In vitro Synthesis of Progesterone by Swine Corpora Lutea.* (25711)

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Nearly 4 centuries have elapsed since original morphologic descriptions of the corpus luteum were made by sixteenth century anatomists(1). During this interval endocrine relationships have been established between this organ and various aspects of reproduction, including lactation. It is well known that time between periods of estrus in polyestrous animals is dependent upon secretory activity of corpus luteum. However, factors responsible for development, maintenance and regression of the corpus luteum need to be determined because findings of current research argue against the theory that life span of corpora lutea is self-limiting(2). Armstrong and Hansel(3) reported that daily administration of oxytocin to post-puberal non-lactating heifers, during first week of estrous cycle, resulted in marked shortening of the diestrual period. They concluded that observed effects of oxytocin were caused by inhibition of normal corpus luteum function. This report presents a method for *in vitro* evaluation of corpus luteum which may prove useful in elucidation of control of this organ.

Materials and methods. Cross-bred gilts, weighing 225-300 lb, were checked daily with vasectomized boars to determine occurrence of estrus and duration of estrous cycles. Average duration of estrous cycles in gilts is 21 days and pregnancy is 113 days. Animals for pregnancy studies were mated one to 3 times. Ovaries were removed at slaughter, corpora were dissected and placed in chilled containers. Average number of corpora lutea was 14, range 10 to 20/animal. Five hundred to 800 mg of representative tissue slices with maximum thickness of 0.3 mm were transferred to 20 ml beakers containing Krebs Ringer bicarbonate buffer medium (pH 7.4) with 200 mg % glucose at 1 ml/100 mg tissue. Slices were incubated 2 hours in Dubnoff Metabolic Shaking Incubator at 37.5°C under atmosphere of 95% O_2 and 5% CO_2. Gas flow was 4 c.f.h. Preliminary work indicated that progesterone was synthesized by luteal tissue throughout incubation of 6 hrs. Control samples for determination of initial endogenous progesterone concentration were prepared at the same time as those to be incubated but were extracted immediately with 95% ethanol. Following incubation, tissue was homogenized in medium and extracted 3 one hour periods with 75 ml of 95% ethanol at 75°C. Pooled ethanol extracts were evaporated under reduced pressure at 60°C for column adsorption chromatography. A column was prepared by pouring a suspension of 4.5 g of aluminum oxide (Merck 71707) in n-hexane,† into chromatographic tube 10 mm diameter and 300 mm long. The residue was dissolved in 20 ml of n-hexane and applied to the column, followed by two 15 ml rinses. Column development and subsequent countercurrent distribution of eluate fraction containing α, β unsaturated steroids followed procedure of Loy *et al.*(4). Solutions contained in center 5 funnels, theoretically containing 93% of progesterone introduced in first funnel, were combined and subjected to paper partition chromatography to preclude possibility of contaminating substances. The solvent systems were those of Bush(5) and Savard(6). Area of chromatogram containing progesterone, as identified by ultraviolet scanning and a reference chromatogram, was eluted and concentration calculated by equation of Allen(7) from observed absorbancies at 230, 240 and 250 mμ. A standard curve was prepared from absorbancy readings of progesterone in 95% ethanol ranging from 1 to 30 μg/ml. Molar absorbancy index (ϵ) was 16,700 at 240 mμ. Average recovery value of 84.7% was obtained by adding 25 to

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† Technical grade, Phillips Petroleum Co., Bartlesville, Okla.

TABLE I. *In Vitro* Synthesis of Progesterone by Swine Corpora Lutea.

Day	No. of animals	Avg wt total luteal tissue per animal, g	Progesterone concentration, μg/g			Avg luteal progesterone per animal, μg
			Initial	After 2 hr incubation	Increase	
Of cycle						
4	3	1.11	21 ± 5.4*	158 ± 17.4*	137	23
8	3	5.32	40 ± 4.3	113 ± 17.7	73	213
12	4	5.49	61 ± 4.3	135 ± 13.3	74	335
16	3	4.20	74 ± 3.1	146 ± 22.9	72	311
18	3	1.40	0	0	0	0
Of gestation						
16	3	5.82	82 ± 3.0	114 ± 12.1	32	477
24	4	5.18	84 ± 4.7	145 ± 24.1	61	435
48	4	5.50	105 ± 7.6	141 ± 6.4	36	578
72	4	5.30	68 ± 4.3	106 ± 14.5	38	360
96	3	5.61	52 ± 3.1	98 ± 8.6	46	292
102	3	5.71	21 ± 9.2	68 ± 13.9	47	120

* Mean ± S.E.

100 μg of progesterone to 8 different tissue samples ranging 400 to 600 mg. Total hormone content of these samples ranged from 55 to 206 μg.

Results. Data in Table I show average weights of luteal tissue/animal and progesterone concentrations, prior to and following 2 hour incubation period. Slices of swine corpora lutea were capable of synthesizing progesterone *in vitro* whereas homogenates of the organ did not produce the hormone under similar conditions. Furthermore, little if any, progesterone diffused into incubation medium from the tissue. Enzymic nature of the reaction is shown by complete inhibition of synthesis when luteal tissue was heated at 95°C for 30 minutes. Intermediate rates of biosynthesis were observed at room temperature (28°C). For example, tissue which synthesized 44 μg/g during 2 hr incubation synthesized 25 μg/g during 6 hr at room temperature. Similarly, tissue synthesizing 70 μg/g during incubation synthesized 12 μg/g during 2 hr at room temperature.

Pregnenolone, a probable precursor in biosynthesis of progesterone, was added to medium in concentrations ranging 117 to 794 μg/g of luteal tissue. Progesterone synthesis increased to 208% above incubated controls in samples containing pregnenolone. Maximum conversion of pregnenolone to progesterone during 2 hr incubation was 74%. DPN also stimulated progesterone synthesis. Concentration of progesterone was increased by 89 μg/g when 6 μM of DPN was added. Addition of 500 μg of pregnenolone to another aliquot of tissue increased concentration 95 μg/g. Addition of both 500 μg of the precursor and 6 μM of DPN to another tissue aliquot increased progesterone concentration 235 μg/g.

Discussion. In evaluating functional aspects of the corpus luteum it is desirable to consider briefly morphologic development and retrogression of the organ(8). During week following ovulation, corpora lutea attain a diameter of 8-10 mm. If the animal is pregnant there is further growth until an average diameter of 10-11 mm is reached. Histologically it has not been possible to distinguish between corpora of the active luteal phase of non-fertile cycle and those of early pregnancy. At approximately day 16 of cycle, a sudden change occurs in appearance of corpora lutea in non-pregnant animals. By day 18 the diameter decreases to 6 mm and color changes from pink of active capillary circulation to whitish of scar tissue indicating retrogressive changes in the nature of fibrous involution. Eventually all that remains of the site of an ovarian follicle, and subsequently a corpus luteum (of either a nonfertile cycle or of pregnancy), is a small mass of scar tissue, a corpus albicans.

Concentration of progesterone in luteal tissue increased from 21 μg/g at day 4 to 74 by day 16 of the cycle. However, at day 18 (late diestrum) no detectable progesterone was present. Increase in hormone concentration was found after 2 hr incubation with the ex-

ception of tissue from day 18. The greatest increase, 137 μg/g, was observed in luteal tissue from animals at day 4 of cycle. This increase was nearly twice that found in tissue from animals at days 8, 12, and 16. Initial progesterone concentrations in corpora lutea from animals at days 16, 24 and 48 of gestation were higher than those observed in tissue from gilts during the cycle. However increase in progesterone concentration following incubation was greater in corpora lutea from non-pregnant than from pregnant animals. A difference of 8 μg/g between mean initial progesterone concentration at day 16 of cycle and day 16 of pregnancy, is not statistically significant at 5%. However, increases in concentration following incubation within each of these 2 stages are statistically significant at 5%. Data summarized in Table I indicate a decrease in hormone concentration during latter part of pregnancy. The average quantity of luteal progesterone/animal was 23 μg at day 4 of cycle, 335 μg at day 12 and 0 by day 18. The greatest amount of luteal progesterone/animal during pregnancy was 578 μg at day 48. This quantity decreased to 120 μg by day 102. Morphologic development and regression of the corpus luteum parallel capacity of luteal tissue to synthesize progesterone as indicated by results of this study.

Kimura and Cornwell(9), using ovariectomized rabbit assay of Allen(10), reported a similar trend for progestin concentration in corpora lutea of pregnant sows during last stages of gestation. They reported a concentration of 50 μg/g in sows pregnant 40 to 75 days. Loy *et al.*,(11) investigated progesterone concentration in corpora lutea from gilts slaughtered at 70th day of gestation. Hormone concentration ranged from 20 to 91 μg/g. Their data are in agreement with the range of 36 to 76 μg/g reported by Allen(10). Elden(12) using the Allen assay found a range from 41 μg/g during early pregnancy to 16 μg/g in late gestation in the sow. Gawienowski(13) used a chemical procedure and reported progesterone range of 5.9 to 37.9 μg/g of corpora lutea from sows 55 days pregnant. Short(14) reported progesterone concentration in plasma of sows during luteal stage of cycle and at day 90 of pregnancy as 0.83 and 0.34 μg/100 ml respectively. No progesterone was detected 4 days postpartum.

Summary. A method is described for investigation of *in vitro* synthesis of progesterone by swine luteal tissue Heating tissue for one half hour at 95°C inhibited *in vitro* production of hormone. Addition of pregnenolone and DPN to tissue medium increased progesterone synthesis. Average weight of luteal tissue/animal was 1.11 g at day 4 of estrous cycle, 5.49 at day 12 and 1.40 at day 18. Average weight of corpora lutea during gestation was relatively constant. During estrous cycle, concentration of progesterone in luteal tissue increased from 21 μg/g at day 4 to 74 μg/g by day 16. At day 18 no detectable hormone was present. An increase in hormone concentration was found after 2 hr incubation in Krebs Ringer bicarbonate buffer medium except with tissue from day 18; greatest increase was observed in tissue from day 4 of cycle. During gestation, initial progesterone concentrations in luteal tissue from animals at days 16, 24 and 48 were higher than those in non-pregnant animals. However, increase in progesterone concentration following incubation was less in corpora lutea from pregnant than from non-pregnant animals.

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