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# Alterations in Follicular Steroid Hormones During the Preovulatory Period in the Ewe[1]

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## ABSTRACT

Progesterone, testosterone and estradiol-17β were quantified within the thecal layer, granulosal layer and fluid of the preovulatory follicle and in ovarian and jugular venous sera of sheep. Prior to the preovulatory surge of luteinizing hormone (LH), the total content of estradiol-17β was elevated within each follicular constituent. These levels diminished rapidly to minimal values concurrent with the surge of LH. Follicular tissue and fluid contents of testosterone were low at all times, but were higher before the surge of LH and subsequently (like estradiol-17β) declined. The initial decrease in follicular estradiol-17β occurred before the first detected decline in testosterone. Changes in sera concentrations of estradiol-17β in the ovarian vein draining the ovary with the preovulatory follicle or in the jugular vein were similar to follicular variations in estradiol-17β. Likewise, changes in concentrations of testosterone in ovarian venous sera were indicative of follicular changes. Testosterone was undetectable in jugular venous sera. During the period leading up to the anticipated time of ovulation, and approximately 8 h after follicular testosterone and estradiol-17β had fallen, content of progesterone within thecal tissue, granulosal tissue and follicular fluid increased. Alterations in follicular progesterone were not reflected by hormone changes in either ovarian or peripheral sera.

## INTRODUCTION

Ovarian steroid hormones appear to have multiple roles in the ovulatory mechanism. Progesterone (Rondell, 1974) and testosterone (Mori et al., 1977) have been implicated as intrafollicular intermediates of pituitary gonadotropic action in this process. By stimulating the preovulatory surge of gonadotropin, estradiol is thought to participate indirectly in causing ovulation. In addition, estradiol may act at the level of the preovulatory follicle to enhance its responsiveness to LH (Richards, 1980). Blood levels of estradiol are known to decline precipitously in a number of species at about the time that LH is reaching peak concentrations (Hansel and Echternkamp, 1972, Butcher et al., 1974; Knobil, 1974; Moor, 1974; Kletzky et al., 1975; Baird, 1978; Saidapur and Greenwald, 1978a).

During the preovulatory stage of the reproductive cycle (shortly after or coincident with the surge of LH) increasing concentrations of progesterone have been noted in the peripheral and/or ovarian venous blood of rabbits (Shaikh and Harper, 1972), rats (Butcher et al., 1974), guinea pigs (Feder et al., 1968), hamsters (Saidapur and Greenwald, 1978a), dogs (Concannon et al., 1977), pigs (Eiler and Nalbandov, 1977), monkeys (Johansson et al., 1968; Kirton et al., 1970; Monroe et al., 1970) and human beings (Neill et al., 1967; Runnebaum and Zander, 1967; Johansson and Wide, 1969; Lloyd et al., 1971). Progesterone was also acutely elevated in fluid or tissue from preovulatory follicles of rabbits (YoungLai, 1972; Patwardhan and Lanthier, 1976; Bahr, 1978; Goff and Henderson, 1979), rats (Szoltys, 1976), pigs (Eiler and Nalbandov, 1977; Ainsworth et al., 1980) and women (Friedrich et al., 1974; Sanyal et al., 1974; McNatty et al., 1975; Fowler et al., 1977; Bomsel-Helmreich et al., 1979). The ewe and cow may be unique in that in these species blood concentrations of progesterone remained low and did not fluctuate significantly during the period of time preceding the expected occurrence of ovulation (Short et al., 1963; Moore et al., 1969; Thorburn et al., 1969; Henricks et al., 1970; Kazama and Hansel, 1970; Bjersing et al., 1972; Murdoch et al., 1981). This certainly does not preclude a

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lack of follicular synthesis of steroid hormone at corresponding times.

In a limited number of studies, preovulatory secretion of androgens by the ovary has been examined. A rise was detected in peripheral blood of the rat (Mori et al., 1977) and hamster (Saidapur and Greenwald, 1978a). Prior to ovulation, increased concentrations of androgens were not found in the utero-ovarian vein (Eiler and Nalbandov, 1977) or in the follicular fluid (Ainsworth et al., 1980) of the pig. Blood androgens exhibited only small changes during the menstrual cycle (Goebelsmann et al., 1974; Ribeiro et al., 1974).

Clearly, ovarian steroid hormone production has been an area of investigation which has received a good deal of attention. In spite of this, further work is needed to define precisely the in vivo changes in steroid hormones within follicles destined to ovulate. The purpose of the following experiment was to determine concomitant alterations in progesterone, testosterone and estradiol-17$\beta$ within the thecal layer, granulosal layer and fluid components of the dominant follicle and in ovarian and jugular venous effluent of periovulatory sheep.

## MATERIALS AND METHODS

Forty mature ewes of mixed breeding were synchronized to estrus with a single intramuscular injection of 2 ml Lutalyse (5 mg dinoprost tromethamine/ml; generously donated by Dr. J. W. Lauderdale, Upjohn Co., Kalamazoo, MI). Occurrence of estrus was monitored twice daily with the use of vasectomized rams. The average cycle length of sheep is 17 days.

Beginning on Day 15 following the induced estrus (first day of observed standing estrus=Day 0) animals were housed indoors and jugular blood samples to be assayed for serum LH (as described by Niswender et al., 1969 and modified by Forrest et al., 1981) were obtained at each observation for estrus every 4 h until laparotomy. Ewes were assigned randomly to receive a mid-ventral abdominal incision at 1 of 8 times (5 animals/group): 0, 4, 8, 12, 16, 20, 24 and 28 h after the first detection of estrous behavior. Ewes in the 0-h group were laparotomized immediately upon detection of estrus. Surgery was performed under sodium thiopental anesthesia. Upon exteriorizing the reproductive organs, the ovary containing the largest follicle was identified and a blood sample was collected from the ipsilateral ovarian vein. It had been shown previously (Bherer et al., 1977) that the ovulatory follicle was the largest in either ovary 2–3 days prior to estrus. We have confirmed (unpublished) that the largest follicle in the ovary of the estrous ewes is devoted to ovulation. After ovariectomy, the designated follicle was dissected from surrounding ovarian tissues and separated into thecal (plus some extraneous tissue), granulosal (plus oocyte) and follicular fluid components as described in detail elsewhere (Murdoch et al., 1981). To remove any residual follicular fluid, granulosal cells were washed twice in 1 ml 0.01 M phosphate-buffered saline (pH 7.0) by mixing on a vortex. The washings were aspirated from the granulosal cell pellet after centrifugations and added to the follicular fluid fraction. Follicular tissues, follicular fluid, and ovarian and jugular venous sera were stored awaiting hormone analyses at $-20^{\circ}$C.

Thecal and granulosal tissues were homogenized in 2 ml 0.01 M phosphate-buffered saline using hand-operated, Ten Broeck tissue grinders. Steroid hormones were extracted from follicular tissue homogenates, follicular fluid, and ovarian and jugular (the sample taken just prior to surgery) venous sera with anhydrous diethyl ether. Progesterone (Niswender, 1973) and estradiol-17$\beta$ (Korenman et al., 1974) were quantified in the extracts by specific radioimmunoassays as modified for use in our laboratory (Moseley et al., 1979). Column chromatography was omitted for estradiol-17$\beta$ determinations because the correlations between hormone content in duplicate aliquots of extracts of a pool of preovulatory follicles (tissue plus fluid) or of a pooled serum (ovarian plus jugular) sample ($\leq$0.5 ml extracted) with or without chromatography were 0.92 and 0.94, respectively (n=30). Testosterone was measured directly in extracted samples by radioimmunoassay. Radioactive testosterone (1,2,6,7-$^3$H; specific activity 98.8 Ci/mM) was purchased from New England Nuclear (Boston, MA) and purified on a column of Sephadex LH-20 within a week of use. Testosterone used for standard preparations was obtained from Sigma Chemical Co. (St. Louis, MO). Antiserum to testosterone (S-250) was a gift from Dr. G. D. Niswender, Colorado State University, Fort Collins, CO. Although the antibody cross-reacts significantly with dihydrotestosterone (69%), the values from these analyses are expressed as testosterone since isolation of testosterone from follicular or serum extracts by Sephadex LH-20 chromatography using a hexane:ethyl acetate:methanol (90:5:5) solvent system (Carr et al., 1971) produced values similar to those for duplicate unchromatographed samples (r=0.90 for the follicular pool and 0.91 for the serum pool; n=20; b's $\simeq$ 1). The usable range of the testosterone standard curve was 0.0078–2 ng. The intra- and inter-assay coefficients of variation on the pooled follicular and serum sample for each of the 3 steroid hormone assays were <10% and <16%, respectively. Recovery after extraction of tritiated progesterone, testosterone and estradiol-17$\beta$ was >80% for follicular tissue homogenates and >90% for follicular fluid and serum. Values were corrected to reflect hormone losses due to the extraction procedure. Solvent blanks in all assays were below the sensitivity of detection.

After determinations for peripheral LH were made, the data were normalized to the first detected rise in the preovulatory surge of LH (i.e., the ascending limb). This initial elevation was defined as the first serum sample collected with a concentration of LH at least 2 standard deviations greater than the mean of the previous baseline concentrations of LH. The preovulatory surge of LH in the ewe is approximately 12 h in duration, with the peak normally occurring during the first 12 h of estrus (Niswender et al., 1968). Differences in levels of steroid hormones in follicles and sera due to time of collection were evaluated by

one-way analysis of variance and least significant difference.

## RESULTS

A fresh ovulation point was observed in each of 2 animals at laparotomy 24 and 28 h after the first detected rise in LH. The newly-ovulated structures were dissected from the ovaries and analyzed for content of steroid hormones. Based on a visual appraisal, it appeared that in both instances the follicular wall had begun to luteinize. No attempt was made to separate the thecal and granulosal layers of these structures and therefore the hormonal data for these tissues were not used in any statistical comparisons.

The total content of progesterone within the thecal tissue, granulosal tissue and fluid of the preovulatory follicle did not vary significantly either before or during the first 12 h following the onset of the surge of LH. However, in all these follicular components there was a rise ($P<0.05$) in content of progesterone at 16 and 20 h. Subsequent to follicular rupture, the increased tissue content of progesterone appeared to be sustained (Fig. 1). Over these same times there were no significant changes in either ovarian or systemic venous concentrations of progesterone (Fig. 1).

The patterns of change in follicular contents of testosterone and estradiol-17$\beta$ were strikingly different from that of progesterone. Testosterone was at its highest in both follicular tissues and fluid collected prior to a detected increase in serum LH, remained elevated in follicular samples collected at the time of the first detected rise in LH (0 h), then declined ($P<0.05$) in 4-h samples. At 8 h, follicular testosterone decreased ($P<0.05$) further to an apparent baseline (Fig. 2). Similar alterations in ovarian venous concentrations of testosterone were found (Fig. 2). Testosterone was consistently undetectable in 1 ml jugular serum. Thecal layer, granulosal layer and follicular fluid contents and ovarian venous serum concentrations of estradiol-17$\beta$ were maximal prior to the surge of LH. Unlike the situation for testosterone, estradiol-17$\beta$ had already declined ($P<0.05$) in the samples obtained at the beginning of the surge of LH (0 h). Levels continued to decrease in samples collected subsequently and, by 8 h after the surge of LH commenced, had reached a low point ($P<0.05$). Follicular and ovarian venous estradiol-17$\beta$ were depressed through the time of ovulation (Fig. 3). A statistically significant decrease ($P<0.05$) in peripheral venous concentrations of estradiol-17$\beta$ was not exhibited until 8 h and thereafter (Fig. 3).

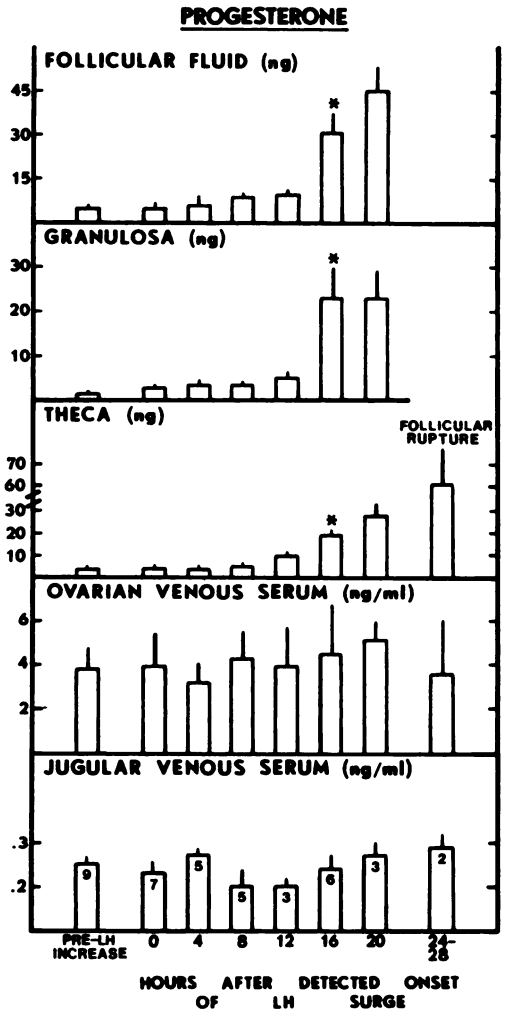


FIG. 1. Changes in follicular fluid and tissue contents and in ovarian and jugular venous sera concentrations of progesterone at corresponding times before and after the surge of LH in sheep. Numbers within the bars denote the number of animals/group. In the 0-h group, follicular and sera samples were collected at the time of the first detected elevation of serum LH. Ovulation occurred in two ewes, 24 and 28 h after the first detected rise in LH. Means + standard errors are plotted. An *asterisk* indicates a difference ($P<0.05$) from the previous time point.

## DISCUSSION

In the present study in the ewe, a dramatic change in the steroidogenic profile of the

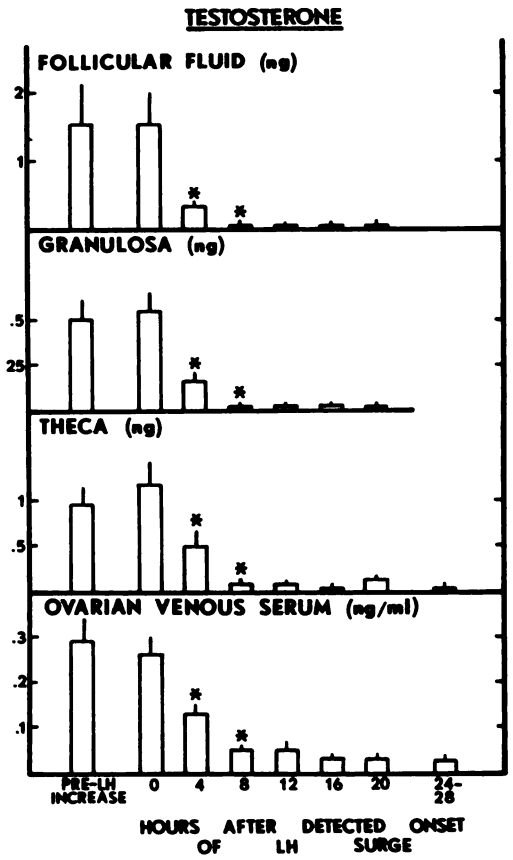


FIG. 2. Changes in follicular fluid and tissue contents and in ovarian venous serum concentrations of testosterone at corresponding times before and after the surge of LH in sheep.

preovulatory follicle was observed after the surge of LH and prior to the time of ovulation. Immediately before the surge the follicle was predominantly an estrogen-producing gland. Since follicular progesterone remained low and did not vary over these times, the $\Delta^5$ steroid hormone biosynthetic pathway was apparently favored. The rapid drop in follicular and blood levels of estradiol-17$\beta$ which occurred in close association with the start of the surge of LH is in agreement with the previous findings that blood levels of this hormone fell attendant with an increase in preovulatory LH in this species and in others (see *Introduction* for references). Such a decline in estrogen could be the consequence of a direct inhibitory effect of LH on aromatase activity (Katz and Armstrong, 1976) or the result of diminished synthesis of testosterone (Hamberger et al., 1978). Both possibilities exist in the ewe. The fact that estradiol-17$\beta$ declined at a time when the highest levels of testosterone prevailed (at the first detected increase in LH) may indicate, that at least initially, LH was suppressing the conversion of testosterone to estradiol-17$\beta$. Nevertheless, the sustained fall in follicular estradiol-17$\beta$ was accompanied by a similar decrease in testosterone. Thus, at this later time, lack of substrate availability for estrogen synthesis could have been the limiting factor. Because the absolute levels of follicular testosterone (ng) were low in comparison to estradiol-17$\beta$ (Fig. 2 vs. Fig. 3), it is assumed that the testosterone

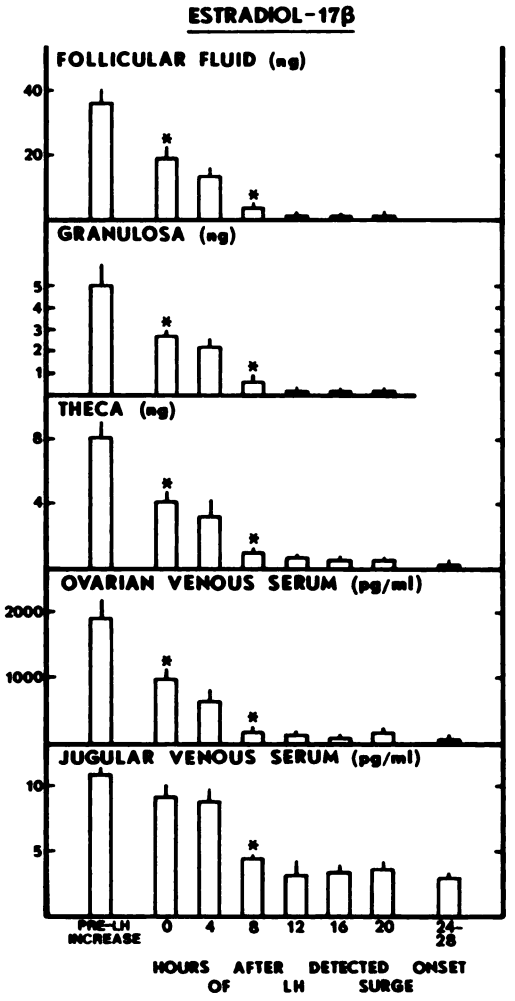


FIG. 3. Changes in follicular fluid and tissue contents and in ovarian and jugular venous sera concentrations of estradiol-17$\beta$ at corresponding times before and after the surge of LH in sheep.

was efficiently aromatized. The predominant estrogen produced by the sheep follicle is estradiol-17$\beta$ (Moore et al., 1969). The data for testosterone in the ewe is inconsistent with the view in the rat (Mori et al., 1977) that testosterone acts as a mediator of gonadotropic involvement in the mechanism of follicular rupture.

The augmented progesterone content of the preovulatory ovine follicle, which appeared to be maintained through the time of ovulation, occurred after the surge of LH and the decline in follicular testosterone and estradiol-17$\beta$. Generally speaking, this lag in production of progesterone contrasts with the rapid blood and/or follicular increases seen in some species in conjunction with the surge of LH. Furthermore, in most of the previously cited studies (see *Introduction*) this progesterone rise was short-lived; progesterone actually decreased again in the follicle and blood before ovulation. It is unknown in the ewe whether the preovulatory elevation in follicular progesterone was merely associated with an early stage of follicular luteinization, or if such a progesterone increase is a prerequisite of follicular rupture. Ovulation was prevented in the rat by treatment with cyanoketone (Lipner and Greep, 1971), an inhibitor of 3$\beta$-hydroxysteroid dehydrogenase, or with antiserum against progesterone (Kohda et al., 1980).

Due to the potential in vivo interaction of follicular cell-types, no definitive statement regarding the cellular site(s) of steroidogenesis can be made from the study at hand. The fact that similar changes in steroid hormones were found within the thecal layer, granulosal layer and follicular fluid indicates that these hormones do accumulate in all these follicular components, regardless of where their particular site of synthesis might have been.

In contrast to the circumstances for testosterone and estradiol-17$\beta$, serum concentrations of progesterone were not indicative of follicular changes in content (i.e., the increase at 16 and 20 h after the initiation of the surge of LH). The reason for the lack in secretion of progesterone into the ovarian vein by the ovine follicle approaching ovulation, or by the newly ruptured follicle, is unknown. Interestingly, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (a potent vasoconstrictor) was elevated within the wall of the preovulatory follicle of the ewe at 16–20 h after the onset of the surge of LH (Murdoch et al., 1981). Although speculative, an inhibitory action of $PGF_{2\alpha}$ on local thecal blood flow during the later stage of the preovulatory period could be responsible for the failure of accumulated progesterone from being transported away from the follicle and into the ovarian drainage system (in this respect, reduced blood flow could also persist within the young corpus luteum). Indeed, at this time blood steroid hormone levels (progesterone as well as testosterone and estradiol-17$\beta$) may represent basal secretion from ovarian tissues other than the preovulatory follicle. This may be possible since levels of progesterone in ovarian venous effluent of estrous sheep were not very much unlike those found in anestrous sheep (Murdoch and Inskeep, 1981). In the ewe, ovarian stromal tissue was steroidogenically inactive (Moor et al., 1975). However, in some species, such as the rabbit (Hilliard et al., 1967) and hamster (Saidapur and Greenwald, 1978b), the ovarian interstitium may be a major source of circulating steroid hormones. Thus, the common assumption that the primary source of blood progesterone in those species exhibiting a preovulatory peak is the follicle (Mikhail, 1970; Friedrich et al., 1974; Hillensjo et al., 1976; Patwardhan and Lanthier, 1976; Szoltys, 1976; Hamberger et al., 1978) may not be entirely correct. Curiously, and in opposition to this, and other studies in the ewe done with the preovulatory ovary in situ (Short et al., 1963; Moore et al., 1969; Bjersing et al., 1972; Murdoch et al., 1981), Wheeler et al. (1975) reported a preovulatory increase in secretion of progesterone from ovaries autotransplanted to the neck.

It is concluded that during the preovulatory phase of the ovine estrous cycle, and in association with the surge of LH, there is a distinct shift in steroid hormone production by the dominant follicle—from a state of estrogen to progesterone dominance. This transition was characterized by an intermediate period of relative steroidogenic quiescence. Data for venous estradiol-17$\beta$ is in accordance with the contention that the preovulatory follicle is the source of the peripheral peak in blood estrogen which precedes the surge of LH (Hay and Moor, 1975; Moor et al., 1975). However, the rise in follicular progesterone seen just prior to ovulation was not represented by analogous changes within sera. It is therefore apparent that care must be taken when formulating strict conclusions regarding follicular production of steroid hormone based solely on the measurement of a blood parameter.

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