General and Comparative Endocrinology 203 (2014) 250–261

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Review

# Reproduction in hens: Is testosterone necessary for the ovulatory process?

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ARTICLE INFO

*Article history:*
Available online 6 April 2014

*Keywords:*
Laying hens
Ovulation
Avian
Follicle
Granulosa
Endocrinology

ABSTRACT

Avian reproduction entails complex endocrine interactions at the hypothalamic and ovarian levels. The initiation of the reproductive season is due to the reduction in melatonin and GnIH production as day length increases. The decline in GnIH permits GnRH and gonadotropin secretion starting follicle growth. Follicular steroids stimulate sexual activity and have important roles for the induction of ovulation. Progesterone ($P_4$) is an inductor of the preovulatory surge of LH, while estradiol ($E_2$) acts as a hypothalamic primer to allow $P_4$ receptor development, as well as a stimulator of yolk production. Conversely, the role of testosterone (T) has been more controversial; however, there is now enough evidence, which demonstrates an essential action of T in the ovulatory process. For instance, blockage of endogenous T, by passive or active immunization or by the use of a specific antagonist of T, inhibits ovulation and the preovulatory surges of $P_4$ and LH. This information is supported by the fact that there is a positive correlation between the occurrences of the T preovulatory surge and those of $P_4$ and LH, in which the absence of T caused a lack of $P_4$ and LH increase in almost 90% of the cases. Additionally, it has been observed that T has a paracrine action within the ovary, to promote $P_4$ secretion by granulosa cells from the larger follicles. This has been related with an increased mRNA expression of StAR and P450scc enzymes, which are essential for $P_4$ production, as well as with LH-R mRNA expression in granulosa cells of preovulatory follicles, an effect that should enhance the positive feedback between $P_4$ and LH necessary for ovulation. Lastly, endocrine activity of hierarchical follicles occurs as a result of a complex interaction between the larger follicles (F1–F3) and the smaller follicles (F4–F6), which is necessary to achieve an adequate preovulatory milieu.



## 1. Introduction

The purpose of this review is to collect original information regarding the reproductive process in laying hens. In particular, we focus on the information pertaining the role of testosterone, a steroid that is normally assumed as a male hormone, in the process of ovulation. In doing so, we searched for the original paper that refer to laying hens, and where data was not available, we included literature gathered in other avian species.

Avian species are mostly seasonal, and their reproductive process is initiated by environmental queues (Sharp, 1996). In the jungle fowl, a predecessor of the domestic hen, the increase in day length initiates gonadotropin secretion (Ono et al., 2009). Similarly in the domestic hen, the artificial lengthening of the day times the start of oviposition at the shortest age at which they can reach sexual maturity (Lewis et al., 2007, 2008). The endocrine control of seasonal reproduction has been extensively studied and the role of melatonin was controversial for many years (Wilson, 1990; Sharp, 1993; Chakraborty, 1995), now it has been proposed that melatonin stimulates GnIH production and release, which in turns inhibit GnRH and LH secretion during the non-reproductive season (Ubuka et al., 2005).

Follicle development and ovulation depends on the coordinated action of hypothalamic, pituitary and ovarian hormones. At the hypothalamic level two relevant hormones participate in the stimulation of follicular development. Whilst in mammals GnRH is solely responsible for controlling gonadotropin secretion, in birds both GnRH and a novel inhibitory hormone call GnIH (Sharp et al., 1990; Wilson et al., 1990; Tsutsui et al., 2009) have equally important and opposing roles in controlling pituitary gonadotropin secretion.

Driven by gonadotropins, follicular development is characterized, at first, by the slow growth of prehierarchical follicles, and later by the rapid growth of hierarchical follicles resulting in the daily ovulation of the preovulatory follicle (Johnson, 1990, 1993).

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http://dx.doi.org/10.1016/j.ygcen.2014.03.040
0016-6480/

Concurrent with follicular development, the production of gonadal steroids takes place in a three-cell model in which progesterone is mainly secreted by granulosa, oestradiol by the external theca and testosterone by the inner theca cells (Porter et al., 1989). Progesterone ($P_4$) (Wilson and Sharp, 1976a) and estradiol ($E_2$) (Wilson and Sharp, 1976b; Kawashima et al., 1979) have known actions in the induction of the preovulatory LH surge. In contrast, testosterone (T) action has been more controversial (Wilson and Cunningham, 1984; Etches, 1996), and is not until recently that its roles both at the endocrine and intraovarian level have been demonstrated. In this review, we show enough evidence to suggest an essential action of T in the ovulatory process of the domestic hen.

## 2. Ovarian follicles

### 2.1. Follicular development

Follicular development within the reproductive process in laying hens comprises 2 main groups of follicles: the first group, called the *prehierarchical follicle* group, includes small white follicles (less than 2 mm in diameter), large white follicles (from 2 to 4 mm in diameter) and small yellow follicles (from 4 to 8 mm in diameter). The second group, the *hierarchical follicle* group, is formed by large yellow follicles, usually between 5 and 7 rapid growing follicles, with diameters ranging from 9 to 35 mm, which reach preovulatory size in an average of 9 days (7–10 d) (Johnson, 1990, 1993). Each one of the follicles belonging to the latter group is identified by a decreasing digit according to its size (i.e. the second largest follicle is called F2, the largest follicle F1, etc.). Once a follicle enters the hierarchical follicle category it cannot suffer atresia (Gilbert et al., 1983; Johnson, 1996), and every day the largest follicle is ovulated, while a new follicle is recruited to enter the hierarchy, from a pool of 10 to 15 follicles that are 6 to 8 mm in diameter.

### 2.2. Role of FSH

It is well known in mammals that FSH stimulates small follicle development and steroid hormone production. In avian species, there is evidence that FSH does not directly regulate steroidogenesis (Johnson, 1996), because while FSH administration 14 h prior to ovulation accelerated steroidogenesis in the follicular wall, it did not concurrently increase steroid hormone concentrations in peripheral circulation (Imai and Nalbandov, 1978). In addition, FSH was unable to induce steroid secretion in prehierarchical follicles (Robinson et al., 1988). Further, FSH plasma concentrations are relatively constant during the ovulatory cycle, with the exception of a small but significant increase 12 h before ovulation (Krishnan et al., 1993).

Notwithstanding, the presence of FSH receptors (FSH-R) has been found in theca and granulosa cells of follicles of all sizes, with greater concentrations in prehierarchical follicles (6–8 mm) during their transition to hierarchical status (Bahr and Johnson, 1984; Yamamura et al., 2001; You et al., 1996). In addition, FSH-R concentrations decrease with follicle maturation (Bahr and Johnson, 1984; Yamamura et al., 2001; You et al., 1996), and are lower in atretic follicles (You et al., 1996). Further, ovine FSH injected to laying hens was able to stimulate $P_4$ production by granulosa cells of F3 and F4 follicles *in vitro* (Hertelendy et al., 1982). Moreover, FSH induces the expression of P450scc (cholesterol desmolase) and $P450_{17c}$ (17α-hydroxylase) mRNAs in granulosa cells for the onset of $P_4$ production (You et al., 1996; Hernandez and Bahr, 2003). Maximum FSH binding is seen in ovarian stroma and small follicles (Etches and Cheng, 1981), the main sites of $E_2$ production (Armstrong, 1984), which would suggest that FSH stimulates production of this steroid as it occurs in mammals. In addition, Tilly et al. (1991b) and Li and Johnson (1993) considered FSH as a primary factor to initiate steroidogenesis by granulosa cells, a view strengthened by the fact that granulosa cells in prehierarchical follicles are not responsive to LH (Tilly et al., 1991b). Therefore, granulosa cell differentiation evidenced by $P_4$ production (Johnson, 1996; Zhang et al., 1997), and, driven by FSH stimulus, is the limiting factor for follicular entry to the hierarchical category. Thus, FSH-R expression within granulosa cells is key for cell differentiation prior to follicle selection into hierarchy. Regulation of FSH-R expression is supported by bone morphogenetic protein 4 (BMP4), prior to and immediately after follicular hierarchization (Kim et al., 2013).

Seemingly, other roles of FSH are to recruit and promote growth of small follicles (Imai, 1973; Palmer and Bahr, 1992; McElroy et al., 2004), to prompt small follicle maturation (Bahr and Johnson, 1984; Zhang et al., 1997) and to stimulate granulosa cell proliferation, possibly promoting follicular growth (Velázquez et al., 1997; McElroy et al., 2004). Epidermal growth factor (EGF), which showed high expression in granulosa cells from prehierarchical follicles, mediated FSH-induced cell proliferation and delayed cell differentiation, stimulating follicular growth before follicular selection into hierarchy (Lin et al., 2011).

Other hormones may be involved in preovulatory follicle growth. It seems that $E_2$ plays a dual role by stimulating growth of the follicular wall in reproductive aged hens, while inhibiting it in young birds (Lebedeva et al., 2010). The stimulatory effect of $E_2$ on follicular growth cannot be attributed to induction of FSH release, as a recent study conducted in quails showed that subcutaneous injections of $E_2$ (0.1 or 0.2 mg twice per week) failed to increase FSH serum concentrations (Ciftci, 2012). Testosterone and LH apparently participate in final follicular growth during the preovulatory surge in young hens (Lebedeva et al., 2010).

### 2.3. Steroidogenesis

Ovarian follicles are able to secrete steroid hormones and this ability changes according to their developmental status (Porter et al., 1989; Hernández-Vértiz et al., 1993; Rodríguez-Maldonado et al., 1996). Hence, prehierarchical follicles secrete the largest amount of estrogens (Armstrong, 1984; Robinson and Etches, 1986), because small yellow follicles and ovarian stroma have 50% of the aromatase capacity present in the ovary (Armstrong, 1984). Prehierarchical follicles also secrete marginal quantities of androgens, however their granulosa cells do not express P450scc hence are unable of releasing $P_4$ (Tilly et al., 1991a). Conversely, preovulatory follicles secrete large amounts of $P_4$ (Robinson and Etches, 1986), increasing their secretory ability as they mature, the preovulatory follicle being the main source of $P_4$ prior to ovulation (Yu et al., 1992). The ability of the F1 follicle to secrete the preovulatory surge of $P_4$ is induced by LH, probably due to an increased responsiveness of this follicle to LH (Calvo and Bahr, 1983). The shift in $P_4$ production capacity between prehierarchical and hierarchical follicles is driven by FSH (Li and Johnson, 1993), and this promotes follicular recruitment and entrance to follicular hierarchy (Tilly et al., 1991b; Yu et al., 1992; Hernandez and Bahr, 2003). Concurrently, hierarchical follicles gradually increase T production, the F3 secreting the greatest amounts, while the F1 loses its ability to transform $P_4$ to androgens around 12 h before ovulation (Robinson and Etches, 1986). In opposition, the capacity of hierarchical follicles to secrete estrogens decreases progressively and is almost null in the preovulatory follicle (Armstrong, 1984).

Steroid hormones produced by ovarian follicles have critical roles in the control of the ovulatory process of birds, and a three cell theory has been put forward which indicates that the main products of secretion are $P_4$ for granulosa cells, T for inner theca cells and $E_2$ for outer theca cells (Porter et al., 1989). In addition,