

Development of Corpus Luteum *Verum* During Multiple Pregnancy in Cattle.*† (28844)

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Studies in the induction of multiple pregnancy in monotocous species by exogenous gonadotropins drew attention to the heavy loss in embryos that occurs in early pregnancy in animals that are stimulated to release additional ova. A relationship has been noted between incidence of early embryonic mortality and number of ova shed following gonadotropin treatment; the higher the number of induced ovulations the lower is the ability of the animal to maintain pregnancy, regardless of the number of embryos that develop in the early stages of gestation. Information is lacking on induced multiple corpora lutea in the cow.

The rate of development of the corpus luteum varies with the species and the reproductive cycle. During the second month of pregnancy in the bovine, the luteal tissue is more compact, and the lutein cells are larger and more spherical in shape, with the cytoplasm being more lightly stained than during the first month of pregnancy(1). The size of the corpus luteum reaches a maximum about the fourth month of pregnancy, whereas the retrogressive changes begin prior to parturition. The first stage of retrogression is marked by fatty degeneration of the lutein cells. This is shown by accumulation in the lutein cells of fat droplets of irregular size which are represented by conspicuous vacuoles in the cytoplasm. The second stage is characterized by reabsorption of the fat and shrinkage of cells, which eventually disappear.

Several morphological, clinical and histopathological studies have been made on the bovine corpus luteum by Warbritton(2),

Bertschneider *et al*(3), Hofliger(4), McDonald *et al*(5), Cupps *et al*(6), Dawson(7) and Erb and Stormshak(8). In dairy cattle, corpora lutea of single ovulations were no heavier at 16 to 89 days (5.9 g) than at 90 to 179 days (5 g) or after 180 days (5.5 g)(9). Moss *et al*(10,11) have undertaken extensive histochemical studies during the estrous cycle and pregnancy. The microscopic structure of the corpus luteum *verum* between 30 to 60 days of gestation is similar to that of the cyclic corpus luteum at diestrus(11). Other studies have dealt with the progesterin content of bovine corpus luteum during the estrous cycle and single pregnancy(12,13,14). Progesterone and Δ^4 -pregnene-20-ol-3-one content of the bovine corpus luteum reaches a maximum at 14 to 16 days of estrus, then declines prior to the following estrus(8). During pregnancy, the average level was 161 μ g at 25 to 34 days and 250 μ g at 37 to 42 days(8). The differences are reflected in concentration, since the weight of the corpus luteum during early pregnancy is essentially constant.

Very few studies have been made of the morphology or histology of multiple corpora lutea. The purpose of this investigation was to study the development of corpora lutea in beef cattle during multiple pregnancy induced by exogenous gonadotropins. An attempt was made to correlate the morphology of corpora lutea with their histological characteristics.

Materials and methods. Nulliparous 1-year-old Hereford heifers weighing from 600 to 1,000 lb were checked twice daily with a vasectomized Holstein bull to detect estrous periodicity. The heifers were divided into 2 groups as follows:

Group 1: Superovulation. The animals were injected subcutaneously 3,000 i.u. of pregnant mare serum (PMS, Equinex) (Ayerst) on day 16 of estrus. They were then

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TABLE I. Weight and Diameter of Bovine Corpora Lutea in Relation to Number of Corpora Lutea per Ovary.

Stage of pregnancy (days)	No. of corpora lutea/ovary	Total No. of corpora lutea measured	Wt of corpus luteum (mg)		Avg wt of corpora lutea/ovary (mg)	Diameter of corpus luteum (mm)	
			Range	Mean		Range	Mean
3	1	6	453- 820	657	657	11-14	12.3
	2-10	35	80-1240	649	3894	7-13	10.2
	11-20	24	72-1343	612	7344	5-14	10.5
	20	90	97- 911	424	15230	7-12	9.0
30	1	7	4005-5240	4752	4752	19-23	20.8
	2	4	3340-3374	3357	6714	19-20	19.5
	3	12	1582-4047	2845	7535	12-20	15.7
	5	10	632-2563	1820	9100	10-18	15.1
45	1	6	2328-4686	3470	3470	17-21	19.3
60	1	3	3962-4970	4326	4326	21-22	21.5
	2	10	900-3304	2994	5988	19-22	18.1
	3	6	1951-2239	2053	6159	16-17	16.7
	4	8	2246-2940	2707	10828	18-20	19.3
	7	7	330-2500	1237	8659	9-18	13.7
90	1	6	3117-4570	3839	3839	19-22	20.5

injected with 2,000 i.u. of human chorionic gonadotropin (HCG, Upjohn) 4 days later. At the following estrus the animals were bred to a fertile bull and inseminated with frozen semen. Three to 4 days after the HCG treatment the ovaries were palpated *per rectum* to detect the incidence of ovulation. The animals were autopsied 3 days *post ovulation*.

Group 2: Mild doses of gonadotropins: The animals were injected with 1,500 i.u. of PMS and in a few cases with 1,000 i.u. of HCG 4 days later. The animals were bred to a fertile bull, inseminated with frozen semen and palpated *per rectum* to detect ovulation and to diagnose pregnancy. They were then autopsied 30, 45, 60 and 90 days *post ovulation*.

The corpora lutea were removed from all ovaries and dissected free from the adjacent connective tissue. The average diameter and weight of each corpus luteum were recorded. A portion of the corpus luteum was fixed in 10% formalin, sectioned serially at 5 μ and stained with hematoxylin-eosin, VanGieson's or Mallory. Four to 6 slides were examined microscopically to study the types of lutein cell as described by Foley and Greenstein (15), and to measure the maximum size of these cells. The histological characteristics of the corpus luteum were correlated with its size, stage of pregnancy and number of corpora lutea per ovary.

Results and discussion. The number of corpora lutea was not significantly different in the right and left ovary, whether the animals were treated with mild doses (1500 i.u.) or large doses (3000 i.u.) of PMS. In sheep Hunter(16) observed corpora lutea more frequently in the right ovary than in the left.

At 3 days *post ovulation* the weight of corpus luteum in ovaries with one ovulation ranged from 453 to 820 mg with an average of 657 mg (Table I). There was wider variability in the weight of the corpus luteum in ovaries with multiple corpora lutea than in those with single corpus luteum. The right and left ovaries showed a similar variability in the weight of corpus luteum. There was no significant correlation between the weight of the corpus luteum and the number of corpora lutea per ovary (Fig. 1). However, the weight of corpus luteum was appreciably decreased when the number of corpora lutea increased over 20. The weight of all corpora lutea per ovary rose slightly with the increase in number of corpora lutea. The diameter of corpus luteum at 3 days *post ovulation*, in ovaries with a single corpus luteum ranged from 11 to 14 mm with an average of 12.3 mm. There was a slight but insignificant decrease in the diameter of corpora lutea with the increase in number of corpora lutea per ovary.

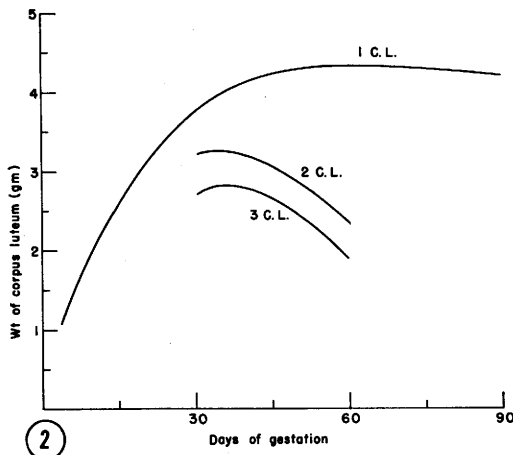
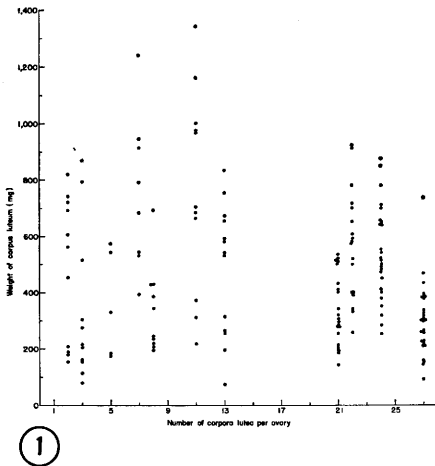


FIG. 1. Weight of corpus luteum, 3 days *post ovulation*, in relation to number of corpora lutea per ovary in cattle superovulated with PMS and HCG.

FIG. 2. Developmental changes in weight of the corpus luteum *verum* in Hereford cattle. Smoothed curves represent 1, 2 or 3 corpora lutea per ovary.

Multiple corpora lutea at 30 to 90 days of pregnancy. At 30 days of pregnancy the weight of the corpus luteum, in ovaries possessing a single corpus luteum, ranged from 4005 to 5240 mg with an average of 4752 mg; this weight was unaffected by stage of pregnancy from 30 to 90 days. However, there was a marked decrease in weight of the corpus luteum with the increase of number of corpora lutea with advancing pregnancy (Fig. 2). The total weight of corpora lutea per ovary rose progressively with their increase in number. At 30 days of pregnancy

the diameter of corpus luteum, in ovaries with a single corpus luteum, ranged from 19 to 23 mm, with an average of 20.8 mm. This diameter was unaffected by the stage of pregnancy from 30 to 90 days. In pregnant animals with multiple corpora lutea there was a slight decrease in the diameter of corpora lutea and a wide variation in the developmental morphology of the corpus luteum (Fig. 3).

Histological characteristics. There was no evidence to show that the histology of multiple corpora lutea was different from that of single corpus luteum. The histological characteristics of the different types of lutein cells and the average size of these cells were not correlated with the weight of individual

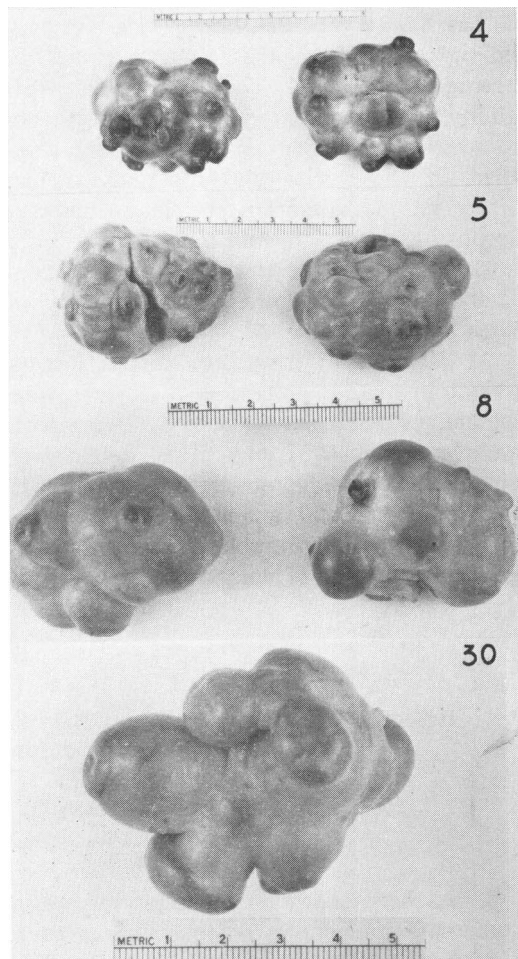


FIG. 3. Bovine ovary with multiple corpora lutea at 4, 5, 8 and 30 days of pregnancy.

corpus luteum, number of corpora lutea per ovary or stage of pregnancy. An increase in lutein cell size from 30 to 90 days of pregnancy as reported by Foley and Reece(1) and Foley *et al*(17) was not observed. Hofliger(4) reported that the corpus luteum at 30 days of pregnancy contained equally-sized colloid inclusions and granulosa cells filled with fatty droplets.

Summary. Nulliparous heifers were injected with 1500 or 3000 i.u. of PMS on day 16 of estrus, bred to a fertile bull and autopsied 3, 30, 45, 60 and 90 days *post ovulation*. The corpora lutea were dissected and the diameter and weight of each were recorded. A portion of each corpus luteum was sectioned serially and examined microscopically. At 3 days *post ovulation* the weight of corpus luteum in ovaries possessing one ovulation, ranged from 453 to 820 mg with an average of 657 mg. There was wider variability in the weight of the corpus luteum in ovaries possessing multiple corpora lutea than in those with single corpus luteum. There was no significant correlation between corpus luteum weight and number of corpora lutea per ovary. The weight of corpus luteum was decreased when the number of corpora lutea increased over 20.

At 30 days of pregnancy corpus luteum weight in ovaries possessing a single corpus luteum, ranged from 4005 to 5240 mg with an average of 4752 mg. This weight was unaffected by stage of pregnancy from 30 to 90 days. There was a marked decrease in weight of the corpus luteum with the increase in number of corpora lutea with ad-

vancing pregnancy. There was a progressive increase in weight of luteal tissue per ovary with increase in number of corpora lutea. The histology of multiple corpora lutea was similar to that of single corpus, and the size of lutein cells was not correlated with the weight of individual corpora lutea, number of corpora lutea per ovary or stage of pregnancy.

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Effect of Cholestyramine, a Bile Acid Binding Polymer, on Vitamin K₁ Absorption in Dogs. (28845)

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It has been shown that cholestyramine, a quaternary ammonium anion exchange resin of high molecular weight, will bind bile acids *in vitro*(1). Several experimental and clin-

ical reports indicate that this resin will lower plasma cholesterol levels in several animal species and in man(2-5). It has been suggested that the lowered plasma cholesterol