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EARLY EFFECT OF CONCEPTUS ON PLASMA PROGESTERONE LEVEL IN THE COW

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Progesterone levels in jugular venous blood are being measured by us in cycling (unmated) and inseminated (pregnant or non-pregnant) cows as part of a comparative study of cows with normal and difficult breeding histories. Hawk, Wiltbank, Kidder & Casida (1955) reported a high incidence of embryonic death in cows during the period 16 to 25 days after insemination. The 3rd week of gestation may indeed be a critical phase in the regulation of luteal function, since towards the end of this week regression of the corpus luteum normally occurs in the absence of a conceptus. This note records that in a herd of normal Friesian cattle with a cycle length of 21·2 days $\pm$ 1·5 S.D. the presence of a conceptus in the uterus first exerted a significant effect on the peripheral plasma progesterone level on the 19th day after insemination.

Samples of blood were collected between 07.30 and 08.00 hours from the jugular veins of cows free from venereal infection or demonstrable lesions of the reproductive tract, at known stages of the oestrous cycle and pregnancy. The blood (about 150 ml) was drawn into vessels containing sodium citrate and cooled rapidly to 4° C. The plasma was separated by centrifugation at 4° C and stored at $-18°$ C until analysis, performed usually within 2 weeks. Plasma samples (80 ml) were extracted as described by Lindner, Lunenfeld & Shelesnyak (1967), and the concentration of progesterone in these extracts was determined by the method of Lindner & Zmigrod (1967), which is based on sequential paper- and gas-chromatography with a hydrogen-flame detector, and use of a radio-active internal recovery standard.

Jugular venous blood collected from a cow 7 days after ovariectomy contained a detectable, if very low, concentration of progesterone (0·17 μg/100 ml); 3 weeks after the operation the progesterone level was undetectable ($<$0·15 μg/100 ml). The circulating progesterone present 1 week after ovariectomy was probably of adrenal origin (Balfour, Comline & Short, 1957), and surgical stress may have temporarily enhanced such adrenal progesterone secretion. Release of progesterone from body fat depots may also have been a contributing factor (cf. McCracken, 1963). The reliability of the assay procedure was tested by replicate determinations of the recovery of progesterone added to plasma from the ovariectomized cow. The results (Table 1) indicated that the procedure was satisfactory.

Plasma progesterone concentration (μg/100 ml±S.E.) during Days 10 to 18 after ovulation was similar in cycling cows (0·43±0·035) and in inseminated cows, whether the latter were pregnant (0·48±0·043) or non-pregnant(0·44±

TABLE 1

RECOVERY OF PROGESTERONE ADDED TO PLASMA FROM AN OVARIECTOMIZED COW

No. of samples	*Days after ovariectomy*	*Progesterone (μg/100 ml plasma)*		
		Endogenous	*Added*	*Total found ± S.E.*
7	7	0·17 (pooled sample)	0·50	0·75±0·03
5	21	<0·15 (undetectable)	0·50	0·49±0·02

0·068). However, a striking difference between pregnant and non-pregnant cows was evident on the 19th day after ovulation (Table 2): only two of twelve non-pregnant cows had detectable blood levels of progesterone, and these were very low (0·16 to 0·22); in contrast, eight cows with normal pregnancies

TABLE 2

INFLUENCE OF CONCEPTUS ON PLASMA PROGESTERONE LEVEL IN COWS

Day after ovulation	*Reproductive state*	*No. of cows*	*Progesterone (μg/100 ml plasma±S.E.)*
10 to 17*	Non-pregnant	41	0·43±0·035
	Pregnant	30	0·48±0·048
18	Non-pregnant	7	0·47±0·074
	Pregnant	4	0·47±0·067
19	Non-pregnant	10	<0·15 (non-detectable)
	Non-pregnant	2	0·16; 0·22
	Pregnant	8	0·47±0·03
19	Disintegrating embryo, found Day 19	1	0·16
	Suspected embryonic death (delayed return to oestrus)	2	0·55; 0·67

All samples were collected between 07.30 and 09.00 hours.

* No significant day-to-day differences detected.

all had plasma progesterone levels above 0·34 on Day 19 (mean 0·47±0·03). One cow with a low plasma progesterone level on Day 19 (0·16) was found at autopsy on the same day to carry a disintegrating embryo. Two other cows, which showed elevated progesterone levels on Day 19 (0·55 and 0·67), are believed to have suffered embryonic death at a later date: one returned to heat with a purulent discharge 39 days after insemination, the other 54 days after insemination.

It thus appears that a peripheral plasma progesterone level above 0·3 μg/100 ml on the 19th day after ovulation is indicative of the presence of a live conceptus, if not necessarily a viable one, at that time. This may constitute the earliest non-surgical means of pregnancy diagnosis in the cow. Some caution is required, however, in view of reports that factors other than pregnancy, such as intra-uterine instillation of cultures of *Vibrio foetus* or *Corynebacterium pyogenes* (Coudert & Short, 1966), may result in prolongation of the life of the corpus luteum in sheep. Also, cycle length may be more variable in some herds.

While a divergence of the blood levels of progesterone in pregnant and non-pregnant cows became manifest only on Day 19 after ovulation, it is likely that the 'anti-luteolytic' action of the conceptus is, in fact, initiated several days earlier: this is indicated by the work of Moor & Rowson (1966a, b), who analysed the effects of blastocyst removal or transplantation on various days of the cycle on the life span of the corpus luteum in the sheep.

Preliminary studies by us indicate that plasma progesterone levels were below the limit of sensitivity of our method (<0·15 μg/100 ml) on the day of oestrus, and became measurable only 2 days after ovulation.

Our findings are in accord with those of Armstrong & Black (1966), who observed a precipitous decline towards the 18th day of the cycle, in the ability of bovine corpora lutea to form progesterone *in vitro* from endogenous substrates. Erb & Stormshak (1961) also found a marked decline in the progesterone and total progestin content of bovine corpora lutea just before oestrus. In ewes, a similar drop in the progesterone concentration in ovarian venous blood occurs on the 16th day of the cycle (Edgar & Ronaldson, 1958).

McCracken (1963) studied the effects of enucleation of the corpus luteum and of ovariectomy in two cows on the 12th day of the oestrous cycle: within 30 min the plasma level of progesterone declined from between 0·85 and 0·98 μg/100 ml to less than half its initial level; thereafter the decline was more gradual, but progesterone could not be detected 24 hr after surgery. The limit of sensitivity of the assay was not stated, but appears to have been below 0·2 μg/100 ml.

The plasma progesterone levels during progestation reported in this study are in agreement with those of Sullivan (1960), who found values ranging from 0·25 to 0·65 μg/100 ml in cycling cows, using a spectrophotometric method. However, our values are very much lower than those of Plotka, Erb, Callahan & Gomes (1967), who used a double isotope derivative method and reported progesterone levels of 1·0 to 2·6 μg/100 ml in cows during the oestrous cycle, including the day of oestrus.

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