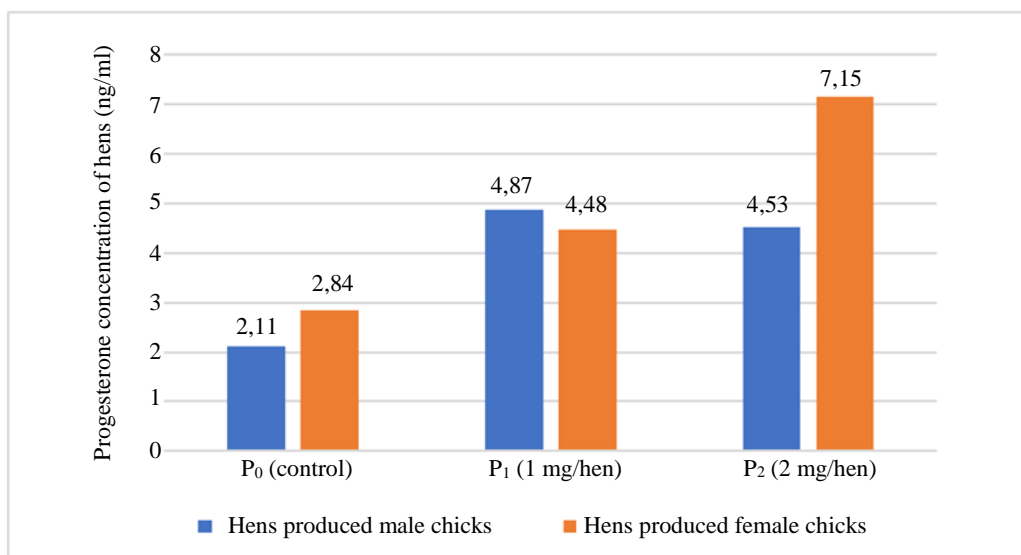
Arabic chicks show secondary sex signs phenotypically: Male chicks comb appear thicker and red, while female chicks do not show comb growth or thin comb, which seen in Fig. 2.

The result of the analysis of the Progesterone concentration of the Arabic hen is shown in Table 3.

The *T-test* analysis of the progesterone concentration of hens produced male and female chicks of the three treatment groups, as shown in Fig. 3.



**Fig. 2:** The differences in the secondary sex signs of 4 weeks old Arabic Gold chicks. Male Arabic chicks show red and thicker combs, while female Arabic chicks have thinner combs



**Fig. 3:** Blood progesterone concentration in the Arabic hens based on produced offspring sex: in the P<sub>0</sub> and P<sub>1</sub> group are not significant; while in P<sub>2</sub> group is significant ( $P < 0.05$ )

**Table 1:** Target egg data after progesterone administration

Treatment	Target eggs		
	Presentation of expectation (%)	Laying not at the target time (%)	No oviposition (%)
P <sub>0</sub>	76.88±10.67	14.37±6.42	8.75±3.96
P <sub>1</sub>	80.63±7.28	11.25±5.27	8.12±3.70
P <sub>2</sub>	75.62±10.15	15.00±6.79	9.38±4.24

P<sub>0</sub>: Control, P<sub>1</sub>: Progesterone injection 1 mg, P<sub>2</sub>: Progesterone injection 2 mg

The target eggs are eggs on day 1 after Progesterone injection

**Table 2:** Effect of progesterone administration on number of hatching eggs, hatching percentage and sex ratio of chicks

Treatment	No. hatching eggs	Infertile eggs (%)	Mortality of embryos and not hatching (%)	No. chicks hatched	Female (%)	Male (%)	Sex ratio (Female: Male)	value $X^2$ (T-test)
P <sub>0</sub>	123	11.78	12.04	95	54.74	45.26	1.2:1	0.72
P <sub>1</sub>	129	16.38	15.89	90	53.33	46.67	1.1:1	0.32
P <sub>2</sub>	121	11.94	15.24	89	62.92	37.08	1.7:1	6.17*

\* Significant ( $P < 0.05$ )

**Table 3:** Hen's blood progesterone concentration after progesterone induction

Treatment	Progesterone concentration (Mean ± SEM)
P <sub>0</sub>	2.47±0.41 <sup>a</sup>
P <sub>1</sub>	4.67±0.52 <sup>b</sup>
P <sub>2</sub>	5.84±0.57 <sup>b</sup>

Description: Different superscript in the same column indicate significant differences (P<0.05)

## Discussion

### *The Target Eggs After Progesterone Induction*

Table 1 shows different target eggs among treatments. The difference of target eggs is due to the effect of the egg-laying cycle (clutch) of hen, which directly affects daily egg production. Egg per clutch is influenced by hen's genetics (Idowu *et al.*, 2019). Current research shows that the target egg was relatively high at peak production in Arabic chickens. This study indicated that P<sub>2</sub> treatment had a higher number of non-oviposition than P<sub>1</sub> and P<sub>0</sub>. This is related to Progesterone's effect on egg production, which causes a decrease in egg production (Zaghari *et al.*, 2009). P<sub>2</sub> shows the highest number of oviposition beyond the expected target period. These eggs were not included in the analysis. Exogenous Progesterone injection affects ovulation and oviposition, not at the expected period. The earlier oviposition time was probably due to Progesterone, causing a surge in Luteinizing Hormone; therefore, ovulation was earlier. The slower oviposition than the expected period was perhaps attributed to the eggs restrained for a longer duration in the oviduct, especially in the uterus. This reason refers to the results of (Bacon and Liu, 2004) that high Progesterone levels can inhibit uterine smooth muscle contraction from locking in oviposition and retaining eggs for a longer time in the shell gland (uterus).

### *The Sex Ratio of Chicks*

Table 2 shows that infertile eggs and dead embryos were not included in the sex ratio data analysis. An incision was made in the chicks' abdomen, which died during the rearing period (up to 4 weeks) for gonadal observation and then the sex was identified. The dead chicks during rearing in each group were 8 (P<sub>0</sub>), 6 (P<sub>1</sub>) and 3 (P<sub>2</sub>). The sex ratio analysis counted from live chicks until the 4th week and dead chicks whose sex was identified (Al-Maksousi *et al.*, 2019).

Table 2 shows that the treatment produced a percentage of female offspring at P<sub>0</sub>, P<sub>1</sub> and P<sub>2</sub>, namely 54.74, 53.33 and 62.92%. This result different from the work of (Correa *et al.*, 2005), who obtained 75% female offspring of the primary sex ratio when Progesterone was administered (2 mg/hen) in laying hens. The difference in this study's results was assumed to be due to the sex ratio in the current research did not include embryos that died at hatching because the secondary sex

ratio performed at four weeks of age chicks was not the embryonic sex ratio or primary sex ratio. The sex-specific mortality was not included in the analysis; whether more male or female embryos died during hatching, only chicks that hatched were included in the sex ratio analysis. The injection method also was thought to affect the results. Different injection times can be involved in different ways, as in the study of (Correa *et al.*, 2005; Pinson *et al.*, 2011a), who did hormone induction with a single dose. In contrast, the present study was carried out with repeated doses in the next egg-laying cycle.

The sex ratio of offspring in birds generally follows the 50:50 (1:1) hypothesis. Sex ratio manipulation with the hens' hormonal treatment was expected to affect the sex ratio of the offspring as a bias sex ratio that deviates from the expected sex ratio (Pike and Petrie, 2005). The sex ratio of female to male offspring was tested by chi-square (X<sup>2</sup> test). It has been shown that in treatment P<sub>0</sub>, the sex ratio of 1.2: 1 and 1.1: 1 in P<sub>1</sub> were not significantly different from hypothesis (1: 1). However, P<sub>2</sub> showed a sex ratio of 1.7: 1 differing significantly from the sex ratio of 1: 1, with a value of X<sup>2</sup> = 6.71 greater than X<sup>2</sup> table (0.05) = 3.84 (Table 2).

Based on this study's results, the increase in female offspring's ratio by 2 mg/hens of progesterone induction was expected to provide practical applications for layer chicken breeders. Increasing the percentage of female offspring compared to males has a vital role in reducing maintenance resources in the poultry industry. Male offspring from laying hens are less economically beneficial if raised as producers of meat. It also reduces the culling of male chicks after hatching, which often creates animal welfare conflicts in laying hens (Galli *et al.*, 2016).

Determination of the sex ratio of Arabic chicks was not carried out when DOC hatched because the newly hatched Arabic chickens were almost monomorphic, could not be distinguished between male and female and according to previous studies, sexing using feather sexing on the freshly hatched Arabic chicks only had an accuracy of 80.47% (Iswati *et al.*, 2020). Sexing of Arabic chicks was performed at four weeks of age (Fig. 2), which had shown secondary sex signs as the study of (Al-Maksousi *et al.*, 2019), who analyzed the sex ratio of Iraqi chickens at four weeks of age.

### Progesterone Concentration in Blood

Progesterone concentration in hens was measured at 2 h after hormone injection, or it coincides with 3 h before the estimated time of ovulation. This confirms that maternal hormones play a role in the first meiosis by influencing the segregation of non-random chromosomes segregation. According to (Correa *et al.*, 2005), high Progesterone during meiosis is a mechanism for sex manipulation of offspring in bias the primary sex ratio.

Table 3 shows that the concentration of the hormone progesterone in the blood showed a significant difference ( $P < 0.05$ ) between the treatment group and the control group ( $P_0 = 2.47 \pm 0.41$  ng/ml). Progesterone concentrations were  $4.67 \pm 0.52$  and  $5.84 \pm 0.57$  ng/ml in  $P_1$  and  $P_2$ , respectively with non-significant difference ( $P > 0.05$ ). When associated with the sex ratio of chicks produced, group  $P_2$  resulted in higher female offspring having higher progesterone concentrations.

The control group ( $P_0$ ) which assumed as a physiological condition showing progesterone levels 2-3 h before ovulation, where the chromosome segregation process occurs. Physiologically, the progesterone concentration in Arabic chickens in this study was in the normal range of Progesterone concentrations in poultry is 2-3 ng/ml (Reece, 2009). The physiological level does not cause a biased sex ratio.

Even though the progesterone concentration of group  $P_1$  was higher than control ( $P_0$ ), it did not cause a significant sex ratio bias towards the number of female offspring, because the progesterone concentration at the  $P_1$  level has not made the random sex allocation lead to the female sex. Progesterone concentration of the  $P_2$  group exhibited the highest value. It makes the sex ratio significantly different ( $P < 0.05$ ) from the expected sex ratio of 1:1, or there was a biased sex ratio towards females and a decrease in the proportion of male offspring. This finding follows the study by (Correa *et al.*, 2005), who stated that layer hens have higher Progesterone levels and a decreased proportion of male offspring.

Progesterone concentration of hens, which produced male and female chicks of the three treatment groups, showed that only the  $P_2$  group resulted in a significant difference ( $P < 0.05$ ) (Fig. 3). Hens of the  $P_2$  treatment group that produced female offspring showed a higher progesterone concentration than the hens with male offspring ( $P < 0.05$ ). This is following (Correa *et al.*, 2005) study, who found that hens laid female eggs had a higher concentration of Progesterone than hens laid male eggs. Among other treatments, progesterone concentration between hens that produced male and female chicks was not different. Moreover, it has been shown that the relatively low progesterone levels tested in the present study have not been able to bias sex toward females.

### Conclusion

Treatment of progesterone injection in hens resulted in a higher blood concentration of Progesterone than the untreated group. Meanwhile, Progesterone's administration in the Arabic hens at a dose of 2 mg/hen caused an increase in the proportion of female chicks, therefore becoming an opportunity for manipulation of the sex ratio of the Arabic chicks after hatching.

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### Author's Contributions

**Iswati and Trinil Susilawati:** Designed and conducting the experiment and writing the manuscript.

**Muhammad Halim Natsir and Gatot Ciptadi:** Analyzed the data and participated in the revision of the document.

### Ethics

This study was approved by the Animal Ethics of Brawijaya University with the number 001-KEP-UB-2020 and declared to fulfill the study's ethical feasibility. All procedures and maintenance of chickens are by institutional guidelines for the use of animals in research.

### References

- Abdellatif, M. A. (2001). Sexing on down and feather colour in the Dandarawi Egyptian breed. *British Poultry Science*, 42(3), 327-332. <https://doi.org/10.1080/00071660120055287>
- Afriani, T., Udin, Z., Jaswandi, D., Asmairicen, S. (2011). Pengaruh Waktu Pelapisan Spermatozoa Sapi Pada Media TALP yang Disuplementasi bovine serum albumin (BSA) Terhadap Jenis Kelamin Embrio In vitro. *Jurnal Peternakan Indonesia*, 13 (2); 141-148. <https://doi.org/10.25077/jpi.13.2.141-148.2011>

- Al-Maksousi, S. K., Al-Hayani, W. K., & Hussein, F. M. (2019). Effect of the level of corticosteron hormone in the blood of local iraqi chicken mothers on the sex ratio of the produced offsprings. *Plant Archives*, 19(2), 1411-1415.
- Alonso-Alvarez, C. (2006). Manipulation of primary sex-ratio: an updated review. *Avian and Poultry Biology Reviews*, 17(1), 1. <https://doi.org/10.3184/147020606783437930>
- Aslam, M. A., Hulst, M., Hoving-Bolink, R. A., Smits, M. A., de Vries, B., Weites, I., ... & Woelders, H. (2013). Yolk concentrations of hormones and glucose and egg weight and egg dimensions in unincubated chicken eggs, in relation to egg sex and hen body weight. *General and Comparative Endocrinology*, 187, 15-22. <https://doi.org/10.1016/j.ygcen.2013.02.045>
- Bacon, W. L., & Liu, H. K. (2004). Progesterone injection and egg production in turkey hens. *Biology of Reproduction*, 71(3), 878-886. <https://doi.org/10.1095/biolreprod.104.027672>
- Correa, S. M., Adkins-Regan, E., & Johnson, P. A. (2005). High progesterone during avian meiosis biases sex ratios toward females. *Biology Letters*, 1(2), 215-218.
- Galli, R., Preusse, G., Uckermann, O., Bartels, T., Krautwald-Junghanns, M. E., Koch, E., & Steiner, G. (2016). In ovo sexing of domestic chicken eggs by Raman spectroscopy. *Analytical Chemistry*, 88(17), 8657-8663. <https://doi.org/10.1021/acs.analchem.6b01868>
- Gam, A. E., & Navara, K. J. (2016). Endogenous corticosterone elevations five hours prior to ovulation do not influence offspring sex ratios in Zebra Finches. *Avian Biology Research*, 9(3), 131-138. <https://doi.org/10.3184/175815516X14495923605779>
- Habibullah, M., Hashem, M. A., Rana, M. S., & Islam, M. H. (2015). Effect of Artificial Insemination on different production parameter in Hubbard classic broiler parent stock. *Journal of the Bangladesh Agricultural University*, 13(1), 71-77. <https://doi.org/10.3329/jbau.v13i1.28720>
- Hens, E. H. (2001). cocks and avian sex determination. A quest for genes on Z or W. *EMBO Reports*, 2(3), 192-196. <https://doi.org/10.1093/embo-reports/kve050>
- Idowu, P. A., Mpayipheli, M., & Muchenje, V. (2019). A Survey Study on Productive and Reproductive Performance of Indigenous Poultry. <https://doi.org/10.3844/ajavsp.2019.33.39>
- Indra, G. K., Achmanu, A., & Nurgartiningih, A. (2013). Performans produksi ayam Arab (*Gallus turcicus*) berdasarkan warna bulu. *Ternak Tropika Journal of Tropical Animal Production*, 14(1), 8-14.
- Iswati, M. Natsir, H., Ciptadi, G., & Susilawati, T. (2020). Sexing on a Day-Old Chick of Gold Arabic Chicken (*Gallus turcicus*) Using Feather Sexing Method. *Journal of Animal & Plant Sciences*, 44 (3); 7708-7716. <http://www.m.elewa.org/Journals/wp-content/uploads/2020/06/3.Iswati.pdf>
- Krautwald-Junghanns, M. E., Cramer, K., Fischer, B., Förster, A., Galli, R., Kremer, F., ... & Bartels, T. (2018). Current approaches to avoid the culling of day-old male chicks in the layer industry, with special reference to spectroscopic methods. *Poultry Science*, 97(3), 749-757. <https://doi.org/10.3382/ps/pex389>
- Kuroiwa, A. (2017). Sex-determining mechanism in avians. In *Avian Reproduction* (pp. 19-31). Springer, Singapore. [https://doi.org/10.1007/978-981-10-3975-1\\_2](https://doi.org/10.1007/978-981-10-3975-1_2)
- Mohammadi, H., & Ansari-Pirsaraci, Z. (2014). Changes in some blood parameters and production performance of old laying hens due to growth hormone and testosterone injection. *Journal of Animal Physiology and Animal Nutrition*, 98(3), 483-490. <https://doi.org/10.1111/jpn.12095>
- Mohan, J., Sharma, S. K., Kolluri, G., Tyagi, J. S., & Kataria, J. M. (2015). Selection of Diluent for Short Term Preservation of Guinea Fowl Semen. *Asian Journal Animal Veterinary Advance*, 10(8), 360-364. <https://doi.org/10.3923/ajava.2015.360.364>
- Navara, K. J. (2018). *Choosing sexes*. Cham, Switzerland: Springer International Publishing. <https://doi.org/10.1007/978-3-319-71271-0>
- Pike, T. W., & Petrie, M. (2005). Maternal body condition and plasma hormones affect offspring sex ratio in peafowl. *Animal Behaviour*, 70(4), 745-751. <https://doi.org/10.1016/j.anbehav.2004.12.020>
- Pinson, S. E., Parr, C. M., Wilson, J. L., & Navara, K. J. (2011a). Acute corticosterone administration during meiotic segregation stimulates females to produce more male offspring. *Physiological and Biochemical Zoology*, 84(3), 292-298. <https://doi.org/10.1086/659373>
- Pinson, S. E., Wilson, J. L., & Navara, K. J. (2011b). Elevated testosterone during meiotic segregation stimulates laying hens to produce more sons than daughters. *General and Comparative Endocrinology*, 174(2), 195-201. <https://doi.org/10.1016/j.ygcen.2011.08.020>
- Reece, W. O., (2009). *Functional Anatomy and Physiology of Domestic Animals*. 4th edition, Wiley-Blackwell, Ames, Iowa. ISBN-13: 978-0813814513
- Smith, C. A. (2007). Sex determination in birds: HINTs from the W sex chromosome?. *Sexual Development*, 1(5), 279-285. <https://doi.org/10.1159/000108934>
- Susilawati, T. (2014). *Sexing Spermatozoa*. UB Press. Malang. Indonesia. <https://fapet.ub.ac.id/wp-content/uploads/2017/10/Sexing-spermatozoa-bu-trinil.pdf>

- Tamzil, M., Ni, H., Haryani, K. D., & Ny, I., Jaya, S. (2018). Polymorphism of Qualitative Traits of Arabic Chicken: A Case Study in Istiqomah Farmer Group, Dasan Cermen, Mataram, West Nusa Tenggara, Indonesia. *International Journal of Poultry Science* 17 (8); 378–384. <https://doi.org/10.3923/ijps.2018.378.384>
- Wilson, K., & Hardy, I. C. (2002). Statistical analysis of sex ratios: an introduction. *Sex Ratios: Concepts and Research Methods*, 1, 48-92. <https://doi.org/10.1017/CBO9780511542053.004>
- Zaghari, M., Taherkhani, R., & Honarbakhsh, S. (2009). Effects of progesterone injection on performance, plasma hormones and ovarian morphology of ad libitum and restricted fed broiler breeder hens. *African Journal of Biotechnology*, 8(22); <https://doi.org/10.1017/S175275620003043X>