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REVIEW PAPER

A H Zakaria

Egg Production is a Reflection of Ovarian Physiology: A Review

Abstract: Under ordinary daylight (14L:10D), the occurrence of oviposition and ovulation in the domestic hen is normally restricted to an 8-10 h period of the day. The mechanism(s) which restricts the occurrence of oviposition and ovulation to a certain period of the normal day is not understood. Among factors proposed are a small daily surge of LH occurring at the onset of darkness; graded facilitatory influences associated with the open period; and products of post ovulatory follicles (POF).

There is considerable evidence supporting the role of gonadotrophins and progesterone on follicular growth and maturation and functional maintenance of the ovary in the hen. Recently, however, there is an ever-expanding list of factors that may be involved in establishing follicular growth and ovulation, currently including the germinal disc region, growth factors, macrophages, plasminogen activator, ornithine decarboxylase, inhibin, activin, follistatin, relaxin, arginin vasotosin, oxytocin, prostaglandines, vasoactive intestinal peptide, and their interactions.

Based on the previous considerations and the data presented in this review, it may be suggested that control of follicular development in laying hens is the result of several physiological changes which still need to be determined. In conclusion, however, ovarian function of laying hens is the end result of a complex series of events accomplished by a major control (FSH, LH, P4) and its modulators (growth factors, inhibin, ornithine decarboxylase, etc). The role of these factors as potential modulators of follicular growth and ovulation needs further investigation.

Egg production by laying hens declines with the progress of age. This decline in egg production with age is an indication of decrease of ovulation. Further, as the hen ages, the interval between ovulation increases and the result is a shorter sequence and a decrease in egg production. There is strong evidence for the role of proteolytic enzymes on follicular rupture in the domestic fowl. However, mechanism(s) controlling sequence termination is poorly understood. In this respect, there are several old theories for the explanation of the mechanism relating to sequence termination. Briefly, one of these systems regulates follicular maturation and the second system regulates the timing of the preovulatory surge of LH. Some recent data, however, suggest differences in follicular growth and maturation between genotypes of birds. It has also been suggested that the activity of the pituitary-ovarian axis in white laying hens is greater than that of brown laying hens. Additionally, the adrenal gland has been given a role in timing of ovulation in the hen.

Keywords: Egg production, oviposition, ovulation, ovarian function, follicular development modulators, aging.

إنتاج البيض في الدجاج الأليف هو انعكاس للفيزيولوجيا المبيضية: مراجعة

عبدالحميد زكريا

المستخلص: ينحصر وقت وضع البيض أو الإباضة في الدجاج الأليف في فترة معينة من النهار تتراوح بين 8 – 10 ساعات. وذلك تحت ظروف إضاءة عادية مكونة من 14 ساعة و 10 ساعات إظلام. و لم تفهم بعد الآليات التي يتم بموجبها ضبط وضع البيض و الإباضة بفترة محدده من اليوم، بيد أن بعض العوامل المقترحة قد تشمل، إما حدوث تغيرات طفيفة في تركيز هرمون LH عند بدء الظلمة، أو ظهور تأثيرات تسهيلية مرتبطة بالفترة المفتوحة، أو بروز آثار منتجات الجريبات المبيضية المنفجرة نتيجة الإباضة.

هناك أدلة جديرة بالإعتبار تدعم دور الهرمونات المحفزة للمناسل (الغونادوتروفينز) و هرمون البروجستيرون في نمو الجريبات المبيضية ونضجها والمحافظة على الوظيفة المبيضية في الدجاج. ومن ناحية أخرى، تتوافر قائمة طويلة و متجددة من العوامل التي قد ترتبط بنمو الجريبات المبيضية و الإباضة، منها في الوقت الحاضر منطقة القرص الإنتاشي، عوامل النمو، البلعميات، محفز مولد البلَزْمين، الإنهيبين، الأكتيفين، الفوليستاتين، الريلاكسين، الأرجنين فاسوتوسين، الأكسيتوسين، البروستاغلانديات، الببتيد المعوي القابض و تداخلاتها.

آخذين في الاعتبار المعطيات السابقة، ثم نتائج البحوث التي قدمت في هذه المراجعة، فانه يمكن الإقتراح بأن التحكم في التطور الجريبي المبيضي في الدجاج البياض هو نتاج تغيرات وظيفية عدة ما زالت بحاجة إلى مزيد من التمحيص. بيد أنه يمكن الوصول إلى إستنتاج مفاده أن الوظيفة المبيضية في الدجاج البياض هي محصلة نهائية لسلسلة معقدة من الحوادث الناجمة عن تحكم عامل رئيس، نواته الهرمون. المنبه للجريب (FSH) والهرمون الملوتن (LH) و البروجستيرون (P_4). وعامل ثانوي، قوامه عوامل النمو المنشطة والمثبطة وبعض الأنزيمات نازعة الكربوكسيل و غيرها. لذا يتطلب توضيح دور العوامل المشار إليها آنفا دراسات إضافية. ينخفض إنتاج البيض مع تقدم العمر ومَرَدّ ذلك إلى النقص في حدوث الإباضة، إضافة إلى أن تقدم الدجاج في العمر يكون مترافقا مع زيادة طول الفترة الزمنية بين إباضتين متتاليتين، مما ينجم عنه قصر سلسلة وضع البيض، و من ثم قلة إنتاج البيض.

تتوافر أدلة داعمة لدور الإنزيمات الحالة للبروتينات في إنفجار الجريبات المبيضية في الدجاج الأليف. بيد أن الآليات التي تتحكم في انقطاع سلسلة وضع البيض غير مفهومة بشكل كافٍ. وفي هذا السياق، ظهرت نظريات حاولت شرح تلك الآليات، و هي باختصار ترتبط إما في مراقبة النمو الجريبي المبيضي و إما في التحكم في وقت تحرر هرمون LH لما قبل الإباضة. غير أن الدراسات الجديدة تشير إلى وجود اختلافات بين سلالات الدجاج فيما يختص بنمو الجريبات المبيضية و نضجها. كما يُبدي محور الغدة النخامية، المبيض في الدجاج البياض أبيض اللون نشاطاً أكبر، مقارنةً مع الدجاج البياض، بني اللون. إضافة لذلك فقد أعطى بعض الباحثون دوراً لغدة الكظر في إتمام عملية الإباضة في الدجاج.

الكلمات المدخلية: وضع البيض، الإباضة، الوظيفة المبيضية، التطور الجريبي.

Abdel Hamid Zakaria
Department of Animal Science, Faculty of Agriculture
Damascus University
Damascus P.O. Box 30414, Syria
E-mail: Zakaria@scs-net.org

Introduction

It is well established that sex hormones are synthesized by the ovary of the domestic hen (Huang *et al.* 1979; Robinson and Etches, 1986; Lee *et al.* 1998). In addition, Johnson (1999) summarized the ever-expanding list of endocrine/paracrine/autocrine factors such as growth factors, prostaglandins, inhibin, activin, relaxin, oxytocin, vasoactive intestinal peptide, vasotocin, and neurotropins. Many of these factors act within the ovary itself to regulate follicle development, whereas others serve as agents that provide feedback information to the hypothalamus and pituitary. The function(s) for many of these has yet to be fully established. Because egg production is a reflection of ovarian activity (Su *et al.* 1999; Zakaria, 1999ab; Hocking and Robertson, 2000; Renema *et al.* 2001), studies on ovarian follicular growth involve information of both basic and applied interest.

It is the objective of this paper to review and speculate on some of the known and presumed mechanisms regulating ovarian function focusing primarily on follicular growth. Recent research information on control of ovarian function reviewed in this article has introduced additional factors as potential modulators of follicular growth and ovulation. In this respect, the current state of knowledge of the role of some of the previously mentioned factors is reviewed. In addition, the effect of aging on ovarian follicular development and theories of ovulation will be discussed. According to my interpretation of the literature, I have subdivided the subject into several categories. Obviously these categories are overlapping.

a. Egg laying characteristics of the domestic hen

It is well known that the domestic hens lay eggs on successive days, after which they will not lay for one or more days. A series of eggs laid on successive days is known as a "sequence" or "clutch", while days on which no eggs are laid are "pause" days. The number of eggs per sequence may vary from 1 to more than 200 before a day is missed (Bahr and Johnson, 1991). Furthermore, an exceptionally prolific hen can forego the pause day and can lay 365 eggs in a year (Johnson, 2000). However, most layers of a commercial strain produce between two and eight eggs per sequence (North and Bell, 1990; Zakaria, 1999b).

Figure 1 illustrates some of the egg laying characteristics of the domestic hen. Briefly, on a conventional lighting schedule of 14 h light and 10 h dark with light off during the night, the first egg (C_1) of the sequence is generally laid in the morning and the last egg (C_t) is laid late in the afternoon. This implies that on each day of the sequence, the egg is laid at a progressively later time until the pause day intervenes.

The first oviposition of a sequence occurs about 1-3 h after the onset of light. Subsequent eggs are laid progressively later each day by the period of lag (the interval between successive ovipositions minus 24 h) until an egg is laid about 3-5 h before the onset of darkness before the next sequence starts. Hens which lay long sequences of eggs generally lay the first egg earlier in the day than the layers with a short sequence on the same lighting schedule. The time intervals between successive ovipositions in the sequence varies from about 24 to 29 h depending on the sequence lengths, decreasing as the sequence length increases. Therefore, the longer the sequence, the shorter the duration of the oviposition cycle. (Fig.1).

In general, the time interval between the C_1 and C_2 (C_1+ 1) ovipositions is the second greatest, then the interval decreases among the midsequence (C_s) ovipositions and the greatest is found between the last and penultimate (C_t-1) ovipositions. As a consequence, the period of lag is greatest between C_t-1 of a sequence and also greater between C_1+1 than between C_s eggs (Fig. 1).

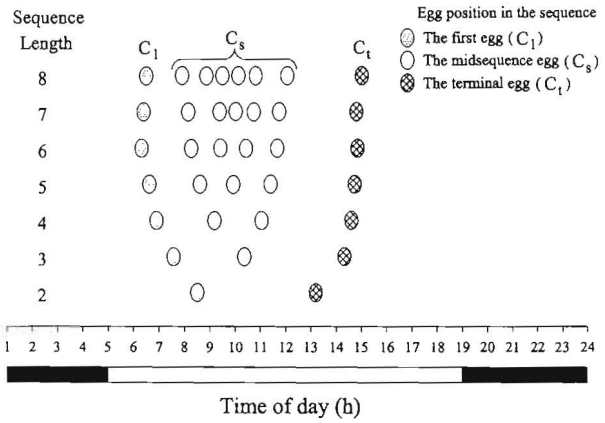

Fig. 1: Times of oviposition of sequences of 2 to 8 eggs produced by hens of a commercial strain subjected to 14 h of lighting (0500 to 1900 h). Time of oviposition was recorded for each bird by an automatic recorder. The black horizontal lines represent the hours of darkness (Drawn from Zakaria, 1991).

b. The relationship between the ovipository and the ovulatory cycles

Warren and Scott (1935) demonstrated a relationship between the ovipository and the ovulatory cycles. They found that each oviposition was followed by ovulation (the release of an ovum from the ovary) in 14 to 75 min., with an average of about 30 min., except for the C_1 ovulation. Melek *et al.* (1973) found the interval to be 10 to 50 min. in hens subjected to 14 h of light and 10 h of darkness. Melek *et al.* (1973) set up the following regression equation: $y = -14.57 + 6.69X$, where y = interval from oviposition to ovulation in min. and X = mean intersequence interval between oviposition in h. It should be noted that the time interval between ovipositions is not equal between sequences and within sequences (Fig. 1). Warren and Scott (1935) and Fraps (1955, 1961) estimated that the C_1 ovulation usually occurred 14 to 18 h after the C_t oviposition of the preceding sequence.

The mechanism(s) which restricts the occurrence of oviposition and ovulation to a certain period of the normal day is not understood but is of practical interest because it places a constraint on egg production. Johnson and van Tienhoven (1980) found a small daily surge of LH occurring at the onset of darkness in birds subjected to 14 h of light and 10 h of darkness with light on between 0600 and 2000 h. These authors proposed that this surge initiates the sequence of events leading to C_1 ovulation. This small rise in plasma LH admittedly, however, does not explain the remaining ovulations in the sequence. Further, the claim that the small rise in LH at the onset of darkness initiates the beginning of the open period was not found in a hen of a short (8 h of light and 16 h of darkness) photoperiod.

Etches and Schoch (1984) developed a mathematical representation, of two equations, for the control of the ovulatory cycle of the hen. These authors postulated that a circadian system controls the restriction of ovulation to an 8h-period of the day under conventional 14 h light and 10 h dark regimes; and ovulation was assumed to occur when a mature follicle was present in the ovary during the appropriate phase of the circadian-linked system. However, fundamental biological properties of laying hens are not easily elucidated using a mathematical representation, thus revealing that the mechanism(s) controlling the ovipository and ovulatory cycles needs further studies of both a fundamental and applied nature.

Cunningham (1987) assumed that there are graded facilitatory influences associated with the open period (the open period is identified as the time during which the pituitary is activated by a neural signal to release LH and considered to be of 8-10 h daily). These influences are minimal at the beginning and end and maximal at the midpoint of the open period. Thus, the extended lag between ovulations arises from LH release occurring either early or late in the open period, and the comparatively small lag between ovulations results from LH release initiated at the midpoint of the open period. Furthermore, Cunningham (1987) presumed that the distribution of ovulations and ovipositions in a sequence can also be explained by the possibility that undefined endocrine or neuroendocrine changes during the open period exert either an accelerating or delaying effect on follicular maturation, depending on the stage of maturity at which the follicle is exposed to these assumed hormonal changes.

On the other hand, Saito *et al.* (1993) suggested a role of prostaglandins in initiation of C_t oviposition and C_1 ovulation. In addition, Johnson (1999) summarized data suggesting that relaxin, oxytocin, steroids and prostaglandinds, are a few of the products that are produced by the post-ovulatory follicles (POF). Thus, it is possible that one or more of these hormones, produced by the POF, is involved in regulating the time of egg laying. Undoubtedly, further studies are required to clarify the physiological mechanism restricting the ovipository and the ovulatory cycle in the hen to 8-10 h under ordinary daylight.

c. Changes in egg laying as a functional of age

In general, egg production of the domestic hen can be divided into three periods; the period of the onset of laying; the main laying period and the end of laying. Fig. 2 demonstrates production standards for White Leghorn and medium-size commercial layers in cages. Production indices include hen-day egg production (eggs produced as a percentage of live hens/day), hen-housed egg production (eggs produced as a percentage of hens housed) and egg weight based on hen-day egg production. The onset of laying (at about 20-21 weeks of age) is of short duration, 3 to 4 weeks, extending from the onset of reproductive activity as represented by the first laying until the beginning of the normal laying. This period is characterized by irregularities in the laying pattern. In the main laying period, egg production

becomes regular and the egg laying pattern is well established. This laying period is the longest among the others and sustains a high production level for several months. Egg weight increases with the progress of age. Furthermore, some poultry producers integrate recycling programs into their regular replacement schedules by force-molting following their first year of production (Fig. 3).

Egg production by laying hens declines over time (Fig. 2 and 3). This decline in egg production with age is an external indicator that the frequency of ovulation has decreased and is one of the major reproductive problems in the domestic hen. As the hen ages, the interval between ovulation increases and the result is a shorter sequence and a decrease in egg production.

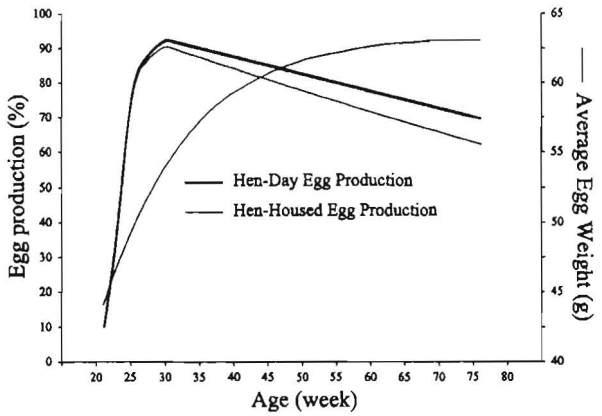

Fig. 2: Hen-day and hen-housed egg production and egg weight of commercial layers maintained in cages (Drawn from data published by North and Bell, 1990).

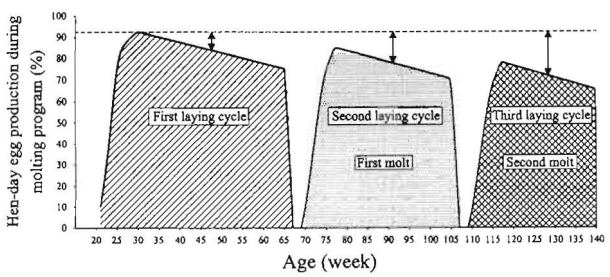

Fig. 3: Hen-day egg production during first, second and third laying cycles of commercial layers maintained in cages. Molting started at 66 and 106 weeks of age for first and second molting program, respectively (Drawn from data published by North and Bell, 1990).

Control of ovarian activity

The left ovary, the functional ovary, of a laying hen generally contains 4-8 yellow growing follicles (follicles in the rapid growth phase) arranged in a hierarchy based on maturation (Fig. 4) and numerous small follicles (follicles in the resting stage) that have not entered the follicular hierarchy. The yellow growing follicles (YGF) constitute most of the mass of the hen's ovary and recruit from smaller follicles. In laying hens, the number of YGF is different between strains (Su *et al.* 1999; Renema and Robinson, 2001) and age of birds (Zakaria, 1999a). Additional information on the structure of the ovary and control mechanism of ovarian function of a laying hen (i.e. FSH, LH, local factors) can be taken from previous reports (Etches, 1990; Zakaria, 1999a; Johnson, 2000).

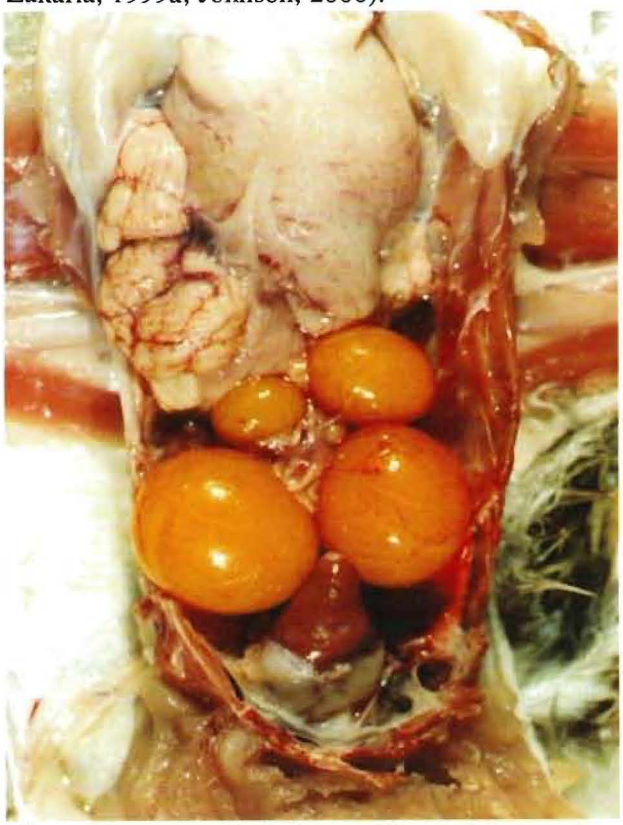

Fig. 4: The ovary of a laying hen. Note the hierarchical arrangement of follicles of the rapid growth phase (yellow growing follicles). The ovary and oviduct of a laying hen occupy a large portion of the abdominal cavity (Photo from Zakaria).

In contrast to laying hens, the ovaries of broiler breeders contain about twice as many YGF (Hocking *et al.* 1987). The reason for the recruitment of additional YGF to the hierarchy is not known; however, it is proposed that follicular tissue at this stage of growth is more sensitive to stimulation by endogenous pituitary gonadotrophins in broilers than in layers (Hocking and McCormack, 1995). Attempts to control ovarian follicular activity in broiler breeders and turkeys can be found in the report of Hocking and Robertson (2000) and Buchanan *et al.* (2000).

a. Control of follicular transformation from the resting stage to the rapid growth phase

The number of YGF in the ovary of laying hens is generally considered to be stable in the individual

so that one small follicle (SF) would enter into the rapid growth (RG) phase after the largest follicle is ovulated. Nalbandov (1959) suggested that follicular transformation (FT) to the RG phase could be a simple matter of chance and depended completely on the presence of blood vessels in the follicle wall. Nalbandov (1959) assumed that the pituitary gland released a constant amount of gonadotrophic hormones.

An earlier conception that gonadotrophins were released at constant and unvarying levels from the pituitary during the ovulatory cycle was proven incorrect (Zakaria 1982; Krishnan *et al.* 1993). The two works shared the view that the profiles of plasma FSH and LH levels during the chicken ovulatory cycle showed marked differences in secretion of the two hormones. Zakaria (1982) demonstrated changes in plasma of young (8 month) SCWL laying hens at 2 h intervals for a 24 h ovulatory cycle and found an increase in plasma FSH level during 8 to 14 h after ovulation, then a relatively steep decrease to 18 h, whereas the highest concentration of LH was found 4 h before ovulation (Fig. 5). Krishnan *et al.* (1993) reported that a preovulatory peak of LH occurred 3h before oviposition, where the highest plasma level of FSH occurred 15-12 h prior to oviposition.

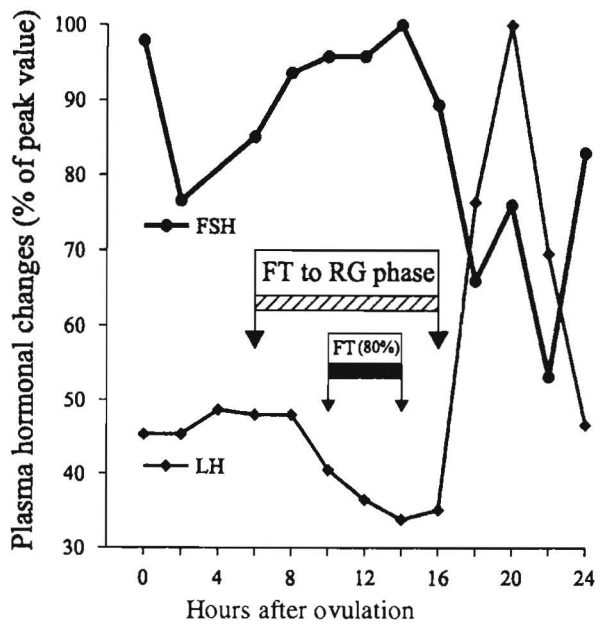

Fig. 5: Plasma FSH and LH (as a percentage of peak value) of hens with sequences of 8 or more eggs and a time interval of about 24 h between intraclutch ovulations. Follicular transformation (FT) from the resting stage to the rapid growth phase (RG phase) occurred at a high frequency (about 80%) within the period of 10 to 14 h after ovulation (Black horizontal line). Hatched horizontal line represents time of FT after ovulation (Drawn from Zakaria 1982 and Zakaria *et al.*, 1984a).

Zakaria *et al.* (1984a) found that FT occurred at a high frequency within the period of 8 to 14 h after ovulation. This period corresponded to the time between 1700 and 2400 h and to high plasma FSH during the ovulatory cycle (Fig. 5). Further, Zakaria *et al.* (1984a) found that yolk deposition in the dark period was higher than in the light period. Therefore, FSH may act as the limiting factor determining the transformation of a follicle from the resting to the RG phase. However, LH seems to play no role in the FT and is more closely associated with ovulation.

On the other hand, Johnson (1996) concluded that the primary signal for follicle selection may not consist of a novel endocrine/autocrine/paracrine stimulus, but rather entail the removal of some inhibitory influence within a single follicle per day which enables differentiation inducing factors (i.e., FSH and vasoactive intestinal peptide, VIP) to be functional. Johnson *et al.* (1993) showed that plasma immunoreactive inhibin decreased with the removal of the largest follicles (Fig. 6). Furthermore, removal of the F1 follicle resulted in a decrease in plasma inhibin and an increase in plasma FSH, but not LH (Johnson, 1997). These findings may led to the conclusion that a negative relationship between inhibin and FSH may exist.

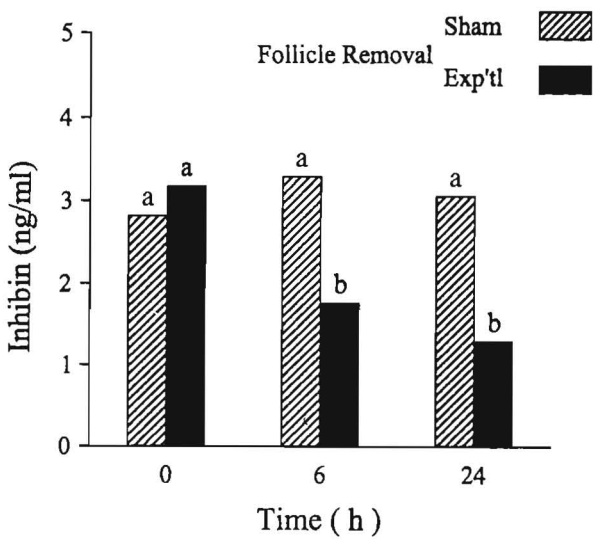

Fig. 6: Plasma inhibin levels (ng/ml) in hens with 3-4 of the largest follicles removed (n=5 hens) or sham-operated (n= 4 hen) hens (Redrawn form Johnson *et al.*, 1993).

In contrast, in mammals there is evidence for an intra-ovarian Insulin Growth Factor-1, IGF-1, system involved in the regulation of the normal follicular recruitment and development (Adashi *et al.* 1985) and data of Gong *et al.* (1991) has shown that exogenous growth hormone, GH, increased the population of small antral follicles in mature heifers.

It was suggested that this was mediated by the increase in peripheral IGF-1 or insulin concentration, and the effect was not mediated through changes in circulating gonadotrophins, gonadotrophin receptor level (Gong *et al.* 1991) or through the mechanisms by which the dominant follicle inhibits a subordinate follicle (Gong *et al.* 1993).

Zakaria *et al.* (1984a), Tilly *et al.* (1991) and Hocking and Robertnson (2000) suggested that increased recruitment into the follicular hierarchy is dependent on the numbers of follicles 6 to 8mm diameter from which recruitment occurs. In the follicles of 6-8mm diameter, there are physiological changes that are consistent with the suggestion that the selection to the hierarchy occurs at this stage. It has been proposed that the process of follicle recruitment is associated with changes in granulosa cells (Tilly *et al.* 1992; Li and Johnson, 1993; Chen and Johnson, 1996; Johnson, 1997; Davis and Johnson, 1998). In this respect, Tilly *et al.* (1992) found that: 1) granulosa cell DNA synthesis and proliferation are 5 to 10-fold higher in prehierarchial follicles compared to granulosa cells from hierarchial follicles; 2) and DNA synthesis is 2-fold higher in granulosa cells within the germinal disc region when compared to cells from the outer layer region. Moreover, plasminogen activator activity is 5.8-fold higher in granulosa cells of 6 to 8 mm follicles when compared to cells from 3 to 5mm follicles (Tilly *et al.* 1992). Li and Johnson (1993) stated that the process of follicle recruitment is related to the acquisition of functional P450Scc enzyme (the first step in converting cholesterol to pregnenolone involves P450Scc enzyme) activity within the granulosa layer. Furthermore, Li and Johnson (1993) found that the expression of P450Scc mRNA and the initiation of enzyme activity in vitro is induced by culture with FSH, and the induction is prevented by co-culture with TGF_ or EGF and no effect for IGF-1. Fig 7 illustrates relative levels of FSH receptor and LH receptor mRNA in granulosa cells from follicles of different stages of development. It is evident that granulosa cells express mRNA at all stages of development, but the levels are highest in 6-8mm diameter follicles.

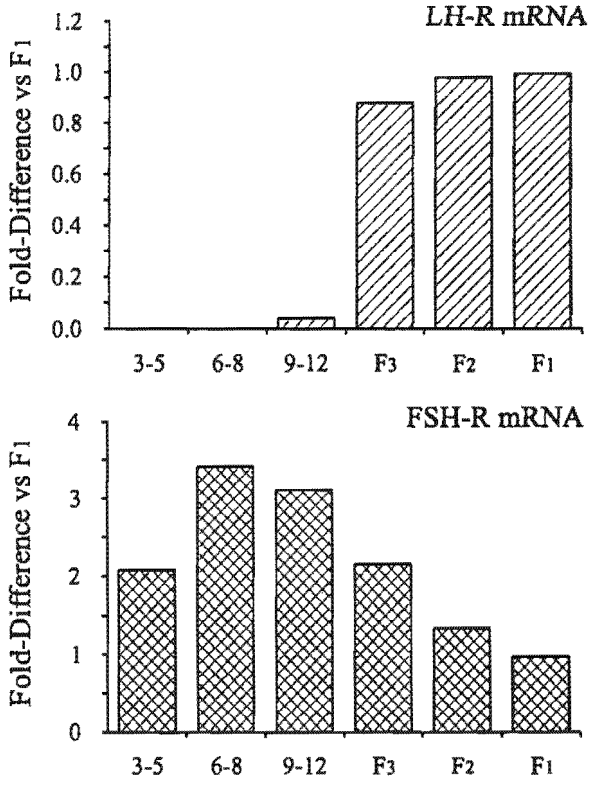

Fig. 7: Relative levels of FSH receptor and LH receptor mRNA in granulosa cells of follicles of different stages of development (Redrawn from data published by Johnson, 1999).

Table 1. Mean activity of ornithine decarboxylase in follicles of different diameter from broiler and layer chickens (n=32) after i.v. injection of 0.2 ml saline kg^{-1} body mass (control) or 200 iu pregnant mares' serum gonadotrophin (PMSG)

		Ornithine decarboxylase activity							
Follicle size (mm)	Strain	(pmol CO_2 h^{-1} per follicle) control		PMSG		(pmol CO_2 h^{-1} mg^{-1} protein) Control		PMSG	
2-3	Broiler	2.8	(16)	4.1	(63)	3.5	(32)	4.6	(97)
	Layer	2.3	(10)	3.9	(50)	3.2	(24)	4.4	(78)
5-6	Broiler	3.5	(34)	5.8	(313)	2.6	(13)	4.8	(125)
	Layer	3.6	(35)	5.2	(179)	2.8	(16)	4.0	(56)
8-10 (yellow)	Broiler	5.0	(141)	5.8	(337)	3.2	(24)	3.9	(52)
	Layer	5.0	(150)	5.7	(285)	3.5	(32)	3.8	(46)

Results are presented as natural logarithms and back transformed values are in parentheses (Modified from Hocking and McCormack, 1995).

Ornithine decarboxylase has been utilized as a marker of gonadotrophin sensitivity in studies of folliculogenesis in domestic fowl (Armstrong, 1994). Hocking and McCormack (1995) found greater activity of ornithine decarboxylase of follicles of 5-6 mm diameter from PMSG-stimulated broiler parent stock compared with that in follicles of similar size from laying hens. These authors proposed that follicular tissue at this stage (5-6mm diameter) of growth is more sensitive to stimulation by endogenous pituitary gonadotrophins in broilers than it is in layers (Table 1 and Fig. 8).

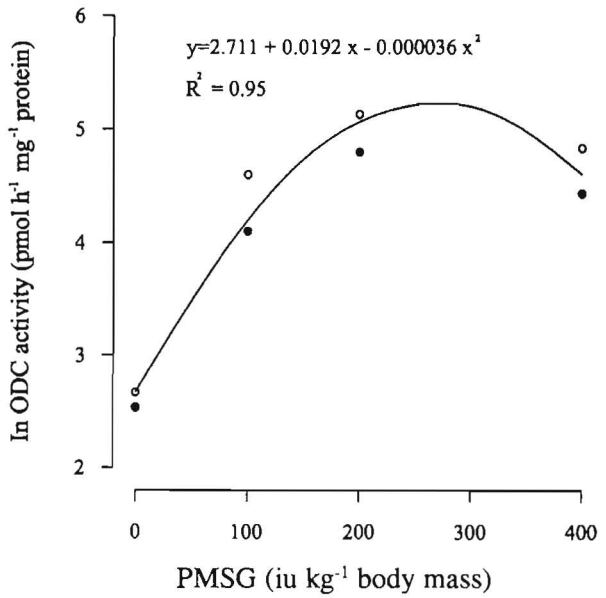

Fig. 8: The activity of ornithine decarboxylase (ODC) tissue from small white follicles of two sizes from ovaries of broiler parents 3 hr after i.v. injection with different doses of PMSG. (_) follicles 2-3 mm diameter, (_) follicles 5-6 mm diameter. The injection volume was 0.2 ml Kg-1 body mass (Redrawn from Hocking and McCormack, 1995).

On the other hand, inhibin/activin β_B subunit and follistatin are expressed in the granulosa cells of prehierarchial follicles and may have a role in follicle recruitment, while β_A subunit either as inhibin-A or activin –A may be critical for ovulatory events (Fig 9). Inhibin is a dimeric protein composed of α and β subunits. Activin, on the other hand, is composed of homodimers or heterodimers of the β subunits, resulting in activin-A(β_A-β_A), activin-B(β_B-β_B), or activin AB(β_A-β_B). Follistatin is structurally unrelated to inhibin/activin subunits. It is a monomeric glycosylated soluble binding protein capable of binding activin and, with less affinity, inhibin (Chen and Johnson, 1996; Johnson, 1997; Davis and Johnson, 1998).

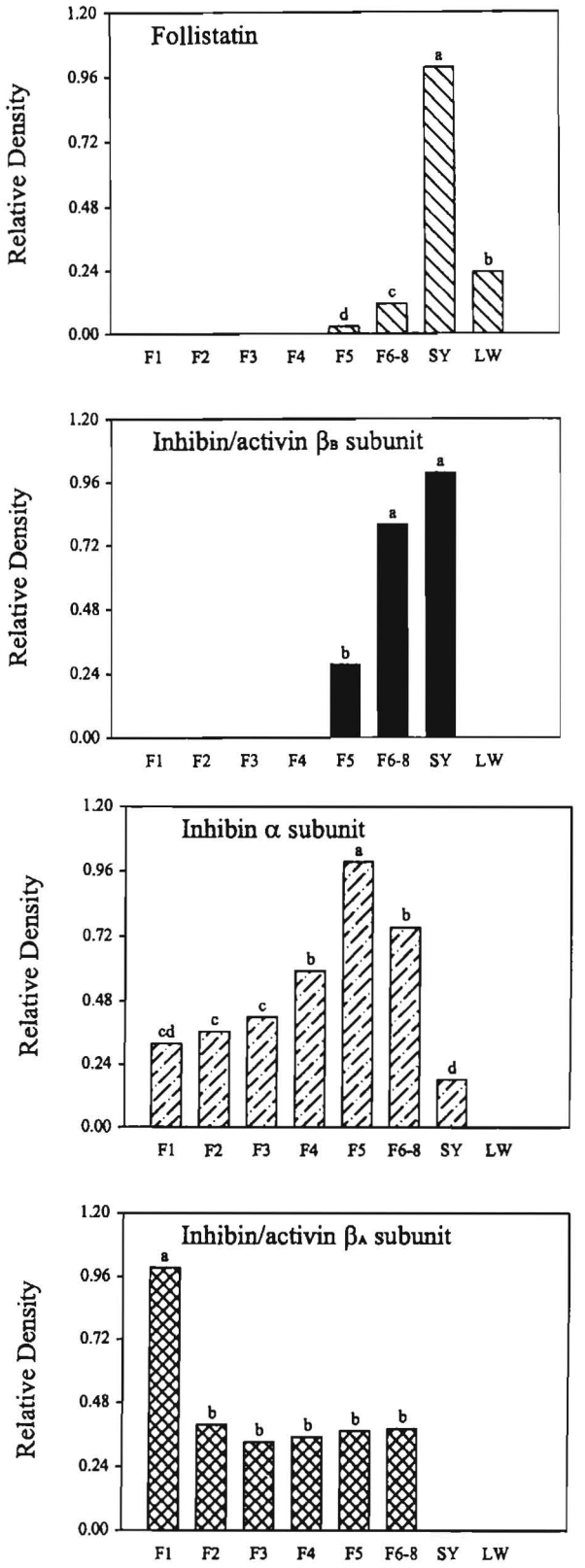

Fig. 9: Expression pattern of mRNA for Follistatin, Inhibin/activin b_B subunit, Inhibin a subunit and inhibin/activin b_A subunit. F follicle (the number represents the hierarchical follicle order); SY small yellow follicles; Lw, large white follicles (Redrawn from Davis and Johnson, 1998).

An alternative hypothesis proposed by Gilbert and Wells (1984) is that selection of follicles which will eventually ovulate occurs at the white-yolky stage of follicular development by a process of differential atresia. Thus, the ovarian follicular hierarchy forms before the formation of the smaller (about 8mm) YGF.

Ovarian follicular selection can be thought of as a balance between follicle growth and atresia (Johnson, 1996). Johnson (1996) added that immediately following follicle selection, granulosa cells differentiate and concomitantly lose their ability to proliferate, except with the germinal disc region. However, the specific signal(s) for the recruitment into the hierarchy and the initial site(s) of its actions have yet to be fully identified.

***b.** Follicular growth during the rapid growth phase*

The avian preovulatory follicle experiences a period of rapid growth in the final 7-9 days preceding its ovulation (Zakaria 1999ab; Su *et al.* 1999). This period is influenced by age and genotypes and lengthened from 15 to 23 months of age (Zakaria *et al.* 1983) and shortened in albino compared to non-albino hens (Su *et al.* 1999). Characteristics of this RG phase include increase in follicular diameter, because of continuous and rapid incorporation of large quantities of yolk from the blood (reviewed in Zakaria, 1999ab; Johnson, 2000) and rapid increase in granulosa and theca cells (Etches *et al.* 1983; Etches, 1984; Tischkau *et al.* 1996).

***b-1.** Yolk deposition*

The growth during the RG phase is due to the deposition of yolk materials, mainly lipids, into the follicle (Smith, 1959; Mackenzie and Martin, 1967). The lipid is in the form of lipoprotein (mainly very low density lipoprotein, VLDL) in the laying hen (Gilbert, 1971; Bacon *et al.* 1978; Griffin *et al.* 1984). There is general agreement that yolk lipoprotein is synthesized in the liver of laying hens and transported by the circulation to the ovary where it is deposited into growing follicles (Schjeide *et al.* 1963; Gruber *et al.* 1976). Perry *et al.* (1978 ab) and Perry and Gilbert (1979) have suggested that the lipoprotein is transported to the surface of the oocyte by widening the spaces between granulosa cells and the meshwork of the perivitelline layer, forming intercellular channels for the passage of yolk lipoprotein. It is thought that the rate of yolk formation is dependent more on the rate of recycling of VLDL receptors on the oocyte membrane than plasma concentration of VLDL (Griffin and Hermier, 1988; Shen *et al.* 1993).

Hepatic synthesis of yolk precursor is mainly controlled by estrogens (Schjeide *et al.* 1963; Deeley *et al.* 1975; Mullinix *et al.* 1976; Griffin *et al.* 1984). Furthermore, it has been suggested that FSH controls yolk deposition (see Palmer and Bahr, 1992 and Zakaria, 1999ab for additional information).

***b-2.** Granulosa and theca cells*

The granulosa layer and theca layer comprise the follicular wall which surrounds the oocyte (Fig. 10). Huang *et al.* (1979) introduced the two-cell theory that granulosa cells of the preovulatory follicles primarily produce progestins that are required as substrate for the production of androgens and estrogen by the theca cells. It has been suggested that progesterone (P_4) was produced in the follicular granulosa cells and diffused into the theca cells where it was converted to testosterone (T) and further estradiol (E_2) and estron (Huang *et al.* 1979). Furthermore, the production of P_4 by the granulosa cells is well documented in studies by many investigators (see Zakaria, 1999ab; Johnson, 2000 for references).

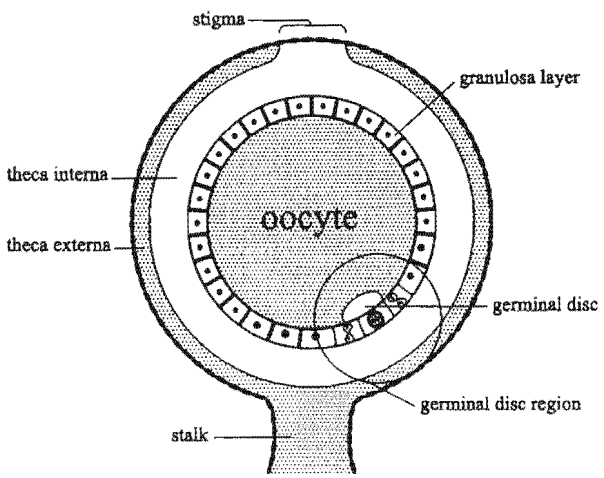

Fig. 10: A diagrammatic drawing of a cross section of the hen preovulatory follicle. The germinal disc region consists of the germinal disc and granulosa cells that are tightly associated with it (Modified and redrawn from Volentine *et al.*, 1998).

Recently, an alternative hypothesis proposed by Lee *et al.* (1998) is that both Δ^5 and Δ^4 pathways are functional in theca and granulosa cells. However, the theca layer preferentially metabolizes steroids via the Δ^5 pathway regardless of the maturational stage of the follicle, whereas the granulosa layer of preovulatory follicles preferentially metabolizes steroids via the Δ^4 pathway. In this respect, there are two pathways for the metabolism of pregnenolone (P_5) into T and E_2. One is the Δ^5 pathway involving $P_5 \longrightarrow$ dihydroepiandrosterone (DHEA) $\longrightarrow$ androstenedione (A) $\longrightarrow$ T $\longrightarrow$ E_2 and the other is the Δ^4 pathway involving $P_5 \longrightarrow P_4 \longrightarrow A \longrightarrow T \longrightarrow E_2$.

Control of follicular growth and maturation

There have been many improvements in our knowledge of pituitary hormones and avian steroids because of the utilization of radioimmunoassay (RIA) technique and the ability to isolate steroidogenic cells that enable the steroidogenesis of each type of cell to be more clearly defined. Hence, many of the endocrine events associated with follicular growth, maturation and ovulation have been defined. The results of RIA have shown one preovulatory surge of LH occurring 7 to 2 h (with the average of about 4 h) before ovulation. During this period a rise in the plasma concentration of progesterone (P_4), testosterone (T), and estradiol (E_2) occurs almost simultaneously (Fig. 11, and 12).

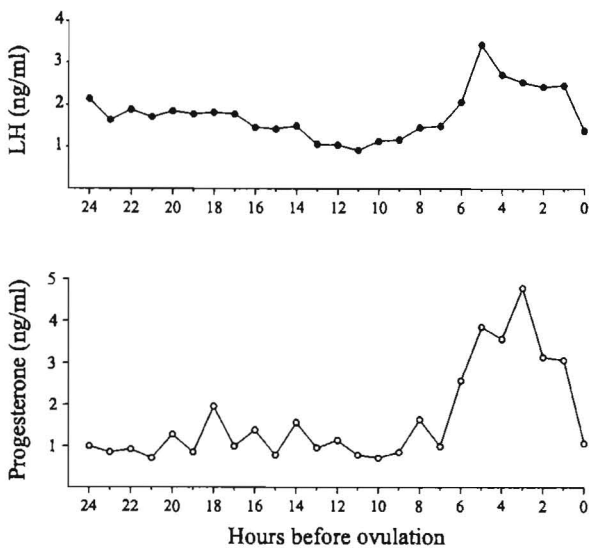

Fig. 11: Plasma concentration of LH and progesterone of laying hens during a 24 h cycle (Redrawn from Furr *et al.*, 1973).

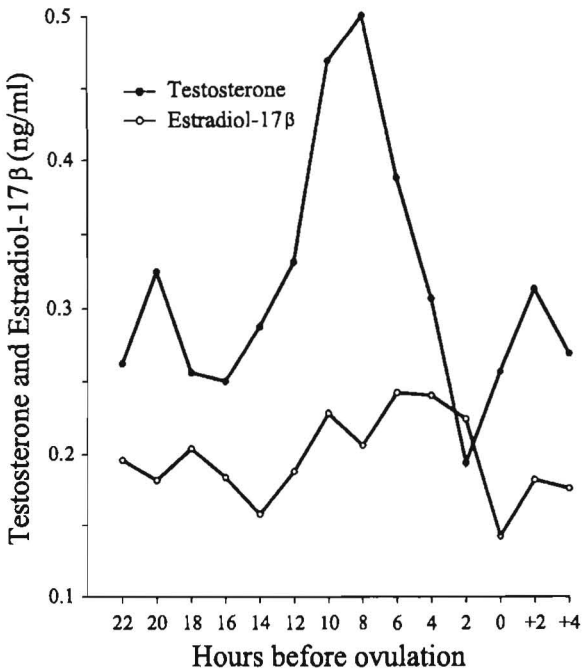

Fig. 12: Plasma concentration of testosterone and estradiol relative to time of ovulation (Redrawn from Johnson and van Tienhoven, 1980).

Although the physiological significance of the rise of each steroid is not completely clear, there is considerable evidence suggesting that P_4 is involved in LH release during the preovulatory period (reviewed in Wells and Gilbert, 1984; Scanes *et al.* 1984; Johnson, 1986; Cunningham, 1987; Bahr and Johnson, 1991 and Johnson, 2000). In brief, P_4 and LH interact in a cascade of events which lead to a preovulatory release of LH (Fig. 11). Additionally, the concentration of E_2 increases 1-2 h before the preovulatory surge in LH, and the concentation of T increases 1-2 h before those of E_2. Thus the sequence of events which leads to the release of the LH preovulatory peak may be as follows: T $\longrightarrow$ E_2 $\longrightarrow$ P_4 $\longleftrightarrow$ LH. Additional information can be taken from previous reviews (Etches, 1984; Wells and Gilbert, 1984; Johnson, 1986 Cunningham, 1987; Bahr and Johnson, 1991, and Johnson, 2000) and from data depicted in Fig. 5, 11, and 12 in this review.

1-. Role of gonadotrophins

There is considerable evidence supporting the role of gonadotrophins on follicular growth and maturation and functional maintenance of the ovary in the hen. Treatments with chicken pituitary preparations to hypophysectomized (Opel and Nalbandov, 1961; Mitchell, 1967) molting (Imai *et al.* 1972) and fasting (Imai, 1972) hens with a completely regressed ovary resulted in maintenance

of follicular hierarchy. Additionaly, injections of chicken pituitary gonadotrophins to immature pullets approaching sexual maturity induced follicular growth in their ovaries (Taber *et al.* 1958).

Although a mass of evidence indicates that the ovarian function is under the regulation of pituitary gonadotrophins, the precise hormone(s) controlling follicular growth is still in obscure. According to some investigators follicular growth is under the control of FSH. In this respect, Krishnan *et al.* (1993) measured FSH and LH by RIA from White Leghorn chickens 30 weeks of age at each 3 h interval for 24 h and concluded that the profiles of plasma FSH and LH levels during the chicken ovulatory cycle showed marked differences in secretion of the two hormones. These authors reported that a preovulatory peak of LH occurred 3 h before oviposition, whereas the highest plasma level of FSH occurred 15-12 h prior to oviposition.

Scanes *et al.* (1977) found that FSH concentration was higher in sexually maturing than immature birds. Additionally, the development patterns of FSH and LH for commercial Babcock B300 female chickens from week 1 to 19 were demonstrated by Johnson and Brooks (1996). Their results showed that the level of FSH was elevated at 13, 15 and 17 weeks compared to earlier ages, whereas no significant change was found in LH throughout the 19 weeks. Further, immuno-active inhibin was significantly elevated at 17 and 19 weeks compared to earlier ages (Fig. 13). The results of Johnson and Brooks (1996) suggest that a negative relationship between inhibin and FSH may be found at the onset of puberty. In addition, it appears that the rise in FSH precedes the rise in inhibin, suggesting that stimulation of gonadal development may increase inhibin production in the hen.

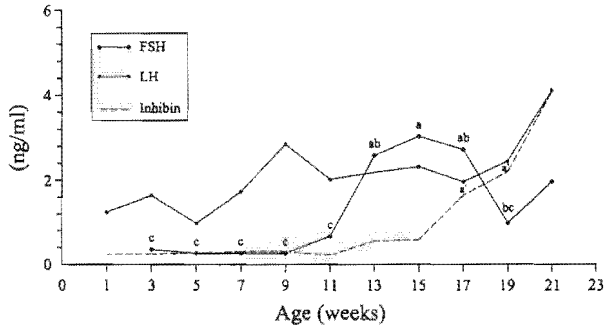

Fig. 13: Plasma levels of FSH, LH and inhibin (ng/ml) in female chickens from week 1 posthatch through week 21. Differences among times are indicated by different letters. Inhibin levels were only different from other times at 17 and 19 weeks. There were no differences in LH levels within times. FSH showed a significant rise at weeks 13, 15, and 17 compared to earlier times (Redrawn from Johnson and Brooks, 1996).

Palmer and Bahr (1992) injected SCWL old hens, aged 80 to 100 weeks that had been laying 2 to 3 eggs per sequence, with saline containing 0.1% bovine serum albumin or 12.5, 50, 200, or 400 micrograms of pFSH for five consecutive days. These authors showed that treatment with FSH increases serum estradiol 17β concentrations, number of growing follicles, and yolk deposition in aging hens with decreased egg production.

Yolk deposition is not the only factor in follicular growth and certainly not the most important factor. There have been published works (Etches, 1984; Wells and Gilbert, 1984; Johnson, 1986; Cunningham, 1987; Bahr and Johnson, 1991, Johnson, 2000, and data depicted in Fig. 5, 11, and 12 in this review) which show that the ability to aquire competency to ovulate is a primarily a function of follicles mounting a P_4 surge in response to a preovulatory surge of LH. Besides, difference in yolk size between hierarchical follicles in albino and non-albino hens suggests differences in follicular growth and maturation between the genotypes (Su *et al.* 1999). Su *et al.* (1999) found that preovulatory LH was higher in albino hens and suggested that the activity of the pituitary-ovarian axis in albino hens was greater than that in non-albinos. These authors concluded that the extra sensitivity of ovarian follicles of albino hens to LH causes them to ovulate earlier at a smaller size. At this size, the follicles have matured not because they have accumulated a greater amount of yolk but because the granulosa cells have differentiated and are able to produce enough P_4 to induce ovulation. Thus, several physiological factors influence follicular development.

2-. Role of germinal disc region

During follicular growth, there is not only a rapid accumulation of yolk, but the number of granulosa and theca cells also increases rapidly (Fig. 10). Furthermore, Perry *et al.* (1978b) demonstrated that the germinal disc region has a higher mitotic activity than do cells in other regions of the granulosa layer. Tischkau *et al.* (1996) concluded that the granulosa layer is the key that coordinates the growth and ovulation of the follicle. Tischkau and Bahr (1996) suggested that the avian germinal disc region secretes factors, presumably growth factors, affecting granulosa cells and hence participates in the regulation of the proliferation and differentiation of the developing follicle.

3-. Role of growth factors

Hormone actions may be mediated by the local production of growth factors. Peddie *et al.* (1993) summarized some actions of growth factors as modulators of the ovarian function of birds, some of which were that epidermal growth factor (EGF) and transforming growth factor α (TGFα) have inhibitory effects on P_4 production by the granulosa cells; EGF and insulin-like growth factor-I (IGF-I) cause proliferation of granulosa and theca cells; and IGF-I and EGF-like factor are synthesized in the ovary.

Recently, Yao *et al.* (1998) found that destruction of the germinal disc region of an immature preovulatory follicle caused atresia, apoptosis and blocked ovulation. Furthermore, Volentine *et al.* (1998) suggested that EGF in the germinal disc regulated several functions of granulosa layer explants from the F_2 follicle by stimulating proliferation, inhibiting apoptosis, and decreasing basal progesterone production in laying hens. It should be taken into consideration, however, that granulosa cells of the immature preovulatory follicles are the primary targets for FSH (see Zakaria, 1999a for references).

4-. Role of macrophages

In rats, macrophages enhanced follicular growth by regulating the secretion of EGF (Fukumatsu *et al.* 1995). In chickens, macrophages produce interleukin (IL)-1, tumor necrosis factor (TNF)-a and a myelomonocytic growth factor (Qureshi, 1998). According to Klasing (1998) macrophages are specially suited for a regulatory role because they are widely dispersed throughout the bird's body fluids and within its tissues, and because of their capacity to secrete an extensive variety of communication molecules that include cytokines, cytokine inhibitors, endocrine hormones, eiczosanoids, neurotransmitters, and reactive oxygen intermediates. Thus, Klasing (1998) suggested that macrophages affect the growth, reproduction and wellbeing of poultry. Barua *et al.* (1998) concluded that macrophages increase in association with follicular development and the regression of postovulatory follicles and may play important roles in the events of follicular growth and postovulatory regression. Furthermore, Qureshi (1998) stated that dietary, genetic and environmental manipulations can modulate macrophage activity. Antioxidants, such as vitamin E, selenium and ascorbic acid (AA) may play a role in modulating macrophage activity. In this respect, *in ovo* injection of vitamin E (Gore and Qureshi, 1996) improved macrophage functions at posthatch. Dietert and Golemboski (1998) added that cells in close proximity to macrophages producing large quantities of reactive oxygen species (ROS) may be damaged in the absence of adequate levels of antioxidants.

5. Role of plasminogen activator

As cited before, follicular growth during the rapid growth phase is characterized by a rapid increase in follicular diameter (from about 7mm to more than 30mm in about a week). Plasminogen activator, a serine protease, has been proposed to play an important role in the remodeling of the extracellular matrix, which is necessary to accommodate the rapid increase in size (Tilly *et al.* 1992; Wang *et al.* 1993; Jackson *et al.* 1994; Tischkau *et al.* 1996). According to Tischkau *et al.* (1996) the granulosa layer produced a high amount of plasminogen activator (PA) in response to a stimulatory factor, produced by the theca layers, that is inhibited by LH. Further, these authors suggested that the granulosa layer is the site of mRNA and/or protein regulation of PA production by LH. A previous report of Tilly and Johnson (1987) stated that prostaglandin stimulates both cell-associated and secreted PA. Based on the previous considerations, it may be suggested that control of follicular development is the result of several physiological changes which still need to be determined.

The effect of aging on the ovarian follicular growth in laying hens

As cited before, in normally laying hens there are several numbers of YGF hierarchically arranged in the ovary and such an arrangement produces the ovulatory and ovipository cycles. Each follicle requires about 7-9 days for its growth to reach ovulation (Zakaria *et al.* 1983; 1984b; Zakaria, 1999ab; Su *et al.* 1999). It may be suggested that the length of the RG period and the growth pattern during the period affect sequential ovulation and consequently give an influence to the egg production performance in the hen. Gilbert (1972) and Williams and Sharp (1978) found that the growth period did not change with the progress of age, except during the first 2 months after the

initiation of egg laying, and that the larger size of the ovulated yolk with aging was due to an increase in the efficiency of the hen in depositing yolk into the follicles.

On the other hand, Zakaria *et al.* (1983) studied the growth pattern of the ovarian follicles in layers aged from 5 to 23 months of age. Results indicated:

1) there was a continuous increase in follicular volume at ovulation with the progress of age
2) the growth period shortened from 5 to 11 months; then lengthened from 15 to 23 months of age (Fig. 14)
3) during the first year of age, yolk deposition increased and remained constant thereafter (Fig. 15)
4) the growth rate was low at 5 months, increased from 8 to 11 months and remained constant thereafter (Zakaria *et al.*, 1983).

Additionally, follicular arrangement, number and size were studied in young and old laying SCWL and local layers (Zakaria, 1999a). The hierarchy of the ovarian follicle observed in young layers was disrupted in old layers (Fig. 16). This disruption may be determined by the failure of a follicle to enter into the rapid growth phase, a decrease in the rate of follicular maturation or an increase in atresia of smaller follicles.

Johnson and Wang (1993) found that plasma immunoreactive inhibin levels were significantly higher in laying hens with short sequences (3-7 eggs) compared to those in hens with long sequences (>20 eggs). In addition, these authors suggested that a fall in inhibin levels resulted in an acute rise in FSH with no change in LH. Hence, the amount of FSH in old layers may be inadequate to maintain the follicular hierarchy observed in young layers with long sequences.

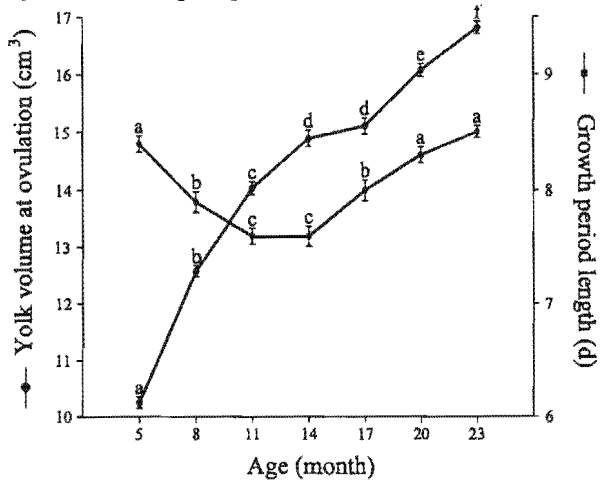

Fig. 14: Length of the rapid growth period and follicular volume at ovulation of laying hens of a commercial strain 5 to 23 months of age (Redrawn from Zakaria *et al.*, 1983).

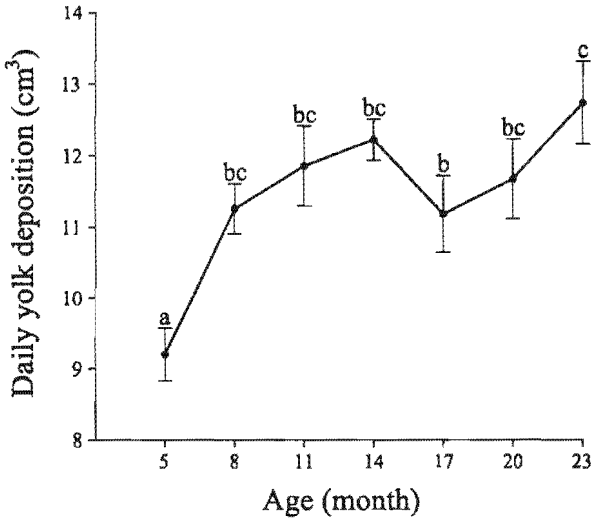

Fig. 15: Total amount of daily yolk deposition (cm^3) of laying hens of a commercial strain 5 to 23 months of age (Drawn from Zakaria *et al.*, 1983).

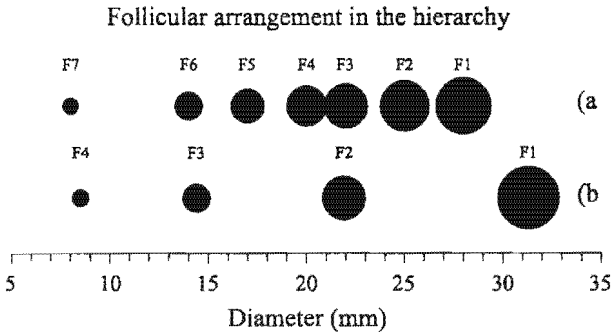

Fig. 16: A diagrammatic comparison of the arrangement, size, and number of yellow growing follicles in young (32 weeks) and old (78 weeks) SCWL hens. Egg production rate (%) about 3 weeks preceding death for young (a) hen was 100% and for old (b) hen was 52%. Each circle represents the size of a follicle in a given position in the follicular hierarchy. The yellow growing follicles of the hierarchy are identified according to size with F_1 follicle being the largest and the next to ovulate, followed by the F_2 follicle, the second largest, etc. (Modified from Zakaria, 1999a).

On the other hand, the response to injection of 25 µg of GnRH (Etches *et al.* 1983), the sensitivity to 25-50µg bovine LH *in vitro* (Moudgal and Razdan, 1985), or the sensitivity to LH by measuring basal and LH-stimulated activity of the adenylyle cyclase enzyme system of the granulosa layer of the F1 and F2 follicles (Johnson, 1986) decreases with age. In this respect, Bahr and Balmer (1989) have written an excellent account of several major changes in ovarian functions associated with aging. These changes include decreased ovulation rate, decreased recruitment of follicles into the hierarchy and increased atresia, production of larger eggs, increased incidence of soft-shelled and shell-less eggs, and internal laying. Also, information on the effect of aging on ovarian follicular activity can be obtained from Zakaria (1999ab).

Theories of rupture of the follicle

Nakajo *et al.* (1973) were the first to demonstrate that commercial proteolytic enzymes (Pronase, Nagarse, Collagenase) administered either by injection into the follicle wall or by infiltration from filter paper pads cause a rapid induction of follicular rupture in the hen. Additionally, the stigma was invariably found to be more responsive than the nonstigma region to the enzyme administration (Table 2).

Nakajo *et al.* (1973) presumed that the stigma contains structural constituents which are relatively easily attacked and disintegrated by proteases. From the histological basis, Yoshimura and Fugii (1981) also confirmed the role of the proteolytic enzyme on follicular rupture in the domestic fowl. Furthermore, there were specific changes in the stigma region prior to ovulation (Yoshimura and Koga, 1982; Jackson *et al.* 1991) that included changes in collagen fibers from dense, ordered bundles to loose, widely dispersed fibrils (Yoshimura and Koga, 1982) and a decrease in interfibrillar proteoglycans/glycosaminnoglycans (Jackson *et al.* 1991) compared to the nonstigma region.

Jackson *et al.* (1993, 1994) and Tischkau *et al.* (1996) found that plasminogen activator (PA) participates in follicular growth and ovulation. PA converts plasminogen to plasmin, which then acts to cause tissue remodeling or to convert latent collagenase to active collagenase. Plasmin and collagenase may cause degradation and/or dissociation of theca connective tissue especially in the stigma region resulting in ovulation. Tischkau *et al.* (1996) added that the granulosa layer is the site of messenger Ribonucleic acid and/or protein regulation of PA production by LH in the avian ovary. Saito *et al.* (1993) suggested a supportive role for prostaglandin in causing follicular rupture because prostaglandin stimulates both cell-associated and secreted PA (Tilly and Johnson, 1987). In mammals, Espey (1980) postulated that prostaglandins stimulated the production of proteolytic enzymes.

Although detailed studies on the hormonal control of ovulation have been carried out, surprisingly little is known about the cause(s) of termination of the sequence in laying hens. In this respect, there are several old theories for the explanation of the mechanism relating to sequence termination. These theories will be summarized without much debate pro and contra for these old theories.

Table 2. Effects of enzymes injected locally into the ovarian follicles or applied on filter paper placed on the stigma or the non-stigma regions of follicles of the domestic fowl

Enzyme	Injection or site of application	No. of follicles			Time from treatment to rupture (min)		
		Treated	Ruptured	%	Mean	Range from	to
Pronase	Shallow inj.*	7	7	100	25.4	11	60
	Deep inj.**	8	8	100	13.3	8	20
Nagarse	Shallow inj.	6	4	67	15.0	14	16
	Deep inj.	11	11	100	13.9	10	16
Collagenase	Shallow inj.	6	4	67	51.8	26	69
	Deep inj.	16	12	75	45.9	7	126
Hyäluronidase	Deep inj.	4	0	0			
Lysozyme	Deep inj.	4	0	0			
Saline	Shallow or Deep inj.	6	0	0			
Pronase	Stigma	5	5***	100	64.5	10	92
	Non-stigma						
Collagenase	Stigma	5	4***	80	104.0	78	137
	Non-stigma						
Saline	Stigma	4	0	0			
	Non-stigma						

* Shallow injection was probably made into the theca externa.
** Deep injection was probably made into the theca interna and/or stratum granulosum.
Follicles injected locally or administered on filter paper with 100mg (0.1 ml of the 1mg enzyme/ml solution) of the proteolytic enzymes.
*** Rupture occurred at stigma region (Modified from Nakajo *et al.* 1973).

1-. Asynchronous theory

Bastian and Zarrow (1955) advanced their hypothesis, called the asynchronous theory, concerning rhythmic maturation of the ovarian follicles at every 24 + a h intervals and LH release at every 24h interval. They considered that LH was released over a period of 8h each night and that a follicle matured every 26h. When these two events coincided, ovulation occurred. According to this concept, lag is caused by successive follicles maturing later in the period of LH release until the two events are not coincident and ovulation fails to occur. The mature follicle in the ovary is duly ovulated during the period of LH release the next day.

Bastian and Zarrow (1955) believed that follicles showed variation in the sensitivity to LH that was not synchronous with LH release. They showed that the C_1 follicle was sensitive to 0.5mg of LH about 10 to 12h before the normal ovulation and its sensitivity was approximately 10 times higher 4 to 36h before its normal ovulation. This high level of LH exceeded the amount released endogenously every night. On the other hand, the threshold for intraclutch follicles was lower than the daily amount of released LH (about 3mg daily according to this study). Bastian and Zarrow (1955) found that the follicular size at ovulation was graded and each succeeding follicle within a sequence was smaller than the preceding follicle within the same sequence length of 2 to 3 eggs.

2-. Fatigue theory

Nalbandov (1959) postulated his hypothesis named the fatigue theory. After ovulation, the ovulated ovum is engulfed into the infundibulum, then passes through it and enters into the magnum. When the ovum is present in the magnum and in the isthmus a neural inhibition on LH secretion is performed. Thereafter, the ovum leaves the magnum and enters the uterus, and LH secretion resumes again. The LH secretion slowly recovers until it returns to the pre-inhibition level and the next ovulation can occur. Due to the repetition of this inhibition and release during several succeeding cycles, the recovery rate becomes slower as the egg position of the sequence advances, until the pituitary gland does not recover in time to cause ovulation of the next egg and the sequence is interrupted.

3-. Daily variation in excitability of a neural component (excitation hormone theory)

Fraps (1955, 1961, 1965) proposed that a hypothetical "excitation hormone" acting via a neural pathway stimulates the release of the increased LH from the pituitary. The threshold of the hypothalamus in response to the excitation hormone is believed to be subject to a diurnal rhythm associated with the light-dark cycle. The excitation hormone is generally accepted to be progesterone by Fraps.

It is proposed that progesterone increases until it reaches the threshold level for the release of LH and consequently ovulation occurs. In cases of delayed ovulation, a relatively higher threshold may occur on the day preceding the terminal egg in the sequence. This makes it impossible for the release of LH to occur with a given concentration of the excitation hormone in the blood. In other words, the C_1 follicle is forced to wait longer before it is ovulated because it arrives at maturity outside of the daily "open period" governing pre-ovulatory LH release. In this respect, the open period is identified as the time during which the pituitary is activated by a neural signal to release LH and is considered to be of 8-10h daily.

In general terms, each of the previous hypotheses proposes that the failure of LH release is the cause of the missed oviposition. They differ in attributing the cause(s) of failed LH release to different levels of the hypothalamo-pituitary-gonadal axis; especially to the hypothalamus (Fraps, 1955), the follicles of the ovary (Bastian and Zarrow, 1955), or the oviduct (Nalbandov, 1959).

The results of RIA concerning the LH concentration during the ovulatory cycle revealed that LH is not released for a prolonged period as suggested by Bastian and Zarrow (1955) or inhibited as proposed by Nalbandov (1959). Now almost all reports on LH during the ovulatory cycle show that LH is maintained without noticeable change through the cycle except about 4h before ovulation where a preovulatory surge is found (Furr *et al.* 1973; Zakaria, 1982; Krishnan *et al.* 1993).

With regard to the hypothesis proposed by Fraps, the main point is that on the day of missed ovulation a gradual increase in the secretion of progesterone accompanies the cycle of follicular maturation. No such increase has been reported. Furthermore, administration of progesterone outside the open period will induce an increase in the plasma concentration of LH, and in response to an adequate

LH stimulus the ovary is able to synthesize and secrete steroids by 12h after the previous ovulation (Etches and Cunningham 1976). Thus, the normal restriction of the spontaneous preovulatory release of LH to the open period of the day is not due to a failure of the positive feedback mechanism either at the hypothalamus-pituitary level or at the ovary. Also, it is argued that an extra stay of C_1 follicle in the ovary as proposed by Fraps might influence all the growing follicles, leading to disturbance of the follicular hierarchy (Gilbert and Wood-Gush, 1971; Zakaria *et al.* 1983).

Fraps's hypothesis (1955) is based on the understanding that the maturation time is similar for all follicles. However, Zakaria (1999b) found differences in maturation time in C_1 and C_t follicles and came to the conclusion that C_t follicles acquire competency more quickly than C_1 follicles. Furthermore, Zakaria (1999a) found that yolk deposition in YGF is a continuous process and the arrangement, size, numbers and maturation time of YGF in young and old hens were different. Zakaria (1999ab) proposed that the pause day between two successive sequences is either caused by a delay of the C_1 follicle to enter the rapid growth phase or by extension of the growth phase of the predestined C_1 follicle (see Zakaria, 1999ab for further information). However, the theory of Fraps that there is a daily rhythm in the sensitivity of the hypothalamus to progesterone feedback has received the most attention, although this theory has been neither proven nor disproven (Bahr and Johnson, 1991).

4-. The role of adrenal gland

Etches *et al.* (1984) and Cunningham (1987) proposed important roles for the adrenal gland in the timing of ovulation in the hen and they introduced a variety of anatomical and physiological evidence supporting this proposition. The evidence included the anatomical juxtaposition of the left ovary and adrenal gland and innervation of steroid-producing cells within the follicle by nerve tracs passing through the adrenal gland. Furthermore, treatments of deoxycorticosterone, corticosterone or adrenocorticotrophic hormone (ACTH) can induce premature ovulation in hens. Additional evidence included the ability of an injection of metyrapone (a drug which inhibits 11 b-hydroxylase activity in the adrenal gland and reduces corticosterone production) to alter the timing of preovulatory LH release, and the ability of an injection of dexamethasone (a synthetic corticosteroid which inhibits ACTH secretion and which leads to a reduction in the plasma concentration of corticosterone and blocks the preovulatory release of LH) or infusions of corticosterone to block ovulation. Moreover, infusions of a small amount of corticosterone for an extended period of time caused ovarian regression.

Bahr *et al.* (1986) suggested that catecholamines may have some role in the ovulatory process of the domestic hen. Their suggestion was based on the findings that the level of norepinephrine (noradrenaline) and epinephrine (adrenaline) in the F_1 follicle was elevated at 6 h before ovulation. Detailed information on the role of the adrenal gland on ovulation of the hen can be taken from the reports of Etches *et al.* (1984), Cunningham (1987), and Johnson (2000).

In conclusion, FSH, LH, and progesterone have been shown to regulate follicular growth and development. However, other factors may be involved in establishing follicular growth and ovulation, including the germinal disc region, growth factors, inhibin, macrophages, plasminogen activator and their interactions, etc. While the exact role and function of these factors are not clear it is assumed that these factors have paracrine/autocrine effects that amplify the action of gonadotrophins, especially FSH, on follicular development. Thus, the role of growth factors, macrophages, and plasminogen activator on follicular growth may warrant further investigation.

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