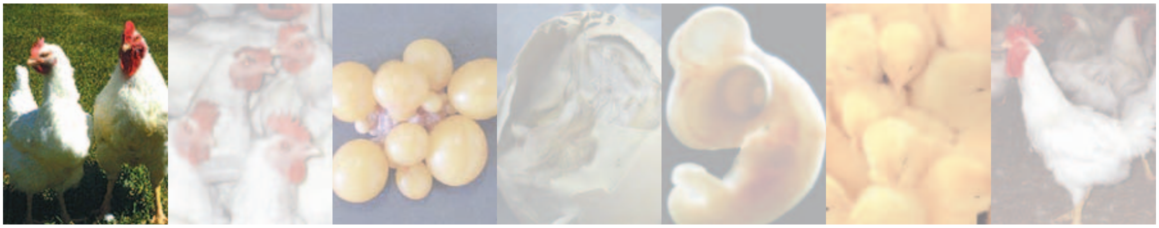

2 Female Reproduction: Control of Ovarian Function

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Introduction

The control of the release of mature yolks from the ovary, a process known as ovulation, is similar in broiler breeder hens and egg-type hens in principle, but sometimes not in practice. The potential difference is that table-egg producing hens have been selected for reproductive efficiency and meat-type hens have been selected to a greater extent for conformation and growth rate. The means that egg-type hens have been selected to truly excel in egg output. These hens are able to lay well because they have highly efficient and organized recruitment of follicles in the ovary. Meat-type hens have been intensively selected for growth

Other reviews of the avian reproductive system:

Etches, R J. 1996. Reproduction in Poultry. CAB International, Wallingford, UK

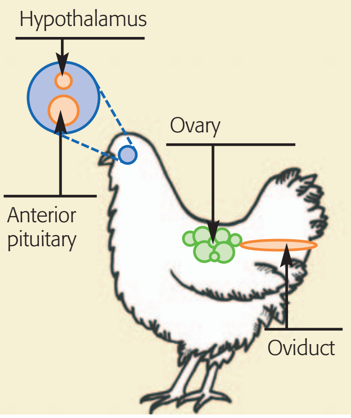
Proudman, J A. 1995. The biology of egg production and fertility. Proc. First International Symposium on the Artificial Insemination of Poultry. Bakst, M R, and G J Wishart, Eds. Poultry Science Association, Savoy Ill.

Ovulation

This is the act of a mature follicle (yolk) being released from the ovary. It will travel down the oviduct having albumen, shell membranes and shell applied around it.

Figure1

The main reproductive organs of a laying hen



rate, feed efficiency, and breast meat yield traits, and they are prone to excessive and erratic follicular recruitment. This results in a high numbers of unsettable eggs for broiler breeders.

This review of the control of ovulation is written from a viewpoint of matching “form” with “function.” The hen’s reproductive system consists of a highly organized system that results in the formation of the yolk. Ideally, it is only a single yolk per day, each and every day. This system involves the brain, the adenohypophysis (also called the anterior pituitary) and the ovary. We could consider the liver to be an integral part of that system because that is where the egg yolk lipid is formed (covered in Chapter 4). Another system (the oviduct) plays the role of adding albumen around the yolk. Shell membranes are added around the yolk and shell is added around these membranes (Chapter 5). Another requirement of the reproductive system is that it must facilitate the passage of spermatozoa from the site of semen deposition to the site of fertilization. Considering that sperm can be stored in this tract for 1-2 weeks, this is an important role too for the production of fertile hatching eggs. This chapter focuses on how the hormonal system of the hen works such that she may release a mature yolk each day.

The Hypothalamus

The hypothalamus is the reproductive controller of the hen. This very small organ (Figure 1) deep in the brain is a key component to the hen’s reproductive output. It receives input from other brain centers through tiny neuro-transmitters, as well as signals directly from the environment. Some studies have shown that the hypothalamus must be “mature” to respond to light stimulation prior to the onset of laying the first egg.

The reproductive system is driven by hormones. Hormones are very small chemicals that are produced and released in one organ, but are transported by the blood stream to make something happen in another organ. For a hormone to elicit a response in a specific cell or tissue, that cell or tissue must have the necessary “receptor” for the hormone. It acts like a “key” and “lock” model. It gets more complicated, however, because as the number of hormones and receptors changes with time. It is as if the number of “keys” and “locks” changes with time as well. The precise concentrations of these hormones can measured using sophisticated tests called radioimmunoassays.

The purpose of the hypothalamus in the control of reproduction is to release very small protein hormones (gonadotrophin releasing hormones also known as GnRH) that stimulate the release of "gonadotrophin" hormones from the neighboring adenohypophysis (also called the anterior pituitary). There are two types of GnRH in chickens. There are special cells in the hypothalamus that are thought to receive light energy at photostimulation in response to the days becoming longer. Contrary to what is thought, this recognition of day length does not involve the eyes. The light response involves the stimulation of specialized cells within the brain. That means that light intensity must be adequate to have the light energy penetrate the feathers, the skin, the skull and the brain.

There is a network of blood vessels that link the hypothalamus and the adenohypophysis. This means that the message from the hypothalamus (GnRH) is sent directly to the adenohypophysis in a rapid manner.

The Adenohypophysis

The adenohypophysis is the organ that produces the hormones that travel to the gonads to actually stimulate reproductive function in the long term (through life cycles) as well as the short term (through each day). The adenohypophysis receives messages from the hypothalamus and, if the timing is right, it releases two important gonadotrophin hormones.

One hormone is luteinizing hormone (LH) which is essential for sexual maturation and for daily egg production to occur. This hormone is also produced by the adenohypophysis of mammals, but there are some differences in LH make-up between species. The output of LH into the blood stream is directly related to the output of GnRH reaching the adenohypophysis. The role of LH is to stimulate the production of sex-steroids (steroidogenesis) in the follicles in the ovary. It also stimulates androgen hormone production from the testes of males. This hormone is an integral component of the day-to-day events of ovulation.

The second hormone is follicle stimulating hormone (FSH). As its name suggests, this hormone is thought to be involved in the development of many tiny follicles days or weeks before they become competent to ovulate. The role of FSH in promoting sex steroid production in the ovary is not thought to be significant, as small amounts of LH can do as much as larger amounts of FSH.

How do you measure hormone concentrations in blood?

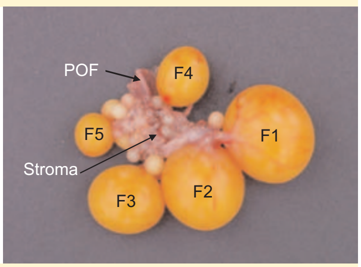
Hormones are too small to isolate from blood plasma and weigh to determine concentration. Early researchers injected blood from one animal into another animal to see if they could elicit a "biological response." These "bio-assays" require a lot of blood and many animals. Furthermore, they are not very precise. For the past 30 years researchers have used radio-immunoassays to monitor levels of many hormones in blood and other biological fluids. These techniques involve a known amount of radio-active tracer hormones and a limited amount of antibody to the hormone being studied. The displacement of the radio-active hormone from the binding complex is proportional to the amount of hormone in the sample.

Oviposition
This is the act of laying an egg. The egg is released from the shell gland into the world. Oviposition normal proceeds with the large end of the egg appearing first.

Luteinizing hormone (LH)
A glycoprotein hormone released from the adenohypophysis. The name of this hormone does not make sense in chickens, but it is called that because it is very similar to the mammalian hormone that does 'luteinize.' It is very important in stimulating sex-steroid production from the ovary.

Follicle Stimulating Hormone (FSH)
A glycoprotein hormone released from the adenohypophysis. It stimulates small follicle development. The number of follicles a hen has is determined before she hatches as a chick. Hens have many thousand follicles and only a few of them actually go so far as to become eggs.

Figure 2
The ovary of a laying hen showing the stroma (pool of undifferentiated follicles) , the large yellow follicles (F1-F5) and a post-ovulatory follicle (POF)



The Neuro-Hypophysis
The neurohypophysis lies adjacent to the adenohypophysis. The neurohypophysis (also know as the posterior pituitary) differs from the adenohypophysis in that it communicates with the higher brain by nerves. The neurophypophysis produces argininne vasotocin and mesotocin. Not as much is know about the role of these hormones as there is about adenohypophyseal hormones, but arginine vasotocin is presumed to be involved in the smooth muscle contraction needed at oviposition.

The Ovary
Hens only have a single functional ovary and oviduct (the left one). The ovary of an adult hen in excellent laying condition is seen in Figure 2. In a juvenile breeder female, the ovary is small and undeveloped. It normally stays in this condition until the hen approaches sexual maturity. The factors that determine when a hen will reach sexual maturity are poorly defined. It is believed that hens have to meet a minimum threshold for body weight to start laying. There is also evidence to suggest that hens that are too lean (inadequate carcass fat or carcass fleshing) will be delayed in reaching sexual maturity. Another criteria is "age." We have observed that some strains of hens will reach sexual maturity sooner when they are fed *ad libitum* than when they are feed restricted. However, other strains will not start laying any sooner when they are fed *ad libitum*, suggesting that these hens were not limiting in body weight, carcass fat or carcass protein, but rather, they were needing to wait until they were "old enough." This age requirement likely refers to the maturation of the hypothalamus to release GnRH in a proper manner.

The ovary is made up of the stroma, which is the base supporting structure. It is also composed of the vast pool of small undifferentiated follicles. The pool of follicles is established prior to the chick hatching. Some people have recorded counting several thousand of these follicles. It would not be likely that a hen would ever deplete herself of follicles. The follicles can be classified by size and color as seen in Figure. 3 The classification of ovarian follicles currently used at the University of Alberta is shown in Table 1. The smallest follicles contain "white" yolk. As they are recruited into the pool of larger follicles they become yellow. Most of our attention has been placed on the yellow follicles that are greater than 1 cm in diameter. The typical numbers of each

Table 1
Classification of Ovarian Follicles

Classification	Abbreviation	Color	Minimum diameter	Maximum diameter	Typical number per ovary in a breeder hen at sexual maturity
Large Yellow Follicles	LYF	Yellow	10 mm		7-9
Small Yellow Follicles	SYF	Yellow	5 mm	10 mm	5-15
Large White Follicles	LWF	White	2-5 mm	5 mm	5-15
Small White Follicles	SWF	White		1 mm	1000 to 12,000 or more

classification of follicle found at sexual maturity are given in Table 1 as well. It is well known that not all follicles that start to develop actually develop through to ovulation. Some follicles are said to be come "atretic" meaning that they have stopped accruing egg yolk (Figure 4).

Each follicle is made up of concentric rings of egg yolk (Chapter 4). The wall of the follicle consists of a single layer of "granulosa" cells which lie closest to the yolk (Figure 5). These cells in the largest (F1) follicle produce the steroid progesterone in response to circulating LH levels. The significance of this will be discussed later. On the outside of the granulosa layer is a layer of "theca" tissue. The theca tissue is a mixture of structural tissue, nerves, blood vessels and other specialized cells that secrete other steroid hormones. This tissue is very important for the production of female sex steroid (estrogen) that are important in the transformation of an immature pullet into a hen. As plasma levels of estrogens increase, externally visible features include reddening and enlargement of the comb and wattles, a prenuptial feather molt (feather drop) and a widening of the pubic bones to permit egg passage. Internally, estrogen stimulates liver production of egg yolk lipids with a significant change in the color and size of the liver. Finally, the oviduct enlarges and becomes a secretory organ for deposition of albumen.

Androgens are thought of as being "male" sex steroids. However, the ovary of the hen produces them as well. The theca cells of the very small follicles produce estrogens and androgens in response to photostimulation. Androgen hormones are thought to be involved in feather patterns and comb growth in females.

The ovary shown in Figure 2 is one from a hen with a high rate of egg production. A "perfect" ovary does not have too many large follicles and does not have too few large follicles. The large follicles are arranged in a

Figure 3
Classification of small ovarian follicle by size and yolk color

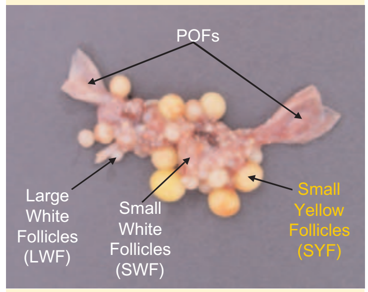


Figure 4
An atretic large yellow follicle

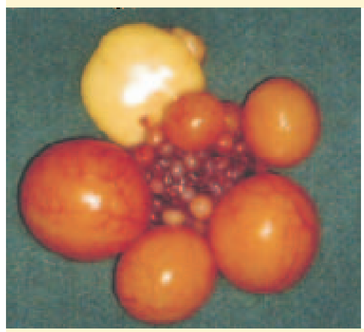


Figure 5
A schematic representation of the cross section through a follicle wall showing theca cells, granulosa cells and the centered yolk

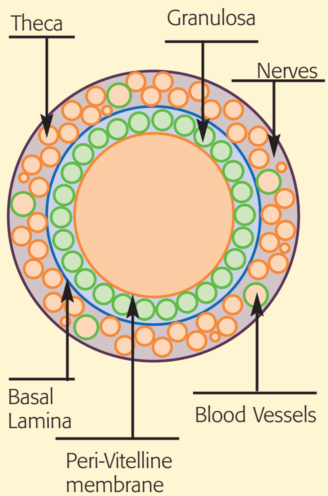


Figure 6
A normal ovarian hierarchy of large yellow follicles

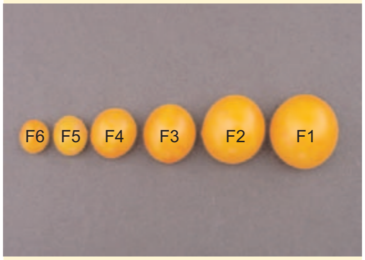


Figure 7
An abnormal double hierarchy of an *ad libitum*-fed hen

hierarchy, with the largest follicle (F1) being the closest one to ovulating (Figure 6). The spacing of follicles in the hierarchy is just as important as is the number of large follicles. Results from many University of Alberta research trials suggest that the ideal number of large follicles at sexual maturity is in the range of 7 to 8. Having too many follicles is associated with over feeding (see Chapter 7). Basically, when there are too many follicles the hen has some that are of identical maturational status as other follicles and double hierarchies can result (Figure 7). This can result in double-yolked eggs, or the laying of two eggs in one 24 hour period.

Having too few follicles is usually a problem with under weight pullets. Such birds often become hens that have poor persistency of lay as gaps appear in the follicular hierarchy. The number of follicles decreases with age, so under normal conditions, hens that have too few follicles at sexual maturity are thought to always have a decreased large follicle component.

When a hen ovulates, the layers of cells that surrounded the follicle are known as a post-ovulatory follicle (POF) as seen in Figure 2. This structure is not thought to serve a useful purpose after ovulation. There is minimal blood supply to it. It does not produce significant amounts of hormones. It is very unlike the analogous structure (corpus luteum or CL) in mammals in all of these three ways. In about 5 to 7 days, it regresses and disappears. Therefore a laying hen may have four to seven POF sacs in varying stages of regression. Sometimes when pullets are coming into egg production, the development of the oviduct lags behind the ovary, and internal laying results (see Chapter 6).

How do double-yolked eggs happen?

When two ovarian follicles are developing at a similar rate, both will ovulate at the same time (identical functional maturity) resulting in a double-yolked egg. This condition is normally seen at a rate or 2-3% during the first 6 weeks of the laying period. As flocks are over-fed, the incidence increases. Some hatching egg producers monitor the incidence of double-yolked eggs to determine if they are under-feeding or over-feeding the flock.

Control of Pattern of Ovulation

Chickens lay eggs in sequences or clutches. Normally, this egg laying pattern follows certain rules. The first rule is that the first egg of a sequence is laid within 1-2 hours after the house lights come on (see Table 2). Secondly, each egg in a sequence is laid later in the day. Thirdly, the last egg of a sequence is typically laid about 9-10 hours after the lights come on. We call the delay in time of lay from one day to another "lag." Hens that lay long sequences must lay them at close to 24 hour intervals so that there is minimal lag. Each sequence is separated by a non-laying day known as a "pause". This pause is typically of 40 to 44 hours duration.

It is the release of GnRH from the hypothalamus that results in laying sequences. About 6 hours before a hen ovulates she experiences a surge of GnRH which results in a surge of LH. This hormone activity is limited to a finite period of the day. This is called the "open period for LH release." If there is a mature pre-ovulatory follicle it will respond to this burst of LH release and will produce progesterone. This progesterone will stimulate further LH release and so on. This is a "positive feedback" where the release of one hormone triggers further release of another hormone. Ovulation is the end point, about 6-8 hours after this initial LH surge. These hormonal interactions are illustrated in Figure 8.

Normally ovulation follows oviposition by a period of about 15-45 minutes. This is not the case when a hen lays an egg late in the afternoon, many hours after the first egg of a sequence was laid. In this case, the hen does not ovulate soon after oviposition because it is too late in the day (has exceeded the time limit of her open period for LH release). She will not lay an egg the next day (pause day). The hen will

Table 2

Times of egg laying

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
9:05	13:30					
8:44	11:26	15:15				
8:01	10:00	12:03	15:23			
7:24	9:22	10:26	11:46	15:05		
7:33	9:20	10:34	11:30	12:34	15:40	
7:45	9:12	10:10	10:51	11:39	12:38	15:26

Can a hen lay two eggs per day?

Yes, a hen can lay more than one egg per day. In one University of Alberta trial, one group of *ad libitum*-fed hens peaked at 107% egg production. However, many of the eggs did not have shells. A hen can normally only put shell on a single egg per day properly, as the shell gland only holds one egg at a time, and it takes about 20 hours to apply the shell. When two eggs are laid on a single day, one usually will have ovulated a few hours before the other and is already becoming calcified when the second egg arrives in the shell gland. The point where the two eggs contact is usually poorly calcified, and both eggs show a flat side. Both eggs would be unsettable due to poor shell quality, as they would dehydrate too much during incubation.

How do blood spots in eggs happen?

Blood spots are normally a result of a problem that occurs at the time of ovulation. A normal follicle ruptures at the stigma which is a stripe on the follicle wall that is quite devoid of blood vessels. Sometimes ovulation can occur outside the stigma, involving rupturing a small blood vessel which can leak blood to a small or to a large extent.

Ad libitum
A term meaning to eat to full consumption. *Ad libitum*-fed burds have feed available to them continuously.

Do hens ever have two working oviducts?
The occurrence of a broiler breeder hens having two oviducts is quite common (2-3%). Some lines have a greater frequency of having two oviducts than others. We have never encountered a hen with an egg in the second (right side) oviduct. For more information see Chapter 6.

Sequence
A sequence is a period of several days of continuous laying of one egg per day.

Prime Sequence
The longest sequence a hen lays. It is typically seen about the time of peak egg production.

hold the mature F1 follicle, overnight, and ovulate it at the very start of the next open period for LH release she experiences. This means she will lay early in the day and a new sequence will begin. This explains why hens that have long sequences do so well. They do not take many pause days. The fact that the hens holds the follicle over night (approximately an extra 16 hours) means that the first yolks of a sequence are heavier as she still applies egg yolk in that time period. Since yolk size affects egg size, first of sequence eggs are heavier than are subsequent eggs in a sequence.

To record sequence length for a population, individual laying records must be obtained. We have done this for a group of broiler breeder hens and plotted the average sequence length for hens during lay (Figure 9). Sequences start out being relatively short. In egg-type hens managed under ideal conditions laying sequences can be very long, sometimes greater than 100 days. In feed restricted broiler breeders the longest laying sequence (prime sequence) is typically about 18 to 20 days duration. After peak egg production laying sequences decline in length, to as low as two egg sequences.

Figure 8
Hormonal interactions between the hypothalamus, adenohypophysis and ovary that result in ovulation

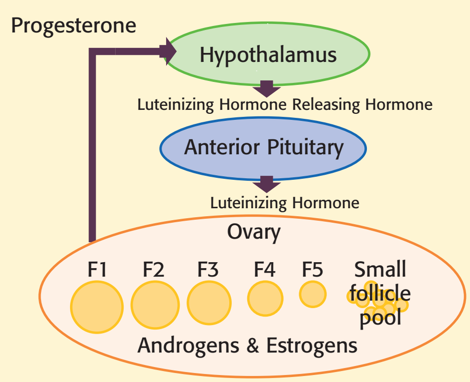


Figure 9
An illustration of the average length of laying sequences of broiler breeder hens at 2-week intervals throughout a laying period