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Effect Of Human Ovarian Stimulating Hormone (FSH) On The Ovarian Fertility Of Laying Hens

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Abstract

Reproductive hormones are called gonadotropins, and examples include FSH. Increased ovulation and follicle growth in chickens may lead to more eggs being laid each cycle if the right amount of FSH is administered. The action of follicle-stimulating hormone occurs early in the development of follicles. The synergistic effect of FSH ensures healthy follicular development. Follicles develop in hens with insufficient FSH, however this does not lead to successful egg production. The purpose of this research was to examine the levels of FSH in laying hens that produce eggs at various intervals. Fifty serum samples were taken from ISA brown strain chickens aged 54 weeks and split into five groups according on how often they lay eggs: (i) daily, (ii) once every two days, (iii) twice every three days, (iv) three times every four days, and (v) never. Using an enzyme-linked immunosorbent test, levels of follicle-stimulating hormone in the samples were determined, and the results were evaluated using multivariate analysis of variance. Among ISA brown strain hens, the level of FSH was strongly correlated with the number of eggs laid per day (p 0.05), with the highest FSH level being seen in daily-laying chickens. In ISA brown strain chickens, a high level of FSH was related to daily egg laying.

Keywords: *Egg, Follicle-Stimulating Hormone, Reproductive, Enzyme-Linked Immunosorbent Test.*

1. Introduction

Egg production in a hen takes a whole day, and some hens go for weeks at a time without producing any eggs at all. In addition, not all of the hens in a particular cage will begin producing eggs at the same time. Some of the hens in the cage may never produce an egg, while others may begin laying eggs earlier than the rest of the hens in the cage. [1] In some very unusual instances, hens who normally produce eggs may just stop doing so entirely. Egg production is affected by a variety of factors, including age, genetics, and environm[1]ental conditions, among others. [2] Egg production is influenced by environmental factors like as temperature and brightness of the surrounding light. [3] Egg production is significantly correlated with the age at which pullets reach sexual maturity; nevertheless, the optimal time for this milestone to be reached varies from genetic stock to stock in order to maximize egg production. [4]The lighting program that a flock of laying hens is exposed to throughout the development and production phases has a considerable influence on the timing of sexual maturity in the flock as a whole.[5] The time of when plants attain their optimum production is affected, to some degree, by the schedule of lighting since this factor is connected to the growth of reproductive organs. It has been shown that exposing animals, especially poultry, to artificial light at night speeds up the maturation process of their reproductive organs and genitalia. When there is no artificial

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light present, the darkness at night causes chickens to begin molting earlier than normal. [6,7]

Since long-wave light can more easily penetrate epidermal tissue, it elicits a greater response from the pituitary gland, which is responsible for reproduction regulation. Some of these hormones, known as gonadotropins, are responsible for ovulation [8]. They accomplish this by encouraging the formation of follicles, which in turn results in the generation of eggs. [9] The presence of optimal levels of FSH is capable of quickly stimulating the development of follicles, which in turn leads to an increase in egg production as a consequence of the increased number of follicles that have developed to the point of ovulation. [10] The management of gonadotropins, which is unique to poultry, is what maintains the continued existence of the follicular hierarchy while also controlling the development of the largest follicles in preparation for ovulation. FSH collaborates with other hormones to encourage the growth of healthy follicles. [11.12]

Demand for chicken products, most notably eggs, continues to climb around the world, demanding continuous study into methods to boost the productive capacity of laying hens as well as their reproductive effectiveness. [13] In order to accomplish this goal, researchers have looked at a variety of potential methods that may boost the reproductive potential of these bird populations [14]. An interesting and potentially fruitful area of research would be to investigate the influence that human ovarian stimulating hormones like follicle-stimulating hormone (FSH) have on the ovarian fertility of laying hens.[15,16] This novel technique has the potential to give new strategies for enhancing egg production in industrial chicken farming by bringing together the domains of human endocrinology and avian reproductive biology.[17,18]

Eggs laid by laying hens, also known as Gallus gallus domesticus in the scientific world, are an essential food source due to the high levels of protein that they contain. The steady expansion in the population of the globe has resulted in a meteoric rise in demand for chicken products, particularly eggs.[19] As a direct consequence of this need, chicken farming has evolved to meet it, and as a result, it has expanded from small-scale household operations to large-scale industrial farms. Because of this change, there is a need for improved reproductive efficiency as well as increased egg output. [20]

In laying hens, the level of reproductive success is closely related to both the ovarian follicular hierarchy as well as the ovulatory process. In birds, as opposed to mammals, the ovary includes follicles of varied sizes that develop concurrently. [21] These follicles are referred to as hierarchal follicles. These follicles go through a succession of stages, starting with the pre-hierarchical follicles, which are smaller, and ending with the mature follicles, which are larger. Only the F1 follicles, also known as pre-ovulatory follicles, are capable of ovulating and releasing an egg. Other follicles do not have this ability. When trying to maximize egg production, it is essential to both manipulate the follicular hierarchy and increase the number of F1 follicles. [22]

When looking at birds, one of the hormones that is often investigated in human subjects also comes into play: FSH. Because it regulates both the rate at which and the number of ovarian follicles grow, FSH is an extremely important hormone in humans. Because the procedures of ovarian function in people and birds are fairly comparable to one another, researchers have pondered the possibility that FSH may be used to hasten the formation of ovarian tissue and boost the amount of F1 follicles in laying hens. The objective of this introductory paper is to offer a high-level overview of the possible benefits, challenges, and ethical concerns of this fascinating new area of research. [23]

It is imperative that the significance of ovarian fertility in laying hens be underlined before moving on to describe the role of FSH. The pace at which hens can lay eggs is the single most critical aspect in determining the efficacy of their reproductive capabilities. Increasing

the amount of eggs produced in commercial hen houses has a number of beneficial benefits, both on the environment and on the bottom line. Because of this, chicken farmers and poultry industry experts have spent years seeking for ways to increase ovarian fertility in chickens. [24]

The follicular hierarchy and the process of ovulation are very important to the fertility of the ovary in birds. Laying hens, much like many other kinds of birds, have a complicated system of ovarian follicles that consists of several follicles that are all at various stages of development. The most developed of these follicles is called the F1, and it is responsible for ovulation as well as the generation of eggs. The challenge is in the production of more F1 follicles, which, if successful, may result in a significant increase in the hen's fertility. [25]

Increasing egg production in laying hens by injecting them with human FSH, a hormone that is well known for its involvement in regulating ovarian function in humans, is a novel approach. In the field of human reproductive medicine, FSH has shown to be a useful tool for expanding the size of ovarian follicles and initiating the release of an egg. The usage of FSH has been investigated by researchers in the context of birds, with the goal of identifying parallels between humans and other species. [26]

The use of FSH as a method for controlling ovarian fertility in layer hens has the potential to result in several advantageous consequences. Altering the follicular hierarchy and increasing the number of F1 follicles may be able to result in a substantial rise in egg output. This is a distinct possibility. When there is a greater output of eggs, there is a greater likelihood that poultry farmers will make more money, and consumers will have access to a more consistent source of protein. In addition, from a scientific point of view, this sort of development would provide insight on avian reproductive biology and endocrinology, which may lead to new breakthroughs in poultry research. [27]

However, before utilizing FSH to improve ovarian fertility in laying hens, there are a number of challenges and ethical issues that need to be taken into consideration. It is necessary to address concerns about the health of humans, the well-being of the birds, and the ethics of messing with the birds' reproductive processes. Because this research involves giving the birds a hormone injection, which may disrupt their natural reproductive cycles, concerns regarding the birds' ethics and wellbeing are of the utmost significance.[28]

2. Review of literature

Davis & Johnson (2022) [29] Inhibin B, also known as inhB, is a protein that is released by hen G cells in the F1-F3 follicle. Despite the fact that the chicken ovary has not been demonstrated to synthesize inhB, activin AB, activin B, and follistatin (FS) proteins, RNA molecules for both Bsubunit and FS are expressed there, with maximal expression in the G layer of early hierarchy follicles (F6-F8) and tiny yellow follicles.
Lovell et al. (2021) [30] In primary cell cultures of G cells derived from preovulatory (F1, F2, and F3) follicles that have been produced in vitro, the secretion of inhA is controlled by the hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as insulin-like growth hormone-I (IGF-I), and actA.
Chen & Johnson (2020) [31] There are additional mRNAs for the inhibin/activin -, A-, and B-subunits in chicken ovarian follicles. The involvement of inhibin-related proteins in controlling ovarian activity in birds is being more supported by research. Preovulatory follicles of chickens contain both inhibitory (inhA) and activating (actA) proteins in their G and T layers, respectively.
Gilbert et al., (2020) [32] Atresia is very uncommon after a follicle has reached a size of 9 mm, and the follicle is thus committed to the fast rising preovulatory hierarchy (usually the biggest 5–10 follicles.

3. Research methodology

- **Ethical approval**

The ethics committee for the area's experimental animal care was consulted before any animal was harmed in the name of science.

- **Study period and location**

From January through May of 2022, researchers gathered data in the hot, dry season. The samples for this investigation were from a Lucknow-based chicken farm. Levels of reproductive hormones were analyzed in blood samples at a lab in Lucknow.

- **Meteorological data**

A thermometer was hung in the hen house at a height of 1.5 meters, right next to the laying chickens. From 07:00 to 19:00 every day for 8 weeks, both dry-bulb and wet-bulb temperatures were recorded.

In this research, "we utilized ISA brown laying hens who had been in the breeding program for 54 weeks and had a mean live weight of 1.85 0.3 kg. The diet the flock was provided with contained nutrient values of 17.5% crude protein, 8.0% crude fiber, 5.0% crude fat, 35.0% ash, 4.0% Ca, 0.5% phosphorus, and 2750 kCal/kg metabolizable energy, and water was available ad libitum." The laying hens received the usual preventative treatments, such as immunizations and medicines.

The chickens were monitored for 14 days before any blood was taken from any of them. Each hen's cage was examined three times a day and labeled with a "1" if the hen had laid an egg, or a "0" if it had not. Blood samples were taken from hens that had laid eggs at least twice in a row.

The selection of hens for blood sample is seen above. The hen labeled as a "T2 sample" had a consistent pattern of laying one egg every other day. The hen with the X was disregarded because of its erratic egg-laying patterns. The hens that were selected for blood sample were also marked.

- **Experimental design and animal management**

This research used a totally random methodology. Fifty chickens were randomly assigned to one of five groups:

- T1: laying hens that produce eggs on a daily basis.
- T2: laying hens that produce an egg every other day.
- T3: laying hens that produce two eggs every three days.
- T4: hens that produce eggs at a rate of three every four days.
- T5: Chickens that is infertile.

For the duration of the study, layer hens were kept in a typical cage within a barn and given a commercially available feed. The laying hen chamber was kept at a constant 26 3 degrees Celsius, with a light/dark cycle of 16 hours on, 8 hours off. "After keeping track of how often the chickens laid eggs for 14 days, blood samples were obtained to analyze their health.

One milliliter of blood was drawn from the brachial vein of each chicken's wing and placed in a vacutainer plain tube, which was then placed in a cold box with ice gel and ice cubes maintained at 4 degrees Celsius. After the blood samples were obtained and preserved as mentioned above, the delivery standard was modified to conform to the Decree of the Minister of Health of the Republic of Lucknow."

- **Follicle-stimulating hormone**

Blood samples from hens were examined using ELISA, in which the samples competed with antibodies housed in a microplate to detect FSH. "After leaching, reagents or unbound materials were removed from the microplate and a substrate was added, causing color to develop at the interaction of the antibodies with the conjugate enzymes. The OD was then read off using an ELISA reader."

Statistical analysis

The levels of follicle-stimulating hormone were evaluated using an ANOVA in SPSS 20. Duncan's test was used to identify the significant group if a statistically significant difference was detected at the p 0.05 level.

4. Results

Daily layer chickens had the highest average FSH levels, whereas those that laid eggs three times per week had the lowest.

Table 1: FSH levels of activity.

FSH	**Hormone level**
T1	869.05
T2	429.13
T3	277.335
T4	52.543
T5	193.168

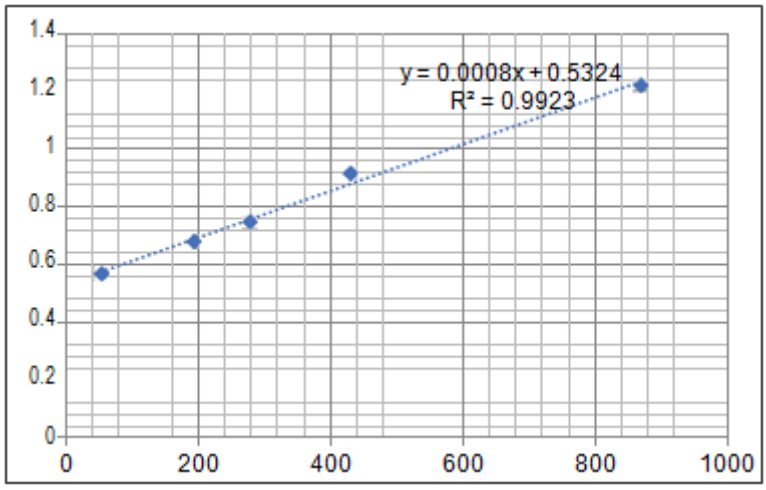
One-egg-a-day layers had the highest rate of absorption, whereas those that laid eggs three times in four days had the lowest.

Table 2: The typical absorbance readings for FSH concentrations

FSH	**Hormone level**
T1	1.221
T2	0.919
T3	0.747
T4	0.567
T5	0.68

Duncan found that the absorbance values and FSH levels of the T1 hen group were much greater than those of the other hen groups. The degree of reactivity of a sample may be calculated from its absorbance value. When the amount of analytes is large, the OD will read high because of the strong hue. We see the standard curve for FSH. An R2 of 0.9699 indicates a degree of accuracy in the curve of 99.99 percent.

Figure-1: Standard curve determination for FSH.



5. Discussion

Both male and female animals, including humans, are dependent on adequate amounts of follicle-stimulating hormone in order to successfully reproduce. The synthesis of GnS originates in the anterior pituitary in response to the brain's release of GnRH. Granulosa cells are collected from surrounding follicles of varying sizes and put through their developmental process in the hen when FSH is present. It has an impact on the larger follicles as well as the smaller ones, notably the granulosa layer of the yellow follicles. "The level of reproductive hormones in a hen will determine not only whether or not she is able to produce eggs, but also the quality of those eggs. One of the ways in which follicle-stimulating hormone increases follicular development is by an increase in angiogenesis in the follicles of the theca externa of laying hens with low egg production rates. This is one of the mechanisms through which follicle-stimulating hormone works."

According to the data, daily-laying ISA brown hens (T1) had the highest average FSH levels in comparison to the other types of chickens. The average levels of FSH in hens who laid eggs once every two days (T2) and even in hens that laid eggs twice every three days (T3) were lower, coming in at 429.130 and 277.335 pg/mL, respectively. The amount of FSH, measured as an average of 52.543 pg/mL, was much lower in the T4 chickens than it was in the other groups of hens. The average FSH levels of chickens who did not lay eggs (T5) were higher than those of T4 hens but lower than those of T3 hens, coming in at 193.168 pg/mL. This was in contrast to the levels of T4 hens, which were lower than those of T3 hens. Due to the fact that T5 hens were given a break in between egg production, the FSH levels in the T5 chickens were much higher than those seen in the T4 hens. There is some evidence to suggest that elderly chickens that have stopped laying eggs have higher levels of FSH than laying hens do. The levels of FSH that were measured at the peak of production and those that were recorded during a rest time following a stressful period were shown to have no significant difference when compared in earlier studies.

The hypothalamus is responsible for the secretion of GnRH, which then causes the anterior pituitary gland to be stimulated into producing FSH. The production of steroids and the development of follicles in the ovary both need the presence of follicle-stimulating hormone.

FSH, often known as FSH, is a hormone that plays a significant role in the process of follicle formation and differentiation." In addition to this, it encourages the proliferation and differentiation of granulosa cells, as well as the generation of steroid hormones and progesterone. An increase in the total number of follicles present in the ovary is often accompanied by a rise in FSH levels [33]. During pre-ovulation (F1), the follicle-stimulating hormone has an effect on the steroidogenesis of immature yolk follicles and little yellow follicles, but it has no effect on the steroidogenesis of large follicles." Granulosa cells are able to differentiate into more mature forms with the assistance of follicle-stimulating hormone in pre-hierarchical follicles. This hormone is also responsible

for the increased production of steroid hormones by granular cells. Their disproportionately large percentage in laying hens with low egg production is due, in part, to the fact that atretic follicles have a lower number of blood arteries, as well as that they express a lower level of FSH and FSH receptors.[34]

The development of eggs needs a certain amount of prolactin, but producing too much of it might have a detrimental impact on the levels of FSH. Due of the hormone's influence on GnRH secretion inhibitors, egg production may be inhibited when prolactin levels are too high. This is due of the effect. The hypothalamus is susceptible to stimulation by outside elements, such as light, which ultimately leads to an increase in the synthesis of GnRH. The lighting program, which has an influence on the reproductive organs of laying hens, causes gonadotropin hormones to be released. Examples of these hormones include FSH. Laying hens are able to view their surroundings because their eyes include photoreceptors, and the non-visual photoreceptors, also known as extraretinal photoreceptors, are the ones that detect the photoperiod and enable the hen to adjust her physiology in response to it. Due to the fact that the wavelength determines the color of light, exposure to red light may cause increased levels of FSH.

Light is responsible for regulating the production of elatonin in the retina and the pineal gland. Exposure to artificial light throughout the evening reduces the amount of melatonin that is secreted. An rise in the concentration of melatonin may lead to a reduction in egg production. This, in turn, can lead to a drop in the concentration of GnRH, which influences inhibition of the reproductive axis and increases the concentration of GnIH in the plasma.

According to the results of our research, FSH and the frequency at which laying hens produce eggs may differ even when the chickens are exposed to the same photoperiod. As a direct consequence of this, there is a pressing need for improved lighting, nutrition distribution, and pullet quality monitoring of laying hens. [35]

6. Conclusion

Researching how human ovarian stimulating hormone FSH affects the ovarian fertility of laying hens is an exciting new frontier in the field of poultry science. This overview has laid the groundwork for a more in-depth knowledge of the possible advantages and problems of bringing FSH into the setting of laying hens, as well as avian ovarian fertility and the hierarchical organization of follicles. Whether or not FSH can be used to increase the reproductive potential of these vital avian species will be explored in greater detail in the following sections of this study, along with the ethical and welfare implications of doing so. The research found that ISA brown chickens with higher FSH levels had more frequent egg lying.

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