

PERIPHERAL PLASMA PROGESTERONE LEVELS IN PIGS DURING THE OESTROUS CYCLE

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Summary. Progesterone levels were determined in the peripheral plasma of four gilts on each day of the oestrous cycle. An initial rise was observed at Day 3 or 4 of the cycle (oestrus = Day 1) followed by a very rapid increase up to Day 7 or 8 and a slower rate of increase until Day 14 or 15 of a 20-day cycle. The highest levels of progesterone during the luteal phase were about 35 ng/ml plasma and the average concentration during Days 10 to 15 was approximately 27 ng/ml plasma. The decline in progesterone levels after Day 15 was precipitous, a thirty-fold decrease occurring in most cases within 48 hr. Progesterone levels remained low (about 0.5 ng/ml) for about 7 days during the phase of follicle growth and ovulation.

A rather consistent time interval (7 days) was observed between the decline in progesterone concentration and the onset of overt oestrus. The high levels of circulating progesterone in the gilt may suppress follicle development sufficiently during the luteal phase so that approximately 1 week is required between corpus luteum regression and ovulation.

The concentration of plasma progesterone in nine castrated boars was 0.9 ng/ml suggesting an extra-gonadal source of progesterone, possibly adrenal in origin.

INTRODUCTION

An adaptation of the technique described by van der Molen & Groen (1965), whereby progesterone was measured as a chloroacetate derivative using gas-liquid chromatography (GLC) with electron capture detection, enabled progesterone levels in the peripheral plasma of the cow to be determined daily during the oestrous cycle (Stabenfeldt, Ewing, Patton & McDonald, 1969a; Stabenfeldt, Ewing & McDonald, 1969b). A close correlation was demonstrated between peripheral plasma progesterone concentration and corpus luteum (CL) function. It thus appeared feasible to study CL function in other domestic animals by this approach. This report presents changes of progesterone levels in the peripheral plasma of gilts during the oestrous cycle.

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MATERIALS AND METHODS

Animals and collection of blood

Four Yorkshire gilts were used in this study with three of the animals being litter mates (Nos. 241, 244 and 245). Oestrous cycles of normal length were observed in all gilts before the start of the experiment. A diet of mixed grain with unrestricted water was calculated to maintain the gilts at a weight of about 125 kg. The animals were housed outdoors with access to indoor shelter; a portion of the pen was shaded. As samples were collected during July 1967, the animals were cooled by an electric fan as well as by water spray. Signs of thermal stress were not evident during the experiment. Sexual receptivity was determined daily by the use of a vasectomized boar with the first day of receptivity designated as Day 1 of the oestrous cycle. Blood was obtained each morning before 07.00 hours by anterior vena cava puncture from animals held in the standing position (Carle & Dewhirst, 1942); local anaesthetic was not used. Blood (25 to 50 ml) was collected in 5 ml 10% potassium oxalate using a 50-ml syringe. After measuring the volume of the sample and haematocrit determination, the plasma was removed by centrifugation for 20 min at 0° C. Progesterone was extracted from plasma samples within 24 hr after collection. Of ninety-six plasma samples, sixty-four were analysed in duplicate giving a total of 160 determinations. It was found that progesterone concentration could be determined daily for most of the oestrous cycle by using 10 ml plasma for each determination; as little as 5 ml could be used from Day 5 to Day 15.

Blood obtained at slaughter from nine castrate male pigs was analysed for progesterone concentration to assess the non-gonadal endocrine contribution.

Progesterone determination

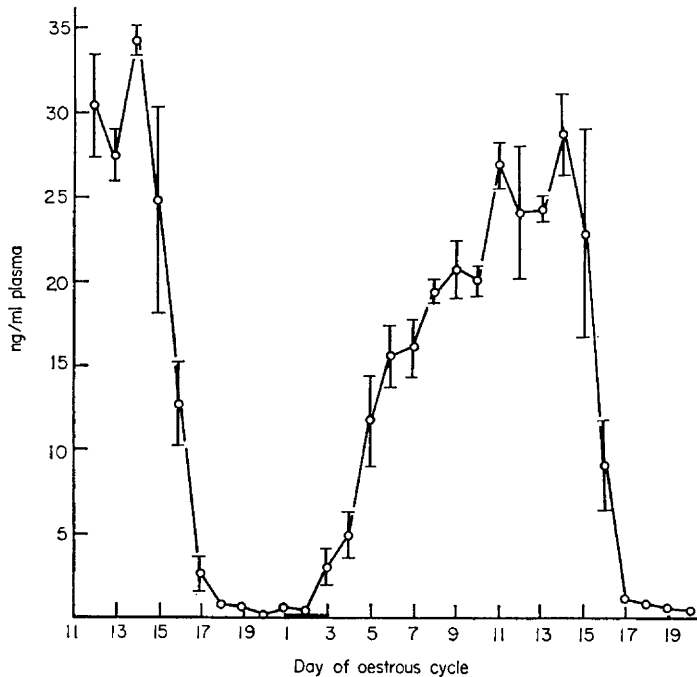
The method used to measure progesterone was adapted (Stabenfeldt *et al.*, 1969a) from the chloroacetate derivative technique reported by van der Molen & Groen (1965). The basic steps included (1) extraction of progesterone from plasma with dichloromethane, (2) saponification of the solvent residue, (3) isolation of progesterone by thin layer chromatography (TLC), (4) enzymatic conversion of progesterone to 20 β -hydroxypregn-4-en-3-one, (5) acetylation of 20 β -hydroxypregn-4-en-3-one with monochloroacetic anhydride, (6) isolation of 20 β -hydroxypregn-4-en-3-one monochloroacetate by TLC and (7) estimation by GLC with electron capture detection and by liquid scintillation spectrometry.

A trace amount of [7-³H]progesterone was added initially to the plasma to determine procedural losses while testosterone chloroacetate was added before GLC to monitor losses incurred during GLC. No progesterone was found in water blanks containing [7-³H]progesterone (0.2 ng). Chemical identification of the monochloroacetate derivative of 20 β -hydroxypregn-4-en-3-one was by mass spectrometry for which a molecular weight of 392 was observed (Waller, 1967).

RESULTS

Blood was taken daily from gilts Nos. 241, 244 and 245 for 29 days without

ill-effects as judged by gross appearance, appetite and haematocrit values. Plasma progesterone levels during the cycle were similar in these animals, the standard error of the mean for each day of the oestrous cycle being relatively small (Text-fig. 1). In general, an initial rise in progesterone levels was observed at Day 3 or 4 of the cycle followed by a very rapid increase until Day 7 or 8 and a slower rate of increase until Day 14 or 15 of a 20-day cycle. Peak luteal progesterone levels were about 35 ng/ml plasma. The decline in progesterone levels after Day 15 was precipitous with a thirty-fold decrease occurring in most cases within 48 hr. Progesterone levels remained low (about 0.5 ng/ml) for about 7 days during the phase of follicle growth and ovulation. The results observed in each animal are shown in Text-fig. 2.



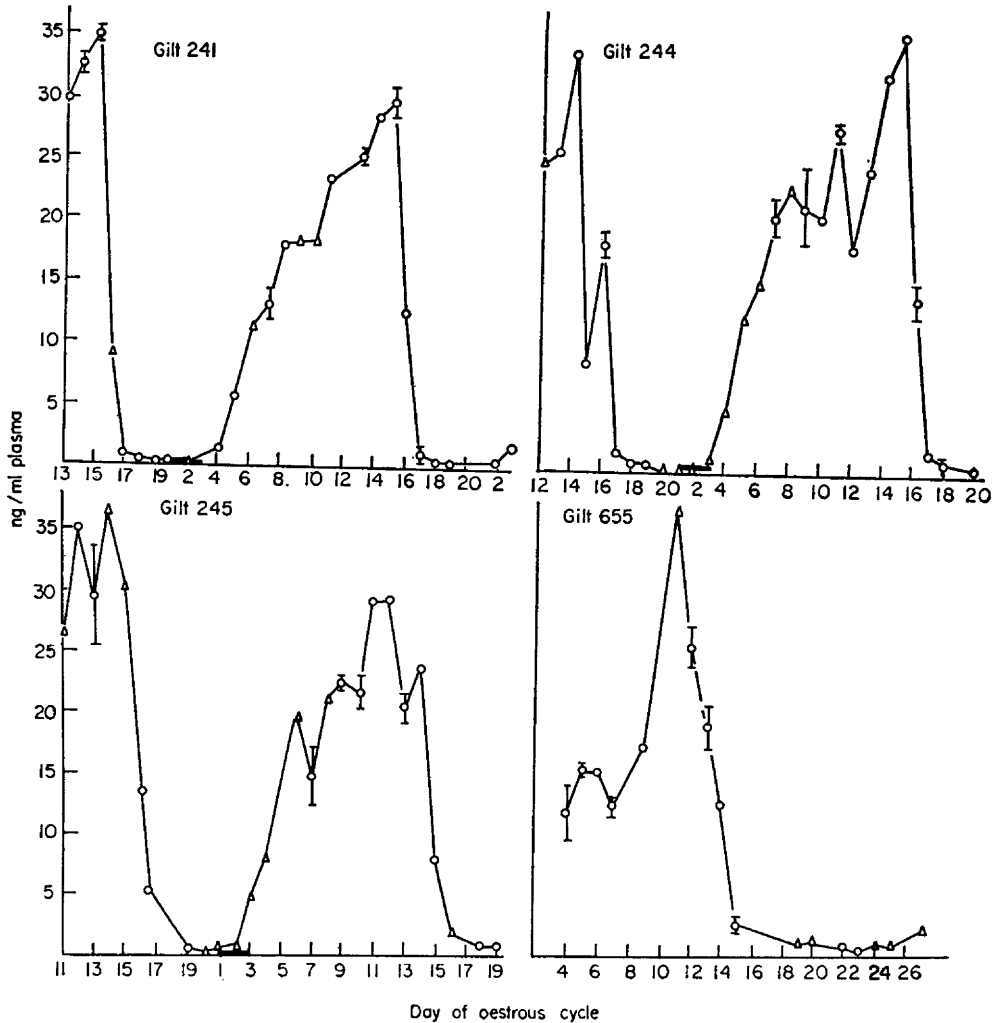
TEXT-FIG. 1. Progesterone concentration (ng/ml) in jugular venous plasma during the oestrous cycle of the pig (oestrus = Day 1). The data represent the average values obtained in three non-pregnant, cyclic gilts sampled daily for 30 days. Vertical bars represent the standard error of the mean.

Gilt No. 655 resisted blood collection strenuously throughout the experiment; she became lame and anorectic about 2 weeks after the start of the experiment. Blood collection was continued for another 10 days until it became apparent that oestrus had been delayed. Progesterone levels reached a peak on Day 11 followed by a decline in concentration until Day 15 (Text-fig. 2). The cyclic pattern of progesterone in this animal suggests that CL function, once established during the oestrous cycle, is not terminated by outside factors such as stress. The stress of blood collection, however, did have a deleterious effect on the occurrence of the next oestrous cycle.

Plasma progesterone levels observed in castrated boars averaged 0.9 ± 0.25

ng/ml (mean \pm S.D.). The level in one animal was exceptional, with a value of 3.7 ng/ml. These levels are slightly higher than those observed in the gilts during the follicular phase of the oestrous cycle (0.5 ng/ml plasma).

The 95% confidence interval on duplicate samples containing less than 1.0 ng progesterone/ml plasma was $\bar{x} \pm 0.3$ ng where \bar{x} was the average of two duplicate



TEXT-FIG. 2. Progesterone concentration (ng/ml) in jugular venous plasma of individual gilts during the oestrous cycle (oestrus = Day 1). Vertical bars indicate the range of duplicate determinations. O, Duplicate determinations; Δ , single determinations.

samples, $S_{\bar{x}}$ was the estimate of the standard deviation of the mean of two duplicate samples and t was Student's $t_{0.05}$ associated with fifteen degrees of freedom (number of duplicate sets). The 95% confidence interval on duplicate samples containing 1 to 35 ng progesterone/ml plasma was $\bar{x} \pm 1.9$ ng ($n = 49$).

The presence of yellow pigments in bovine plasma (apparently carotenoids

of dietary origin) have complicated the initial isolation of progesterone on TLC (Stabenfeldt *et al.*, 1969a). In contrast, fewer pigments were found in the extraction residue of pig plasma. An effective separation of progesterone from the pigments by TLC was obtained by the use of the solvent system, benzene: ethyl acetate (4:1, v/v).

DISCUSSION

Cyclic patterns of peripheral plasma progesterone have been reported during the oestrous cycle of the guinea-pig (Feder, Resko & Goy, 1968), cow (Plotka, Erb, Callahan & Gomes, 1967; Schomberg, Coudert & Short, 1967; Gupta & Pope, 1968; Stabenfeldt *et al.*, 1969b), sow (Tillson & Erb, 1967), ewe (Plotka & Erb, 1967) and the menstrual cycle of monkeys (Neill, Johansson & Knobil, 1967) and women (van der Molen & Groen, 1965; Neill, Johansson Datta & Knobil, 1967). Tillson & Erb (1967) found about 7 ng of progesterone/ml plasma at oestrus in the sow which increased to 25 ng at Day 10 of the cycle. In the present study, values ranged from about 0.5 ng at oestrus to 35 ng/ml at Day 14 or 15 of the cycle with an average of 27 ng during the luteal phase. The discrepancy of the follicular phase values may be due to the presence of high blank values sometimes associated with the double isotope derivative technique used by Tillson & Erb (1967). Short (1957, 1961) found 17.0 and 16.2 ng of progesterone/ml plasma in two sows during the luteal phase of the oestrous cycle; these levels are comparable to those found in this study at about Day 7 or 8 of the cycle.

A cyclic pattern of progesterone concentration has been observed in the ovarian venous effluent of non-pregnant sows (Gomes, Herschler & Erb, 1965). The pattern, slightly skewed toward the early part of the cycle, is similar to that reported here for peripheral plasma. Progesterone concentrations were about thirty times greater in the ovarian venous effluent as compared to peripheral plasma.

Duncan, Bowerman, Hearn & Melampy (1960) and Akins (1968) found that luteal progesterone concentration in the pig remained elevated until Day 15 of the cycle. Other studies, however, on ovarian venous plasma progesterone and/or CL progesterone in the cyclic pig (Rombauts, Pupin & Terqui, 1965; Masuda, Anderson, Hendricks & Melampy, 1967; Cook, Kaltenbach, Norton & Nalbandov, 1967; Brinkley & Young, 1968) have led to the concept that peak luteal function is attained during the early to middle part of the cycle. The data reported in the current study do not support this concept. In general, CL function, as indicated by peripheral plasma progesterone levels, increased continuously until Day 15 of a 20-day cycle. An abrupt drop in progesterone concentration was found on Day 16. Increased ovarian blood flow between Days 7 and 13 may be the reason for the decline in progesterone concentration found in the ovarian venous blood (Masuda *et al.*, 1967) while an increase in the rate of progesterone synthesis and release could compensate for the decline of total luteal progesterone observed. Duncan, Bowerman, Hearn & Melampy (1960), however, found synthesis rates were highest early in the cycle. It appears that ovarian venous progesterone and/or total luteal progesterone concentra-

tions should be viewed with some caution as indicators of the amount of progesterone circulating in the peripheral plasma. The concentration of progesterone in the peripheral plasma is important because it is the best indicator of the amount delivered to the main target organs such as the uterus and mammary glands. There is no evidence at the present time to support the idea of a direct influence of ovarian venous progesterone on the uterus.

The rapid development of functional CL after ovulation in the pig is illustrated by the significant increase in progesterone concentration observed in peripheral plasma on Day 3 of the cycle (Text-fig. 1). As the gilts were in oestrus for approximately 2 days, it can be assumed that ovulation took place on Day 2. Thus, significant increases of progesterone occurred in the peripheral plasma within 24 hr after ovulation.

Bjersing (1967) suggested the development of whorled endoplasmic reticulum in pig CL during the first half of the oestrous cycle (up to Day 10) might be indicative of active progesterone synthesis while its lack of further development (after Day 10) suggested the possibility of a decline in progesterone synthesizing activity. That the development of a stationary phase of growth of endoplasmic reticulum in the second half of the cycle does not indicate lower capability to synthesize progesterone is indicated by the finding of a continuous increase of progesterone in the peripheral plasma up to Day 15 of the cycle.

The secretion of progesterone from sources other than the gonads is suggested by the finding in castrated boars in which a peripheral level of 0.9 ng/ml was found. Blood samples were collected at slaughter. These levels may be higher than those found under normal physiological conditions since a level of 50 ng/ml has been observed in a cow which was 8 months pregnant at the time of slaughter (unpublished observations). This is five to six times higher than the 7 to 10 ng/ml plasma usually observed in the cow (Short, 1961). The stress of killing may have caused a release of progesterone by the adrenal cortex. Recently, Harrison & Heap (1968) showed transplanted adrenals (left carotid artery-jugular vein skin loop) secreted low levels of progesterone throughout gestation in the ewe. Thus the adrenals may not only release large amounts of progesterone during stress, but also may serve as a main source of progesterone in normal physiological situations, for example, the follicular phase of the oestrous cycle.

The longer interval observed between cessation of CL function and manifestation of oestrus in gilts (7 days) as compared with cows (2 to 5 days) (Stabenfeldt *et al.*, 1969b) may be due to the higher circulating levels of progesterone (four to five times greater) in the pig during the luteal phase of the cycle. The result may be a more effective suppression of follicle (or ovum) development in the pig as compared to the cow during the luteal phase. Burger (1952) did find follicles destined for ovulation (pigs) increased slowly in size during most of the cycle with a rapid growth period occurring after CL regression.

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