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CYTOKINE IL-1β MODULATION OF REPRODUCTIVE FUNCTION IN HEAT STRESSED HENS

by

Mohammed Abdullah Alodan

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Animal Science

(Physiology)

Under the Supervision of Professor Mary M. Beck

Lincoln, Nebraska

December, 2001

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DISSERTATION TITLE

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SUPERVISORY COMMITTEE:

	APPROVED	DATE
Signature	Mary M. Beck	10/29/2001
Typed Name	Dr. Mary M. Beck	
Signature	Dennis E. Feely	Oct 29 2001
Typed Name	Dr. Dennis E. Feely	
Signature	Thomas M. Petro	10/29/01
Typed Name	Dr. Thomas M. Petro	
Signature	Sheila E. Scheideler	10/29/01
Typed Name	Dr. Sheila E. Scheideler	
Signature		
Typed Name		
Signature		
Typed Name		

GRADUATE COLLEGE
UNIVERSITY OF NEBRASKA

CYTOKINE IL-1β MODULATION OF REPRODUCTIVE FUNCTION IN HEAT STRESSED HENS

Mohammed Abdullah Alodan, Ph.D.

University of Nebraska, 2001

Advisor: Mary M. Beck

The effect of heat stress on reproduction is a complex interaction of all systems in the animal body. Because the granulosa cells are the main source for one of the main hormones (progesterone) involved in reproduction, this series of studies was focused on the effect of heat stress on: 1) the ability of granulosa cells to produce progesterone; 2) The involvement of other mediators from other systems in progesterone production under heat stress; 3) Morphology of granulosa cells; 4) The ability of granulosa cells from heat stressed hens to recover over time. Study 1 was conducted to examine the effect of heat stress and cytokine interleukin-1 on the ability of granulosa cells to secrete progesterone. It was found that both heat stress and interleukin-1 significantly reduce the amount of progesterone secreted by chicken granulosa cells. In study 2, the effect of heat stress and interleukin-1 on 3β-hydroxysteroid dehydrogenase (3β-HSD), a key enzyme in progesterone synthesis, was determined. A significant reduction in 3β-HSD activity in response to heat stress and interleukin-1 was observed. Study 3 was conducted to examine the effect of heat stress on granulosa cells morphology, found in earlier studies to be altered. The area covered by lipid droplets in heat stressed hens granulosa cells was significantly greater than that in granulosa cells of thermoneutral hens. In study 4,

granulosa cells from heat stressed hens were examined for their ability to recover over time. It was found that these cells have the ability to regain their activity and normal morphology over time, a finding that appears to parallel the systemic return of circulating hormone concentrations during acclimatization long-term heat stress.

We conclude that both heat stress and Interleukin-1 have the ability to reduce the production of progesterone by granulosa cells, perhaps by reducing 3β-HSD activity. In addition, heat stress alters granulosa cells morphology. However, the effects of heat stress on granulosa cells in terms of enzyme activity, steroidogenesis, and morphology return to near normal over time.

ACKNOWLEDGEMENT

I would like to thank my advisor, Dr. Mary M. Beck for her patience, support, guidance, and true friendship throughout this research project.

I would also like to thank my committee members, Dr. Dennis E. Feely, Dr. Thomas M. Petro, and Dr. Sheila E. Scheideler for their support, comments, and suggestions.

I thank Lyle Robeson, the farm manager, for making our job easier.

I extend my thanks to Debra Clopton and Candy Toombs for their technical assistance and help.

In addition, I would like to thank my colleagues and friends at work, Marianna Burks, Dr. Samar Elnagar, Danilo Franco, Kimberly Franzen, Farrah Madison, and Hiroko Taira, for their help and friendship.

Additionally, I would like to thank King Saud University at Riyadh, Saudi Arabia, for providing me with the financial assistance.

Finally, my special appreciation and sincere gratitude is expressed to my wonderful parents, brothers, sisters, and wife for their support and encouragement.

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General introduction

In poultry, there has been intensive genetic selection for productivity traits such as egg production, egg size, internal and external egg quality, body size, and feed efficiency. Because there is unfavorable genetic correlation between productivity traits and resistance to heat stress, genetic selection for productivity has adversely affected the chickens ability to resist thermal challenges. In the poultry industry, a rise in environmental temperature above normothermia zone is considered one of the most serious problems that adversely affects productivity in commercial flocks, leading to large economic losses. The reduction in poultry productivity in response to high ambient temperature is mainly due to a decrease in feed intake as birds attempt to decrease heat generated by metabolism. Birds also respond to high environmental temperature by increasing respiration rate as a method of cooling themselves. As a consequence of high respiratory rate, CO_2 in blood is depleted, which leads to a reduction in HCO_3^-. HCO_3^- is directly involved in eggshell formation. Any reduction in HCO_3^- results in poorer shell strength and quality. In addition to behavioral changes to high ambient temperature, there are physiological consequences such as disruption of reproductive hormones, steroids and Luteinizing hormone, which directly affect egg production.

In 1999, the farm value of U.S. poultry production was $22.4 billion, of which $4.3 billion were attributed to commercial egg production. The 328 million laying hens in the U.S. in 2000, laying at the rate of 257 eggs/hen produced 84.4 billion eggs. The size of this industry is not insignificant. Heat stress during summer months for only partially climate controlled houses has devastating effects. For example, in 1995 in Iowa alone, some 1.8 million hens died during a 2-week heat wave; this amounted to a

monetary loss of $9 million (Xin, 1998). In addition to mortality, heat stress significantly impacts egg production, including decreases in the number of eggs produced as well as loses due to poor shell and interior quality.

Although it is difficult to isolate costs to producers that are specific to heat stress as a single factor, losses from calcification failures (of which heat stress would be one cause) range from $256 million (Bell. 1999) to $400 million (Roland, 1988) annually. Understanding mechanisms by which heat stress affects reproductive function (as manifested by egg production decreases) is thus of considerable importance to the layer industry.

For better understanding of this problem, a series of experiments was conducted to investigate the effect of acute heat stress on progesterone production and the enzymes involved in steroidogenesis. In addition, a link was sought between the immune system, in particular mediators such as cytokine IL-1, in the reduction of progesterone formation. The effect of heat stress on the morphology of the main source of progesterone, granulosa cells and the ability of those cells to recover over time in terms of activity and morphology, was investigated.

Review of literature

I. Female reproductive system in chickens

The reproductive system in chickens is different from mammals. These differences include that their offspring will develop outside the female body. The ovulatory cycle is shorter, about 26h in length, whereas in cow and mare is about 21 days

and about 17 days in ewe. Only the left half of Mullerian duct persists. The sperm retain their fertilizing ability for at least a week within the oviduct.

A. Anatomy of the female reproductive system

Chickens normally have only one ovary and one oviduct located on the left side. The right ovary and oviduct are present in the embryonic stages, and by the time of hatching they have degenerated.

1. Ovary

The ovary is located at the left side of the body at the cephalic end of the left kidney and attached to the body by mesovarian ligament (Parker, 1972; Parkhurst and Mountney, 1988). In the mature active female, the ovary consists of medulla covered with cortex. It is a cluster of many follicles; up to 2000 follicles can be seen by the naked eye (Sturkie, 2000). The functional mature ovary is arranged in hierarchy of follicles, usually 4-6 large yolk-filled follicles (2-4 cm) with a larger number of 2-10 mm small yellow follicles, many small white follicles, and postovulatory follicles (Etches, 1984). The ovary's blood supply is received from the ovarian artery. The developed large follicles are surrounded by layers of (perivitelline membrane, granulosa cells, basal lamina, theca interna and theca externa) and many other layers (Figure 1) (Aitken, 1971; Etches, 1984; Etches, 1995; Etches, 1996).

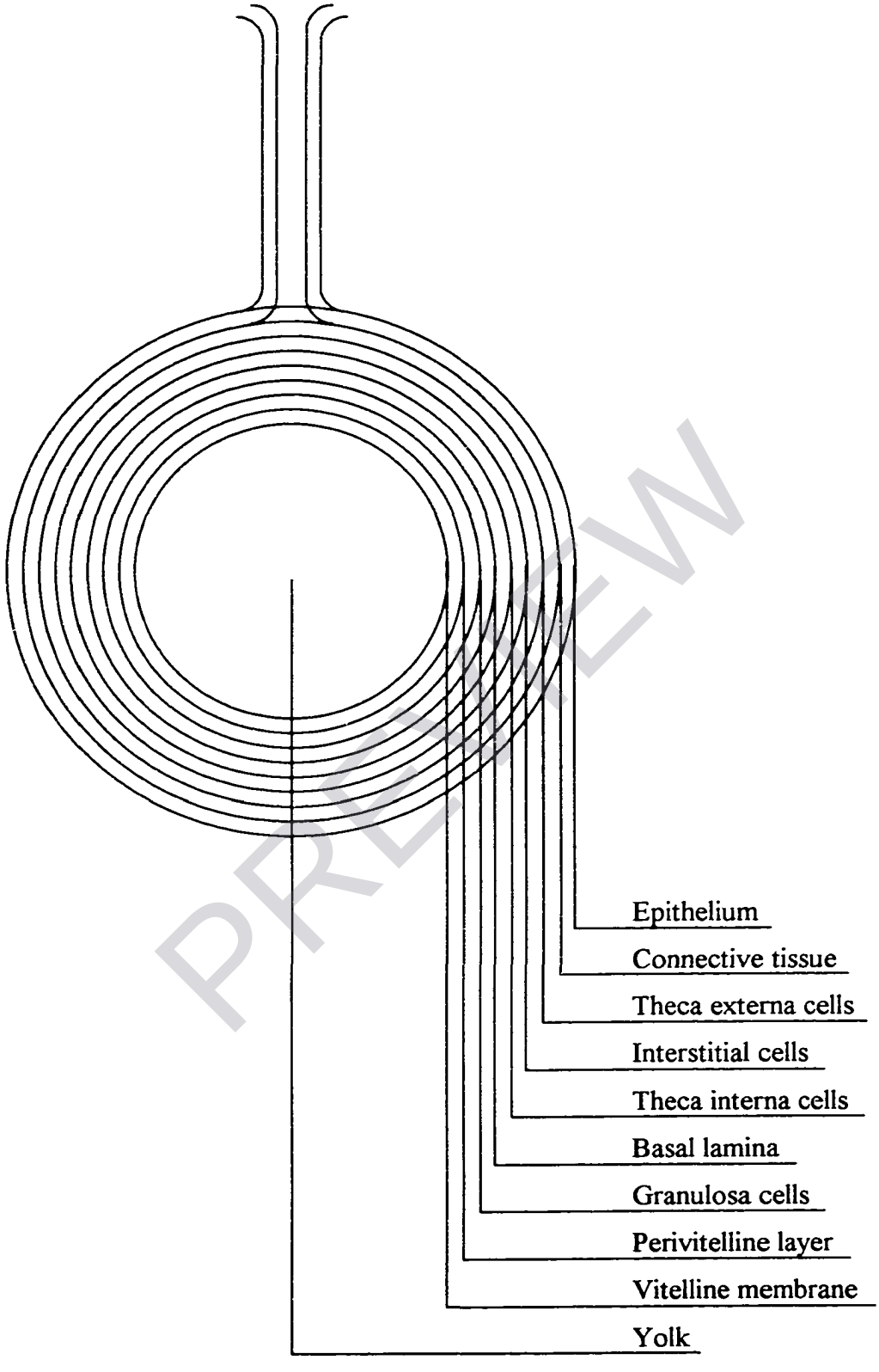

Figure 1. Schematic diagram representing the layers of mature laying hen follicle.

2. Oviduct

The oviduct is a long folded tube that occupies the majority of the left side of the abdominal cavity (Parker, 1972; Parkhurst and Mountney, 1988). The oviduct starts near the ovary and ends at the cloaca. The wall of the oviduct is well supplied with blood vessels and consists of an outer layer of longitudinal muscles, a layer of circular muscles, and a glandular epithelial lining (Parker, 1972). It is about 70 cm in length and 1-5 cm in width. There are five distinguishable regions in the oviduct. The first is infundibulum followed by magnum, isthmus, shell gland (uterus), and vagina (Sturkie, 2000).

a. Infundibulum

The infundibulum is about nine cm in length and has a funnel shape to assist in engulfing the ovum. Also, fertilization occurs in this region (Parkhurst and Mountney, 1988). The wall of infundibulum is folded and these folds are covered by pseudo-stratified columnar ciliated epithelium at the tops and sides, but at the end of glandular groove, the epithelial layer is composed of simple columnar cells (Aitken, 1971). The wall of infundibulum also contains the infundibular sperm tubules where sperm are stored (Parkhurst and Mountney, 1988; Etches, 1995).

b. Magnum

The magnum is the longest part of the oviduct (30 cm). The magnum is the place where the most of the albumen is added to the ovum (yolk). The wall of the magnum is thicker, lighter in color and greater in diameter than the infundibulum (Aitken, 1971).

The epithelial layer of the magnum consists of secretory and ciliated cells. Two types of glands are present in the epithelial layer (tubular and epithelial glands) (Aitken, 1971).

c. Isthmus

The isthmus is about 10 cm in length. Its main function is to secrete inner and outer shell membranes. The wall has thick circular muscles and the glandular tissue is less developed when compared to magnum (Johnson, 1986).

d. Shell gland (uterus)

The shell gland is about 10 cm in length. Its wall is characterized by thick longitudinal muscle, and tubular and epithelial glands (Johnson, 1986; Parkhurst and Mountney, 1988). The function of the shell gland is to secrete the shell of the egg and its pigment (brown color in some breeds) ((Johnson, 1986; Parkhurst and Mountney, 1988). Also, it is responsible for oviposition of the egg by increasing the contraction of its muscles.

e. Vagina

The vagina is relatively short and it has no role in the formation of the eggs, but it participates with the uterus in the expulsion of the egg. The epithelial layer of the vagina is a mix of pseudostratified and columnar ciliated and mucus secreting cells (Aitken,

1971). There are sperm storage tubules also located at the junction of shell gland and vagina (Etches, 1995).

B. Physiology of female reproductive system

1. Oviposition and ovulatory cycle

Hens start laying eggs at the age of ≈22 weeks. They usually lay eggs in sequences (series) separated by one day pause under the usual lighting schedule (16L: 8D). The first egg is laid 1-2 hours after light and the last egg is laid about 6-7 hours before dark, limiting the oviposition to about an eight hour (open period) (Etches, 1995; Etches, 1996). Ovulation occurs 15-75 minutes after oviposition (Sturkie, 2000). The shorter the time between oviposition-ovulation, the longer the sequence is.

The asynchrony of oviposition and ovulation are controlled by two systems; one controls the timing of laying, which is affected by light (open period for oviposition), and the other control the follicular maturation that is not effected by light (Etches et al., 1984). Follicular maturation and the open period cause oviposition to occur in sequence. When a mature follicle secretes progesterone (P4), it stimulates the production of gonadotrophin releasing hormone (GnRH) from the hypothalamus, which then stimulates the anterior pituitary to produce luteinizing hormone (LH), and follicle stimulating hormone (FSH) (Figure 2) (Etches, 1984; Lang et al., 1984; Shimada et al., 1984; Etches, 1995). These two gonadotrophins bind to receptors on granulosa and theca cells of the follicles and stimulate P4 production from large follicles and estrogen and androgen from small follicles. Progesterone and LH initiate the enzymatic activity of the

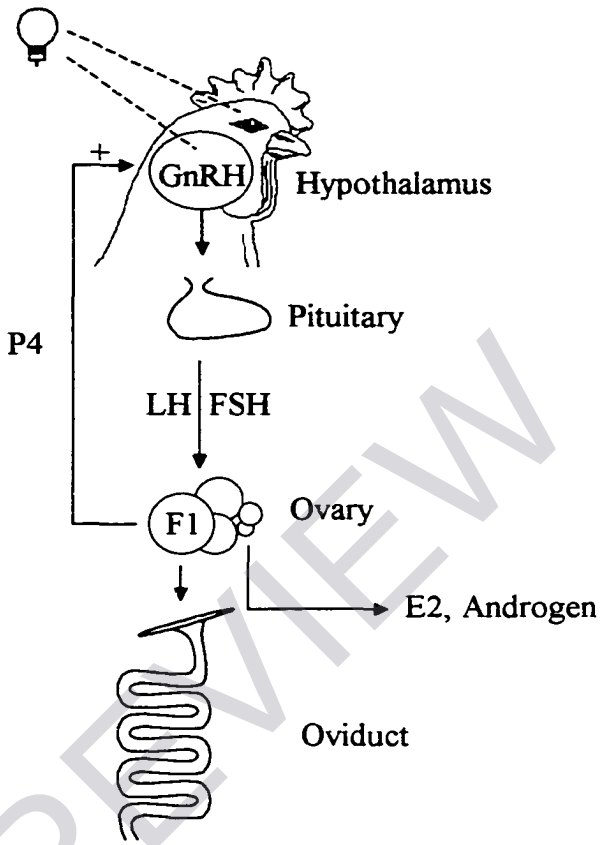

Figure 2. Schematic diagram of the hormonal control of reproductive system, ovulation in chickens. Gonadotrophin releasing hormone (GnRH), Progesterone (P4), Luteinizing hormone (LH), Follicle stimulating hormone (FSH), largest follicle (F1), Estrogen (E2).

theca cells that causes the rupture of the stigma on the follicle wall (Etches, 1995). If the follicle fails to secrete enough P4 in the open period, then there will be no response from the anterior pituitary because its ability to secrete LH is restricted to the open period (Etches, 1995). Therefore, if the mature follicle does not secrete P4 in the open period, then there will be no LH surge to stimulate ovulation. Thus, the chicken will not lay on the next day. Ovulation will then occur at the beginning of next open period (Etches 1995).

a. Steroidogenesis

Steroid production occurs in several organs of the animals. One of these organs is the ovary and its follicles. Progesterone production from ovarian follicles is produced in granulosa cells (Huang et al., 1979; Johnson et al., 1987; Novero et al., 1991; Li and Johnson, 1993), whereas estrogen is synthesized in the theca cells (Huang et al, 79; Bahr et al., 1983). Synthesizing steroids depends on the follicular stage of maturation. At early stages of maturation, follicles produce estrogen and androgen. As the follicles mature, they start producing large amount of progesterone (Bahr et al., 1983; Etches, 96). A three-cell model for steroidogenesis in avian follicles has been described (Figure 3); progesterone is synthesized in granulosa cells, testosterone in theca interna, and estrogen in theca externa (Porter et al., 1989; Nitta et al., 1991).

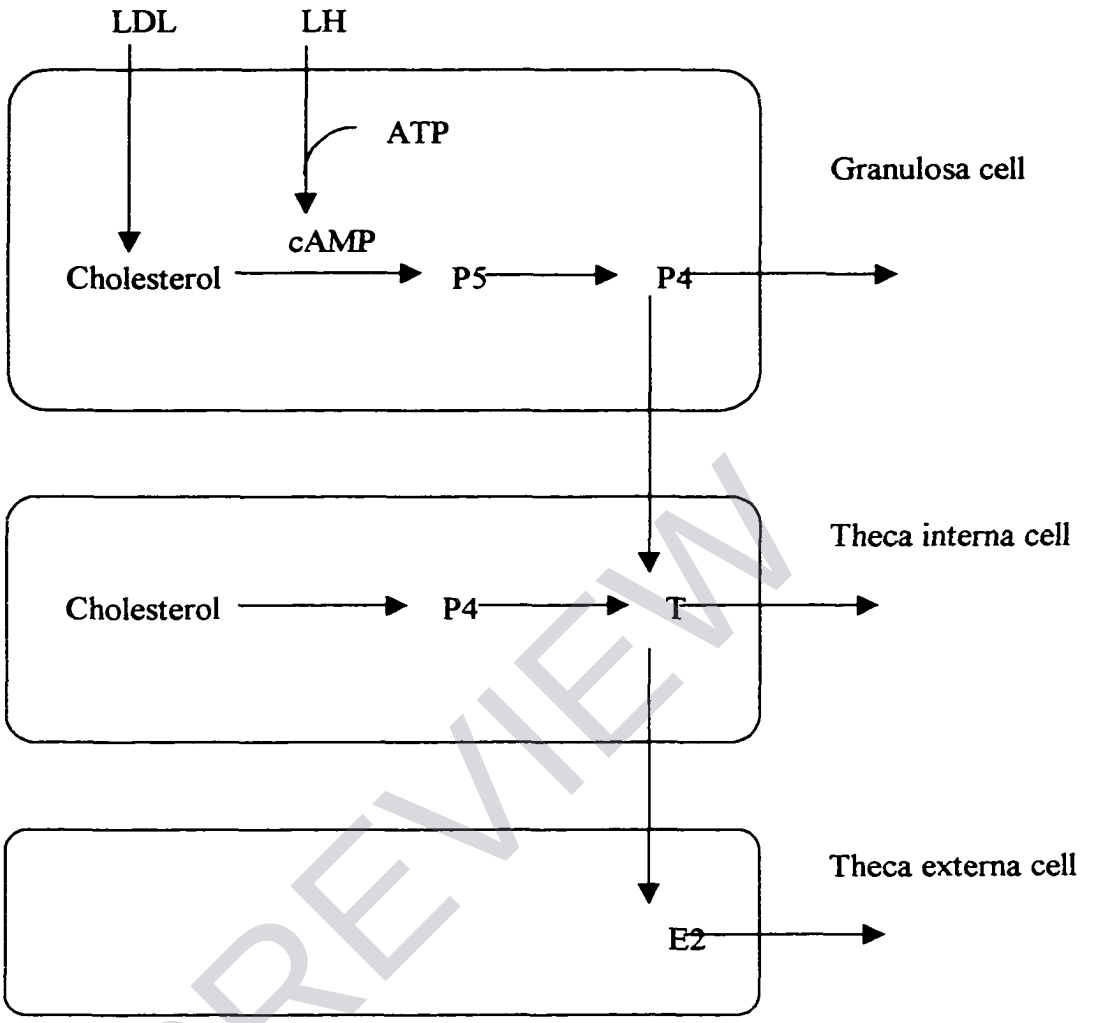

Figure 3. Schematic diagram of three-cell theory for steroidogenesis in the follicle of chicken ovary. Low-density lipoprotein (LDL), Luteinizing hormone (LH), Pregnenolone (P5), Progesterone (P4), Testosterone (T), Estrogen (E2). After Huang et al. (1979); Porter et al. (1989); Nitta et al. (1991).