IMMUNOLOGICAL STUDIES OF THE ORIGIN, STRUCTURE AND FUNCTION OF BOVINE CORPUS LUTEUM

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment for the Degree of

Doctor of Philosophy

by

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January, 1984

INTRODUCTION

The bovine corpus luteum (CL) of the estrous cycle has a short and well defined lifespan lasting approximately 21 days. During its formation from a ruptured follicle after ovulation, it undergoes rapid cellular rearrangement and growth. There is a rapid intermixing of cells from theca and granulosa layers. In a mature bovine CL, as in the CL of other mammals, distinct cell types of different diameters, broadly classified as large (25-40μ) and small (10-22μ)), can be recognized. Thus, the fate of theca and granulosa cells after ovulation is directly relevant to understanding the function and lifespan of the CL. There is controversy about the follicular origin of the cells of the CL. Enders (1973) reviewed the literature on this subject and concluded that many workers considered the cells of the CL to be mainly derived from granulosa cells, despite the work by Corner (1919) showing that both theca and granulosa cells contributed to the formation of the pig CL.

There are a number of reasons for the differences of opinion about the contribution of theca and granulosa cells to formation of luteal cells. In bovine CL there is such a rapid intermixing of theca and granulosa cells that these cells may not be easily distinguished after ovulation (Donaldson and Hansel, 1965). It is also not known whether real species differences exist in the extent of the contributions of the follicular elements to formation of the CL.

Histological and histochemical studies are, at present the best sources of information on the origin of different luteal cell types. The rapid invasion of theca cells into granulosa cells during CL formation may hinder accurate recognition of these cells by histological methods. In some species, such as the rat and rhesus monkey the small and large cells cannot be easily differentiated because the small cells are usually obscured by the large cells in tissue sections (Wilkinson et al., 1976; Gulyas et al., 1979). These cells are more readily recognized after the cells have been dissociated.

Despite the generally accepted dogma that small cells are theca-derived and large cells are granulosa-derived, some workers have provided a different opinion. Donaldson and Hansel (1965) suggested that small luteal cells in cow CL differentiated into large cells. This opinion was based on the fact that mitotic activity in granulosa-derived cells ceased early during CL formation, whereas small cells continued to divide (Moss et al., 1954; Donaldson and Hansel, 1965, Priedkalns et al., 1968). Numbers of large cells increased as the CL matured. Large cells were almost non-existent in the CL early in the cycle but they increased in number as the age of the CL increased (Thwaites and Edey, 1970; Fitz et al., 1981). Similar opinions have been expressed for other species such as the rat (Wilkinson et al., 1976), kob antelope (Morrison, 1971), and sheep (Warbritton, 1934; Fitz et al., 1982). In the rat, the size of luteal cells appeared to depend on the hormonal milieu (Enders and Lyons, 1964).

Alkaline phosphatase (AP) has been utilized in histochemical studies to determine the fate of theca cells in formation of the CL of artiodactyls (Corner, 1948; Lobel and Levy, 1968; O'Shea et al., 1980).

AP activity was demonstrated in theca interna but not in granulosa cells of pigs (Corner, 1948), cattle (Lobel and Levy, 1968) and sheep (Hadek, 1958; O'shea et al., 1980). This distinction was absent in the rabbit, guinea pig, dog, rhesus monkey and man (Corner, 1948). AP was present in small cells up to day 18 in pigs (Corner, 1948), day 8 in sheep (O'Shea, 1980) and day 9 in cow CL (Lobel and Levy, 1968). Thereafter, large cells considered to be granulosa-derived also acquired AP activity. The loss of discrete localization of AP in small cells during subsequent days of the CL development was considered a limitation for the use of AP as a marker in these studies. However, it can also be interpreted as evidence that small cells developed into large cells.

It has been demonstrated that there are interactions and differences in the function of small and large luteal cells in the cow (Ursely and Leymarie, 1979; Koos, 1980), pig (Lemon and Loir, 1977; Lemon and Mauleon, 1982) and sheep (Fitz et al., 1981; Fitz et al., 1982). Small cells were six times more responsive to LH added in vitro than large cells (Koos and Hansel, 1981). Large cells secreted more progesterone per cell in vitro than small cells in the absence of stimulation. Co-incubation of both cell types resulted in greater net progesterone than the sum of progesterone produced by both cell types when they were separately incubated. Large cells from sheep CL had more receptors for prostaglandins ($PGF_2\alpha$ and PGE_2), while the small cells had more receptors for LH or hCG (Fitz et al., 1982). The different steroidegenic capabilities of the two cell types may be important in the regulation of function and lifespan of the CL.

It is therefore important to study the origin of the different cell types in the CL. The extent to which the granulosa and theca cells

contribute to the formation of the CL must be known if accurate extrapolations of functional interactions demonstrated for follicular cells can be made. In the two-cell model proposed for follicular function (Armstrong and Dorrington, 1977; Hansel and Fortune, 1978), theca interna and granulosa cells are believed to cooperate in estradiol-17β biosynthesis. According to this model, androgens synthesized by theca interna in response to LH are transferred to granulosa cells where, under FSH control, they are aromatized to estradiol 17β. Both theca and granulosa cells produce progesterone. During luteinization the granulosa cells lose their ability to aromatize androgens to estradiol in some species, including cattle. In these species, the mature CL does not synthesize estradiol 17β (Savard, 1973). It was originally thought that bovine CL could not produce androgens and estrogens because they lacked the 17-hydroxylase and 17-,20-lyase enzyme systems, as well as the 17-hydroxylase - aromatase complex (Savard and Telegdy, 1965). Microsomal cytochrome P450, the necessary component of the hydroxylase systems, was also reported to be absent in bovine CL (McIntosh et al., 1971). Like the bovine CL, CL of several species, including sheep, horse, and rabbit are incapable of estrogen synthesis; but in contrast to the cow CL, they continue to synthesize progestins as well as androgens (Savard, 1973). Sheep CL produce androsteredione, since the levels of this hormone in the ovarian vein were reduced significantly after the removal of the CL (Baird and Scaramuzzi, 1976).

In another group of species, including man, monkey and pig, the CL continue to produce all steroids produced by the preovulatory follicle (estadriol 17β, androgens and progesterone). Only the young bovine CL

(up to 5 days old) produces all of the steroids produced by the follicle (Henderson and Moon, 1979). Testosterone (2-6ng/g of tissue) was present in CL of unknown age. Shemesh et al., (1975) reported synthesis of testosterone in response to LH or $PGF_2\alpha$ in vitro, but no response to added LH or arachidonic acid was detected by Lukaszewska and Hansel (1980). Mid-cycle bovine CL were unable to synthesize estradiol 17β, even with the addition of testosterone (Lukaszewska and Hansel, 1980; Koos, 1980). The reasons for these differences between species are not known, although the extent of contribution of granulosa and theca cells to the formation of the CL may be partly responsible.

Studies based on histology or histochemistry have inherent limitations. The rapid cellular rearrangement that occurs after ovulation makes it difficult to accurately distinguish the cells as they develop. Another approach has been used in this study; monoclonal antibodies to theca and granulosa cells were used as reagent markers during formation, development and involution of the CL during the estrous cycle and pregnancy. The hybridoma technique developed by Kohler and Milstein (1975) has proved useful in dissecting complex cellular development in several tissues. To date the technique has been successfully used in studies of spermatogenesis (Bechtol et al., 1980), development of intestinal cells (Quaroni, 1982) and nerve cells (Raff et al., 1983). For this reason, we decided to utilize the technique to study the contribution of granulosa and theca cells to formation of the bovine CL.

Since tissue samples were available, we have also studied the in vitro functions of the CL at different stages of the estrous cycle and pregnancy. Luteal tissue progesterone synthesis in vitro has been a

useful model for studying CL function in vivo (Hansel et al., 1973). Histology and ultrastructure have also been investigated, since information on these topics, especially during pregnancy, is limited.

Lymphocytes which infiltrate the CL beginning on day 14 (Lobel and Levy, 1968), have been of particular interest in these studies. Lymphocyte numbers increase with age of the CL, unless pregnancy ensues, in which case only moderate infiltration occurs. The presence of lymphocytes was considered the first sign of incipient luteolysis by Lobel and Levy (1968). Generally, lymphocytic infiltration is considered an immunological process. In this study, we have attempted to define the roles of lymphocytes at the luteal tissue level, and in one study, the effects of administering anti-lymphocyte serum were determined.