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Progesterin in the Pregnant Mare

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The high unitage of gonadotropin in the blood¹ and especially the placenta² of the mare during the second, third and fourth months of pregnancy when the fetus is growing from about 2 to 20 cm. (crown-rump length) would seem to be responsible for the secondary, multiple luteal bodies of the maternal ovaries,³ and the remarkable overgrowth of the fetal gonads.⁴ The primary corpus of pregnancy regresses at about the end of the first month, and from then until about the end of the fourth month multiple corpora are found in both ovaries. Cole and others³ found rupture points in some of the secondary corpora, leading them to the belief that these bodies followed a true ovulation as normally, but histologic studies have led us to believe that the secondary corpora may also be formed by hyperplasia of the theca interna with accompanying degeneration of the ovum and granulosa (Fig. 1). Such theca-luteal bodies may be produced in rodents by injections of pregnant mare's gonadotropin, and appear similar to the aberrant corpora of rhesus monkeys.⁵

The secondary corpora usually regress by mid-pregnancy, and the maternal ovaries become atrophic and fibrotic. It was of interest, therefore, not only to determine the progesterin-content of the corpora lutea, but also to investigate other possible sites of production or storage of that hormone, especially the placenta in the latter half of pregnancy, since some workers^{6, 7, 8} have found progesterin in the human placenta. Unfortunately the work on the detection of pro-

¹ Cole, H. H., and Hart, G. H., *Am. J. Physiol.*, 1930, **93**, 57.

² Catchpole, H. R., and Lyons, W. R., *Am. J. Anat.*, 1934, **55**, 167.

³ Cole, H. H., Howell, C. E., and Hart, G. H., *Anat. Rec.*, 1931, **49**, 199.

⁴ Cole, H. H., Hart, G. H., Lyons, W. R., and Catchpole, H. R., *Anat. Rec.*, 1933, **56**, 275.

⁵ Corner, G. W., Bartelmez, G. W., and Hartman, C. G., *Am. J. Anat.*, 1936, **59**, 433.

⁶ Ehrhardt, K., *Münch. Med. Wchnschr.*, 1934, **81**, 869.

⁷ Adler, A. A., Fremery, P., and Tausk, M., *Nature*, 1934, **133**, 293.

⁸ McGinty, D. A., McCullough, N. B., and Wolter, J. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 176.

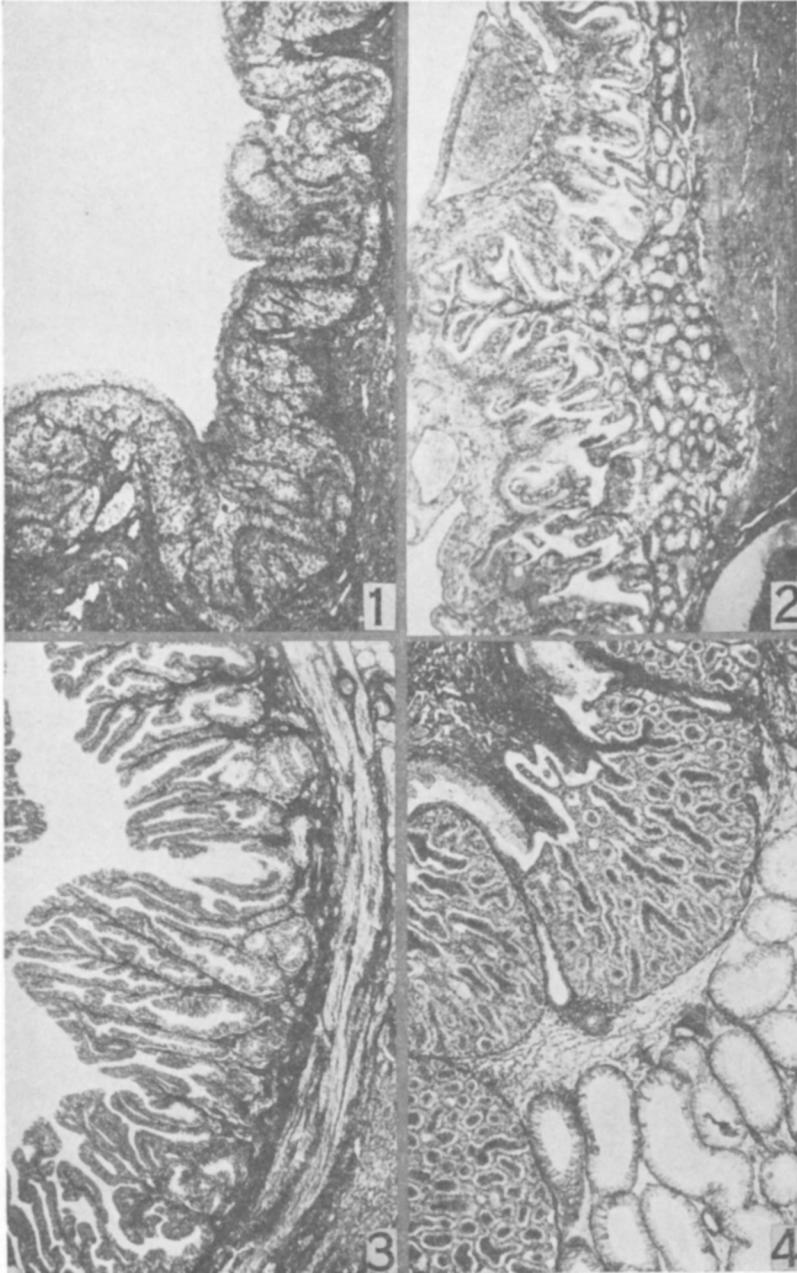


Fig. 1.

Section of the wall of a newly-forming secondary corpus luteum of a pregnant mare (fetal crown-rump length = 10 cm). The thin layer of granulosa (nearest the cavity) shows degenerative changes, but the underlying theca interna is hyperplastic. $\times 48$.

FIG. 2.

Section of chorion-endometrial juncture from a mare with a 17.5 cm. fetus. The endometrium (right) shows villous proliferation not unlike that produced in the rabbit by injections of progestin (see Fig. 3). $\times 48$.

FIG. 3.

Progestational proliferation in uterus of immature rabbit treated with 18.9 mg. (11.7 gm. fresh tissue) of progestin from secondary corpora of a mare with a 10 cm. fetus. Note formation of vascular, primary, and secondary villi, almost to the exclusion of uterine glands. $\times 48$.

FIG. 4.

Section of chorion-endometrial juncture from a mare with an 83 cm. fetus. The uterine glands (lower right) are hypertrophic and distended. No connection could be seen between them and the lumen. $\times 48$

gestational hormone in the adrenal cortex^{9, 10} had not come to our attention until this study was completed.

Through the kindness of Mr. J. H. Rowell, we were able to collect material at his Dublin Canyon abattoir. All of the mares were small, wild mustangs from the southwestern states. The stage of gestation was estimated by the fetal crown-rump length. Ovaries, chorion, endometrium, blood and urine were collected immediately after shooting the animal. Corpora lutea, dissected out of the ovaries, were handled separately in each case, as were the chorions, and separated endometria (see Fig. 2 for the histology of these layers). The blood was citrated, and the plasma used for bio-assay. The urine was collected from the bladder after death.

The tissues and fluids were stored in twice their weight of 95% ethanol, and the finely ground tissues or precipitates extracted with this same ethanol for 4 hours in a Soxhlet. Essentially the same procedure as described by Allen and Meyer¹¹ was used for extracting progestin, hexane being substituted for petroleum ether. The oestrin fraction was separated for future assay. The weighed crude progestin was dissolved in sesame oil, and usually 90% of an individual yield was injected in 5 daily doses into a single 2-month-old female rabbit previously treated for 6 days with 10 R.U. of oestrin daily.^{12, 13} If a strongly positive reaction was obtained, the remaining 10% was tested on another rabbit.

The results included in Table I show that while good yields of progestin were obtained from the corpora, no definite progestational proliferation was observed in any of the rabbits treated with extracts of the other tissues or fluids. This does not necessarily mean that there was no progestin in these tissues. It may have been

⁹ Engellhart, E., *Klin. Wchenschr.*, 1930, **9**, 2114.

¹⁰ Callow, R. K., and Parkes, A. S., *J. Physiol.*, 1936, **87**, 28P.

¹¹ Allen, W. M., and Meyer, R. K., *Am. J. Physiol.*, 1933, **106**, 55.

¹² Allen, W. M., *Am. J. Physiol.*, 1930, **92**, 612.

¹³ McPhail, M. K., *J. Physiol.*, 1934, **83**, 145.

TABLE I.
Assays for Progesterin Carried Out on 2-month-old Rabbits Primed for 6 Days with 10 R.U. Oestrin per Day. The Following Equivalents of the Original Tissues or Fluids Were Injected Over a Period of 5 Days.

C. R. Length cm.	Blood cc.	Reaction	Urine cc.	Reaction	Chorion gm.	Reaction	Endometrium gm.	Reaction	C.L. gm.	Reaction
1	225	—	225	—			49	—	4.9	+
2.5	225	—	225	—			29	—	3.6	+
4							48	—	7.6	+
5	225	—	225	—			49	—	7.6	+
8	225	—	54	—			103	—	19.8*	+
10	225	—	225	—	17	—	72	—	11.7*	+
10									1.1	+
14	225	—	225	—	60	—	69	—	24.3*	+
14									2.4	+
30	405	—	405	—	216	—	153	—	11.7*	+
30									1.1	+
40	360	—		—			310	—	5.8*	+
43	225	—	225	—			159	—	6.9*	+
47	315	—	360	—	342	—	193	—		+
52					70	—	40	—		+
76	90	—	90	—	90	—	90	—	27.0†	+
									None	

*Secondary multiple corpora. †Whole ovaries.

there in small traces masked by oestrin incompletely removed in the extraction procedure. The progestin fractions were not assayed for oestrin. Fig. 3 illustrates the degree of uterine proliferation produced with 18.9 mg. (11.7 gm. of fresh tissue) of crude progestin from the secondary corpora of a mare with a 10 cm. fetus. It will be noted that the surface epithelium has proliferated and formed villi quite similar to those produced in the mare's endometrium as the maternal contribution to the equine placenta (Fig. 2). The endometrial glands of the mare appear to be shut off from the uterine lumen, and late in pregnancy when the oestrin titre is still high they become greatly enlarged and dilated (Fig. 4).

As far as we are aware, no one has previously reported on the progestin content of an individual corpus luteum or "crop" of corpora in the case of any animal. Our results show that as little as 1.1 gm. of a pregnant mare's corpus luteum contains an amount of progestin detectable by the immature rabbit test. The assays reported herein, as well as further data obtained from a study of mixed lots of pregnant mare's secondary corpora, indicate that these structures constitute the best "natural" source of progestin yet discovered.

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First Estrus in Rats in Relation to Age, Weight, and Length.*

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The experiments here reported represent an attempt to determine whether a relation exists between the growth rate and first estrus in the rat.

It has been common knowledge for a decade that rats and mice may be retarded in growth rate and in size at weaning by the amount of available nourishment. Many breeders have found that optimal conditions of growth and size in an animal colony may be attained by limiting the size of the nursing litter to 5 or 6 animals.

Litters of rats were selected for this study over a period of more

* Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council.

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